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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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 polymorphysm 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 detemined 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

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

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I. Introduction

Matrix metalioproteinases contribute to vascular remodeling by breaking down extra-matrix while new matrix is synthesized. By breaking the exracellular matrix, MMPs may allow smooth muscle cells to invade and migrate, which contributes to pathologic processes such as atherosclerosis and restenosis after angioplasty.[1] Of the variety of MMPs, recently common variants in the promoter polymorphism of stromelysn-1 gene and gelatinase B gene have been reported to be associated with atherosclerosis.[2-4]

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.[5-6] 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).[2,7] In vitro assays of promoter activity revealed that 5A allele had 2-fold higher promoter activity than 6A allele.[8]

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.[9-11] Zhang et al suggested that a 9-bp sequence (GCGCAC/GCC, -1567 to –1559) containing the C-1562T polymorphic site was an important regulatory element as a binding site for a transcription

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.[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.[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 wihout 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 artey 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.[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/μll exonuclease (USB Corporation) for one hour at 37 ℃, followed by the deactivation of the enzymes for 15 minutes at 72 ℃. 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 amplication 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 amplication 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-AGA-GAC-TCT-ACA-CCC-3' (Figure 1C).

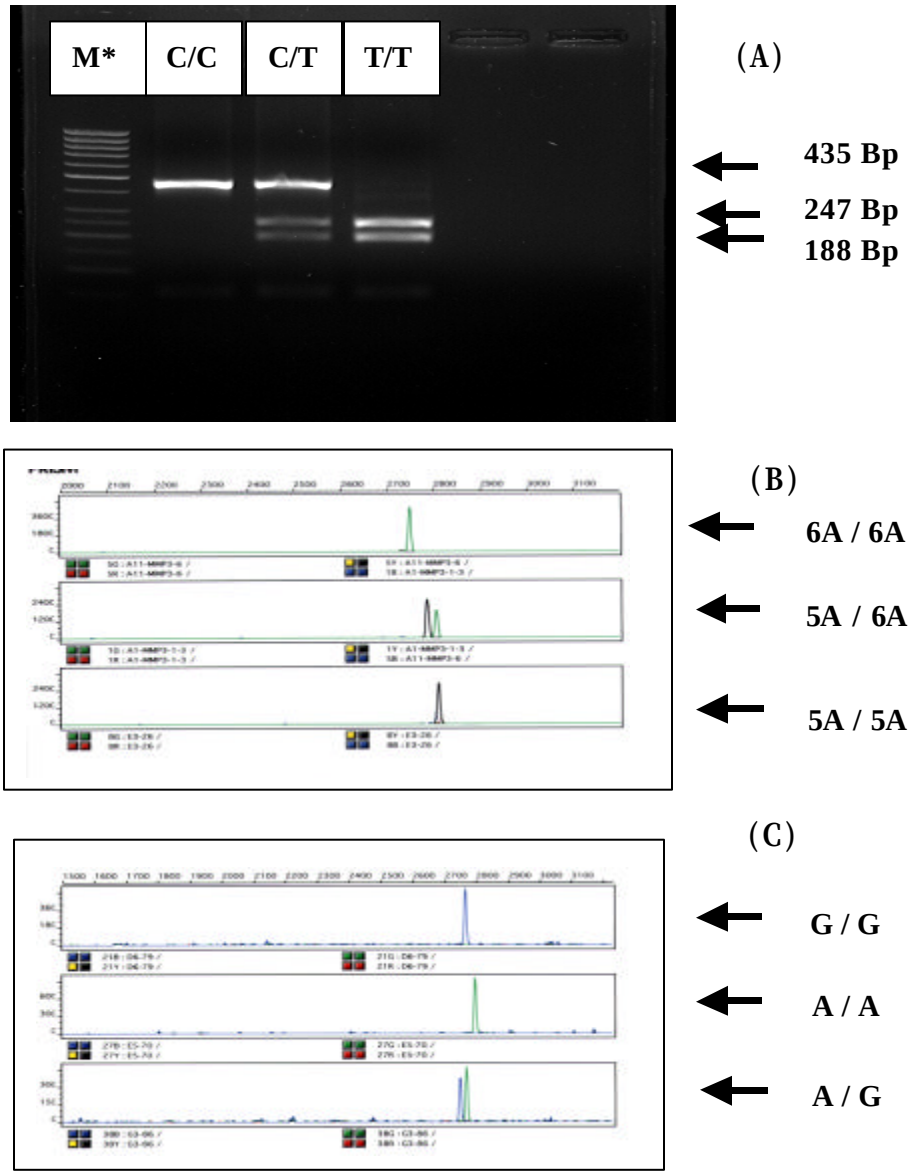

Figure 1 (A) Polymorphism of gelatinase B gene promoter was analyzed using restriction fragment length polymorphism (RFLP). Stromelysin-1 gene (B) and gelatinase B gene exon 6 (C) was analyzed by single base pair extension assay using SnaPshot™ kit.

* 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, χ^2 test, respectively. χ^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

	Stable angina (n = 131)	Control (n=117)	p - value
Age (years)	**61.3 ±7.9**	**59.3 ±8.5**	**p =0.04**
Gender (M/F)	**89 / 42**	**59 / 58**	**p =NS**
BMI (kg/m^2)	**25.7 ±3.5**	**25.2 ±4.3**	**p = NS**
Smoking (n)	**40 (30.8%)**	**39 (34.2%)**	**p =NS**
Hypertension (n)	**62 (47.3%)**	**52 (45.6%)**	**p =NS**
Diabetes (n)	**31 (23.7%)**	**18 (15.5%)**	**p =NS**
Total Cholesterol[1]	**198.0 ±35.8**	**176.7 ±33.3**	**p < 0.001**
LDL-cholesterol[1]	**120.0 ±27.6**	**102.0 ±27.3**	**p < 0.001**
HDL-cholesterol[1]	**42.6 ±12.9**	**45.8 ±12.9**	**p =NS**
Triglyceride[1]	**166.6 ±107.7**	**142.6 ±75.1**	**p =NS**
Fibrinogen[1]	**367.8 ±93.5**	**381.1 ±127.5**	**p =NS**
ESR (mm/hr)	**19.9 ±14.9**	**20.0 ±17.1**	**p =NS**
Lipoprotein (a)[1]	**28.2 ±23.0**	**27.2 ±20.8**	**p =NS**

[1]: mg/dl

* Student t-test (for continuous variables) and χ^2 test (for discrete variable) were used to compare the values for stale 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

age. 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.[5] 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.[4] 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, respetively. The frequencies were close to the values reported in European studies.[12] The distributions of genotypes of all three gene polymorphisms were compatible with Hardy-Weinberg equation.

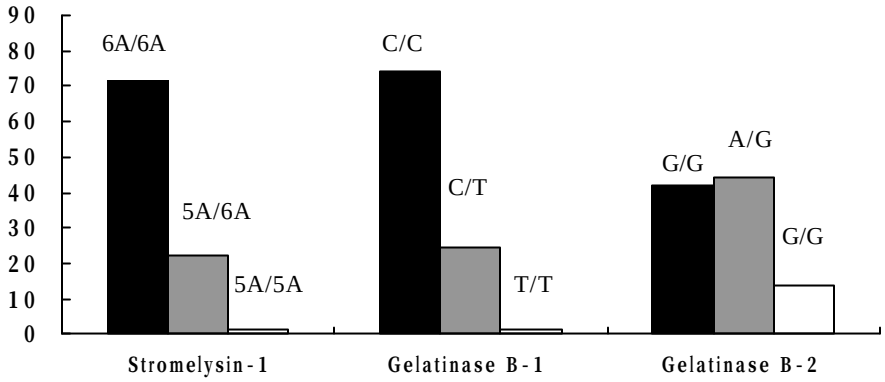

Figure 2. Distribution of genotye frequencies of stromelysin-1 and gelatinase B genes.

*Gelatinase B-1: polymorphism of promoter region, Gelatinase B-2: polymorphism of exon 6 region.

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).

