The Role of Stromelysin-1 Gene and Gelatinase B Gene Polymorphism in Coronary Artery Disease

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The Role of Stromelysin-1 Gene and Gelatinase B Gene Polymorphism in Coronary Artery Disease

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Finally I would like to dedicate this article to my wife and daughter.

Written by Author
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Abstract

The Role of Stromelysin-1 Gene and Gelatinase B Gene Polymorphisms in Coronary Artery Disease

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(Directed by Professor Yangsoo Jang)

Background: Matrix metalloproteinases contribute to vascular remodeling by breaking down extra-matrix while new matrix is synthesized. Of the variety of MMPs, stromelysin-1 gene and gelatinae B gene may have a key role in coronary artery atherosclerosis. The 5A/6A polymorphism in the promoter of the stromelysin-1 gene can be a pathogenetic risk factor for acute myocardial infarction. Gelatinase B gene (92-kDa type IV collagenase and MMP-9) is one of the MMPs found to be highly expressed in the disruption-prone regions of atherosclerotic plaques. The C-to T substitution at the promoter site (1562) resulted in a higher promoter activity of T-allelic promoter. The R279Q polymorphism in exon 6 led to substitution of adenosine by guanine and was common polymorphism in the population. We evaluated the relation of these polymorphisms with stable angina, severity of atherosclerosis in coronary artery, and instent restenosis after percutaneous coronary angioplasty. Method: The study populations were composed of 131 patients with stable angina (mean age 61.3 years, 89 males) and 117 control subjects (mean age 59.3 years, 59 males). For all patients, coronary angiography was performed in Yonsei University Cardiovascular Hospital from February 1998 to June 2000. Genotype for each polymorphism was determined using SnaPshot™ kit and restriction...
fragment length polymorphism (RFLP). **Results:** the prevalence of 5A containing polymorphism of stromelysin-1 gene was higher in stable angina group than control patients, but there were no difference in two polymorphisms of gelatinase B gene between two groups. In multiple logistic analysis, 5A-allele of stromelysin-1 gene was independent risk factor for stable angina with odds ratio 2.29 (95% CI; 1.19-4.38). However, the severity of atherosclerosis in coronary artery or instent restenosis was not related to any polymorphisms of stromelysin-1 and gelatinase B.

**Conclusion:** Our data showed functional genetic variation of stromelysin-1 could be significant risk factor for stable angina. It might be play a important role of initiation for coronary atherosclerosis involving vascular remodeling.

Key Words: Stable angina, Polymorphism, Stromelysin-1, Gelatinase B
I. Introduction

Matrix metalloproteinases contribute to vascular remodeling by breaking down extra-matrix while new matrix is synthesized. By breaking the extracellular matrix, MMPs may allow smooth muscle cells to invade and migrate, which contributes to pathologic processes such as atherosclerosis and restenosis after angioplasty. Of the variety of MMPs, recently common variants in the promoter polymorphism of stromelysin-1 gene and gelatinase B gene have been reported to be associated with atherosclerosis. Of the variety of MMPs, recently common variants in the promoter polymorphism of stromelysin-1 gene and gelatinase B gene have been reported to be associated with atherosclerosis.

Stromelysin gene can play a key role in the rupture of atherosclerotic plaque, and the 5A/6A polymorphism in the promoter of the stromelysin-1 gene can be a pathogenetic risk factor for acute myocardial infarction. The polymorphism is located 600 bp upstream from the start of transcription in which one allele has a run of 6 adenosines (6A), whereas the other has only 5 adenosines (5A). In vitro assays of promoter activity revealed that 5A allele had 2-fold higher promoter activity than 6A allele.

