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Association between cytochrome P450 promoter polymorphisms and ischemic stroke

SU KANG KIM[1], SUNG-VIN YIM[1] and BYUNG-CHEOL LEE[2]

[1]Kohwang Medical Research Institute; [2]Department of Internal Medicine, College of Oriental Medicine, Kyung Hee University, Seoul, Republic of Korea

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Abstract. The human cytochrome P450 (CYP) superfamily includes at least 57 genes that encode enzymes with diverse metabolic and biosynthetic functions. This study was conducted in order to investigate the associations between polymorphisms in CYP superfamily genes (*CYP11B2*, *CYP17A1*, *CYP2B6*, *CYP2C9*, *CYP2E1* and *CYP7A1*) and ischemic stroke (IS). Six single nucleotide polymorphisms (SNPs) of CYP superfamily genes were selected and genotyped by direct sequencing in 121 patients with IS and 321 control subjects. The genetic data were analyzed using SNPStats and SPSS 18.0. Multiple logistic regression models (codominant 1, codominant 2, dominant, recessive and log-additive) were used to evaluate odds ratios (ORs), 95% confidence intervals (CIs) and p-values. The rs179998 SNP of *CYP11B2* was significantly associated with IS (p=0.0336 in a log-additive model). The rs3813867 SNP of *CYP2E1* was significantly associated with smoking in IS (p=0.0336 in a log-additive model). The rs1799998 SNP of *CYP11B2* and rs3808607 of *CYP7A1* were related to diabetes mellitus in IS ($p<0.05$). *CYP11B2*, *CYP2E1* and *CYP7A1* SNPs were associated with IS in the population studied. Further study is required to confirm these associations and to determine their biological significance.

Introduction

Stroke is the third leading cause of mortality and disability in Western countries and a major health problem worldwide. Almost 800,000 strokes occur annually in the United States, 80% of which are ischemic (1). Ischemic stroke (IS) results from the abrupt interruption of focal cerebral blood flow (2). The causes of IS include an embolic or thrombotic occlusion or a decrease in cerebral blood flow resulting from the abrupt occlusion of arteries and arterioles, poor blood circulation, arteritis, venous occlusion, anemia or hyperviscosity (2-5). Potent independent risk factors for IS include abdominal obesity and body mass index (BMI) (6,7). Insulin resistance, as indicated by elevated waist-to-hip ratios and insulin levels, may also contribute to IS risk (8). A decrease in blood pressure during the acute phase of IS is associated with brain injury and poor stroke outcome (9). A low level of high-density lipoprotein (HDL) cholesterol is a risk factor for mortality from coronary artery disease and stroke in advanced age (10). Spousal smoking may also be a significant risk factor for IS (11).

The human cytochrome P450 (CYP) superfamily comprises 57 genes and these code for enzymes with diverse metabolic and biosynthetic activities. Mutations in *CYP* genes may cause inborn errors of metabolism and contribute to clinically defined conditions (12). The *CYP11B2* genotype significantly affects the risk of hypertension in association with alcohol consumption (13), and also interacts with BMI and waist circumference in the Chinese Mongolian population (14). Variants of *CYP17A1* contribute to variations in blood pressure and hypertension risk (15), and the *CYP17A1-CNNM2-NT5C2* region is associated with hypertension (16). Genetic variants of *CYP2B6* may be related to the pathogenesis of leukemia (17) and to the metabolism of nicotine and cotinine (18). Polymorphisms of *CYP2C9* can effect the risk of bleeding complications (19) and the response to the antihypertensive effects of losartan (20). Polymorphisms of *CYP2E1,* together with cumulative smoking and alcohol consumption, and polymorphisms of *NQO1* and *ALDH2* have been synergistically associated with lung cancer in Koreans (21). Polymorphisms of *CYP7A1* have been associated with baseline low-density lipoprotein (LDL) and HDL cholesterol levels and risk of coronary artery disease (22).

Recent studies have shown the relationships between genetic polymorphisms and IS. The P213S polymorphism in L-selectin and levels of the gene product sL-selectin have been associated with IS in a Chinese population (23), polymorphisms in AdipoQ in a Korean population (24), and variants of the adiponectin gene in a Han population (25). Increased PAI-1 levels in plasma (26), as well as the *ACE* (DD) genotype and D allele, significantly increase susceptibility to IS (27). A significantly increased risk of adverse cardiovascular events has been associated with polymorphisms of CYP2C19 (28), and the V433M variant of CYP4F2 has been associated with IS in Swedish males, beyond its effect on blood pressure (29).

