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Plasminogen Activator Inhibitor-1 4G/5G Promoter Polymorphism and Coagulation Factor VII Arg353→Gln Polymorphism in Korean Patients with Coronary Artery Disease

An increased risk for arterial thrombosis is associated with high plasma levels of coagulation and fibrinolytic factors such as PAI-1 and FVII. In this study, the 4G/5G polymorphism in the promoter of PAI-1 gene and Arg353→Gln polymorphism in the FVII gene were analysed in 139 normal adults and 158 patients with coronary artery disease (CAD), and their association with plasma lipid traits was investigated. There were no significant differences in the allele frequencies of *PAI-1* and FVII polymorphisms between control and patient groups. The allelic distributions of both polymorphisms in Koreans were similar to those in Japanese but significantly different from those in Caucasians. In the CAD group, the 4G homozygotes of PAI-1 polymorphism showed significantly higher levels of total (p=0.0250) and LDL cholesterol (p=0.0335) with individuals having other genotypes. However, FVII polymorphism showed no association with lipid levels. In conclusion, the 4G/5G PAI-1 promoter polymorphism and Arg353→Gln FVII polymorphism are not major genetic risk factors for CAD in Koreans. However, 4G allele of PAI-1 polymorphism revealed to be associated with the levels of cholesterol, especially LDL cholesterol levels in CAD patients.

Key Words: *Plasminogen Activator Inhibitor 1; Factor VII; Polymorphism (Genetics); Coronary Disease*

Junghan Song, Yeo Min Yoon, Hyun Jin Jung, Seung Ho Hong, Hyosoon Park*, Jin Q Kim

Department of Clinical Pathology, Seoul National University College of Medicine and Sungkyunkwan University School of Medicine*, Seoul, Korea

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Address for correspondence
Jin Q Kim
Department of Clinical Pathology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea
Tel: +82.2-760-3326, Fax: +82.2-745-6653
E-mail: jqkim@plaza.snu.ac.kr

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INTRODUCTION

Plasminogen activator inhibitor-1 (PAI-1) is a 50 kDa glycoprotein that belongs to the serine protease inhibitor superfamily. Although there is controversy surrounding the implication in coronary heart disease (CHD) (1, 2), reduced plasma fibrinolytic activity, due mainly to elevated PAI-1 activity, was found to be associated with CHD. The *PAI-1* gene has been cloned and localized to q21.3-q22 of chromosome 7 (3, 4). Within the *PAI-1* gene, several polymorphisms have been described (1). Among them, a common single base pair insertion (5G)/deletion (4G) polymorphism, 675 bp upstream from the start of transcription of the *PAI-1* gene, has been studied extensively. In patients with myocardial infarction (MI), non-insulin dependent diabetic mellitus (NIDDM) and healthy control subjects, studies of 4G/5G polymorphism have shown higher plasma PAI-1 activity in subjects with the 4G than with the 5G allele (5-9).

Coagulation factor VII is a serine protease found in plasma and is a vitamin K-dependent coagulation factor, along with prothrombin (factor II), factors IX and X and proteins C and S. Several cross-sectional studies have also reported increased FVIIc levels in groups with manifest CHD or at risk of CHD (10-12). The gene for FVII is located close to the gene for factor X on the tip of the long arm of chromosome 13 (13). A common polymorphism of the FVII gene, which was detected with the presence or absence of a cleavage site for *Msp*I, was reported by Green in 1991 (14). The base change that caused the polymorphism is G to A substitution in the second position of the codon for amino acid 353, which leads to the substitution of arginine in the protein product of the G allele (designed FVII Arg353), with glutamine in the product of the A allele (FVII Gln353). The Gln353 allele was known to be consistently associated with significantly lower levels of FVIIc (15).

