5A/6A Polymorphism of the Stromelysin-1 Gene and Angiographic Restenosis After Coronary Artery Stenting

Kuan-Rau Chiou[1,4], Sheng-Liang Chung[2], Min-Ji Charng[3,4]*

[1]*Division of Cardiology, Department of Medicine, Kaohsiung Veterans General Hospital,* [2]*Division of Cardiology, Department of Medicine, Lo Tung Poh-Ai Hospital,* [3]*Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, and* [4]*Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.*

Background: Coronary stent deployment is a major advance in interventional treatment, but 20–40% of patients still develop in-stent restenosis (ISR) due to neointimal hyperplasia. Genetic factors play a role in restenosis. This study investigated the frequency of 5A/6A polymorphism in the promoter of the stromelysin-1 gene, and the issue of whether it contributes to restenosis among patients receiving coronary stent in the Chinese population in Taiwan.
Methods: We investigated 344 symptomatic patients after successful coronary stent placement. All patients received repeated angiography after 6 months, or earlier if clinically indicated. Angiographic restenosis was defined as ≥ 50% diameter stenosis at follow-up. Genotyping for stromelysin-1 promoter was based on a polymerase chain reaction technique.
Results: The stromelysin-1 gene promoter genotypes 5A5A, 5A6A, and 6A6A were distributed in 3.5%, 22.7%, and 73.8% of patients, respectively. The frequency of the 6A allele was 0.85. There was no significant difference in angiographic ISR between the non-6A6A and 6A6A groups (28.9% and 37.0%, respectively, $p = 0.165$). However, subgroup analysis revealed a significant difference in patients according to angina status. Among the 5A5A and 5A6A genotype groups, patients with unstable angina had significantly higher ISR rates than those with stable angina (48% vs 21.5%, $p = 0.013$). On the other hand, among patients with stable angina, those with a 6A6A genotype had a higher ISR rate than those with a non-6A6A genotype ($p = 0.029$), making the 6A6A genotype an independent predictor of ISR (odds ratio, 2.57; 95% confidence interval, 1.22–5.41; $p = 0.013$).
Conclusion: There is a low frequency of the stromelysin-1 promoter 5A allele in the Chinese population in Taiwan. How stromelysin-1 5A/6A polymorphism affects ISR appears to be linked to angina status. These results merit further study to identify patients carrying genotypes which put them at increased risk of ISR, and which matrix metalloproteinase inhibitors or drug-eluting stents are more effective for those at risk. [*J Chin Med Assoc* 2005;68(11):506–512]

Key Words: angioplasty, genetics, restenosis, stent, stromelysin-1

Introduction

Percutaneous coronary intervention (PCI) with stent implantation significantly reduces the incidence of complications and restenosis compared with balloon angioplasty alone, via an improved post-procedure luminal diameter and an abrogation of the constrictive remodeling of the artery. However, stent-related arterial injury results in intense inflammatory responses, leading to severe neointimal proliferation and matrix accumulation, which, in 20–40% of patients, ends up as clinically significant in-stent restenosis (ISR).[1–3] Several angiographic and patient-related factors, such as small coronary arteries, lesion complexity, the

*Correspondence to: Dr. Min-Ji Charng, Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, 201, Section 2, Shih-Pai Road, Taipei 112, Taiwan, R.O.C.
E-mail: mjcharng@vghtpe.gov.tw • Received: February 17, 2005 • Accepted: September 13, 2005

presence of diabetes mellitus and acute coronary syndrome, have been identified to be associated with ISR. However, these risk factors cannot fully explain the complex process of ISR.[4] Recent information reveals that genetic factors play a role in the initiation or progression of ISR.[5]

The matrix metalloproteinase (MMP) family and their endogenous tissue inhibitors regulate the accumulation of extracellular matrix, and thus contribute to the rate of growth of atherosclerotic plaques.[6] Stromelysin-1, also known as MMP3, is a key member of the MMP family, with wide substrate specificity, degrading collagen types II, IV and IX, proteoglycans, laminin, fibronectin, gelatins and elastin, as well as having a role in activating other MMPs. Its expression is primarily regulated at the level of transcription.[7,8] In addition, animal study revealed that migration and proliferation of vascular smooth cells and neointimal formation after vessel wall injury of rat carotid arteries was substantially inhibited by antisense oligonucleotides to stromelysin-1 mRNA.[9] Thus, stromelysin-1 is a candidate for influencing vascular remodeling, plaque formation and rupture, and restenosis.