Correspondence to: Dr Byung-Cheol Lee, Department of Internal Medicine, College of Oriental Medicine, Kyung Hee University, 1 Hoegi-dong, Dongdaemun-Ku, Seoul 130-702, Republic of Korea
E-mail: hydrolee@korea.com

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In this study, we focused on the associations of single nucleotide polymorphisms (SNPs) of the *CYP* superfamily genes, *CYP11B2*, *CYP17A1*, *CYP2B6*, *CYP2C9*, *CYP2E1* and *CYP7A1,* with IS, and with smoking and diabetes mellitus (known risk factors for stroke) in IS. The aim of this study was to clarify the relationship between *CYP* polymorphisms and IS.

Materials and methods

Subjects and clinical phenotypes. Patients with IS who visited the Stroke Center of the East-West Neo-Medical Center between October 2007 and December 2010 were enrolled in this study. A total of 121 IS patients (64 male/56 female) were recruited. Patients with trauma, vascular malformations, brain tumors and congenital brain disorders were excluded. Each patient was diagnosed using cranial computed tomography (CT), magnetic resonance imaging (MRI), angiography or duplex sonography. Stroke patients were classified by two physicians with specialized knowledge of stroke into clinical phenotypes based on the results of the National Institutes of Health Stroke Survey (NIHSS), the Modified Barthel Index (MBI) and the presence or absence of complex regional pain syndrome (CRPS). Neurological deficit on admission was measured using the NIHSS. The outcome at hospital discharge was assessed using the MBI. The control subjects (n=321, 176 male/145 female) were selected from participants in a general health check-up program. Participants with transient ischemic attack, ischemic heart diseases and any other severe diseases were excluded. The Ethics Review Committee of the Medical Research Institute, School of Medicine, Kyung Hee University (Korea) approved the protocol for this study and all subjects gave written informed consent.

SNP selection and genotyping. We searched the SNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP, build 131) for promoter SNPs in the *CYP* superfamily genes. SNPs with unknown heterozygosity, minor allele frequency (MAF) below 10% and monomorphic genotypes in Asians were excluded. We selected the following six promoter-region SNPs: *CYP11B2*, rs1799998, -344; *CYP17A1*, rs2486758, -190; *CYP2B6*, rs4802100, -1180; *CYP2C9*, rs4918758, -1163; *CYP2E1*, rs3813867, -1272; *CYP7A1*, rs3808607, -278. Genomic DNA was extracted from the whole blood of each subject using the High Pure PCR Template Preparation kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. Blood samples collected from each subject were stored at -20˚C. Genotypes were determined by direct sequencing. Polymerase chain reactions (PCRs) were performed with the following primers (Table I). The PCR products were sequenced using the ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA), and sequence data were analyzed using SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical analysis. SNPStats (http://bioinfo.iconcologia.net/index.php?module=Snpstats) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA) were used to analyze the genetic data. Hardy-Weinberg equilibria (HWE) were calculated using the Chi-square test. Multiple logistic regression analyses (codominant 1, codominant 2, dominant, recessive and log-additive) were performed to determine odds ratios (ORs), 95% confidence intervals (CIs) and p-values. For multiple comparisons, the Bonferroni correction was performed by multiplying the p-value by the number of SNPs analyzed (n=6). The significance level was set at $p<0.05$.

Results

The clinical characteristics of IS patients and control subjects are shown in Table II. The mean ages of patients with IS and control subjects were 64.6±13.1 [mean ± standard deviation, (SD)] and 59.1±11.9 years, respectively. Patients were divided into subgroups according to NIHSS scores for neurological deficit on admission (<6 and >6), MBI scores for outcome at discharge (<60 and >60), CRPS (present and absent), smoking, hypertension, diabetes and dyslipidemia (30).

