Association between ABCB1 Polymorphisms and Ischemic Stroke in Korean Population

Young-Ock Kim¹, Seung-Yu Kim¹, Dong Hwan Yun² and Sang-Won Lee¹*

¹Department of Herbal Crop Research, National Institute of Horticultural & Herbal Science, Rural Development Administration, Eumseong 369-873, ²Department of Physical Medicine & Rehabilitation, Kyung Hee University, Seoul 130-702, Korea

Neuronal expression of ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1) has been demonstrated after brain ischemia. To investigate whether ABCB1 polymorphisms are associated with the development, risk factors (hypertension, dyslipidemia, and diabetes mellitus), severity (National Institutes of Health Stroke Scale, NIHSS), and sequelae (Modified Barthel Index, MBI) of ischemic stroke (IS), four single nucleotide polymorphisms (SNPs) of the ABCB1 gene [rs4148727, promoter, -154T>C; rs3213619, 5'-untranslation region (5'UTR), -129T>C; rs1128503, synonymous, Gly412 (C>T); rs3842, 3'UTR, A>G] were analyzed in 121 IS patients and 291 control subjects. SNPStats and SPSS 18.0 were used to obtain odds ratios (OR), 95% confidence intervals (CI), and p values. Multiple logistic regression models (codominant1, codominant2, dominant, recessive, and log-additive models) were applied to analyze the genetic data. The rs3842 SNP was weakly associated with the development of IS (p=0.020 in codominant1 model and p=0.028 in dominant model). In the analysis of clinical phenotypes, ABCB1 polymorphisms were nominally associated with hypertension (rs3213619 and rs3842, p<0.05), dyslipidemia (rs1128503, p<0.05), diabetes (rs3842, p<0.05), and NIHSS (rs4148727, p<0.05). Interestingly, rs3842 showed statistically strong association between IS with hypertension and IS without hypertension (Fisher's exact p=0.003, OR=0.11, 95% CI=0.03-0.51 in recessive model). These results suggest that the ABCB1 gene may be associated with the development and clinical phenotypes of IS in Korean population.

Key words: ABCB1, clinical phenotypes, MBI, NIHSS, ischemic stroke, single nucleotide polymorphism

INTRODUCTION

Stroke is the second most common cause of death and one of major causes of disability around the world. Stroke may be divided into two major categories by etiology: Ischemic stroke (IS), which is about 80%, and hemorrhagic stroke, about 20% [1]. Environmental factors including hypertension, hyperlipidemia, and smoking are risk factors of stroke, and genetic factors also influence susceptibility to stroke [2-4]. Recently, genetic studies have shown the association between the development of stroke and genetic polymorphisms. Dahabreh et al. [5] reported that paraoxonase 1 (PON1) polymorphism [rs4148727, promoter, (A>G)] contributed to the risk of IS. Lim et al. [6] showed that the synonymous SNP [rs2228048, Asn389 (C>T)] of transforming growth factor, beta receptor II (TGFBRII) might be associated with the development of intracranial hemorrhage (ICH) in Korean population. Park et al. [7] found that the C alleles of rs3804099 [Asn199 (T>C)] and rs3804100 [Ser450 (T>C)] of toll-like receptor 2 (TLR2) gene were associated with National Institutes of
Health Stroke Scale (NIHSS) of IS patients.