Gelatinase B gene (92-kDa type IV collagenase and MMP-9) is one of the MMPs found to be highly expressed in the disruption-prone regions of atherosclerotic plaques. Zhang et al suggested that a 9-bp sequence (GCGCAG/GCC, -1567 to -1559) containing the C-1562T polymorphic site was an important regulatory element as a binding site for a transcription factor.
repressor protein, and this DNA-protein interaction was abolished by the C-to T substitution at the polymorphism site (-1562), resulting in a higher promoter activity of T-allelic variant.\textsuperscript{4} The R279Q polymorphism in exon 6 led to substitution of adenosine (A) by guanine (G) and was common polymorphism in the population. Unlikely C to T substitution polymorphism, there is no study to address possible functional effects on atherosclerosis of this polymorphism.\textsuperscript{12}

The aims of this study are 1) to evaluate the role of the polymorphisms of the stromelysin-1 promoter gene, and gelatinase B gene in stable angina, 2) evaluate relationship of polymorphism to the severity of coronary artery disease and 3) evaluate relationship to instent restenosis.

II. Materials and Methods

A. Subjects

The study populations were composed of 131 patients with stable angina and 117 control subjects. For all patients, coronary angiography was performed in Yonsei University Cardiovascular Hospital from February 1998 to June 2000.

Inclusion criteria of stable angina were patients with coronary artery disease proven by coronary angiography, without 1) history of myocardial infarction or unstable angina, 2) previous coronary angioplasty, 3) peripheral or cerebral artery disease, 4) recent infection, and 5) atrial fibrillation. And inclusion criteria of control subjects were patients without 1) evidence of coronary artery disease proven by coronary angiography, 2) peripheral or cerebral arterial disease, 3) recent infection, and 4) atrial fibrillation.

All subjects enrolled in this study were Korean and gave written informed consent.
B. Assessment of angiographic data

Quantitative computer-assisted angiographic measurements (QCA) were performed on end-diastolic frames before balloon angioplasty, after stenting, and follow-up coronary angiography using an on-line quantitative coronary angiographic system (Hicor, Siemens). Angiography was routinely performed in at least 2 projections. These projections were recorded in our database, and follow-up angiography was performed using the same projections. Operators were unaware of the patients’ genotype. Minimal luminal diameter (MLD), reference diameter, percent diameter stenosis, and lesion length were obtained from QCA. The severity of coronary artery atherosclerosis was evaluated by coronary angiography and graded to 1-vessel, 2-vessel, 3-vessel disease by the number of > 50% occluded coronary artery. Definition of instent restenosis was the classic criteria of more than 50% diameter stenosis during follow-up angiography.\textsuperscript{13,14} Multi-vessel disease included 2-vessel and 3-vessel disease.

C. Baseline data collection

Baseline data was obtained from medical records and asking the patients. Lipid profiles (total cholesterol, low-density lipoprotein cholesterol, triglyceride and high-density lipoprotein cholesterol), lipoprotein (a), other inflammatory markers (ESR, fibrinogen) were obtained right after admission and before angiography in stable angina and control subjects.

D. DNA analysis

1. Stromelysin-1 gene polymorphism

Genomic DNA was extracted from 300 µL of whole blood with a QIAmp
Blood Kit (QIAGEN). Single nucleotide polymorphism (SNP) analysis was performed using the ABI Prism SnapShot ddNTP Primer Extension Kit (ABI) according to the manufacturer’s guideline. Sense and antisense sequences of the primer were 5'- GAT TAC AGA CAT GGG TGA CG-3' and 5'-CAT CAC TGC CAC CAC TCT G -3' respectively. After an initial denaturation at 95°C for 10 minutes, samples were cycled 35 times as follows; 1 minute at 95°C, 1 minute at 62°C, and 1 minute at 72°C. And the final extension was performed for 5 minutes at 72°C. After amplification PCR product were treated with 0.164 U/µl shrimp alkaline phosphatase (USB Corporation) and 0.164 U/µl exonuclease (USB Corporation) for one hour at 37 °C, followed by the deactivation of the enzymes for 15 minutes at 72 °C. SnaPshot thermal cycling was performed with primer 5'- CCT TTG ATG GGG GGA AAA A-3', matching PCR product, and SNapShot Ready Reaction Premix for 25 cycles of 10 seconds at 96 °C denaturation, five seconds at 50 °C annealing, and 30 seconds at 60 °C extension. SnaPshot products were dephosphorylated by incubation at 37 °C (one hour) with 0.15 U/µl shrimp alkaline phosphatase (USB Corporation). The enzyme was deactivated as above and SnaPshot products were analyzed on an ABI310 automated DNA sequencer (ABI)(Figure 1B).