A common adenine insertion/deletion polymorphism (5A/6A) at position –1171 of the human stromelysin-1 gene promoter (National Center for Biotechnology Information SNP identification number rs3025039) influences transcription factor binding and stromelysin-1 promoter activity. *In vitro* promoter activity as well as *in vivo* gene expression of the 6A allele has approximately 50% less promoter strength than that of the 5A allele.[10,11] This lower level of proteolytic activity would favor extracellular matrix deposition because of decreased degradation. Three independent studies found that Caucasian patients carrying the 6A6A genotype had more progression of angiographically detectable lesions in documented coronary artery disease.[12–14] In contrast, increased focal expression and activity of MMP-3 by the 5A allele predispose to plaque instability and rupture in the presence of a high atherosclerotic burden associated with acute coronary syndrome.[15,16] Thus, stromelysin-1 genetic variations contribute to heterogeneity in the presentation and natural history of atherosclerosis.[17] However, little information on the Chinese population has been reported. Accordingly, the purpose of this study was to investigate the allele frequency of stromelysin-1 promoter gene polymorphism (5A/6A) and its possible influences on restenosis after coronary stent implantation in the Chinese population in Taiwan.

Methods

Patient population

The study initially had 435 enrolled patients who underwent successful bare metal stent implantation between January 1999 and July 2002. All stents were implanted using high-pressure adjunct balloon angioplasty (≥ 12 atm) to achieve the targeted stent expansion. All subjects received 300–500 mg ticlopidine (or 75 mg clopidogrel) and 100–325 mg aspirin per day after coronary intervention for at least 3 months. All patients gave written informed consent for the intervention, follow-up angiography and genotype determination. Ninety-one patients (21%) who did not repeat angiography systematically because of advanced age, impaired general condition, or patient preference (without symptoms) were excluded. Therefore, a total of 344 patients were included in the final analysis. The study protocol conformed to the Declaration of Helsinki and was approved by our institution's ethics committee.

Determination of stromelysin-1 genotypes

All blood was collected in Vacutainer EDTA tubes in an overnight fasting state. The stromelysin-1 promoter genotype was analyzed by polymerase chain reaction (PCR) amplification of the genomic DNA extracted from lymphocytes of the stored blood. The sense and anti-sense primers were 5'-GGTTCTCCATTCCTT-TGATGGGGGGAAAGA-3' and 5'-CTTCCTGGA-ATTCACATCACTGCCACCACT-3', respectively. The amplification protocol consisted of an initial denaturation segment at 95°C for 5 minutes. After this, each cycle consisted of 3 segments (94°C for 30 seconds, 61°C for 30 seconds and 72°C for 1 minute). The cycle was repeated 30 times, followed by an additional extension at 72°C for 5 minutes. The amplified fragments were cut with endonuclease TthIII I, which can recognize the sequence 5'-GACNNNGTC-3', in which the DNA template contains 5As (but not 6As) at the polymorphic site. These fragments were then electrophoresed in 4% agarose gel and stained with ethidium bromide.

Angiographic assessment

Quantitative computer-assisted angiographic analysis was performed off-line on angiograms obtained just before and immediately after coronary intervention, and at follow-up, using the automated edge-detection system with CAAS II (Pie Medical, Maastricht, The Netherlands). Operators were unaware of the patients' genotypes. Identical

projections were used for all assessed angiograms. The angiographic parameters determined were interpolated reference diameter, lesion length, minimal lumen diameter, and percent diameter stenosis. Late lumen loss was calculated as the difference between the final post-stenting minimal lumen diameter and minimal lumen diameter measured at follow-up angiography. Lesion morphology was assessed according to the modified American College of Cardiology/American Heart Association grading system, and classified as type A, B1, B2, or C.[18] Lesions of types B2 and C were considered complex lesions. Angiographic restenosis was defined as diameter stenosis of 50% or more on follow-up examination. Patients who had more than 1 lesion treated were defined as having restenosis if at least 1 stented vessel fulfilled the criteria for restenosis.

