Stromelysin Promoter 5A/6A Polymorphism Is Associated With Acute Myocardial Infarction

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Background—Rupture of the fibrous cap of an atherosclerotic plaque is a key event that predisposes to acute myocardial infarction (AMI). Matrix metalloproteinases (MMPs) may contribute to weakening of the cap, which favors rupture. Stromelysin, a member of MMP family, is identified extensively in human coronary atherosclerotic lesions. It can degrade most of the constituents of extracellular matrix as well as activating other MMPs, which suggests that it may play an important role in plaque rupture. Recently, a common variant (5A/6A) in the promoter of the stromelysin gene has been identified. The 5A/6A polymorphism could regulate the transcription of the stromelysin gene in an allele-specific manner.

Methods and Results—To investigate the relation between the 5A/6A polymorphism in the promoter of the stromelysin gene and AMI, we conducted a case-control study of 330 AMI patients and 330 control subjects. The prevalence of the 5A/6A+5A/5A genotype was significantly more frequent in the patients with AMI than in control subjects (48.8% vs 32.7%, P<0.0001). In logistic regression models, the odds ratio of the 5A/6A+5A/5A was 2.25 (95% CI, 1.51 to 3.35). The association of 5A/6A polymorphism with AMI was statistically significant and independent of other risk factors.

Conclusions—The 5A/6A polymorphism in the promoter of the stromelysin gene is a novel pathogenetic risk factor for AMI. (Circulation. 1999;99:2717-2719.)

Key Words: plaque ■ metalloproteinases ■ myocardial infarction ■ risk factors

Plaque rupture with thrombosis is well established as a critical factor in the pathogenesis of acute myocardial infarction (AMI).1,2 Although the mechanisms underlying plaque rupture are unclear, matrix metalloproteinases (MMPs) may contribute to weakening of the cap and subsequent rupture.3 MMPs, such as interstitial collagenase, gelatinase, and stromelysin, can degrade extracellular matrix and are identified extensively in human coronary atherosclerotic plaques.3,4 Stromelysin can play a key role in rupture of atherosclerotic plaque, because it can cleave many of the extracellular matrix components as well as enhancing the activity of other MMPs.5

Recently, a common polymorphism in the promoter region of the human stromelysin gene was described in which 1 allele sequence has 5 adenosines (5A) and the other has 6 (6A).6 In vitro assays of promoter activity revealed that the 5A allele had 2-fold higher promoter activity than the 6A allele.7 We hypothesized that the 5A allele may carry the risk of plaque rupture, leading to AMI. To test our hypothesis, we evaluated the 5A/6A polymorphism in AMI patients and control subjects.

Methods

Subjects

The study population was composed of 330 AMI patients and 330 control subjects. Consecutive AMI patients ≤70 years old were recruited from the inpatients who were admitted to the coronary care unit either in Kobe University Hospital from June 1995 to September 1998 or in the Himeji Cardiovascular Center from January 1998 to September 1998. The diagnosis of AMI was established by World Health Organization criteria and confirmed by coronary angiography and left ventriculography. The control subjects were selected from the inpatients of Kobe University Hospital and matched with the AMI patients for sex and age. They had no evidence of myocardial infarction, angina pectoris, stroke, peripheral vascular disease, or malignancy. All subjects enrolled in this study were Japanese and gave written informed consent. The Ethics Committee of Kobe University and Himeji Cardiovascular Center approved this study.

Patients were considered smokers if they had a smoking index (years of smoking×amount of smoking [No. of cigarettes] per day) >100 and current smoking status. They were considered to have hypertension if they met the criteria of the World Health Organization or were already being treated with antihypertensive agents. They were considered to have hyperlipidemia if their fasting total plasma cholesterol level was ≥220 mg/dL or they had already been treated with cholesterol-lowering drugs. They were defined as having...
diabetes if they met the diagnostic criteria of the World Health Organization or were already under treatment for diabetes.

DNA Extraction and Polymerase Chain Reaction
Genomic DNA was extracted from 2 mL of whole blood with a Genomix kit (Tarent) according to the manufacturer’s instructions. Stromelysin promoter gene containing the 5A/6A polymorphism (−1171 bp) was amplified from genomic DNA isolated from subjects by polymerase chain reaction (PCR). PCR procedures were performed as described by Ye et al.6 We used modified oligonucleotide primers for accuracy: forward primer (−1259 to −1240), 5′-GATTACAGACATGGGTCACG-3′; reverse primer (−879 to −860), 5′-ACAGCATGGCCCATTTGCCC-3′.