2. Gelatinase B gene promoter polymorphism

Sense and antisense sequences of the primer were 5'- GCC TGG CAC ATA GTA GGC CC-3' and 5'-CTT CCT AGC CAG CCG GCA TC-3', respectively. After an initial denaturation at 95°C for 10 minutes, samples were cycled 35 times as follows; 1 minute at 95°C, 1 minute at 62°C, and 1 minutes at 72°C. Final extension was performed for 5 minutes at 72°C. After amplification with annealing at 62°C, the PCR product was restricted with 5 U of the restriction endonuclease SphI over 2 hours at 37°C, and the final product was electrophoresed in 1.5% agarose gel and visualized directly by
ethidium bromide staining. The C-allele lacks of a SphI site that is present in the T- allele and gives rise to a fragment of 435bp rather than 247bp or 188bp (Figure 1A).

3. Gelatinase B gene exon 6 R279Q polymorphism

Sense and antisense sequences of the primer were 5'- CTC GCC CCA GGA CTC TAG AC-3' and 5'-GTG GAG GTA CCT CGG GTC GGG-3' respectively. After an initial denaturation at 95°C for 10 minutes, samples were cycled 35 times as follows; 1 minute at 95°C, 1 minute at 62°C, and 1 minutes at 72°C. Final extension was then performed for 5 minutes at 72°C. After amplification with annealing at 62°C, SNP analysis was performed same method described above using the ABI Prism SnapShot ddNTP Primer Extension Kit (ABI) with primer 5'- GCG-AAG-ACT-ACA-CCC-3' (Figure 1C).
Figure 1 (A) Polymorphism of gelatinase B gene promoter was analyzed using restriction fragment length polymorphism (RFLP). Stromelysin-1 gene (B) and gelatinase B gene exon 6 (C) was analyzed by single base pair extension assay using SnaPshot™ kit.

* M: Marker (50 bp)
E. Statistical Analysis

Data was analyzed with SPSS 10.0 statistical software. The differences between two groups for continuous variables, discrete variables were analyzed by the independent $t$-test, $\chi^2$ test, respectively. $\chi^2$ analysis was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and to determine whether there was any significant difference in allele or genotype frequencies between stable angina and control subjects. Logistic multiple regression was carried out to test for difference in genotype distribution between stable angina and control subjects. And, adjusted estimates of conditioned relative risk and 95% confidence interval were determined. In this study, a value of $P < 0.05$ was taken to be statically significance.
III. Results

A. Baseline characteristics

The baseline characteristics of patients with stable angina and control subjects are shown in table 1.

Table 1. Baseline clinical characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Stable angina (n = 131)</th>
<th>Control (n=117)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.3 ±7.9</td>
<td>59.3 ±8.5</td>
<td>p=0.04</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>89 / 42</td>
<td>59 / 58</td>
<td>p=NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ±3.5</td>
<td>25.2 ±4.3</td>
<td>p=NS</td>
</tr>
<tr>
<td>Smoking (n)</td>
<td>40 (30.8%)</td>
<td>39 (34.2%)</td>
<td>p=NS</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>62 (47.3%)</td>
<td>52 (45.6%)</td>
<td>p=NS</td>
</tr>
<tr>
<td>Diabetes (n)</td>
<td>31 (23.7%)</td>
<td>18 (15.5%)</td>
<td>p=NS</td>
</tr>
<tr>
<td>Total Cholesterol¹</td>
<td>198.0 ±35.8</td>
<td>176.7 ±33.3</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>LDL-cholesterol¹</td>
<td>120.0 ±27.6</td>
<td>102.0 ±27.3</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>HDL-cholesterol¹</td>
<td>42.6 ±12.9</td>
<td>45.8 ±12.9</td>
<td>p=NS</td>
</tr>
<tr>
<td>Triglyceride¹</td>
<td>166.6 ±107.7</td>
<td>142.6 ±75.1</td>
<td>p=NS</td>
</tr>
<tr>
<td>Fibrinogen¹</td>
<td>367.8 ±93.5</td>
<td>381.1 ±127.5</td>
<td>p=NS</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>19.9 ±14.9</td>
<td>20.0 ±17.1</td>
<td>p=NS</td>
</tr>
<tr>
<td>Lipoprotein (a)¹</td>
<td>28.2 ±23.0</td>
<td>27.2 ±20.8</td>
<td>p=NS</td>
</tr>
</tbody>
</table>