Since gene pools, lifestyle and gene-environment interactions differ among populations, the risk should not be assumed to be similar in all populations for a given genetic trait. In some candidate genes, the Korean population showed a quite different pattern in frequency and disease association (16). Therefore, ethnic differences in the allele frequency of the *PAI-1* and FVII polymor-

phisms and in the genetic association with disease and lipid traits may exist. In this study, the 4G/5G polymorphism in the promoter of *PAI-1* gene and Arg353→Gln polymorphism in the FVII gene were analyzed in Korean patients with CAD, and their association with plasma lipid traits was investigated.

MATERIAL AND METHODS

Subjects

One hundred and fifty-eight patients with coronary artery disease (CAD) (CAD group: 99 males and 59 females) and 139 sex- and age-matched normal adults (control group: 86 males and 53 females) were selected from Seoul National University Hospital, Seoul, Korea. The patients with CAD (recent myocardial infarction or angina) were documented by coronary angiography. All of the selected CAD patients were not on therapy to lower lipid at the time of sampling. In myocardial infarction patients, blood samples were obtained two months after the occurrence of myocardial infarction. The adults in the normal control group were randomly selected via health-screening at the same hospital. Those who had a history of chest pain, diabetes, hypertension and general illness were excluded. Body mass index (BMI: calculated by dividing weight by height^2), diabetes and hypertension was also studied. A subject was considered to have hypertension if taking antihypertensive medication or if the diastolic blood pressures was greater than 95 mmHg. Diabetes was considered present if a prior physician diagnosis had been made or if the fasting serum glucose was >140 mg/dL. Detailed subject characteristics are shown in Table 1.

Table 1. Characteristics of the study subjects

Parameters	Control (n=139)	CAD (n=158)	p*
Age	60.4±5.5	60.7±9.2	NS
Male/Female	86/53	99/59	NS
Hypertension (%)	0	53%	-
Diabetes (%)	0	19%	-
BMI (kg/m^2)	23.9±2.8	24.9±3.0	0.0082
Chol. (mmol/L)	5.310±0.877	5.158±1.034	NS
TG (mmol/L)	1.261±0.534	1.527±0.816	0.0023
HDL-C (mmol/L)	1.549±0.385	1.136±0.319	0.0001
LDL-C (mmol/L)	3.188±0.800	3.327±0.931	NS
ApoAI (g/L)	1.386±0.339	1.149±0.282	0.0001
ApoB (g/L)	1.103±0.265	1.111±0.300	NS

Continuous data are expressed by mean±SD.
Control, normal control group; CAD, coronary artery disease group; BMI, body mass index; Chol., total cholesterol; TG, triglyceride; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; NS, not significant
*p value of Wilcoxon rank sum test between CAD and Control groups

Lipid and lipoprotein measurements

Blood samples were collected from all the subjects after they had fasted for 12 hr. The levels of plasma cholesterol and triglyceride were measured by enzymatic methods (Boehringer Mannheim, FRG). HDL-cholesterol levels were measured directly with the determiner HDL-C diagnostic kits (Kyowa Medex, Japan) using a Hitachi 747 or 7170 automatic chemistry analyzer. Using the formula of Friedewald et al., the levels of LDL-cholesterol were calculated. The levels of apo A-I and B were measured with immunonephelometric assay (Behring Nephelometer, Behringwerke AG, Germany).

Genetic polymorphism analysis

PAI-1 4G/5G polymorphism was analyzed by polymerase chain reaction amplification of genomic DNA, using an allele specific primer with insertion 5G allele; 5'-GTC TGG ACA CGT GGG GG-3' and deletion 4G allele UP; 5'-GTC TGG ACA CGT GGG GA-3, each in a separate polymerase chain reaction (PCR) together with the common downstream primer 5'-TGC AGC CAG CCA CGT GAT TGT CTA G-3' and control upstream primer 5'-AAG CTT TTA CCA TGG TAA CCC CTG GT-3'. The PCR condition was as described previously (8). The amplified DNA fragments were separated by agarose gel electrophoresis and viewed after staining with ethidium bromide. The samples were genotyped and classified into one of three possible genotypes 4G/4G, 5G/5G or heterozygous 4G/5G (Fig. 1). FVII polymorphism was determined using PCR amplification followed by *Msp*I (Promega, U.S.A.) digestion. The oligonucleotide primers and PCR condition have been described elsewhere (17). *Msp*I digestion of the amplified DNA yielded 40-, 67-, and 205-bp fragments in Arg353 allele (G allele), and 40- and 272-bp fragments in Gln353 allele (A allele) (Fig. 1).

