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Association of *PAI-1* Polymorphism with Schizophrenia in Korean Population

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Abstract

Several reports have suggested a possible relationship between blood coagulation factors and schizophrenia. Plasminogen activator inhibitor type 1 (*PAI-1*) belongs to a serine protease inhibitor family, which regulates fibrinolysis and proteolysis by inhibiting plasminogen activation. The purpose of this study was to investigate the association of polymorphisms of the *PAI-1* gene with schizophrenia in Korean population. Two important polymorphisms (−675 4G/5G and −844 G/A) located on promoter region of the *PAI-1* gene were analyzed on 178 schizophrenia patients and 226 controls. The genotypic and allelic associations of −675 4G/5G were found significant. Furthermore, haplotype analysis revealed significant result, which suggests that −675 4G/5G polymorphism might confer increased susceptibility for schizophrenia in Korean population.

Keywords: Plasminogen activator inhibitor 1, Schizophrenia, Polymorphism, Association

Schizophrenia is a chronic, severe, and disabling brain disorder that affects approximately 1% of the population worldwide. Although, the exact etiology or pathology of schizophrenia remains unknown, genetic predisposition along with the environmental factors have been suggested to be important in the development of schizophrenia. Wong *et al.*[1], by investigating the changes of serum protein levels, have reported decreased plasminogen levels in Chinese patients with both acute and chronic schizophrenia compared to controls. Decreased level of antithrombin in cerebrospinal fluid (CSF) of schizophrenia patients was reported[2]. In other studies, platelet aggregation and dense granule secretion were significantly higher in schizophrenia patients than normal controls[3]. In a 22q11 deletion syndrome subtype of schizophrenia, low platelet counts were observed[4]. Furthermore, it has been suggested that mental and physical stress not only cause alterations of platelet function, but also decrease fibrinolytic activity[5,6]. These stress-induced thromboses were proposed to be regulated by plasminogen activator inhibitor-1 (*PAI-1*)[7].

PAI-1 is a primary regulator of the fibrinolytic cascade and a rapid inhibitor of both tissue- and urokinase-type plasminogen activators. As a member of the serpin superfamily, it is encoded by a gene of approximately 12.2 kb that is located on chromosome 7q21.3-q22[8]. *PAI-1* is produced in several tissues, such as liver, endothelium, adipose tissue, and vascular smooth muscle cells[9-11]. Interestingly, the presence of *PAI-1* in human CSF has been reported to have a potential role in nervous system homeostasis[12]. Tissue-type plasminogen activator (tPA) has been also known to be involved in neuronal migration and directly influence the brain function[13]. It would be meaningful to investigate the relationship between *PAI-1* and schizophrenia. Therefore, we examined the genetic association between two well-known polymorphisms present in the promoter region of the human *PAI-1* gene: a single guanosine insertion /deletion 4G/5G polymorphism (dbSNP rs#1799768) upstream at −675 bp and a G/A single base substitution polymorphism (dbSNP rs#2227631) −844 bp upstream from the start of transcription, and schizophrenia in individuals residing in Korea.

The genotype distributions in schizophrenia patients and controls had no deviation from Hardy-Weinberg equilibrium (−675 4G/5G: $\chi^2=2.60$, $p=0.107$ for patients, $\chi^2=3.78$, $p=0.052$ for controls; −844 G/A: $\chi^2=0.81$, $p=0.367$ for patients; $\chi^2=3.24$, $p=0.072$ for controls). Allele and genotype frequencies of *PAI-1* polymorphisms −675 4G/5G and −844 G/A are summarized in Table 1. Significant differences were observed in both genotype distribution ($\chi^2=18.63$, $p<0.0001$) and allele frequency [$\chi^2=12.18$, $p=0.0005$, odds ratio (OR)=1.645, 95% confidence interval (CI)=1.243−2.177] of *PAI-1* −675 4G/5G polymorphism. In order to determine whether these

Table 1. Genotypic and allelic frequencies of *PAI-1* polymorphisms in schizophrenia patients and controls.