¹: mg/dl

* Student t-test (for continuous variables) and χ² test (for discrete variable) were used to compare the values for stable angina and control patients.

† Continuous variables are presented as mean ± SD.

LDL: low density lipoprotein, HDL: high density lipoprotein.

There was no significant difference in demographic characteristics except
In analysis of coronary artery risk factors, total cholesterol and low density lipoprotein (LDL) – cholesterol level were significant higher in stable angina patients than those of control subjects. But other risk factors, such as, smoking, hypertension, diabetes and HDL-cholesterol were not significantly different between two groups.

**B. Distributions of stromelysin-1 and gelatinase B genotypes**

The distributions of stromelysin-1 6A/6A, 5A/6A, 5A/5A genotypes were 76.2% (n=189), 22.6% (n=56), and 1.2% (n=3), respectively. They were similar to values reported in other studies on Japan. The distributions of gelatinase B promoter gene CC, CT and TT genotypes were 74.2% (n=184), 24.2% (n=60), and 1.6% (n=4), respectively and they were similar to values reported in European studies. In the cases of gelatinase B genes polymorphism, the frequencies were 14.1%(n=35), 44.0%(n=109), and 41.9%(n=104) for genotype GG, GA and AA, respectively. The frequencies were close to the values reported in European studies. The distributions of genotypes of all three gene polymorphisms were compatible with Hardy-Weinberg equation.
Figure 2. Distribution of genotype frequencies of stromelysin-1 and gelatinase B genes.


In comparison of stable angina group with control group, the frequencies of 6A homozygote was higher in control group than stable angina group and 5A/6A heterozygote was high in stable angina group. But the distribution of genotypes at two polymorphisms of gelatinase B were not significantly different between two groups (Figure 3).
Fig 3. Distribution of the genotypes of stromelysin-1 and gelatinase B between stable angina group and control group.

C. Comparision of genotype frequencies of stromelysin-1 and gelatinase B gene polymorphism between stable angina and control patients

Genotype and allele frequencies of the stromelysin-1 gene, and gelatinase B gene are summarized in table 2. The distributions of each genotype were consistent with Hardy-Weinberg equilibrium. The prevalence of the 5A/5A + 5A/6A genotype was significantly more frequent in sable angina patients than in control patients. The comparison of allele, the 5A allele was more frequent in patients with stable angina. But, the prevalence of C/T+T/T genotype and A/A+G/A genotype in gelatinase B was not significant (Table 2). In multivariate logistic analysis, the 5A/6A + 5A/5A genotype of the stromelysin-1 promoter was independent risk factor with odds ratio of 2.28 (95% CI; 1.19-4.38) (Table 3).
Table 2. Genotype and allele frequencies in patients with stable angina and control subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stable angina (n =131)</th>
<th>Control (n=117)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromelysin –1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A/6A</td>
<td>92 (70.2%)</td>
<td>97 (89.2%)</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>5A/6A + 5A/5A</td>
<td>39 (29.8%)</td>
<td>20 (17.1%)</td>
<td></td>
</tr>
<tr>
<td>5A allele</td>
<td>0.24</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Gelatinase B exon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA + GA</td>
<td>75 (57.3%)</td>
<td>69 (59.0%)</td>
<td>p = 0.44</td>
</tr>
<tr>
<td>GG</td>
<td>56 (42.7%)</td>
<td>48 (41.9%)</td>
<td></td>
</tr>
<tr>
<td>A allele</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Gelatinase B promoter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>99 (75.6%)</td>
<td>85 (72.6%)</td>
<td>p = 0.35</td>
</tr>
<tr>
<td>CT + TT</td>
<td>32 (24.4%)</td>
<td>32 (27.4%)</td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>0.21</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