ATP-binding cassette, sub-family B (MDR/TAP), member 1 (ABCB1) is a protein which confers resistance towards multiple cytostatic drugs (multidrug resistance) in tumor cells. ABCB1 is also known as multidrug resistance 1 (MDR1) and P-glycoprotein (PGY1) [8]. The previous studies about ABCB1 have been mainly focused on drug efflux function associated with tumor chemotherapy including colorectal cancer [9], breast cancer [10], and brain tumor [10]. However, ABCB1 exports endogenous inflammatory mediators such as prostaglandins and leukotrienes, which may aggravate the inflammation and destruction of various size of ischemized tissue, from the initial steps to latent steps of inflammation [11]. In the central nervous system (CNS), ABCB1 is normally expressed in blood-brain barrier (BBB) [12]. In the mouse model of IS, the increased ABCB1 in brain tissue was able to efflux drugs despite of permeable BBB [13]. Neuronal expression of ABCB1 has been demonstrated after brain ischemia [14]. ABCB1 expression was increased in human capillary endothelial cells under hypoxia [15]. In addition, Bochud et al. [16] suggested that ABCB1 is related to hypertension via the renin–angiotensin–aldosterone system. Although ABCB1 may contribute to the pathogenesis or pathophysiology of IS, however, there was no case-control study analyzed the relationship between ABCB1 and IS.

In this study, we assessed the association between four SNPs of the ABCB1 gene [promoter SNP (rs4148727, -154 T>C), 5'-untranlation region (5'UTR) SNP (rs3213619, -129T>C), exon SNP (rs1128503, Gly412(C>T)], and 3'UTR SNP (rs3842, A>G)] and the development, risk factors (hypertension, dyslipidemia, and diabetes mellitus), severity (NIHSS), and sequelae (Modified Barthel Index, MBI) of IS in Korean population.

MATERIALS AND METHODS

Patients with IS and control subjects

One hundred twenty one IS patient and 291 control subjects were included. IS patients were enrolled among participants who diagnosed at the East-West Neomedical Center and Kyung Hee Medical Center (Seoul, Korea), during 2009-2012. The diagnosis of IS were confirmed through computed tomography (CT), magnetic resonance imaging (MRI), angiography, or duplex sonography by well-trained neurologists. Subjects with accidental or iatrogenic stroke, transient ischemic attack, brain tumors, or other cerebrovascular disorders were excluded. Controls were enrolled among participants examined through general health check-up program. Controls with stroke, ischemic heart diseases, neurological diseases, or any severe underlying diseases were excluded. This study was conducted according to the guidelines of the Helsinki Declaration and was approved by the ethics review committee of Medical Research Institute, School of Medicine, Kyung Hee University, Seoul, Korea. Informed consent was obtained from all individuals. If a patient was in incommunicable state, we obtained informed consent from close relatives or legal guardian.

Clinical subgroups of IS

All of IS patients were divided into subgroups in accordance with the clinical features (Table 1). The abnormal range of the clinical features were as following: hypertension, >140 mmHg in systolic blood pressure (SBP) or >90 mmHg in diastolic blood pressure (DBP); diabetes mellitus, >126 mg/dL in fasting plasma glucose or >6.5% hemoglobin A1c (HBA1c); dyslipidemia, >250 mg/dl in total cholesterol (TC), >150 mg/dl in triglyceride (TG), or <40 mg/dl (male) and <50 mg/dl (female) in high-density lipoprotein cholesterol (HDL-C). The severity of IS was scaled by the NIHSS, because it is common and sensitive tool for checking neurological status [17]. NIHSS is a 15-item impairment scale, consisted of the level of consciousness (3 items), extraocular movements, visual fields defects, facial palsy, extremity strength (4 items), sensory deficit, ataxia, aphasia, dysarthria, and neglect [18]. The sequelae, disabilities caused by neurologic damages of IS, was analyzed with the MBI. MBI scale measures 10-items of the self-care and mobility, consisted of bowels (10 points), bladder (10 points), grooming (5 points), toilet use (10 points), feeding (10 points),...
transfer (15 points), mobility (15 points), dressing (10 points), stairs (10 points), and bathing (5 points), with total score 100 [19]. MBI score is known to correlate with infarct volume [20].