PAI-1		Total (%)		Men (%)		Women (%)	
		Patients (n=178)	Controls (n=226)	Patients (n=97)	Controls (n=122)	Patients (n=81)	Controls (n=104)
−675 4G/5G	Genotype						
	4G/4G	45 (25.3)	73 (32.3)	22 (22.7)	33 (27.1)	23 (28.4)	40 (38.5)
	4G/5G	78 (43.8)	123 (54.4)	40 (41.2)	77 (63.1)	38 (46.9)	46 (44.2)
	5G/5G	55 (30.9)	30 (13.3)	35 (36.1)	12 (9.8)	20 (24.7)	18 (17.3)
		χ^2=18.63		χ^2=22.60		χ^2=2.64	
		$p<0.0001$		$p<0.0001$		p=0.2677	
	Allele						
	4G	168 (47.2)	269 (59.5)	84 (43.3)	143 (58.6)	84 (51.9)	126 (60.6)
	5G	188 (52.8)	183 (40.5)	110 (56.7)	101 (41.4)	78 (48.1)	82 (39.4)
		X^2=12.18		χ^2=10.14		χ^2=2.82	
		p=0.0005		p=0.0014		p= 0.0928	
		OR=1.645		OR=1.854		OR=1.427	
		95% CI=1.243−2.177		95% CI=1.266−2.715		95% CI=0.942−2.161	
−844 G/A	Genotype						
	GG	69 (38.8)	67 (29.6)	41 (42.3)	34 (27.9)	28 (34.6)	33 (31.7)
	GA	79 (44.4)	124 (54.9)	42 (49.3)	70 (57.4)	37 (45.7)	54 (51.9)
	AA	30 (16.8)	35 (15.5)	14 (14.4)	18 (14.7)	16 (19.7)	17 (16.4)
		χ^2=4.75		χ^2=5.37		χ^2=0.77	
		p=0.0929		p=0.0682		p=0.6810	
	Allele						
	G	217 (61.0)	258 (57.1)	124 (63.9)	138 (56.6)	93 (57.4)	120 (57.7)
	A	139 (39.0)	194 (42.9)	70 (36.1)	106 (43.4)	69 (42.6)	88 (42.3)
		χ^2=1.23		χ^2=2.44		χ^2=0.003	
		p= 0.2665		p=0.1186		p=0.9561	
		OR=1.174		OR=1.361		OR=0.988	
		95% CI=0.885−1.558		95% CI=0.924−2.004		95% CI=0.652−1.498	

n=number of samples; SD=standard deviation; OR=odds ratio; CI=confidence interval.

associations remain significant when analyzed according to sex, both patient group and control group were divided into men and women. Male population revealed significant association similar to that of total population (genotype: χ^2=22.60, $p<0.0001$; allele: χ^2=10.14, p=0.0014, OR=1.854, 95% CI=1.266−2.715). On the other hand, no significant difference was observed in female population. In case of −844 G/A polymorphism, no significant association was found, although genotype distribution in men was close to being significant (χ^2=5.37, p=0.0682).

The frequencies of the *PAI-1* promoter haplotypes in schizophrenia patients and controls are shown in Table 2. The −675 5G allele occurred mostly with −844 A allele and the −675 4G allele was mostly associated with the −844 G allele in schizophrenia patients. However, in control subjects, results were opposite. Haplotypes with highest frequencies were −675 5G/−844 G and −675 4G/−844 A in controls. Due to these differences in combination of all four alleles, significant result was found (χ^2=110.39, degree of freedom (df)=3, $p<0.0001$).

Table 2. Comparison of *PAI-1* gene haplotypes in schizophrenia patients and controls.

Haplotype	Schizophrenia (n=178)	Controls (n=226)
−675 4G/−844 G	0.4116	0.2327
−675 4G/−844 A	0.1980	0.3325
−675 5G/−844 G	0.0603	0.3608
−675 5G/−844 A	0.3301	0.0740
	df=3, χ^2=110.39	
	$p<0.0001$	

Values given are haplotype frequencies. P values for overall difference in haplotype distribution between schizophrenia patients and controls were calculated using the EH program. (n, number of samples; df, degree of freedom).

Discussion

Present study showed that genotype and allele frequencies of *PAI-1* promoter −675 4G/5G polymorphism were significantly associated with schizophrenia in Korean population. However, the genotype distribution and allele frequency of −844 G/A poly-

morphism were not associated with schizophrenia. Although the genotype and allele frequencies of −844 G/A polymorphism were not associated with schizophrenia, haplotype analysis of two polymorphisms (−675 4G/5G and −844 G/A) revealed a significant association with schizophrenia ($\chi^2=110.39$, $p<0.0001$).