*χ² test were used to compare the values for stable angina and control patients.

Table 3. Multiple logistic analysis of risk factors for stable angina

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromelysin-1, 5A/6A + 5A/5A</td>
<td>2.28 (1.19 – 4.38)</td>
<td>0.01</td>
</tr>
<tr>
<td>Male gender</td>
<td>2.26 (1.29 – 3.93)</td>
<td>0.04</td>
</tr>
<tr>
<td>High LDL-cholesterol¹</td>
<td>3.65 (1.79 – 7.39)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

¹ Higher LDL-cholesterol was defined when the LDL-cholesterol was higher than 130 mg/dl.
D. Comparision of genotype frequencies between single-vessel and multi-vessel disease instable angina subjects

In 131 stable angina patients in whom coronary angiographic data were available, there was no significant association between polymorphism of two genes and severity of coronary atherosclerosis. The severity of coronary atherosclerosis was assessed by an analysis of the number of coronary arteries that had a stenosis more than 50% (Table 4).

Table 4. Genotype frequencies between single and multi-vessel disease

<table>
<thead>
<tr>
<th>Variables</th>
<th>Single vessel (n = 48)</th>
<th>Multiple vessel (n = 83)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromelysin-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A/6A</td>
<td>33 (68.8%)</td>
<td>59 (71.1%)</td>
<td></td>
</tr>
<tr>
<td>6A/5A + 5A/5A</td>
<td>15 (31.3%)</td>
<td>24 (28.9%)</td>
<td>P = 0.46</td>
</tr>
<tr>
<td>Gelatinase B exon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA + GA</td>
<td>27 (56.3%)</td>
<td>48 (57.8%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>21 (43.8%)</td>
<td>35 (42.2%)</td>
<td>P = 0.50</td>
</tr>
<tr>
<td>Gelatinase B promotor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>34 (70.8%)</td>
<td>65 (78.3%)</td>
<td></td>
</tr>
<tr>
<td>CT + TT</td>
<td>14 (29.2%)</td>
<td>18 (21.7%)</td>
<td>P = 0.23</td>
</tr>
</tbody>
</table>

* $\chi^2$ test was used to compare the values for single-vessel and multi-vessel disease.
E. Comparision of genotype frequencies between patients with instent restenosis and patency in stable angina

In 48 patients in whom percutaneous coronary angioplasty was undertaken and 6-month follow-up coronary angiographic data were available, the genotype frequencies were analyzed. there were no significant association between polymorphisms in stromelysin-1 or gelatinase B and instent restenosis (Table 5).

Table 5. Genotype frequencies between patients with instent restenosis and patency

<table>
<thead>
<tr>
<th>Variables</th>
<th>Instent restenosis (n =14)</th>
<th>Patency (n = 34)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stromelysin-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6A/6A</td>
<td>22 (64.7%)</td>
<td>10 (71.4%)</td>
<td></td>
</tr>
<tr>
<td>5A/6A + 5A/5A</td>
<td>12 (35.3%)</td>
<td>4 (28.6%)</td>
<td>P = 0.46</td>
</tr>
<tr>
<td>Gelatinase B exon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA + GA</td>
<td>16 (47.1%)</td>
<td>8 (57.1%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>18 (52.9%)</td>
<td>6 (42.9%)</td>
<td>P = 0.38</td>
</tr>
<tr>
<td>Gelatinase B promotor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>25 (73.5%)</td>
<td>10 (71.4%)</td>
<td></td>
</tr>
<tr>
<td>CT + TT</td>
<td>9 (26.5%)</td>
<td>4 (28.6%)</td>
<td>P = 0.57</td>
</tr>
</tbody>
</table>