Statistical analysis

Discrete variables were expressed as counts or percentages, and continuous variables were expressed as mean ± standard deviation. The Chi-squared statistic with Yates' correction, or Fisher's exact test when appropriate, was used to test associations of discrete variables. Student's *t* test and 1-way ANOVA were used to test differences between mean values of continuous variables. Potential associations among clinical, angiographic, or procedural factors, genotypes and restenosis were tested by univariate analysis (Student's *t* or Chi-squared tests). All variables associated with a p of less than 0.1 were entered into a stepwise multivariate logistic regression model to identify independent predictors. The odds ratios (OR) and 95% confidence intervals (CI) were presented for the final multivariate model. Data were prospectively collected and analyzed using SPSS version 11.0 (SPSS Inc, Chicago, IL, USA). A p value of less than 0.05 was considered to be statistically significant.

Results

The main baseline characteristics of the 344 patients (301 men, 43 women; mean age, 67 years; age range, 27–90 years) are listed in Table 1. The stromelysin-1 promoter genotypes 5A5A, 5A6A, and 6A6A were in 12 (3.5%), 78 (22.7%), and 254 (73.8%) patients, respectively. Thus, the frequency of the 6A allele was 0.85. There were no significant differences among the 3 genotypes in terms of baseline clinical characteristics, lesion variables before coronary intervention, and procedural parameters.

Predictors of ISR

The clinical, angiographic and procedural features, and allele and genotype frequencies according to angiographic restenosis among the whole population are summarized in Table 2. Overall, 120 patients had angiographic restenosis (34.9%). There was no significant difference between the non-6A6A and 6A6A groups (28.9% and 37.0% respectively, $p = 0.165$). Hypercholesterolemia, complex lesions (B2 or C), lesion length, minimal lumen diameter, reference vessel diameter immediately before and after, and diameter stenosis were identified as potential risk factors for ISR by univariate analysis. These factors were entered into a multivariate logistic regression model; lesion complexity (OR, 2.67; 95% CI, 1.55–4.61; $p < 0.001$), reference vessel diameter before angioplasty (OR, 0.35; 95% CI, 0.19–0.64; $p = 0.001$), and minimal diameter stenosis (OR, 0.43; 95% CI, 0.23–0.80; $p = 0.08$) emerged as independent predictors of ISR.

Subgroup analyses

We separately examined the putative effect of the subgroup analyses to determine whether there were associations between genotype and ISR regarding age (< 60 or ≥ 60 years), gender, hypertension, diabetes mellitus, smoking, hypercholesterolemia, history of myocardial infarction, presence of unstable angina pectoris on admission, number of diseased vessels, and target coronary vessel. The subgroup analysis revealed that stromelysin-1 polymorphism had a significant influence on patients with unstable angina or stable angina undergoing stent implantation. The other subgroup analyses failed to show significance.

Table 3 shows a significantly higher ISR rate for the 6A6A genotype in patients with stable angina ($p = 0.029$). Furthermore, when a multivariate logistic regression model was built for this group with stable angina, it was still found that homozygosity for the 6A6A genotype was an independent predictor of ISR (OR, 2.57; 95% CI, 1.22–5.41; $p = 0.013$). On the other hand, Table 4 shows that patients with the 5A5A or 5A6A genotypes and with unstable angina had the highest ISR rates. Compared with those with stable angina, there was a significant difference (48% vs 21.5%, $p = 0.013$).

Discussion

The results of the present study showed that polymorphism of the stromelysin-1 promoter 5A/6A gene does not correlate with the occurrence of ISR in the whole study population. However, subgroup

Table 1. Baseline characteristics of 344 patients with 5A/6A polymorphism of the stromelysin-1 genotype*