SNP selection and genotyping

We searched the promoter and exon regions of the ABCB1 gene in the SNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP, BUILD 135). We selected four SNPs [rs4148727, -154T>C; rs3213619, 5’UTR (-129T>C), rs1128503, Gly412 (C>T), and rs3842, 3’UTR (A>G)] of the ABCB1. Peripheral blood sample of all subjects was collected in EDTA tube, and genomic DNA was extracted using DNA Isolation Kit for Cells and Tissues (Roche, Indianapolis, IN, USA). Genotypes of each SNP were determined by direct sequencing (MACROGEN, Seoul, Korea). PCRs were performed as the following condition: 40 cycles at 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and 1 cycle at 72°C for 5 min for the final reaction. The primer sequences used for PCRs were as following: for rs3842, sense primer GCCACAGAAGGCACTTATTTT, antisense primer CAAGGCAGTCATGTCAGTCCA; for rs1128503, sense primer CCCATCTGAAAAGAAGTATG, antisense primer CATCTCACCATCCCCTCTGT; for rs3213619, sense primer ATTCCTCCTGGAAATTCAACCT, antisense primer CATCTCACCATCCCCTCTGT; for rs3842, sense primer GCCACAGAAGGCACTTATTTT, antisense primer TTGGGAACTGTCCCATAGTAGC; for rs4148727, sense primer TTATCCCAGTACCAGAGGAGGA. The PCR products were sequenced by an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA) and sequencing data were collected in EDTA tube, and genomic DNA was extracted using DNA Isolation Kit for Cells and Tissues (Roche, Indianapolis, IN, USA). Genotypes of each SNP were determined by direct sequencing (MACROGEN, Seoul, Korea). PCRs were performed as the following condition: 40 cycles at 94°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and 1 cycle at 72°C for 5 min for the final reaction. The primer sequences used for PCRs were as following: for rs3842, sense primer GCCACAGAAGGCACTTATTTT, antisense primer CAAGGCAGTCATGTCAGTCCA; for rs1128503, sense primer CCCATCTGAAAAGAAGTATG, antisense primer CATCTCACCATCCCCTCTGT; for rs3213619, sense primer ATTCCTCCTGGAAATTCAACCT, antisense primer CATCTCACCATCCCCTCTGT; for rs3842, sense primer GCCACAGAAGGCACTTATTTT, antisense primer TTGGGAACTGTCCCATAGTAGC; for rs4148727, sense primer TTATCCCAGTACCAGAGGAGGA. The PCR products were sequenced by an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA) and sequencing data were analyzed using SeqManII software (DNASTAR, Madison, WI, USA).

Statistical analysis

Multiple logistic regression models were applied using following models: codominant1 (major allele homozygotes vs. heterozygotes), codominant2 (major allele homozygotes vs. minor allele homozygotes), dominant (major allele homozygotes vs. heterozygotes+minor allele homozygotes), recessive (major allele homozygotes+heterozygotes vs. minor allele homozygotes), and log-additive (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes) models. Odds ratios (OR), 95% confidence intervals (CI), and p-values were determined using SNPStats (http://bioinfo.iconcologia.net/index.php?module=Snpstats) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA). The linkage disequilibrium (LD) block and haplotypes were estimated using Haploview version 4.2 (Daly Lab, Cambridge, MA, USA). The significant p-value was considered below 0.05.

RESULTS

One hundred twenty-one IS patients and 291 control subjects were analyzed. The age of IS group (mean±standard deviation) was 65.7±12.1 years and control was 63.0±9.3 years (Table 1). Table 2 shows the genotype and allele frequencies of the four examined rs4148727, -154T>C; rs3213619, 5’UTR(-129T>C), rs1128503, Gly412 (C>T), and rs3842, 3’UTR (A>G) of ABCB1 in the control and IS groups. The genotype distributions of the four tested SNPs were consistent with the Hardy-Weinberg equilibrium in the control group (p>0.05, data not shown). The rs3842 SNP was weakly associated with the risk of IS (p=0.020, OR=0.58, 95% CI=0.36-0.92 in codominant1 model and p=0.028, OR=0.62, 95% CI=0.40-0.95 in dominant model). However, the allele frequencies of rs3842 showed no difference between the IS group and the control group. Other three SNPs (rs4148727, rs3213619, rs1128503) were not associated with the development of IS (p>0.05).