Statistical analysis

Continuous data are expressed as mean±SD. All statistical analyses were performed using the Statistical Analysis System software (SAS Institute, Inc). Variables in two or three groups were compared with Wilcoxon rank sum test or Kruskal Wallis 1 way ANOVA test. The χ^2 test and Fisher's exact test were performed to test for an independent relationship among the variables.

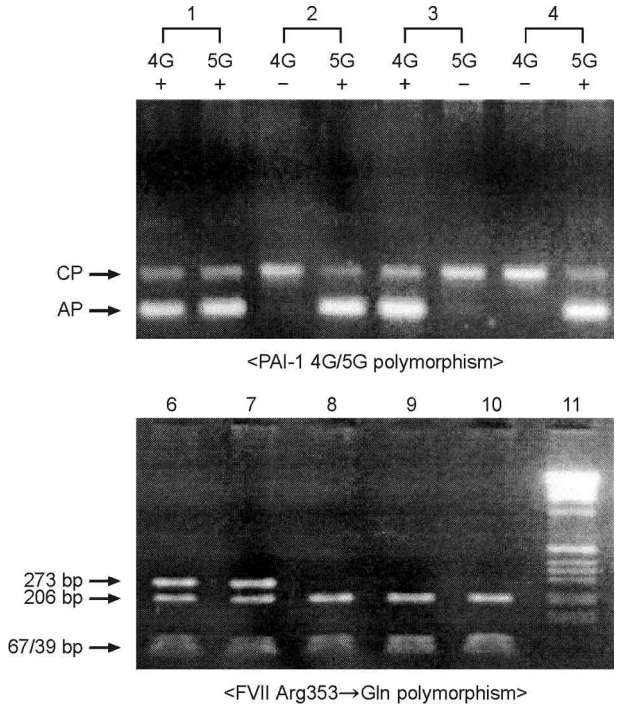

Fig. 1. Gel electrophoresis patterns for the analysis of PAI-1 4G/5G and FVII Arg353→Gln polymorphisms. Lane 1: 4G/5G genotype; lane 2, 4: 5G/5G genotype; lane 3: 4G/4G genotype; lane 6, 7: Arg/Gln genotype; lane 8, 9, 10: Gln/Gln genotype. CP: amplified DNA product by common upstream primer, AP: amplified DNA product by allele specific upstream primer.

RESULTS

The genotypic and allelic frequencies of PAI-1 4G/5G promoter polymorphism and FVII Arg353→Gln polymorphism are given in Table 2. Genotype distributions of the two polymorphisms did not deviate from those expected for Hardy-Weinberg proportion. There were no significant differences in the allele frequencies of PAI-1 and FVII polymorphisms between control and CAD groups. The allelic distribution of PAI-1 4G/5G promoter polymorphism and FVII Arg353→Gln polymorphism in Koreans was similar to that in Japanese but significantly different from that in Caucasians (Table 3). Orientals tended to show a higher frequency of 4G allele of PAI-1 polymorphism and Gln353 (A allele) of FVII polymorphism compared with Caucasians.

We investigated the association between the polymorphisms and serum lipid levels (Table 4). In the CAD group, the 4G homozygous individuals of PAI-1 polymorphism showed significantly higher levels of total (p=0.0250) and LDL cholesterol (p=0.0335) compared with individuals having other genotypes. In control group, however, there was no significant difference in total or LDL cholesterol levels among the genotypes of PAI-1 polymorphism.

