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Genetic Polymorphisms of t-PA and PAI-1 Genes in the Korean Population

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Abnormalities in fibrinolysis system is associated with risk of hypertension. In this report, the *Alu* repeat insertion/deletion (I/D) polymorphism of tissue plasminogen activator (t-PA) and the *Hin*d III RFLP of plasminogen activator inhibitor-1 (PAI-1) genes were investigated in 115 normotensives and 83 patients with hypertension, and their association with anthropometrical data and plasma biochemical parameters were analyzed. There were no significant differences in the gene frequencies of the two candidate genes between normotensives and hypertensives, respectively. Our results indicate lack of associations between the two polymorphisms in t-PA and PAI-1 genes and risk of hypertension in the population under study. However, the *Hin*d III RFLP of PAI-1 gene was significantly associated with plasma glucose level, suggesting its role in glucose metabolism. It needs to be tested whether this RFLP of PAI-1 gene is associated with insulin resistance syndrome or non-insulin dependent diabetes mellitus (NIDDM) in the Korean population.

Hypertension is likely to be a polygenic and multifactorial disorder that results from the inheritance of number of susceptibility genes (Kang et al., 2000). Impaired fibrinolysis with elevated PAI-1 levels and depressed t-PA activity is common in patients with hypertension (Landin et al., 1990; Jeng et al., 1996). Thus, genes involved in fibrinolysis may be good candidates in the etiology of hypertension.

Fibrinolysis constitutes a series of proteolytic reactions regulated by activators and inhibitors, with events partly occurring on the surface of fibrin. Plasminogen, the inactive zymogen, is cleaved to the active enzyme plasmin by enzymes such as tissue plasminogen activator (t-PA), urokinase or plasma derived plasminogen activators. Plasmin degrades fibrin or fibrinogen into well-defined degradation products (Collen, 1980).

Physiologically, the most important plasminogen-activating enzyme is probably t-PA. It is synthesized in endothelial cells and released into the blood by certain stimuli. It has a short half-life in blood, which is due to inactivation by inhibitors and rapid uptake and degradation in the liver. When fibrin is present in the blood, t-PA is adsorbed onto and activated by this fibrin (Collen, 1980).

The gene for t-PA is located on chromosome 8p12-p11.2 (Yang-Feng et al., 1986), and one polymorphism, an *Alu* repeat I/D polymorphism, was found in intron 8 of this gene (Tishkoff et al., 1996). This *Alu* repeat insertion probably arose early in human evolution, and a number of populations have been found to be dimorphic for its presence or absence (Novick et al., 1998).

Although this polymorphism is not significantly correlated with basal endothelial t-PA synthesis (van den Eijnden-Schrauwen et al., 1995), its association with myocardial infarction was reported (van der Bom et al., 1997).

PAI-1 is a 50 kD glycoprotein that belongs to the serine protease inhibitor superfamily (so-called serpin), which occurs in a latent and active form *in vivo*, and is partially bound to vitronectin (Ny et al., 1986). It is synthesized in endothelial cells and the liver. The 50 kD glycoprotein(PAI-1)'s synthesis may be regulated by certain hormones and neurotransmitters (Andreasen et al., 1990; Wiman and Hamsten, 1990). Most of the information regarding the regulation of PAI-1 synthesis is, however, derived from studies on cell cultures. *In vitro* studies suggest that the synthesis of PAI-1 in certain cells is stimulated by insulin (Kooistra et al., 1989). PAI-1 is a rapidly reacting acute-phase reactant and has a pronounced circadian variation with peak values in the mornings and plasma concentrations that increase with age (Teger-Nilsson et al., 1991). Lower values of PAI-1 have been reported in women (Wiman and Hamsten, 1990).

The gene encoding PAI-1 located on chromosome

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7q21.3-q22 (Klinger et al., 1987), and its several polymorphisms have been reported (Falk et al., 1995). Among them, the *Hind* III RFLP has been used as a genetic marker for the PAI-1 activity (Dawson et al., 1991) and the extent of coronary artery disease (Benza et al., 1998; Kim et al., 1998; Grenett et al., 2000).

Although several studies have documented that genetic variations in fibrinolysis system may be the genetic risk factors for ischemic heart diseases (van der Bom et al., 1997; Benza et al., 1998; Kim et al., 1998; Song et al., 2000), there had been few reports on the relationship between hypertension and genetic variations in these genes (Wang et al., 2002).

In this study, the *Alu* repeat I/D polymorphism in the intron 8 of t-PA gene and a *Hind* III RFLP in the 3' end of PAI-1 gene were investigated in the Korean patients with hypertension, and their association with plasma biochemical parameters was tested.