The genotype frequencies of the six selected SNPs are shown in Table III. An association was found between rs1799998, a SNP of *CYP11B2*, and IS. Multiple logistic regression analysis was performed for the promoter SNP (rs1799998, -344, T/C) [codominant 1, T/T (reference) vs. T/C; codominant 2, T/T vs. C/C; dominant, T/T vs. T/C+C/C; recessive, T/T+T/C vs. C/C; and log-additive, T/T vs. T/C vs. C/C]. The parameters for the association of SNP rs1799998 with IS are shown in Table III [(p=0.018; OR, 0.29; 95% CI, 0.11-0.81 in the codominant 2 model), (p=0.0220; OR, 0.60; 95% CI, 0.39-0.93 in the dominant model), (p=0.023; OR, 0.35; 95% CI, 0.13-0.95 in the recessive model) and (p=0.0056; OR, 0.61; 95% CI, 0.43-0.87 in the log-additive model)]. In allele distribution analysis, the frequency of the rs1799998 C allele was lower in the IS group than in the control group (C allele frequency, 32.0 vs. 23.5%, p=0.015; OR, 0.65; 95% CI, 0.46-0.92; data not shown). Following Bonferroni correction, the association was also significant (p=0.0336; Table III).

We analyzed the genetic data in order to evaluate the interactions of traditional IS risk factors (smoking, hypertension, diabetes and dyslipidemia) with the association between IS and polymorphisms of the CYP superfamily genes (*CYP11B2*, *CYP17A1*, *CYP2B6*, *CYP2C9*, *CYP2E1* and *CYP7A1*). When we assessed the genetic relationships between CYP promoter SNPs and IS risk by smoking status, *CYP11B2* (rs1799998) and *CYP2E1* (rs3813867) differed between smoking (-) and smoking (+) [(rs1799998: p=0.022; OR, 14.00; 95% CI, 1.03-190.90 in the recessive model), (rs3813867: p=0.019; OR, 0.28; 95% CI, 0.09-0.87 in the dominant model) and (p=0.0056; OR, 0.30; 95% CI, 0.11-0.79 in the log-additive model) Table IV]. Following Bonferroni correction, the association of rs3813867 of *CYP2E1* was significant (p=0.0336; Table IV). In allele distribution analysis, the frequency of the rs3813867 C allele of *CYP2E1* was lower in the smoking (-) than in the smoking (+) group (C allele frequency, 25.6 vs. 8.6%; p=0.010; OR, 0.28; 95% CI, 0.10-0.73; data not shown). The SNPs rs1799998 of *CYP11B2* and rs3808607 of *CYP7A1* were associated with diabetes mellitus in IS [rs1799998: (p=0.002; OR, 3.65; 95% CI, 1.59-8.41 in the codominant 1 model), (p=0.0011; OR, 3.77; 95% CI, 1.66-8.56 in the dominant model) and (p=0.0014; OR, 3.10; 95% CI, 1.51-6.38 in the log-additive model)] and [rs3808607: (p=0.047; OR, 0.41; 95% CI, 0.17-0.99 in the codominant 1 model), (p=0.013; OR, 0.23; 95% CI, 0.07-0.73 in the codominant 2 model), (p=0.011;

Table I. Primer sequences for each SNP.

Gene	rs number	Forward ('5-'3)	Reverse ('5-'3)	Size (bp)
CYP11B2	rs1799998	GACTCCAGGACCCTGGTTGATA	CAGCCAAAGGTAGATGAAGGAG	390
CYP17A1	rs2486758	GGACAGTCACACCACTGCACAC	TGTGTTATCTCTTGCCTTGTGG	393
CYP2B6	rs4802100	TAGTCAGAGGCAGGGAAGAAAC	AATTTGGCCTACTGCCTTTGTA	375
CYP2C9	rs4918758	CCAGTAAAGCTTTGTGCAACTG	TGAGAAACTAGGGCTTCTCGAC	418
CYP2E1	rs3813867	CACTGGAAAGGAAAGAGAGGAG	ACAATCCAGCCAAATCACTTGT	328
CYP7A1	rs3808607	CTCTCTGGCAAAGCACCTAAAT	AAGGATGCCACTGAAAAGAGAC	440

SNP, single nucleotide polymorphism.

Table II. Clinical characteristics in patients with IS and control subjects.