	5A5A (*n* = 12)	5A6A (*n* = 78)	6A6A (*n* = 254)	*p*
Age (yr)	67.4 ± 8.4	66.9 ± 9.5	66.8 ± 11.3	0.980
Men	10 (83.3)	69 (88.5)	222 (87.4)	0.879
Body mass index	26.5 ± 2.2	26.6 ± 3.2	25.9 ± 3.3	0.247
Hypertension	9 (75.0)	52 (66.7)	231 (90.9)	0.840
Diabetes mellitus	3 (25.0)	26 (33.3)	79 (31.1)	0.829
Hypercholesterolemia	5 (41.7)	26 (33.3)	80 (31.5)	0.743
Previous myocardial infarction	1 (8.3)	26 (33.3)	89 (35.0)	0.160
Current smoker	1 (8.3)	10 (12.8)	39 (15.4)	0.707
Angina status on admission				0.515
Stable	10 (83.3)	55 (70.5)	173 (68.1)	
Unstable	2 (16.7)	23 (29.5)	81 (31.9)	
Number of diseased vessels				0.100
Single vessel disease	1 (8.3)	28 (35.9)	100 (39.4)	
Double vessel disease	4 (33.3)	29 (37.2)	90 (35.4)	
Triple vessel disease	7 (58.3)	21 (26.9)	64 (25.2)	
Stented coronary vessel				0.275
LAD	6 (50.0)	43 (55.1)	122 (48.0)	
LCX	2 (16.7)	7 (9.0)	51 (20.1)	
RCA	4 (33.3)	28 (35.9)	81 (31.9)	
ACC/AHA classification				0.658
A	2	13	42	
B1	4	21	70	
B2	4	25	103	
C	2	19	39	
Lesion length (mm)	11.8 ± 7.2	14.1 ± 7.2	14.0 ± 6.4	0.549
Reference diameter (mm)	2.88 ± 0.68	2.86 ± 0.45	2.89 ± 0.42	0.750
Minimal lumen diameter (mm)	0.77 ± 0.81	0.52 ± 0.37	0.54 ± 0.73	0.189
Diameter stenosis (%)	80.2 ± 14.6	81.5 ± 14.0	81.5 ± 13.6	0.956
Acute gain (mm)	2.33 ± 0.43	2.32 ± 0.64	2.28 ± 0.94	0.939

*Data are presented as mean ± standard deviation or number (%) of patients. ACC/AHA = American College of Cardiology/American Heart Association; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RCA = right coronary artery.

analysis by patients with angina status revealed that there were significantly different effects. Recently, 5A/6A polymorphism and angiographic outcome in patients who underwent stent implantation were examined by Humphries et al[19] and Hoppmann et al.[20] No statistical differences in ISR rates among the whole study population were found. That said, the clinical factors of their study populations, such as age, distribution of risk factors, lesion complexity, and ethnic population, were different from those in our study. In addition, the abovementioned studies did not further explore whether associations existed using subgroup analyses. Furthermore, interaction between stromelysin-1 gene variation and environmental factors plays an important role in clinical significance.[21–23] Therefore, further subgroup analysis for ISR provided valuable information regarding whether it influenced outcomes after coronary stenting.

The reported prevalence of the 5A allele in Caucasians varies from 40% to 50%.[11–15] In this study, the frequency of the 5A allele in the promoter region of stromelysin-1 was only 15%. This allele frequency was similar to that in Chinese patients with normal coronary angiograms in our laboratory (unpublished data, frequency of the 5A allele was 17% and that of the 6A allele was 83%). It was compatible with the Hardy-Weinberg equilibrium. Interestingly, the distribution of the genotype in the Chinese group is similar to that in a Japanese ethnic group[16] and the subjects in the study of Liu et al.[23] These data support the hypothesis that genetic mutations are specific to particular ethnic groups.

Disruption of culprit coronary stenoses in patients who have undergone coronary angioplasty causes inflammatory responses. It triggers more expression of stromelysin-1 by inflammatory cells over unstable coronary plaques than stable plaques. In this regard,

Table 2. Univariate analysis for angiographic predictors of in-stent restenosis in the 344 study patients*