LD block was applied using Haplovip 4.2. The LD block in the four examined SNPs was not constructed (Fig. 1). Therefore we did not showed the haplotype among the four examined SNPs.

In order to investigate correlation between ABCB1 and clinical phenotypes of IS, the IS patients were divided into two subgroups in accordance with the clinical and biochemical features (n=35 IS without hypertension vs. n=81 IS with hypertension; n=86 IS without dyslipidemia vs. n=33 IS patients with dyslipidemia; n=72 IS without diabetes mellitus vs. n=45 IS with diabetes mellitus; n=56 NIHSS score <6 vs. n=57 NIHSS score ≥6; n=71 MBI score <60 vs. n=25 MBI score ≥60) (Table 1).

Table 3 represents the genotype and allele frequencies of the four examined SNPs in IS subgroups in accordance with hypertension. The two SNPs of ABCB1 (rs3213619 and rs3842) were associated with the hypertension in IS (p=0.055, Fisher’s exact p=0.020, OR=0.26, 95% CI=0.07-0.91 in codominant1 model; rs3842, p=0.015, Fisher’s exact p=0.016 OR=0.15, 95% CI=0.03-0.70 in codominant2 model, p=0.0023, Fisher’s exact p=0.003, OR=0.11 95% CI=0.03-0.51 in recessive model). The C allele frequency of rs3213619 SNP was lower in the hypertension (+) group (3.1%) than that in the hypertension (-) group (11.4%). The difference in the allele frequency of rs3213619 was statistically significant (p=0.018, Fisher’s exact p=0.024, OR=0.25, 95% CI=0.28-0.78). Two SNPs (rs4148727 and rs1128503) were not related to hypertension in IS.

One coding SNP of ABCB1 [rs1128503, Gly412(C>T)] showed weak association with dyslipidemia (p=0.040, OR=0.40, 95% CI=0.17-0.96 in codominant1 model, p=0.02, OR=0.37, 95% CI=0.16-0.86 in dominant model, p=0.018, OR=0.45, 95%
CI=0.23-0.90 in log-additive model). The C allele frequency of rs1128503 was lower in dyslipidemia (+) group (25.8%) than that in the dyslipidemia (-) group (41.8%). The difference was statistically significant (p=0.024, OR=0.48, 95% CI=0.26-0.91). Three SNPs (rs4148727, rs3213619, and rs3842) were not related to dyslipidemia in IS (data not shown).

Finally, one promoter SNP of ABCB1 (rs4148727, -154T>C) was marginally associated with NIHSS in IS (p=0.034, Fisher's exact p=0.042, OR=3.31, 95% CI=1.09-10.01 in codominant1 model, p=0.032, OR=2.99, 95% CI=1.05-8.48 in dominant model). Three SNPs (rs3213619, rs1128503, and rs3842) were not related to NIHSS in IS (data not shown).

The four examined SNPs showed no association with MBI in IS (p>0.05, data not shown).