In the present study, all the AA genotype for FVII polymorphism was added to the AG group for analysis

Table 2. Genotypic and allelic frequencies of control, CAD and DM groups of PAI-1 4G/5G polymorphism and FVII Arg343→Gln polymorphism

RFLPs	Groups	Genotypic frequencies number (%)			Allelic frequencies		p*
		4G4G	4G5G	5G5G	4G	5G	
PAI-1 4G/5G	Control	54 (38.8)	60 (43.2)	25 (18.0)	0.604	0.396	
	CAD	62 (39.2)	64 (40.5)	32 (20.3)	0.595	0.405	NS
		GG	GA	AA	G (Arg353)	A (Gln353)	
FVII *Msp*I	Control	122 (87.8)	16 (11.5)	1 (0.7)	0.935	0.065	
	CAD	140 (88.6)	18 (11.4)	0 (0.0)	0.943	0.057	NS

RFLP, restriction fragment length polymorphism; PAI-1, plasminogen activator inhibitor-1; FVII, coagulation factor VII; NS, not significant
*p value of Chi-square test between the allele frequencies of control and disease groups

Table 3. Comparison of the allele frequencies of the polymorphisms in different ethnic groups

RFLPs	Groups	Number	Allelic frequencies		p*
			4G	5G	
PAI-1 4G/5G	Koreans (This study)	139	0.604	0.396	
	ECTIM (21)	701	0.537	0.463	0.040
	Japanese (35)	177	0.599	0.401	NS
	Japanese (19)	148	0.615	0.385	NS
			G (Arg353)	A (Gln353)	
FVII Arg353→Gln	Koreans (This study)	139	0.935	0.065	
	Dravidian Indians (17)	185	0.714	0.286	0.001
	Italian (34)	224	0.786	0.214	0.001
	Japanese (36)	101	0.955	0.045	NS

Abbreviations are same as Table 1 and 2.
*p value of Chi-square test between the allele frequencies of Koreans and other ethnic groups

Table 4. Comparison of the levels of various risk factors among the genotypes of each polymorphism

Risk factors	Group	PAI-I				FVII		
		4G4G	4G5G	5G5G	p*	GG	GA+AA	$p^{\dagger}$
Number	Control	54	60	25		122	17	
	CAD	62	64	32		140	18	
BMI (kg/m^2)	Control	24.0±3.2	23.8±2.4	24.0±3.0	NS	23.9±2.9	23.8±2.9	NS
	CAD	24.9±2.9	24.9±2.8	24.8±3.5	NS	24.9±3.0	24.7±2.7	NS
Chol. (mmol/L)	Control	5.243±0.932	5.318±0.858	5.435±0.8	NS	5.319±0.891	5.244±0.790	NS
	CAD	5.416±1.026	5.018±1.123	4.936±0.743	0.0250	5.193±1.057	4.879±0.803	NS
TG (mmol/L)	Control	1.264±0.483	1.239±0.470	1.310±0.761	NS	1.265±0.551	1.235±0.402	NS
	CAD	1.501±0.754	1.574±0.925	1.483±0.709	NS	1.557±0.843	1.296±0.519	NS
HDL-C (mmol/L)	Control	1.521±0.329	1.550±0.383	1.605±0.498	NS	1.549±0.369	1.545±0.502	NS
	CAD	1.192±0.356	1.064±0.288	1.169±0.281	NS	1.127±0.301	1.199±0.439	NS
LDL-C (mmol/L)	Control	3.148±0.819	3.203±0.789	3.236±0.811	NS	3.195±0.823	3.138±0.620	NS
	CAD	3.542±0.966	3.237±0.976	3.091±0.675	0.0335	3.358±0.962	3.088±0.606	NS
ApoAI (g/L)	Control	1.402±0.399	1.370±0.272	1.392±0.353	NS	1.380±0.327	1.436±0.419	NS
	CAD	1.220±0.319	1.089±0.250	1.132±0.241	NS	1.142±0.276	1.203±0.333	NS
ApoB (g/L)	Control	1.100±0.247	1.086±0.280	1.148±0.270	NS	1.103±0.267	1.104±0.259	NS
	CAD	1.166±0.304	1.083±0.321	1.062±0.233	NS	1.121±0.306	1.034±0.238	NS