*χ² test was used to compare the values for patients with instent restenosis and patients with patency after 6-month follow-up coronary angiography.
IV. Discussion

This study showed the evidence of association between stromelysin promoter 5A/6A polymorphism and stable angina. However, the genetic polymorphisms in gelatinase B, C-1562T polymorphism in promoter region and R279Q polymorphism in exon region, were not found the association with stable angina.

Stromelysin-1 is a key regulator of matrix remodeling and has a broad substrate specificity and can also activate other MMPs.\textsuperscript{15-19} The stromelysin-1 promoter gene 5A/6A polymorphism has been analyzed in relation to atherosclerosis in a number of epidemiologic studies. Terashima et al reported the frequency of 5A allele carriers was significantly higher in acute myocardial infarction (AMI) patients than in control subjects (48.8 % vs 32.7%, \( P < 0.0001 \)).\textsuperscript{5} These data indicated that individuals carrying the 5A allele were genetically predisposed to clinical event commonly caused by the rupture of coronary atherosclerotic plaque.\textsuperscript{20} On the progression of angiographically determined coronary atherosclerosis in man after coronary bypass surgery and in subjects with coronary artery disease, a common polymorphism (6A/6A genotype) in the promoter region for stromelysin-1 has been associated with a faster progression of coronary atherosclerosis.\textsuperscript{3,21} However, this finding was only seen in comparing those with the least stenosis (< 20%) and higher serum LDL-cholesterol (> 160mg/dl).\textsuperscript{2} In our study, the distribution of 5A allele was higher in stable angina group than control group. Minor plaque rupture plays a important role in initiation and progression of coronary artery luminal narrowing to occur stable angina. 5A allele may have a effect on not only deep rupture leading to myocardial infarction\textsuperscript{5} but also pathway that not also lead to minor plaque rupture resulting the progression of atherosclerosis.
Gelatinase B gene possesses proteolytic activity against type IV collagen, a major component of the basement membrane, and has been known to facilitate vascular smooth muscle cell migration.\textsuperscript{22-23} Zang et al reported C-1562T polymorphism was related with the severity of coronary artery atherosclerosis.\textsuperscript{4} One explanation for this finding was that higher T-allele associated higher gelatinase B expression would enhance smooth muscle migration during atherosclerosis. But there is controversy for that finding. When an MMP inhibitor was added to the injured vessel environment where smooth muscle cells reacted to platelet-derived growth factor, invasion of the basement membrane was inhibited, but not migration. Therefore, the invasion of the basement membrane in the postangioplasty state was to be MMP-dependent, but the migration of smooth muscle cells was not, even though smooth muscle cell migration and basement membrane invasion were triggered by platelet-derived growth factors in this environment.\textsuperscript{1,24-27}

However, in recent autopsy study, the gelatinase B promoter with high-activity genotypes compared with the promoter low activity genotype, were found be associated with a large area of coronary complicated plaque.\textsuperscript{28}