	Restenosis (n = 120)	No restenosis (n = 224)	p
Demographics			
Age (yr)	66.0 ± 12	67.2 ± 10.1	0.313
Male	104 (86.7)	197 (87.9)	0.732
Body mass index	26.1 ± 3.3	26.1 ± 3.2	0.921
Risk factors			
Hypertension	81 (67.5)	150 (67.0)	0.920
Diabetes mellitus	38 (31.7)	70 (31.3)	0.137
Hypercholesterolemia	30 (25.0)	81 (36.2)	0.035
Previous MI	45 (37.5)	71 (31.7)	0.278
Current smoker	23 (19.2)	27 (12.1)	0.074
Angina status on admission			0.140
Stable angina	77 (64.2)	161 (71.9)	
Unstable angina	43 (35.8)	63 (28.1)	
Coronary angiography			
Multivessel disease	78 (65.0)	137 (61.2)	0.483
Stented coronary vessels			0.057
LAD	63 (52.5)	108 (48.2)	
LCX	13 (10.8)	47 (21.0)	
RCA	44 (36.7)	69 (30.8)	
Lesion type†			< 0.001
Simple (A/B1)	26 (21.7)	111 (49.6)	
Complex (B2/C)	94 (78.3)	113 (50.4)	
RD pre-PCI (mm)	2.76 ± 0.39	2.95 ± 0.44	< 0.001
% DS pre-PCI	84.9 ± 13.0	79.6 ± 13.7	0.01
MLD pre-PCI	0.41 ± 0.36	0.62 ± 0.47	< 0.001
Lesion length (mm)	16.0 ± 6.7	12.8 ± 6.3	< 0.001
RD post-PCI (mm)	2.70 ± 0.45	2.93 ± 0.45	< 0.001
Acute gain (mm)	2.23 ± 0.56	2.32 ± 0.99	0.341
Alleles			
5A	26 (21.7)	64 (28.6)	0.165
6A	117 (97.5)	215 (96.0)	0.465
Genotypes			0.364
5A5A	3 (2.5)	9 (4.0)	
5A6A	23 (19.2)	55 (24.6)	
6A6A	94 (78.3)	160 (71.4)	

*Data are presented as mean ± standard deviation or number (%) of patients; †simple lesions were defined as type A or B1 and complex lesions were defined as type B2 or C according to the modified American College of Cardiology/American Heart Association grading system. DS = diameter stenosis; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; MI = myocardial infarction; MLD = minimal luminal diameter; PCI = percutaneous coronary intervention; RCA = right coronary artery; RD = reference diameter.

Table 3. Distribution of the 5A/6A polymorphism in a subgroup of patients with unstable or stable angina

	Patients with unstable angina			Patients with stable angina		
	Restenosis (n = 43)	No restenosis (n = 63)	p	Restenosis (n = 77)	No restenosis (n = 161)	p
Genotypes, n (%)						
5A5A + 5A6A	12 (27.9)	13 (20.6)	0.387	14 (18.2)	51 (31.7)	0.029
6A6A	31 (72.1)	50 (79.4)		63 (81.8)	110 (68.3)	

Table 4. Association between 5A/6A polymorphism and angina status for patients with in-stent restenosis

Genotypes	Angina status	Restenosis, *n* (%)	No restenosis, *n* (%)	*p*
5A5A + 5A6A	Unstable	13 (52.0)	12 (48.0)	0.013
	Stable	14 (21.5)	51 (78.5)	
6A6A	Unstable	31 (38.4)	50 (61.7)	0.440
	Stable	63 (36.4)	114 (63.6)	

there is an exaggeration of smooth muscle cell recruitment, migration and proliferation at the neointima during the early weeks, which produces intimal hyperplasia among patients with unstable angina undergoing stent implantation.[24,25] Previous data revealed more expression and activity of stromelysin-1 in patients with the 5A allele.[10,11] Taken together, these support our results that patients with unstable angina and the 5A5A or 5A6A genotype had the highest ISR rate. Based on this, we speculate that patients with unstable angina, especially those with the 5A5A or 5A6A genotype, may benefit from anti-inflammatory agents or inhibitors of MMPs to inhibit the early inflammatory response and reduce neointimal hyperplasia.

In contrast to the early tissue response consisting of aggregated inflammatory cells and loosely structured matrix components, the late tissue response (proliferation phase) consists of large components of extracellular matrix and loose arrays of smooth muscle cells.[26] On the basis of the idea that lower stromelysin-1 expression in patients with the 6A6A genotype causes matrix accumulation and thickening of the neointima,[10,11] we speculate that patients carrying the 6A6A genotype with stable angina would have more pronounced extracellular matrix accumulation by smooth muscle cells in the late proliferative phase of neointima and have higher ISR rates compared with non-6A6A genotype patients with stable angina.