Table 2. Genotype and allele frequencies of ABCB1 SNPs in controls and IS

| SNP          | Type   | Control | IS       | Model   | OR     | p      | Fisher's exact p |
|--------------|--------|---------|----------|---------|--------|--------|------------------|
|              |        | n (%)   | n (%)    |         |        |        |                  |
| rs4148727    | Genotype |         |          |         |        |        |                  |
| -154 T/C     | T/T    | 221     | 76.0     | 99      | 81.8   |        |                  |
|              | T/C    | 64      | 22.0     | 20      | 16.5   |        |                  |
|              | C/C    | 6       | 2.1      | 2       | 1.6    |        |                  |
|              |        |         |          | Codominant1 | 0.71 (0.41-1.25) | 0.24 |
|              |        |         |          | Codominant2 | 0.86 (0.17-4.37) | 0.85 |
|              |        |         |          | Dominant | 0.73 (0.42-1.24) | 0.24 |
|              |        |         |          | Recessive | 0.92 (0.18-4.66) | 0.91 |
|              |        |         |          | Log-additive | 0.77 (0.47-1.25) | 0.28 |
| rs3213619    | Genotype |         |          |         |        |        |                  |
| 5'UTR        | T/T    | 261     | 89.7     | 107     | 88.4   |        |                  |
|              | T/C    | 29      | 10.0     | 14      | 11.6   |        |                  |
|              | C/C    | 1       | 0.3      | 0       | 0.0    |        |                  |
|              |        |         |          | Codominant1 | 1.14 (0.57-2.25) | 0.72 |
|              |        |         |          | Codominant2 | 0.00 (0.00-NA) | NA   |
|              |        |         |          | Dominant | 1.10 (0.56-2.18) | 0.78 |
|              |        |         |          | Recessive | 0.00 (0.00-NA) | NA   |
|              |        |         |          | Log-additive | 1.07 (0.55-2.07) | 0.85 |
| rs1128503    | Genotype |         |          |         |        |        |                  |
| Gly412Gly    | T/T    | 103     | 35.4     | 45      | 37.5   |        |                  |
|              | T/C    | 140     | 48.1     | 60      | 50.0   |        |                  |
|              | C/C    | 48      | 16.5     | 15      | 12.5   |        |                  |
|              |        |         |          | Codominant1 | 0.98 (0.62-1.57) | 0.94 |
|              |        |         |          | Codominant2 | 0.76 (0.39-1.52) | 0.44 |
|              |        |         |          | Dominant | 0.93 (0.59-1.45) | 0.74 |
|              |        |         |          | Recessive | 0.77 (0.41-1.45) | 0.42 |
|              |        |         |          | Log-additive | 0.90 (0.66-1.24) | 0.52 |
| rs3842       | Genotype |         |          |         |        |        |                  |
| 3'UTR        | A/A    | 132     | 45.4     | 69      | 57.0   |        |                  |
|              | A/G    | 134     | 46.0     | 40      | 33.1   |        |                  |
|              | G/G    | 25      | 8.6      | 12      | 9.9    |        |                  |
|              |        |         |          | Codominant1 | 0.58 (0.36-0.92) | 0.020 |
|              |        |         |          | Codominant2 | 0.81 (0.38-1.74) | 0.59 |
|              |        |         |          | Dominant | 0.62 (0.40-0.95) | 0.028 |
|              |        |         |          | Recessive | 1.02 (0.49-2.15) | 0.95 |
|              |        |         |          | Log-additive | 0.75 (0.54-1.05) | 0.09 |
|              |        |         |          |         |        |        |                  |
| Allele       |        |         |          |         |        |        |                  |
| T            | 551    | 94.7    | 228      | 94.2    |        |        |                  |
| C            | 76     | 13.1    | 24       | 9.9     |        |        |                  |
| rs3842       | Genotype |         |          |         |        |        |                  |
|              | A/A    | 134     | 46.0     | 40      | 33.1   |        |                  |
|              | A/G    | 132     | 45.4     | 69      | 57.0   |        |                  |
|              | G/G    | 25      | 8.6      | 12      | 9.9    |        |                  |
|              |        |         |          | Codominant1 | 0.58 (0.36-0.92) | 0.020 |
|              |        |         |          | Codominant2 | 0.81 (0.38-1.74) | 0.59 |
|              |        |         |          | Dominant | 0.62 (0.40-0.95) | 0.028 |
|              |        |         |          | Recessive | 1.02 (0.49-2.15) | 0.95 |
|              |        |         |          | Log-additive | 0.75 (0.54-1.05) | 0.09 |
| Allele       |        |         |          |         |        |        |                  |
| A            | 398    | 68.4    | 178      | 73.6    |        |        |                  |
| G            | 184    | 31.6    | 64       | 26.4    |        |        |                  |

ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1; SNP, singe nucleotide polymorphism; IS, stroke; UTR, untranslated region; OR, odds ratio; CI, confidence interval; NA, not applicable. The p-values were calculated from logistic regression analysis adjusting age and sex.