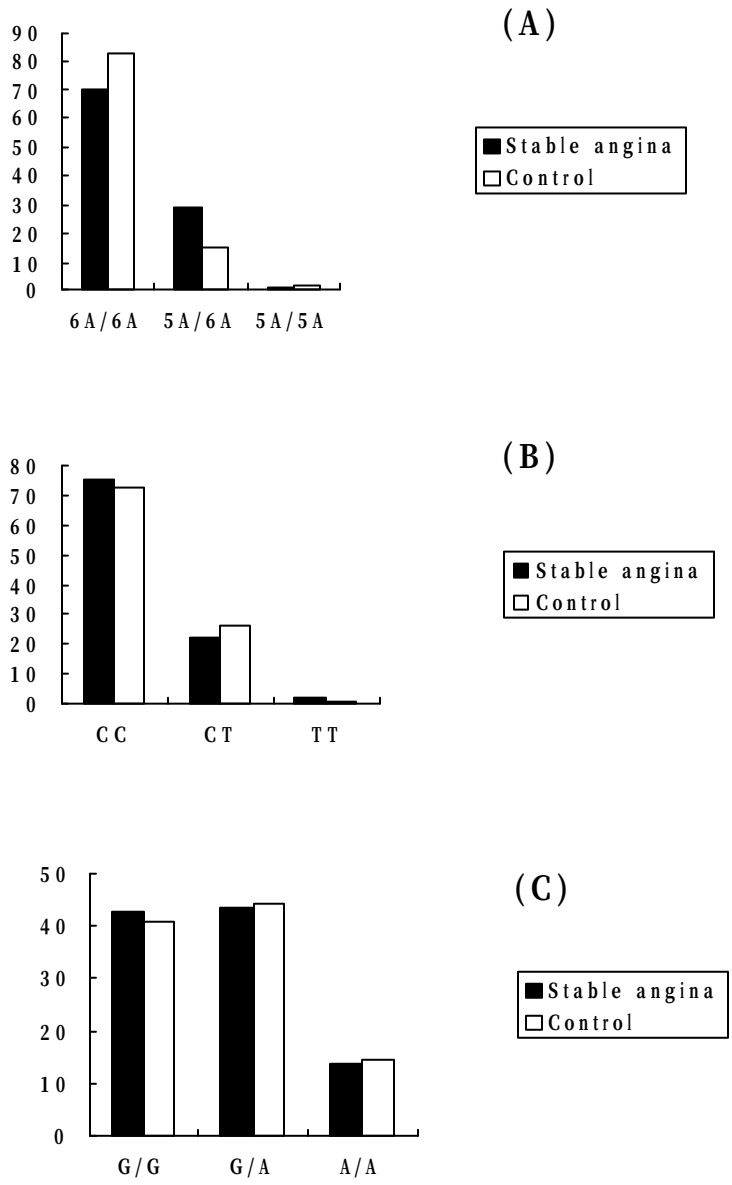

Fig 3. Distribution of the genotypes of stromelysin-1 and gelatinase B between stable angina group and control group.

(A): Stromelysin-1, (B): Gelatinase B promoter, (C): Gelatinase exon-6

C. Comparision of genotype frequencies of stromelysin-1 and geltinase 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 consitent 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 geneotype 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

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

* χ^2 test were used to compare the values for stale angina and control patients.

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

Predictors	Odds ratio (95% CI)	P value
Stromelysin-1, 5A/6A+ 5A/5A	**2.28 (1.19 – 4.38)**	**0.01**
Male gender	**2.26 (1.29 – 3.93)**	**0.04**
High LDL-cholesterol[1]	**3.65 (1.79 – 7.39)**	**< 0.001**

[1] Higher LDL-cholesterol was defined when the LDL-cholesterol was higher than 130 mg/dl.