PAI-1 has been noted as an important factor of the coagulation cascade and its promoter −675 4G/5G polymorphism has been implicated in many diseases. Elevated level of *PAI-1* has been demonstrated to be related to −675 4G/5G polymorphism[14,15]. Margaglione *et al.*[16] found a higher frequency of −675 4G/4G than −675 5G/5G in patients with coronary artery disease. −675 4G/5G polymorphism has been reported to be associated with a higher risk of myocardial or cerebrovascular infarction in Chinese[17]. Pre-eclampsia has been reported to be associated with 4G allele of −675 4G/5G polymorphism[18]. On the contrary, in studies of vascular dementia and autistic disorder, no association between the −675 4G/5G polymorphism and these diseases were observed[19,20].

To best of our knowledge, this is the first to study the relationship between −675 4G/5G polymorphism with schizophrenia. Although, in most cases, the frequency of 4G allele was higher and was closely related to the diseases, results were somewhat different in our study with Korean schizophrenia samples. In our study, −675 5G/5G genotype was found approximately 2.3 times more frequent in schizophrenia patients than controls. When genotype distribution of −675 4G/5G polymorphism of healthy controls of our study was compared with that of Italians, British, and Dutch all mentioned in the study of Persico *et al.*[20], there was some difference. The percentages of 4G/4G, 4G/5G, and 5G/5G were approximately 25%, 50% and 25%, respectively in Europeans; however, in Korean population, they were approximately 33%, 54% and 13%, respectively.

In conclusion, this study examined the possible association of *PAI-1* gene polymorphisms with schizophrenia. There was a significant association between −675 4G/5G and schizophrenia, although no association was observed in −844 G/A polymorphism. Significant differences in −675 4G/5G polymorphism distribution among the schizophrenia patients and controls suggest that −675 4G/5G may be an important genetic risk factor for schizophrenia.

Methods

Subjects

One hundred and seventy-eight unrelated patients [97 men and 81 women; mean age ± standard deviation (SD), 42.3 ± 11.1] with schizophrenia and 226 Korean healthy control subjects (122 men and 104 women; mean age ± SD, 55.3 ± 13.5) were involved in this study. The patients met the criteria for schizophrenia of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV. The controls were healthy volunteers with no history of psychiatric disorders. Written informed consent was obtained from each subject under protocols approved by the ethics review committee of the Medical Research Institute at the Kyung Hee University Medical Center. All studies were carried out according to the Declaration of Helsinki Guideline.

Genotyping

Genomic DNA was extracted from peripheral leukocytes by standard procedure using NucleoSpin DNA isolation kit (Macherey-Nagel GmbH, Germany). Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) assay was performed to genotype the DNA sequence variants of the *PAI-1* gene. The forward and the reverse primer sequences used for analysis of −675 4G/5G were 5′-TCC AAC CTC AGC CAG ACA AG-3′ and 5′-TGA TAC ACG GCT GAC TCA CC-3′, respectively and those of −844 G/A were 5′-CAG GCT CCC ACT GAT TCT AC-3′ and 5′-GAG GGC TCT CTT GTG TCA AC-3′. The amplification conditions were initiated at 95°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at the appropriate primer-pair annealing temperature 59°C for 30 s and extension at 72°C for 30 s, with a final extension step of 7 min at 72°C. The PCR products were digested at 37°C with the corresponding restriction enzyme, *Dra*III (−675 4G/ 5G) and *Xho*I (−844 G/A), respectively, and subsequently electrophoresed on 3.0% agarose gels stained with ethidium bromide. Digestion with *Dra*III generated two fragments of 71 and 19 bp while *Xho*I resulted in two fragments of 364 and 146 bp.

Statistical Analysis

The statistical significance of differences in the genotype distribution and allele frequency between schizophrenia patients and controls were assessed by a chi-square (χ^2) test at a significance level of $p<0.05$. The statistical analysis was carried out using SPSS (Version SPSS 12.0, Inc., Chicago, Il, USA) and the level of pair-wise haplotype between two SNPs, −675 4G/5G and −844 G/A, was analyzed using the EH program[21].

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