Fig. 1. Linkage disequilibrium (LD) blocks and haplotypes are tested using Haploview version 4.2. The LD block is not constructed among rs4148727, rs3213619, rs1128503, and rs3842 (D' and r squared value <1.00).
DISCUSSION

In this study, we investigated the relationships between the four SNPs of ABCB1 [rs4148727, -154T>C; rs3213619, 5’UTR(-129T>C), rs1128503, Gly412(C>T), and rs3842, 3’UTR(A>G)] and IS in Korean population. Our result suggests that ABCB1 might be associated with the development, risk factors (hypertension, dyslipidemia, and diabetes mellitus), and severity (NIHSS) of IS. Some studies documented the association between ABCB1 polymorphisms (rs3213619, rs1128503, and rs3842) and certain diseases. Fujii et al. [21] investigated the relationship between ABCB1 SNPs (rs2188524, rs3213619, rs1128503, rs2032582, and rs1045642) and major depressive disorder (MDD) in the Japanese, they observed that T allele of rs1045642 is risk factor for MDD. Urayama et al. [22] reported that haplotype CGC (rs3213619, rs1128503, and rs2032582) may be less susceptible to the leukemogenic effects of indoor insecticide exposures.

In present study, we observed that rs4148727 was associated with the severity of IS (NIHSS). There was no study about rs4148727 of ABCB1 to date. The T and C allele frequencies in rs4148727 have been reported to be 0.976 and 0.024 in European, 0.950 and 0.050 in Hispanic, 0.917 and 0.083 in Asian, and 0.909 and 0.091 in Sub-Saharan African, respectively (http://www.ncbi.nlm.nih.gov/projects/SNP). In our controls, the T and C allele frequencies of rs4148727 were 0.869 and 0.131. The allele frequency of rs4148727 showed no ethnic difference. To investigate the binding site of transcription factors for the T and C alleles of rs4148727 (-154T>C), we used the online program AliBaba2.1 (http://www.gene-regulation.com/pub/programs/alibaba2). The C allele in rs4148727 has an activation protein (AP)-1 transcription factor (http://gene-regulation.com/pub/programs/alibaba2). The C allele in rs4148727 has an activation protein (AP)-1 transcription factor (http://www.gene-regulation.com/pub/programs/alibaba2).

ABC1, ATP-binding cassette, sub-family B (MDR/TAP), member 1; SNP, singe nucleotide polymorphism; IS, stroke; UTR, untranslated region; OR, odds ratio; CI, confidence interval; NA, not applicable. The p-values were calculated from logistic regression analysis adjusting age and sex.