Materials and Methods

Study samples

Eighty-three patients with hypertension (case group: 26 males, 54 females and 3 unknown) and 115 normal controls (control group: 54 males, 60 females and 1 unknown) were selected from Seoul Hygiene Hospital, Seoul, Korea. Study subject was considered to have hypertension if the systolic blood pressure value was greater than 140 mmHg or diastolic blood pressure value was greater than 90 mmHg.

Biochemical analysis

Blood samples were collected from the subjects who had been fasting for 12-16 h. The levels of plasma total cholesterol (TC), triglyceride (TG) and glucose were measured by enzymatic colorimetric methods with a commercial kit (Boehringer Mannheim) and chemistry analyzer. Plasma HDL-cholesterol level was determined by measuring cholesterol in the supernatant after precipitation of plasma with $MgCl_2$ and dextran sulfate, using a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Also, plasma low-density lipoprotein (LDL)-cholesterol level was calculated by Friedewald's equation (Friedewald et al., 1972).

Genetic analysis

Total genomic DNA was isolated using a commercial genomic DNA purification kit (QIAamp; QIAGEN). An I/D polymorphism resulting from the presence or absence of an *Alu* repeats in intron h of the t-PA gene was identified by PCR using the following primers (Tishkoff et al., 1996):

Sense primer: 5'-GTGAAAAGCAAGGTCTACCAG-3'
Non-sense primer: 5'-GACACCGAGTTCATCTTGAC-3'.

PCR reaction was performed in a 50 μl volume with 10 *p*mol each of primers, 200 μM each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 3 mM $MgCl_2$, 0.25 units *Taq* DNA polymerase (Perkin-Elmer/Cetus), and 100 ng genomic DNA. The samples were subjected to 30 cycles consisting of 1 min at 94°C (denaturation), 1 min at 60°C (annealing) and 1 min at 72°C (extension) in a Perkin-Elmer model 9700 thermal cycler. A 10 μl sample of this reaction was electrophoresed on a 2.5% agarose gel, stained with ethidium bromide (EtBr) and photographed under UV light. PCR amplification produces a 570 bp fragment from chromosomes with the *Alu* insertion and a 260 bp fragment from those without (Fig. 1A).

A *Hind* III RFLP in the 3' end sequence of PAI-1 gene was identified by PCR-RFLP analysis with the following primers (Grenett et al., 2000):

Sense primer: 5'-GCCTCCAGCTACCGTTATTGTACA-3'
Non-sense primer: 5'-CAGCCTAAACAACAGAGACCCC-3'.

PCR reaction was performed in a 50 μl volume with 10 *p*mol each of primers, 200 μM each dNTP, 50 *m*M KCl,

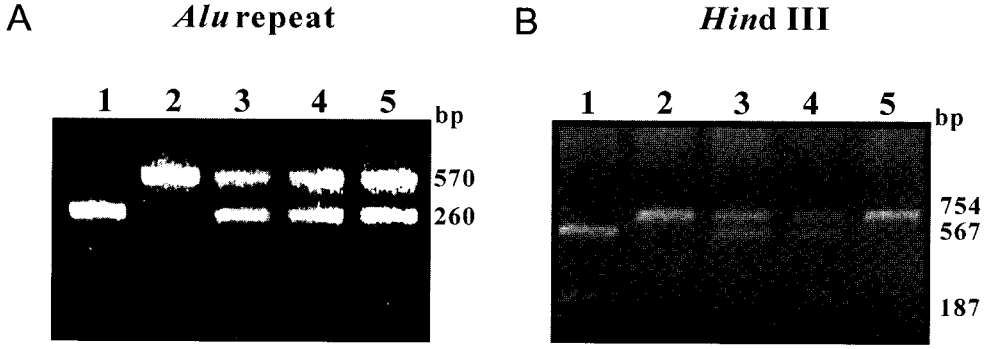

Fig. 1. Electrophoretic patterns of t-PA and PAI-1 gene polymorphisms. A, *Alu* repeat I/D polymorphism of t-PA gene. Lane 1, D/D genotype; lane 2, I/I genotype; lane 3-5, I/D genotype. B, *Hind* III RFLP of PAI-1 gene. Lane 1, H2H2 genotype; lane 2 and 5, H1H1 genotype; lane 3 and 4, H1H2 genotype.