Dot Blot Hybridization With Allele-Specific Oligonucleotide Probes
PCR products (20 to 40 μL) were denatured in 0.3 mol/L NaOH for 60 minutes and transferred onto 2 nylon membranes for allele-specific probes. Two probes hybridizing specifically to the 5A or 6A alleles (5′-GGGAAAAACCATG-3′ and 5′-ACATGGTTT-TYCC-3′) were 5′ end-labeled with [γ-32P]ATP. Labeling of each probe, subsequent hybridization procedures, washing conditions, and exposure were performed as described by Ye et al.6 Samples of known genotype by sequence analysis were run alongside the samples being analyzed as markers. All genotypes were assessed independently by 2 individuals who were blinded to the AMI status of the patients who gave samples.

Statistical Analysis
Data on age are presented as mean±SD. The difference between the groups was analyzed by the unpaired Student’s t test. The differences in frequencies of smoking, hypertension, hyperlipidemia, diabetes mellitus, and stromelysin promoter genotypes were analyzed by Fisher’s exact test. χ² analysis was used to test deviations of genotype distribution from Hardy-Weinberg equilibrium and to determine allele or genotype frequencies between patients and control groups. Multivariate analyses were conducted with multiple logistic regression methods, and adjusted estimations of conditioned relative risk and 95% CIs were done. In this study, a value of P<0.05 was taken to be statistical significance.

Results
The characteristics of AMI patients and control subjects are shown in Table 1. There was no significant difference in age and sex between the groups. The coronary risk factors examined, ie, smoking, hyperlipidemia, diabetes, and hypertension, were significantly pronounced in AMI patients.

Genotypic and allelic frequencies of the 5A/6A polymorphism of stromelysin promoter gene are summarized in Table 2. These data are consistent with the distribution predicted by the Hardy-Weinberg equilibrium. The allele frequency in the Japanese population was different from that in healthy controls of the United Kingdom that was previously reported by Ye et al.6 The prevalence of the 5A/5A + 5A/6A genotype was significantly more frequent in AMI patients than in control subjects. There was also a significant difference in allele frequencies between AMI patients and control subjects. The odds ratio of the 5A/6A + 5A/5A versus the 6A/6A genotype of the stromelysin promoter polymorphism between AMI patients and control subjects was 2.25 (95% CI, 1.51 to 3.35). The association of this polymorphism with AMI patients was statistically significant and independent of other coronary risk factors when subjected to logistic regression analysis (Table 3).

Discussion
The present study provides the first evidence of the association between a common polymorphism in the stromelysin promoter and AMI. We found that the 5A allele in the stromelysin promoter was significantly more frequent in AMI patients than in control subjects, which suggests that patients with the 5A allele have coronary atherosclerotic lesions more prone to rupture.

Stromelysin has a broad substrate specificity and thus can degrade many extracellular matrix proteins. Moreover, it can also activate other MMPs, such as collagenase and gelatinase.9 By in situ mRNA hybridization, the presence of stromelysin was demonstrated in coronary atherosclerotic plaques, particularly at the regions considered prone to rupture.9 Expression of stromelysin is regulated primarily at the level of transcription, where the promoter of the gene responds to various stimuli.9,10 There is a common polymorphism in the promoter sequence of the human stromelysin gene (5A or 6A).6 In transient expression experiments, cultured fibroblasts and vascular smooth muscle cells transfected with the constructs containing 5As expressed a 2-fold higher amount of reporter gene product compared with the transfects of the constructs containing 6As.7

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On the basis of these observations, we hypothesized that the 5A/6A polymorphism could be a genetic risk factor for plaque rupture leading to AMI. We found a strong association between a common polymorphism in the stromelysin promoter gene and AMI. This association was independent of known coronary risk factors, such as smoking, hyperlipidemia, diabetes mellitus, and hypertension. Recently, Ye et al. showed preliminarily that the 5A/6A polymorphism in the stromelysin promoter gene seems to be associated with progression of coronary atherosclerosis. However, it remains to be elucidated whether this polymorphism is related to AMI.

In conclusion, this is the first study to demonstrate that the 5A/6A polymorphism in the stromelysin promoter gene is associated with AMI. To confirm that this polymorphism is a novel genetic marker for plaque rupture, investigations in a larger population and other ethnic populations will be necessary. We would achieve a great advantage in the prevention of AMI by distinguishing patients who have genetically increased susceptibility to plaque rupture.

References
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_Circulation_. 1999;99:2717-2719
doi: 10.1161/01.CIR.99.21.2717
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/21/2717

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