	IS	Control
Male/female (n)	64/56	176/145
Age (mean ± SD)	64.6±13.1	59.1±11.9
NIHSS (score)		
<6	58	
≥6	61	
MBI (score)		
<60	94	
≥60	25	
CRPS		
-	96	
+	23	
Smoking		
-	90	
+	29	
Hypertension		
-	39	
+	80	
Diabetes		
-	74	
+	45	
Dyslipidemia		
-	86	
+	33	

IS, ischemic stroke; SD, standard deviation; NIHSS, National Institutes of Health Stroke Survey; MBI, Modified Barthel Index; CRPS, complex regional pain syndrome.

OR, 0.34; 95% CI, 0.15-0.79 in the dominant model) and (p=0.0067; OR, 0.47; 95% CI, 0.26-0.83 in the log-additive model)]. Following Bonferroni correction, the associations of the rs1799998 SNP of *CYP11B2* and rs3813867 of *CYP2E1* were significant (p<0.05; Table V). In allele distribution analysis, the C allele of rs1799998 of *CYP11B2* and the G allele of rs3808607 of *CYP7A1* were also associated with diabetes mellitus [(rs1799998 of *CYP11B2*: p=0.002; OR, 2.59, 95% CI, 1.40-4.77), (rs3808607 of *CYP7A1*: p=0.012; OR, 0.50, 95% CI, 0.29-0.86)].

We used the online program AliBaba2.1 (www.gene-regulation.com/pub/programs/alibaba2) to evaluate whether the promoter SNPs affect transcription factor binding at the promoter site. The results are shown in Table VI.

Discussion

The results of this study reveal that promoter SNPs of *CYP* superfamily genes are associated with IS, and suggest that these polymorphisms may effect the risk of IS in Koreans. Recently, genetic studies of SNPs from *CYP11B2*, *CYP17A1*, *CYP2E1* and *CYP7A1* in several diseases have been reported. Tu *et al* reported that the promoter SNP of *CYP11B2* (rs1799998, T/C, -344) is associated with IS in both a dominant and a recessive effect in meta-analyses, with a moderate overall effect in the Han Chinese population (31). They suggest that the rs1799998 variant in *CYP11B2* affects susceptibility to stroke. In an Indian population, significant associations were found between the rs1799998 SNP of CYP11B2 and the risk of hypertension and IS (32).

The present study reveals an association between the rs1799998, -344T/C SNP of *CYP11B2* and IS, which suggests that this SNP may effect susceptibility to IS. The C/C genotype and C allele distributions of rs1799998 in IS (C/C genotype, 4.2%; C allele, 23.5%) were approximately 2.3- and 1.4-fold lower than those in the controls (C/C genotype, 9.7%; C allele, 32.0%), respectively. These results may indicate that the C allele of rs1799998 has a protective effect on IS risk.

In a Japanese population, the -344T/C polymorphism in *CYP11B2* was identified as an independent genetic factor associated with hypertension or atherosclerotic disease (33). However, we did not find such an association with hypertension in the present study. Instead, we identified associations with smoking and diabetes mellitus in IS. An association between -344T/C of the *CYP11B2* gene and smoking was identified, but it was not significant. The -1272G/C SNP of *CYP2E1* was significantly associated with smoking and IS in the present study. CYP2E1 metabolizes endogenous substrates, including ethanol, acetone and acetal, and exogenous substrates, including benzene, carbon tetrachloride, ethylene glycol and nitrosamines, which are

Table III. Genotype and allele frequencies of *CYP11B2*, *CYP17A1*, *CYP2B6*, *CYP2C9*, *CYP2E1* and *CYP7A1* polymorphisms in IS patients and control subjects.