10 mM Tris-HCl (pH 8.4), 3 mM $MgCl_2$, 0.25 units *Taq* DNA polymerase (Perkin-Elmer/Cetus), and 100 ng genomic DNA. The conditions for PCR were denaturation of template at 94°C for 2 min, then 30 cycles at the following temperatures: denaturation 94°C, 1 min; annealing 58°C, 1 min; and extension 72°C for 1 min, with one cycle of elongation at 72°C for 3 min. The 754 bp amplified fragments are produced by PCR. Ten μl aliquots of the 754 bp PCR fragments were digested with restriction enzyme, *Hind* III (10 units) in a 20 μl volume, analyzed by 1.2% agarose gel electrophoresis, stained with EtBr, and photographed with UV light. By PCR-RFLP analysis, 754 bp PCR products (H1 allele) were digested to two fragments of 567 bp and 187 bp with restriction enzyme, *Hind* III in the presence of H2 allele (Fig. 1B).

Data analysis

Deviation in genotype distribution from that expected for Hardy-Weinberg equilibrium was estimated by χ^2-fitness test. The significance of differences in allele frequencies between groups was estimated by χ^2-independence test. Continuous data are expressed as mean±SD. Variables in three groups were compared with one-way ANOVA test. P values less than 0.05 were considered to be statistically significant. Statistical analysis was performed using SPSS for Windows V10.0 (SPSS Inc., Chicago, USA).

Results and Discussion

Molecular genetic methods are becoming more and more widespread in medical research (Molnar-Gabor et al., 2000). Thus, considerable progress has been made in identification of genes involved in monogenic forms of hypertension (Lifton et al., 1992; Shimkets et al., 1994; Mune et al., 1995). However, elucidation of those genes that contribute to hypertension as polygenic and multifactor disease has lagged behind (Lander and Schork, 1994). Until now, it has been reported that abnormalities in lipid metabolism, carbohydrate metabolism and blood clotting parameters influence the pathogenesis of hypertension (Luft et al., 1999).

Several reports have indicated that the fibrinolytic system plays a significant role in hypertension (Teger-Nilsson et al., 1991; Jeng et al., 1996, 1998). Fibrinolysis, which is mediated mainly by the t-PA and PAI-1, is a reparative process that occurs in response to haemostatic plug or thrombus formation (Loskutoff, 1991). The fibrin that is formed during haemostasis must be removed when normal tissue structure and function is restored. Thus, fibrin clot that forms quickly in a torn blood vessel to stop hemorrhage is remodeled and then removed to restore normal blood flow (Kim et al., 2003).

In this report, we estimated genotype and allele frequencies to clarify distribution of each polymorphism in the t-PA and PAI-1 genes in the Korean population. Table 1 displays data presenting the genotype and allele frequencies in the I/D polymorphism of the t-PA gene and the *Hind* III RFLP of the PAI-1 gene in Korean normotensives and hypertensive groups, respectively. In the case of I/D polymorphism in the t-PA gene, the genotype and allele frequencies were not significantly different between normotensives and hypertensives (χ^2-independence test; for genotype distribution, df = 2, P = 0.6810; for allele distribution, df=1, P=0.4249). The observed genotype distributions of this polymorphism were not significantly different from those expected for Hardy-Weinberg equilibrium (χ^2-fitness test: for total samples, df=1, P=0.3212; for normotensives, df=1, P=0.4591; for hypertensives, df=1, P=0.5358).

Wang et al. (2002) reported no association of the t-PA gene to hypertension in Chinese Han population using the same *Alu* repeat I/D polymorphism. Thus, this genetic marker in the t-PA may not be appropriate for prediction of hypertension in Chinese as well as Korean population.

For *Hind* III RFLP of the PAI-1 gene, there were also no significant differences in genotype and allele frequencies between the two groups (χ^2-independence test: for genotype distribution, df=2, P=0.9012; for allele distribution, df=1, P=0.9291). The observed genotype distribution was in Hardy-Weinberg equilibrium (χ^2-fitness test: for total samples, df=1, P=0.4692; for normotensives, df=1, P=0.3969; for hypertensives, df=1, P=0.9071). This is probably the first report on the association between a *Hind* III RFLP in the PAI-1 gene and hypertension, but like *Alu* repeat I/D polymorphism in the

Table 1. Genotypic and allele frequencies of control and hypertensive groups of t-PA I/D polymorphism and PAI-1 *Hind* III RFLP

Polymorphism	Group	Genotype number (%)			Allele frequency		P[1]
t-PA		I/I	I/D	D/D	I	D	
I/D	Control	25 (28.7)	40 (46.0)	22 (25.3)	0.52	0.48	
	Case	28 (33.7)	38 (45.8)	17 (20.5)	0.57	0.43	0.4249
PAI-1		H1H1	H1H2	H2H2	H1	H2	
Hind III	Control	25 (21.7)	62 (53.9)	28 (24.4)	0.49	0.51	
	Case	19 (23.5)	41 (50.6)	21 (25.9)	0.49	0.51	0.9291

[1]P value of χ^2-squire test between the allele frequencies of control and case groups.