| SNP                  | Type          | Without hypertension | With hypertension | Model       | OR       | p        | Fisher’s exact p |
|----------------------|---------------|-----------------------|-------------------|-------------|----------|----------|-----------------|
| rs4148727 -154T>C    | Genotype      |                       |                   |             |          |          |                 |
| Allele               | T/T           | 32                     | 91.4              |             | 64       | 79.0     | 2.12 (0.53-8.09) |
|                      | T/C           | 3                      | 8.6               |             | 15       | 18.5     | 0.27 (0.00-0.26) |
|                      | C/C           | 0                      | 0.0               |             | 2        | 2.5      | 1.00             |
| Allele               | T             | 67                     | 95.7              |             | 143      | 88.3     | 2.97 (0.85-10.38)|
|                      | C             | 3                      | 4.3               |             | 19       | 11.7     | 1.00             |
| Allele               | rs3213619 5’UTR| T/T                   | 27                 |             | 77.1     | 93.8     | 0.26 (0.07-0.91)|
|                      | T/C           | 8                      | 22.9              |             | 5        | 6.2      | 2.61 (0.91-7.48)|
| Allele               | rs1128503 Gly412Gly | T/T               | 11                 |             | 31       | 38.8     | 0.25 (0.28-0.78)|
|                      | T/C           | 21                     | 60.0              |             | 38       | 47.5     | 1.43 (0.32-6.48)|
| Allele               | rs3842 3’UTR  | A/A                   | 21                 |             | 60       | 55.6     | 2.61 (0.91-7.48)|
|                      | A/G           | 6                      | 17.1              |             | 33       | 40.7     | 0.15 (0.03-0.70)|
|                      | G/G           | 8                      | 22.9              |             | 3        | 3.7      | 1.18 (0.51-2.75)|
| Allele               | A             | 48                     | 68.6              |             | 123      | 75.9     | 0.71 (0.39-1.32)|
|                      | G             | 22                     | 31.4              |             | 39       | 24.1     | 0.69 (0.37-1.29)|
ABCB1 may play a role in neuronal stress. The rs3213619 SNP was associated with hypertension in IS. The T and C allele frequencies in rs3213619 have been reported to be 0.969 and 0.031 in European, 0.919 and 0.081 in Chinese, 0.953 and 0.047 in Japanese, and 0.934 and 0.066 in Sub-Saharan African, respectively. In our controls, the T and C allele frequencies of rs3213619 were 0.947 and 0.053. The allele frequency of rs3213619 showed no ethnic difference.

The rs1128503 SNP was associated with dyslipidemia in IS. The T and C allele frequencies in rs1128503 have been reported to be 0.451 and 0.549 in European, 0.709 and 0.291 in Chinese, 0.587 and 0.413 in Japanese, and 0.124 and 0.876 in Sub-Saharan African, respectively. The T and C allele frequencies of rs1128503 in our control group were 0.595 and 0.405, those were similar in Japanese population. The allele frequency of rs1128503 showed ethnic difference.

The rs3842 SNP showed association with development, hypertension, and diabetes mellitus. The A and G allele frequencies in the rs3842 SNP have been reported to be 0.858 and 0.142 in European, 0.756 and 0.244 in Chinese, 0.680 and 0.320 in Japanese, and 0.858 and 0.142 in Sub-Saharan African, respectively. The A and G allele frequencies of rs3842 in our control group were 0.684 and 0.316, those were similar in Japanese population. The allele frequency of rs3842 showed ethnic difference.

The rs3213619 and rs3842 SNPs were associated with hypertension in IS. rs3213619 was associated with hypertension in codominant1 model (fisher’s exact p=0.020, OR=0.26, 95% CI=0.07-0.91) and allele frequencies (fisher’s exact p=0.024, OR=0.25, 95% CI=0.28-0.78). rs3842 was associated with hypertension in codominant2 model (fisher’s exact p=0.016, OR=0.15, 95% CI=0.03-0.70) and recessive model (fisher’s exact p=0.003, OR=0.11, 95% CI=0.03-0.51). As hypertension and non-hypertension groups were already affected by stroke, we observed that minor alleles of both rs3213619 and rs3842 SNPs might be contributing to the risk of IS development in non-hypertensive groups.

Blood pressure of the study samples was measured after development of IS. However study result may still have significance, because poststroke BP rise is associated with prestroke BP level, and BP of hypertensive patients are higher than non-hypertensive patients in the first week of stroke [26]. Previous study reported cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5) and ABCB1 polymorphisms are associated with hypertension [16]. The 3435 C>T variant of ABCB1 was associated with angiotensin stimulated aldosterone level [27], and cyclosporin A, an ABCB1 inhibitor, influences the renin–angiotensin–aldosterone system [28]. These results suggest that association of ABCB1 with blood pressure is not only mediated by exogeneous substrates such as ACE inhibitors, and may support the association of rs3213619 and rs3842 with hypertension.