Gene	Genotype	Control		IS		Model	OR (95% CI)	p-value	p^c
SNP		n	%	n	%				
CYP11B2	T/T	146	45.6	68	57.1	Codominant 1	0.67 (0.43-1.06)	0.10	0.60
rs1799998	T/C	143	44.7	46	38.7	Codominant 2	0.29 (0.11-0.81)	**0.0180**	0.11
-344	C/C	31	9.7	5	4.2	Dominant	0.60 (0.39-0.93)	**0.0220**	0.13
						Recessive	0.35 (0.13-0.95)	**0.0230**	0.14
						Log-additive	0.61 (0.43-0.87)	**0.0056**	**0.0336**
CYP17A1	T/T	185	57.8	66	55.5	Codominant 1	1.01 (0.64-1.59)	0.68	1.00
rs2486758	T/C	115	35.9	45	37.8	Codominant 2	1.08 (0.45-2.63)	0.80	1.00
-190	C/C	20	6.2	8	6.7	Dominant	1.02 (0.66-1.58)	0.94	1.00
						Recessive	1.08 (0.45-2.57)	0.86	1.00
						Log-additive	1.02 (0.72-1.45)	0.89	1.00
CYP2B6	C/C	192	60.0	75	63.0	Codominant 1	0.98 (0.62-1.55)	0.70	1.00
rs4802100	G/C	112	35.0	40	33.6	Codominant 2	0.66 (0.21-2.10)	0.44	1.00
-1180	G/G	16	5.0	4	3.4	Dominant	0.94 (0.60-1.46)	0.78	1.00
						Recessive	0.66 (0.21-2.10)	0.47	1.00
						Log-additive	0.91 (0.62-1.33)	0.62	1.00
CYP2C9	T/T	109	34.1	46	38.7	Codominant 1	0.84 (0.52-1.35)	0.55	1.00
rs4918758	T/C	153	47.8	56	47.1	Codominant 2	0.65 (0.34-1.24)	0.27	1.00
-1163	C/C	58	18.1	17	14.3	Dominant	0.79 (0.50-1.23)	0.29	1.00
						Recessive	0.71 (0.39-1.30)	0.26	1.00
						Log-additive	0.81 (0.60-1.11)	0.19	1.00
CYP2E1	G/G	217	67.8	75	63.0	Codominant 1	1.13 (0.70-1.81)	0.58	1.00
rs3813867	G/C	94	29.4	37	31.1	Codominant 2	2.66 (0.92-7.63)	0.12	0.72
-1272	C/C	9	2.8	7	5.9	Dominant	1.25 (0.79-1.96)	0.34	1.00
						Recessive	2.56 (0.90-7.26)	0.09	0.52
						Log-additive	1.32 (0.90-1.93)	0.16	0.96
CYP7A1	T/T	84	26.2	38	31.9	Codominant 1	0.75 (0.45-1.24)	0.23	1.00
rs3808607	T/G	164	51.2	55	46.2	Codominant 2	0.88 (0.48-1.61)	0.45	1.00
-278	G/G	72	22.5	26	21.9	Dominant	0.79 (0.49-1.26)	0.32	1.00
						Recessive	1.05 (0.62-1.77)	0.85	1.00
						Log-additive	0.92 (0.68-1.25)	0.59	1.00

IS, ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NA, not applicable; pc, For multiple comparisons, the Bonferroni correction was performed by multiplying the p-value by the number of SNPs analyzed (n=6). The p-values were calculated from logistic regression analysis. Bold numbers indicate significant associations.

Table IV. Genotype and allele frequencies of *CYP11B2*, *CYP17A1*, *CYP2B6*, *CYP2C9*, *CYP2E1* and *CYP7A1* polymorphisms in IS patients according to smoking status.