Abbreviations are same as Table 1 and 2.
*p value of Kruskal Wallis 1 way ANOVA test between Control and CAD or DM groups
$^{\dagger}p$ value of Wilcoxon rank sum test between Control and CAD or DM groups

Table 5. Comparison of the levels of total and LDL cholesterol among the haplotypes of PAI-1 and FVII polymorphisms in control and CAD groups

Haplotypes of PAI-1 and FVII polymorphisms	Control			CAD		
	No.	Chol. (mmol/L)	LDL-C (mmol/L)	No.	Chol.* (mmol/L)	LDL-C* (mmol/L)
4G4G-AA	1	4.507	3.004	0		
4G4G-GA	11	5.139±0.570	3.017±0.562	8	4.662±0.873	2.929±0.554
4G4G-GG	42	5.289±1.013	3.186±0.886	54	5.527±1.008	3.634±0.984
5G4G-GA	2	4.766, 5.828	2.927, 3.367	4	5.154±0.539	3.328±0.539
5G4G-GG	58	5.317±0.868	3.204±0.803	60	5.009±1.155	3.232±1.000
5G5G-GA	3	5.846±1.484	3.626±1.002	6	4.986±0.896	3.139±0.746
5G5G-GG	22	5.379±0.725	3.186±0.795	26	4.926±0.723	3.080±0.673

Abbreviations are same as Table 1 and 2.
*significantly different in total (p=0.0259) and LDL (p=0.0331) cholesterol among the haplotype by Kruskal Wallis 1 way ANOVA test

purposes, considering the small number of AA genotype. The FVII polymorphism showed no association with lipid and lipoprotein levels.

The lipid and lipoprotein levels according to the haplotypes of PAI-1 and FVII polymorphisms were also investigated. The levels of total (p=0.0250) and LDL cholesterol (p=0.0335) were significantly different among the haplotypes in CAD group (Table 5). These results were similar with those of PAI-1 polymorphism because of low frequency of the 'A' allele of FVII polymorphism.

DISCUSSION

In this study, total and LDL cholesterol concentrations showed no differences between control and CAD groups. There was an report arguing that the average cholesterol levels at which CAD events occurred were substantially decreased with age (18). As the average age of the subjects in this study were relatively high (60.4±5.5 in control and 60.7±9.2 in CAD groups), the difference in total and LDL cholesterol levels between control and CAD groups might be minimized.

Our data indicated some racial differences in the allelic distribution of PAI-1 4G/5G promoter polymorphism and FVII Arg353→Gln polymorphism; Orientals tended to show higher frequencies of 4G allele of PAI-1 polymorphism and Gln353 (A allele) of FVII polymorphism compared with Caucasians. The reason for these differences may be random genetic drift or natural selection

operating at this gene cluster, or a combination of both.

It is clear that an increased risk for arterial thrombosis is associated with high plasma levels of coagulation and fibrinolytic factors. Raised plasma levels of the principal inhibitor of fibrinolysis and PAI-1 have been documented, and impaired fibrinolysis is a strong determinant of vascular ischemic events (1). Impaired fibrinolysis may also accelerate the atherosclerotic process by allowing fibrin deposition and thrombosis within developing lesions. In addition to the markers of insulin resistance and smoking habit, gene variants of PAI-1 account for a significant portion of the between-individual variability of circulating PAI-1 antigen concentrations in a general population without clinical evidence of atherosclerosis.