Table 2. Anthropometrical data and plasma biochemical parameters of subjects according to genotypes of I/D polymorphism in the t-PA gene

Parameter	Genotype		
	I/D (No.)[6]	I/D (No.)	D/D (No.)
Age (year)	61.9±11.7 (53)	58.9±11.2 (75)	57.6±11.3 (38)
BMI (kg/m^2)[1]	23.6± 2.3 (47)	23.8± 2.7 (72)	23.5± 2.8 (35)
Tg (mg/dl)[2]	114.3±67.8 (41)	141.9±89.0 (61)	126.4±69.5 (31)
TC (mg/dl)[3]	143.0±37.6 (41)	153.2±35.2 (61)	152.9±31.3 (31)
LDL-chol (mg/dl)[4]	95.0±32.0 (41)	99.4±37.2 (61)	97.6±33.7 (31)
HDL-chol (mg/dl)[5]	25.1±11.0 (41)	26.0± 7.6 (61)	28.4± 9.3 (31)
Glucose (mg/dl)	71.8±50.7 (20)	90.9±71.5 (42)	109.4±99.1 (20)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Number. Values are mean±SD.

t-PA, this polymorphism may not be suitable as a genetic marker in the diagnosis of hypertension.

Table 2 presents comparison of anthropometrical data and intermediate phenotypes across I/D polymorphism in the t-PA gene. The I/D polymorphism in the t-PA gene was not significantly associated with any anthropometrical data or plasma biochemical parameters. Comparison of the anthropometrics data and plasma biochemical parameters across *Hin*d III RFLP in the PAI-1 gene is shown in Table 3. The *Hin*d III RFLP in the PAI-1 gene was significantly associated with plasma glucose level (one-way ANOVA test, P=0.011). Especially, subjects with H1H1 homozygote had the elevated levels of plasma glucose when compared to subjects with H1H2 and H2H2 genotypes. Some studies reported that the genetic variation in the PAI-1 gene was associated with insulin resistance system or non-insulin dependent diabetes mellitus (NIDDM), suggesting a significant role of this gene in glucose metabolism (Mansfield et al., 1995; McCormack et al., 1996). Thus, it may suggest that the genetic variations in the PAI-1 gene and/or other genetic loci near this gene are also linked to glucose metabolism in the Korean population. It would be interesting to study whether this RFLP in the PAI-gene is significantly associated with the abnormalities in glucose metabolism such as insulin resistance syndrome or NIDDM in Koreans.

Table 3. Anthropometrical data and plasma biochemical parameters of subjects according to genotypes of *Hin*d III RFLP in the PAI-1 gene

Parameter	Genotype		
	H1H1 (No.)[6]	H1H2 (No.)	H2H2 (No.)
Age (year)	58.5± 9.7 (43)	59.3±12.6 (100)	60.2± 8.5 (49)
BMI (kg/m^2)[1]	24.0± 2.3 (41)	23.7± 2.4 (92)	23.4± 2.9 (48)
Tg (mg/dl)[2]	148.1±92.6 (33)	125.4±78.5 (80)	122.3±52.5 (39)
TC (mg/dl)[3]	156.8±34.8 (33)	148.7±37.9 (80)	157.1±39.2 (39)
LDL-chol (mg/dl)[4]	100.8±35.8 (33)	95.6±36.8 (80)	104.2±35.6 (39)
HDL-chol (mg/dl)[5]	26.4± 8.8 (33)	26.8± 9.1 (80)	28.4± 9.4 (39)
[a]Glucose (mg/dl)	119.9±64.4 (23)	69.0±59.7 (51)	103.0±93.0 (25)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol, [6]Number. Values are mean±SD. [a]Statistically significant difference (one-way ANOVA test, P=0.011).

Considering our results, the genetic variations in t-PA and PAI-gene are unlikely to play an important role in the pathogenesis of hypertension in the Korean population. However, the present observations do not necessarily exclude the possibility that some other polymorphisms in these genes influence hypertension. It will be necessary to find a large number of polymorphisms through mutation screening (Orita et al., 1989a,b; Henry et al., 1997) and to perform association studies between these polymorphisms and hypertension in further studies.

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