Gene	Genotype	Smoking (-)		Smoking (+)		Model	OR (95% CI)	p-value	p[c]
SNP		n	%	n	%				
CYP11B2	T/T	51	56.7	17	58.6	Codominant 1	0.55 (0.19-1.56)	0.34	1.00
rs1799998	T/C	38	42.2	8	27.6	Codominant 2	10.51 (0.73-150.38)	0.26	1.00
-344	C/C	1	1.1	4	13.8	Dominant	0.76 (0.29-2.01)	0.57	1.00
						Recessive	14.00 (1.03-190.90)	**0.0220**	0.13
						Log-additive	1.19 (0.53-2.65)	0.67	1.00
CYP17A1	T/T	50	55.6	16	55.2	Codominant 1	1.02 (0.38-2.71)	0.98	1.00
rs2486758	T/C	34	37.8	11	37.9	Codominant 2	0.63 (0.11-3.83)	0.96	1.00
-190	C/C	6	6.7	2	6.9	Dominant	0.94 (0.37-2.37)	0.89	1.00
						Recessive	0.63 (0.11-3.63)	0.60	1.00
						Log-additive	0.89 (0.43-1.82)	0.74	1.00
CYP2B6	C/C	54	60.0	21	72.4	Codominant 1	0.53 (0.19-1.48)	0.35	1.00
rs4802100	G/C	32	35.6	8	27.6	Codominant 2	0.00 (0.00-NA)		
-1180	G/G	4	4.4	0	0.0	Dominant	0.47 (0.17-1.29)	0.13	0.78
						Recessive	0.00 (0.00-NA)		
						Log-additive	0.45 (0.18-1.15)	0.08	0.49
CYP2C9	T/T	34	37.8	12	41.4	Codominant 1	1.19 (0.44-3.26)	0.74	1.00
rs4918758	T/C	43	47.8	13	44.8	Codominant 2	1.21 (0.29-5.16)	0.84	1.00
-1163	C/C	13	14.4	4	13.8	Dominant	1.20 (0.46-3.08)	0.71	1.00
						Recessive	1.11 (0.29-4.27)	0.88	1.00
						Log-additive	1.12 (0.57-2.20)	0.73	1.00
CYP2E1	G/G	51	56.7	24	82.8	Codominant 1	0.42 (0.13-1.35)	0.15	0.90
rs3813867	G/C	32	35.6	5	17.2	Codominant 2	0.00 (0.00-NA)		
-1272	C/C	7	7.8	0	0.0	Dominant	0.28 (0.09-0.87)	**0.0190**	0.11
						Recessive	0.00 (0.00-NA)		
						Log-additive	0.30 (0.11-0.79)	**0.0056**	**0.0336**
CYP7A1	T/T	29	32.2	9	31.0	Codominant 1	1.50 (0.51-4.41)	0.10	0.60
rs3808607	T/G	42	46.7	13	44.8	Codominant 2	1.78 (0.49-6.46)	0.77	1.00
-278	G/G	19	21.1	7	24.1	Dominant	1.59 (0.58-4.33)	0.36	1.00
						Recessive	1.41 (0.46-4.31)	0.55	1.00
						Log-additive	1.35 (0.71-2.54)	0.36	1.00

IS, ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NA, not applicable; pc, For multiple comparisons, the Bonferroni correction was performed by multiplying the p-value by the number of SNPs analyzed (n=6). The p-values were calculated from logistic regression analysis. Bold numbers indicate significant associations.

Table V. Genotype and allele frequencies of *CYP11B2*, *CYP17A1*, *CYP2B6*, *CYP2C9*, *CYP2E1* and *CYP7A1* polymorphisms in IS patients with and without DM.

Gene	Genotype	DM (-)		DM (+)		Model	OR (95% CI)	p-value	p[c]
SNP		n	%	n	%				
CYP11B2	T/T	51	68.9	17	37.8	Codominant 1	3.65 (1.59-8.41)	**0.0020**	**0.0120**
rs1799998	T/C	21	28.4	25	55.6	Codominant 2	5.49 (0.77-39.41)	0.12	0.7200
-344	C/C	2	2.7	3	6.7	Dominant	3.77 (1.66-8.56)	**0.0011**	**0.0066**
						Recessive	2.74 (0.41-18.35)	0.29	1.00
						Log-additive	3.10 (1.51-6.38)	**0.0014**	**0.0084**
CYP17A1	T/T	46	62.2	20	44.4	Codominant 1	2.09 (0.93-4.69)	0.08	0.48
rs2486758	T/C	24	32.4	21	46.7	Codominant 2	2.83 (0.61-13.02)	0.27	1.00
-190	C/C	4	5.4	4	8.9	Dominant	2.18 (1.01-4.74)	0.05	0.28
						Recessive	2.05 (0.47-9.00)	0.34	1.00
						Log-additive	1.86 (1.00-3.45)	0.05	0.28
CYP2B6	C/C	46	62.2	29	64.4	Codominant 1	0.99 (0.44-2.22)	0.90	1.00
rs4802100	G/C	25	33.8	15	33.3	Codominant 2	0.57 (0.05-6.06)	0.59	1.00
-1180	G/G	3	4.0	1	2.2	Dominant	0.95 (0.43-2.08)	0.89	1.00
						Recessive	0.57 (0.05-5.98)	0.63	1.00
						Log-additive	0.91 (0.46-1.81)	0.79	1.00
CYP2C9	T/T	31	41.9	15	33.3	Codominant 1	1.23 (0.53-2.86)	0.38	1.00
rs4918758	T/C	33	44.6	23	51.1	Codominant 2	1.30 (0.40-4.19)	0.53	1.00
-1163	C/C	10	13.5	7	15.6	Dominant	1.25 (0.56-2.77)	0.58	1.00
						Recessive	1.15 (0.40-3.36)	0.79	1.00
						Log-additive	1.16 (0.66-2.02)	0.60	1.00
CYP2E1	G/G	48	64.9	27	60.0	Codominant 1	1.14 (0.49-2.63)	0.46	1.00
rs3813867	G/C	21	28.4	16	35.6	Codominant 2	0.97 (0.17-5.51)	0.70	1.00
-1272	C/C	5	6.8	2	4.4	Dominant	1.11 (0.51-2.44)	0.79	1.00
						Recessive	0.93 (0.17-5.26)	0.94	1.00
						Log-additive	1.06 (0.56-2.00)	0.86	1.00
CYP7A1	T/T	18	24.3	20	44.4	Codominant 1	0.41 (0.17-0.99)	**0.0470**	0.48
rs3808607	T/G	36	48.6	19	42.2	Codominant 2	0.23 (0.07-0.73)	**0.0130**	0.13
-278	G/G	20	27.0	6	13.3	Dominant	0.34 (0.15-0.79)	**0.0110**	0.07
						Recessive	0.39 (0.14-1.10)	0.06	0.37
						Log-additive	0.47 (0.26-0.83)	**0.0067**	**0.0402**