Not all but most of studies reported that 4G allele of *PAI-1* gene is associated with elevated plasma PAI-1 levels. Eriksson et al. (7) suggested that both 4G and 5G allele bind a transcriptional activator, whereas the 5G allele also binds a repressor protein to an overlapping binding site. In the absence of bound repressor, the basal level of PAI-1 transcription is increased, so PAI-1 activity may be higher in 4G allele. It is considered that the risk for MI may be increased in individuals with 4G/4G genotype because of high PAI-1 activity. Several case control studies revealed that 4G allele of the *PAI-1* gene was associated with a high risk of diabetic retinopathy (19), acute coronary syndrome (20) and CAD in patients with NIDDM (21) and premature myocardial infarction. However, there is controversy surrounding the disease association of PAI-1 promoter genotype. Some researchers reported contrary results that the 4G/5G polymorphism in the promoter of the *PAI-1* gene is not a major pathogenic risk factor for MI (22), diabetic retinopathy (23), stroke (24) and arterial or venous thrombosis (25-27).

In this study, there were no differences in allele frequency between CAD patients and controls, suggesting the allele frequency itself is not a major risk factor for CAD in Koreans.

There have been several reports that PAI-1 activity correlated significantly with serum triglycerides (8, 9, 28), and these associations between PAI-1 levels and triglyceride are influenced by genotype in the region of the *PAI-1* gene promoter. However, there were few reports on the association of PAI-1 genotype with other lipid, lipoprotein and apolipoprotein levels. Mansfield et al. (8) reported that there was a weak correlation between PAI-1 activity and cholesterol levels in NIDDM patients. In the study by Broch et al., (29) there was no association between total cholesterol and PAI-1 genotype, although there was a tendency of high cholesterol level in 4G/4G genotype. But, Panahloo et al. (30) reported that there was a significant difference in cholesterol concentration among the genotypes. The levels of cholesterol were significantly higher in 4G/4G group than in other genotypes.

In the present study, individuals with 4G allele had higher levels of total and LDL cholesterol compared with other genotypes in CAD group, which was similar to the report by Panahloo et al. (30). While the 4G allele was known to be associated with higher PAI-1 activity, PAI-1 activity was correlated with cholesterol levels; therefore, 4G allele could be associated with cholesterol levels, especially with LDL cholesterol levels. In Koreans, the 4G allele of PAI-1 promoter polymorphism showing a high LDL cholesterol level might be indirectly associated with CAD. However we could not prove the direct relationship among the PAI-1 genotypes, plasma PAI-1 and cholesterol levels as plasma PAI-1 levels were not measured in the study subjects. Additional study measuring plasma PAI-1 levels would be needed to prove this relationship.

Increased plasma factor VII coagulant activity has been associated with risk for ischemic heart disease (IHD), although not all studies reported the same result (31). The Gln353 allele carrier showed significantly lower plasma factor VIIc and factor VIIag (17, 32-34). The genotype-specific correlation of factor VIIc and factor VIIag with triglyceride levels was stronger in the Gln353 allele carrier. Iacoviella et al. (35) reported that FVII polymorphism may influence the risk of MI. In this study, FVII genetic polymorphism revealed not to be a risk factor for CAD and showed no relationship with triglyceride levels. The allele frequencies of G allele in Koreans is significantly lower than those in Caucasian, which might be an ethnic difference in genetic influence of FVII polymorphism as a risk factor for atherosclerosis.

In conclusion, the allelic distribution of PAI-1 4G/5G promoter polymorphism and FVII Arg353→Gln polymorphism in Koreans was similar to that in Japanese but significantly different from that in Caucasians. The 4G/5G PAI-1 promoter polymorphism and Arg353→Gln FVII polymorphism are not major genetic risk factors for CAD in Koreans. However, 4G allele of PAI-1 polymorphism revealed to be associated with cholesterol levels, especially LDL cholesterol levels in CAD patients.

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