*Cl: Confidence interval

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 a 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

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

* χ^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

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

* χ^2 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.[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 contol subjects (48.8 % vs 32.7%, $P < 0.0001$).[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.[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.[3,21] However, this finding was only seen in comparing those with the least stenosis (< 20%) and higher serum LDL-cholesterol (> 160mg/dl).[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[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 knwon to facilliate vascular smooth muscle cell migration.[22-23] Zang et al reported C-1562T polymorphism was related with the severity of coronary artery atherosclerosis.[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 injuried 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.[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.[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.[12, 29] Our data showed allele frequency similar to previous reports.[4,12] But, the frequency of both polymorphisms are not significant different between two groups. In gelatinase B gene, the frequeny of T allele is higher in control patients than stable angina patients, which finding is contast results to previous European results.[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,[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 signifincant coronary artery stenosis. We hypthesize the mechanism of this finding may be due to beakdown 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.[30] But in our study, there is no differnence of genetic frequecy 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 sudy, the number of population was small, thus further randomized study will be required to prove this hypothesis.

V. Conclusion

This study demostrated 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 gentic variations influencing MMP expression might contribute to disease phenotype.

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국문요약

관상동맥질환에서의Stromelysin-1 유전자와 GelatinaseB 유전자 다형성의 역할

교원단백질 분해제는 새로운 교원질의 생성뿐만 아니라 분해에 관여하여 재형성에 관여하는 중요한 역할을 담당한다. 이 중에서 Stromelysin-1 유전자와 Gelatinase B 유전자는 관상동맥의 죽상경화에 있어 중요한 작용을 한다고 알려져 있다. Stromelysin-1 유전자의 프로모터부위의 5A/6A 다형성은 급성 심근경색의 병인에 있어 위험인자가 될 수 있고 92-kDa의 type Ⅳ 교원단백질 분해제에 작용을 담당하는 Gelatinase B 유전자는 동맥 죽상반의 분열되기 쉬운 병변에 높게 발현이 된다고 보고된 바 있다. 특히 –1562에 위치한 C 대립인자의 T 대립인자로의 치환은 프로모터의 항진을 가져오게 한다. 또한 아직까지는 관상동맥질환에서 실제로 적용되지는 않았지만 Gelatinase exon 6 부위의 R279Q의 흔한 다형성은 새롭게 평가되어야 할 대상으로 대두되고 있다. 이에 본 연구자들은 1) 안정성 협심증에서의 위에 기술한 세가지의 유전자의 다형성과의 관계,2) 관상동맥질환의 중증도에 따른 유전자 다형성과의 관계 3) 관상동맥 스텐트 삽입술 후 6개월 추적관찰상에 스텐트 재협착과 유전자 다형성과의 관계를 알아보고자 하였다.

본 연구에서는 131명의 안정성 협심증 군(평균연령 61.3세, 남자 89명)과 117명의 정상 관상동맥소견을 보인 대조군(평균 연령 59.3세, 남자 59명)을 대상으로 MMP 유전자형을 비교분석하였다. 모든 대상군은 1998년 2월부터 2000년 6월 사이에 연세대학교 심혈관병원에서 관상동맥 조영술을 시행받았다. 환자로부터 얻은 혈액에서 DNA를 축출하였고 SnaPshot™을 이용한 sigle base pair primer extension 방법과 restriction fragmentation length polymorphism(RFLP) 방법을 통하여 stromelysin-1 promoter 유전자의 5A/6A 다형성, gelatinase B 유전자의 –1562 부위의 C/T 다형성과 exon 6 부위의 G/A 다형성을 분석하였다.

분석에 있어 5A를 포함한 stromelysin-1의 다형성은 안정성 협심증 환자군에서 대조군에 비해 유의하게 높았다. 하지만 Gelatinase-B의 두 가지의 유전자 다형성은 두군에서 차이를 보이지 않았다. 로지스틱 회귀 분석에서도 5A를 포함한 Stromelysin-1의 다형성은 유의한 위험인자로 상대적 위험도는 2.29로 나타났다.

관상동맥의 중증도나 스텐트 재협착에 따른 분석에서는 stromelysin-1이나 gelatinase B의 유전자 다형성 모두에서 변이 유전자형과 상관관계를 관찰할 수 없었다.

이상의 결과에서 Stromelysin-1의 기능적인 유전자 변이는 관상동맥의 죽상경화에 중요한 인자가 될 수 있으리라 생각된다.

핵심되는 말 : 안정성 협심증, 다형성, Stromelysin-1, Gelatinase B