IS, ischemic stroke; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; NA, not applicable; DM, diabetes mellitus; pc, For multiple comparisons, the Bonferroni correction was performed by multiplying the p-value by the number of SNPs analyzed (n=6). The p-values were calculated from logistic regression analysis. Bold numbers indicate significant associations.

Table VI. Effects of genetic variations on transcription factor binding.

Gene / SNP	Allele	Transcription factor
CYP11B2 rs1799998 -344	T	NF-1
	C	disappeared
CYP17A1 rs2486758 -190	T	C/EBPα, Oct-1, C/EBPa1p
	C	C/EBPa1p
CYP2B6 rs4802100 -1180	C	C/EBPa1p
	G	disappeared
CYP2C9 rs4918758 -1163	T	Oct-1, HNF-3
	C	C/EBPa1p
CYP2E1 rs3813867 -1272	G	Sp1
	C	disappeared
CYP7A1 rs3808607 -2789	T	disappeared
	G	AP-1, C/EBPa1p

SNP, single nucleotide polymorphism.

premutagens found in cigarette smoke. Through its numerous substrates, CYP2E1 may be involved in such diverse processes, such as gluconeogenesis, hepatic cirrhosis, diabetes and cancer [Online Mendelian Inheritance in Man (OMIM), 124040]. Howard *et al* suggested that CYP2E1 induction in the brain, by ethanol or nicotine, may influence the central effects of ethanol and the development of nervous tissue pathologies observed in alcoholics and smokers (34). The -344T/C of *CYP11B2* and -278T/G of *CYP7A1* were associated with diabetes mellitus in IS. Bellili *et al* found associations of the -344T/C and the 3097 G>A polymorphisms of *CYP11B2* with hypertension, type 2 diabetes and metabolic syndrome in a French population (35). Russo *et al* reported that the C allele of the -344T/C variant of *CYP11B2* increases susceptibility to metabolic syndrome in European males, but not in females (36). Aldosterone is one of the main effectors of the renin-angiotensin-aldosterone system, regulating blood pressure. Previous studies have shown that the aldosterone synthase promoter polymorphism, -344T/C, affects aldosterone levels and is associated with hypertension, a risk factor for the initiation and progression of diabetic nephropathy. The -344T/C of *CYP11B2* may also effect glucose homeostasis and body mass in humans (36,37). CYP7A1 is a rate-limiting enzyme for cholesterol catabolism and bile acid synthesis, and may thereby effect cholesterol homeostasis. The -278T/G of *CYP7A1* is associated with subclinical atherosclerosis, LDL cholesterol level and gallstone disease.

In conclusion, this study, carried out in a Korean population, reveals that variations in the *CYP11B2*, *CYP2E1* and *CYP7A1* genes are associated with IS and may represent genetic risk factors in IS. Larger studies are required to confirm these associations and to evaluate their causal roles in IS.

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