Further studies are needed to confirm this hypothesis in independent populations. Also, it is necessary to investigate the precise timing and location of the influence of stromelysin-1 expression in the different stages (early and late phases) of ISR among 5A/6A genotype patients with different angina status. The potential role of 5A/6A polymorphism in the promoter of the stromelysin-1 gene in the initiation or progression of ISR may be explored by direct measurements of stromelysin-1 levels and degradation and synthesis of extracellular matrix in tissue removed at surgery from patients. This can boost the development of antirestenosis strategies such as MMP inhibitors or antisense oligonucleotide technology.

Recently, the advent of drug-eluting stents (DES) containing the immunosuppressive agent rapamycin or the antimitotic agent paclitaxel has shown encouraging reductions in ISR (4.0–8.9%).[27,28] There is, at present, no information regarding the relationship between genetic profile and restenosis in patients who received DES implantation. However, routine use of DES in all patients during PCI is financially prohibitive. It is worthwhile to analyze the genetic risk profile of an individual patient to identify who will derive particular benefit from the use of DES. In addition, smaller vessels, longer lesions with complex morphologies, and ISR lesions were identified as predictors in patients with high-risk post-DES restenosis (10.3–19.6%).[27] Thus, further investigation to identify patients with these risk factors and stromelysin-1 genotyping to adjust drug doses for DES may help to overcome these limitations in the future.

Some limitations of this study should be mentioned. First, we achieved a follow-up angiography rate of 79%. Previous trials on restenosis have reported similar angiographic follow-up rates. Second, the genetic heterogeneity of complex processes such as ISR is not likely to depend on a single but rather on several polymorphisms that may cluster in the same gene locus or, more probably, in different loci. Third, intravascular ultrasound, which provides more detailed information than quantitative computer analysis, was not available for this study.

In conclusion, the frequency of the 5A allele of the stromelysin-1 gene promoter is low in the Chinese population. There were no significant differences in the whole population of patients receiving stent implantation. However, subgroup analyses revealed that among patients suffering from unstable angina who underwent stent implantation, the non-6A6A genotype group had the highest ISR rate. Also, of patients suffering from stable angina, the 6A6A genotype group had a higher ISR rate. These results merit further study to identify patients' genetic profile and investigate which MMP inhibitors or DES are more effective among those at risk.

Acknowledgments

This work was supported in part by research grants from the National Science Council (NSC-93-2314-B-075-066) and from Kaohsiung Veterans General Hospital (VGHKS 92-24 and VGHKS 93-24).