R279Q polymorphism in exon 6 region was not evaluated in coronary artery disease but might be potential polymorphism related with gelatinase activity. Evidence was follows; first, it was located in the catalytic domain of MMP-9 enzyme, second, the polymorphism led to a substitution of a positively charged amino acid (arginine) by an uncharged amino acid (glutamine) and third, the polymorphism was common in the population.\textsuperscript{12,29} Our data showed allele frequency similar to previous reports.\textsuperscript{4,12} But, the frequency of both polymorphisms are not significant different between two groups. In gelatinase B gene, the frequency of T allele is higher in control patients than stable angina patients, which finding is contast results to previous European results.\textsuperscript{4} In the polymorphism of gelatinase exon 6 gene, the activity of allele should be evaluated in advance. In analysis for combination effect of two polymorphisms, there was no additive effect.
Regarding to the severity of coronary artery atherosclerosis, Zhang et al. reported the carrier of T-allele had higher frequency than C/C homogygotes in 3-vessel disease,\textsuperscript{4} However there was no significant difference between single-vessel and multi-vessel disease in our study. The degree of coronary artery atherosclerosis might not be represented by the number of involved vessel. The polymorphisms might not influence the degree of progressive coronary atherosclerosis when significant coronary artery stenosis was already present, but other risk factors of coronary artery disease may play a role in the progression of coronary atherosclerosis. In this aspect, stromelysin-1 5A/6A promoter gene polymorphism may be important factor in initialtion of coronary artery atherosclerosis from normal or minimal coronary artery stenosis to significant coronary artery stenosis. We hypothesize the mechanism of this finding may be due to breakdown of the basement and progressive minor plaque rupture.

Percutaneous transluminal coronary angioplasty is a widely used treatment for angina pectoris. Smooth muscle cells in an artery for postpercutaneous transluminal coronary angioplasty are in a strikingly different environment than they are in the development of the de novo atherosclerotic plaque. MMPs might play a central role in migration of medial smooth muscle cells to intima. This theory was supported by a rise in gelatinse B synthesis and activity in the vessel at 1 day after balloon injury, and its continued presence at 6 days after injury.\textsuperscript{30} But in our study, there is no difference of genetic frequency between patients with instent restenosis and patency. This finding might explain that the instent restenosis may not related with MMP activity. Main pathophysiology of instent restenosis is neointimal hyperplasia, but MMPs may not closely be related with neointimal hyperplasia. However, in this study, the number of population was small, thus further randomized study will be required to prove this hypothesis.
V. Conclusion

This study demonstrated that 5A/6A polymorphism in stromelysin-1 gene was associated with stable angina proven atherosclerosis. Theses findings supported the hypothesis that connective tissue remodeling, mediated by MMPs, played an important role in progression of atherosclerosis and genetic variations influencing MMP expression might contribute to disease phenotype.
References


15. Schonbeck U, Mach F, Sukhova GK, Murphy C, Bonnefoy JY, Fabunmi RP. Regulation of matrix metalloproteinase expression in human


Stromelysin-1 and Gelatinase B

Stromelysin-1 and Gelatinase B are enzymes involved in tissue remodeling. Stromelysin-1 and Gelatinase B are both matrix metalloproteinases (MMPs) that are involved in the degradation of extracellular matrix proteins. Stromelysin-1 has a preference for degrading type IV collagen, while Gelatinase B degrades gelatin and type IV collagen.

Gelatinase B exon 6 contains a polymorphism at position 1562, with alleles C and T. This polymorphism has been associated with altered activity of Gelatinase B. Stromelysin-1 has two SNPs in the promoter region, 5A/6A and –1562 C/T. These SNPs may affect the expression and activity of Stromelysin-1.

DNA sequencing using SnaPshot™ technology was performed to detect single base pair polymorphisms. The results showed that the 131 bp fragment, with DNA digestion, had restriction fragmentation lengths of 61.3 and 89 bp. For the 117 bp fragment, the restriction fragmentation lengths were 59.3 and 59 bp, and for the MMP fragment, the restriction fragmentation lengths were 1998 bp.

In conclusion, the study highlights the importance of genetic polymorphisms in the regulation of MMP activity and their potential role in tissue remodeling disorders. Further research is needed to understand the functional significance of these polymorphisms.

1) 131 bp fragment (61.3, 89 bp)
2) 117 bp fragment (59.3, 59 bp)
3) MMP fragment (1998 bp)
stromelysin-1
Gelatinase-B
Stromelysin-1
Gelatinase B
2.29
Stromelysin-1
Gelatinase B
Stromelysin-1
Gelatinase B

: stromelysin-1, Gelatinase B