References

1. Mazighi M, Goueffic Y, Scheuble A, Feldmn LJ. Prevention of in-stent restenosis: towards an *in situ* treatment? *Med Sci (Paris)* 2004;20:98–114.
2. King SB. The development of interventional cardiology. *J Am Coll Cardiol* 1998;31:64–88.
3. Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, Detre K, et al. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. Stent Restenosis Study Investigators. *N Engl J Med* 1994;331:496–501.
4. Schwartz RS, Holmes DR Jr, Topol EJ. The restenosis paradigm revisited: an alternative proposal for cellular mechanisms. *J Am Coll Cardiol* 1992;20:1284–93.
5. Lowe HC, Oesterle SN, Khachigian LM. Coronary in-stent restenosis: current status and future strategies. *J Am Coll Cardiol* 2002;39:183–93.
6. Dollery CM, McEwan JR, Henney AM. Matrix metalloproteinases and cardiovascular disease. *Circ Res* 1995;77:863–8.
7. Ye S, Humphries S, Henney A. Matrix metalloproteinase: implication in vascular matrix remodeling during atherogenesis. *Clin Sci* 1998;94:103–10.
8. Quinones S, Buttice G, Kurkinen M. Promoter elements in the transcriptional activation of the human stromelysin-1 gene by the inflammatory cytokine, interleukin 1. *Biochem J* 1994;302:471–7.
9. Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, Lark MW, Amento F, et al. Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix digestion. *Circ Res* 1994;75:181–9.
10. Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 1996;271:13055–60.
11. Medley TL, Kingwell BA, Gatzka CD, Pillay P, Cole TJ. Matrix metalloproteinase-3 genotype contributes to age-related aortic stiffening through modulation of gene and protein expression. *Circ Res* 2003;92:1254–61.
12. Ye S, Watts GF, Mandalia S, Humphries SE, Henney AM. Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. *Br Heart J* 1995;73:209–15.
13. Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. *Circulation* 1999;99:1788–94.
14. de Maat MP, Jukema JW, Ye S, Zwinderman AH, Moghaddam PH, Beekman M, Kastelein JJ, et al. Effect of the stromelysin-1 promoter on efficacy of pravastatin in coronary atherosclerosis and restenosis. *Am J Cardiol* 1999;83:842–6.
15. Sukhova GK, Schonbeck U, Rabkin E, Schoen FJ, Poole AR, Billinghurst RC, Libby P. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation* 1999;99:2503–9.
16. Terashima M, Akita H, Kanazawa K, Inoue N, Yamada S, Ito K, Matsuda Y, et al. Stromelysin promoter 5A/6A polymorphism is associated with acute myocardial infarction. *Circulation* 1999;99:271–9.
17. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* 2002;90:251–62.
18. Ellis SG, Vandormael MG, Cowley MJ, DiSciascio G, Deligonul U, Topol EJ, Bulle TM. Coronary morphologic and clinical determinants of procedural outcome with angioplasty for multivessel coronary disease: implications for patient selection. Multivessel Angioplasty Prognosis Study Group. *Circulation* 1990;82:1193–202.
19. Humphries S, Bauters C, Meirhaeghe A, Luong L, Bertrand M, Amouyel P. The 5A6A polymorphism in the promoter of the stromelysin-1 (MMP-3) gene as a risk factor for restenosis. *Eur Heart J* 2002;23:721–5.
20. Hoppmann P, Koch W, Schomig A, Kastrati A. The 5A/6A polymorphism of the stromelysin-1 gene and restenosis after percutaneous coronary interventions. *Eur Heart J* 2004;25:335–41.
21. Humphries SE, Martin S, Cooper J, Miller G. Interaction between smoking and the stromelysin-1 (MMP3) gene 5A/6A promoter polymorphism and risk of coronary heart disease in healthy men. *Ann Hum Genet* 2002;66:343–52.
22. Murase Y, Yamada Y, Hirashiki A, Ichihara S, Kanda H, Watarai M, Takastu F, et al. Genetic risk and gene-environment interaction in coronary artery spasm in Japanese men and women. *Eur Heart J* 2004;25:970–7.
23. Liu PY, Chen JH, Li YH, Wu HL, Shi GY. Synergistic effect of stromelysin-1 (matrix metalloproteinase-3) promoter 5A/6A polymorphism with smoking on the onset of young acute myocardial infarction. *Thromb Haemost* 2003;90:132–9.
24. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 1994;94:2493–503.
25. Liuzzo G, Buffon A, Biasucci LM, Gallimore JR, Caligiuri G, Vitelli A, Altamura S, et al. Enhanced inflammatory response to coronary angioplasty in patients with severe unstable angina. *Circulation* 1998;98:2370–6.
26. Grewe PH, Deneke T, Machraoui A, Barmeyer J, Muller KM. Acute and chronic tissue response to coronary stent implantation: pathologic findings in human specimen. *J Am Coll Cardiol* 2000;35:157–63.
27. Lemos PA, Hoye A, Goedhart D, Arampatzis CA, Saia F, van der Giessen WJ, McFadden E, et al. Clinical, angiographic, and procedural predictors of angiographic restenosis after sirolimus-eluting stent implantation in complex patients: an evaluation from the Rapamycin-Eluting Stent Evaluated at Rotterdam Cardiology Hospital (RESEARCH) study. *Circulation* 2004;109:1366–70.
28. Hausleiter J, Kastrati A, Wessely R, Dibra A, Mehilli J, Schratzenstaller T, Graf I, et al. Prevention of restenosis by a novel drug-eluting stent system with a dose-adjustable, polymer-free, on-site stent coating. *Eur Heart J* 2005;26:1475–81.