In recently published GWAS some of genes have been candidate. PITX2 and ZNFX3 genes was confirmed to be specific for cardioembolic stroke [29]. NINJ2 gene showed association with all types of strokes and ischemic stroke [30], and HDAC9 is associated with the risk of large vessel ischemic stroke [31]. However, no recent GWAS indicated the ABCB1 as risk factor of IS. Regarding that ABCB1 mainly functions as efflux pump, ABCB1 may not be directly related to development of IS. However, as inflammatory mediators such as prostaglandins and leukotrienes are ABCB1 substrate, we hypothesized that ABCB1 functions in the BBB may contribute to tissue damaging and severity of IS developed by ischemic origin. However, NIHSS and MBI were not associated with the tested ABCB1 SNPs.

In summary, we evaluated the relationships between the four SNPs of ABCB1 (rs4148727, rs3213619, rs1128503, and rs3842) and the development, risk factors, severity, and sequelae of IS. And we are the first to report the association between ABCB1 SNPs and the development, hypertension, dyslipidemia, diabetes mellitus, and NIHSS of IS. These results suggest that ABCB1 may be associated with IS in Korean population. Our sample size was relatively small. Furthermore, some of subgroups in our study included too small cases to conclude the clear association between candidate SNPs and IS. Therefore, the suggested relationship between tested SNPs and the clinical phenotypes of IS still need validation study in larger samples.

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REFERENCES

1. Thrift AG, Dewey HM, Sturm JW, Srikanth VK, Gilligan AK, Gall SL, Macdonell RA, McNeil JJ, Donnan GA (2009) Incidence of stroke subtypes in the North East Melbourne Stroke Incidence Study (NEMESIS): differences between men and women. Neuroepidemiology 32:11-18.

2. Grysiewicz RA, Thomas K, Pandey DK (2008) Epidemiology of ischemic and hemorrhagic stroke: incidence, prevalence, mortality, and risk factors. Neurol Clin 26:871-895.

3. Lanktree MB, Dighans M, Hegele RA (2010) Advances in genomic analysis of stroke: what have we learned and where are we headed? Stroke 41:825-832.
4. Shiber JR, Fontane E, Adewale A (2010) Stroke registry: hemorrhagic vs ischemic strokes. Am J Emerg Med 28:331-333.
5. Dahabreh IJ, Kitsios GD, Kent DM, Trikalinos TA (2010) Paraoxonase 1 polymorphisms and ischemic stroke risk: A systematic review and meta-analysis. Genet Med 12:606-615.
6. Lim YH, Jeong YS, Kim SK, Kim DH, Yun DH, Yoo SD, Kim HS, Baik HH (2011) Association between TGFBR2 gene polymorphism (rs2228048, Asn389Asn) and intracerebral hemorrhage in Korean population. Immunol Invest 40:569-580.
7. Park HJ, Kim SK, Yun DH, Kim DH, Chon J, Kim JW, Chung JH (2012) Association of toll-like receptor 2 polymorphisms with National Institute of Health Stroke Scale scores of ischemic stroke patients. J Mol Neurosci 46:536-540.
8. Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152-162.
9. Cantore M, Capparelli E, Berardi F, Perrone R, Colabufo NA (2011) Clinical pharmacokinetic and metabolism of PET radiotracers for imaging P-glycoprotein in chemoresistant tumor of colorectal cancer. Curr Drug Metab 12:985-988.
10. Agarwal S, Hartz AM, Elmquist WF, Bauer B (2011) Breast cancer resistance protein and P-glycoprotein in brain cancer: two gatekeepers team up. Curr Pharm Des 17:2793-2802.
11. Stanimirovic D, Satoh K (2000) Inflammatory mediators of cerebral endothelium: a role in ischemic brain inflammation. Brain Pathol 10:113-126.
12. Bakos E, Homolya L (2007) Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). Pflugers Arch 453:621-641.
13. Wartenberg M, Ling FC, Müssen M, Klein F, Acker H, Gassmann M, Petrat K, Pütz V, Hescheler J, Sauer H (2003) Regulation of the multidrug resistance transporter P-glycoprotein in multicellular tumor spheroids by hypoxia-inducible factor (HIF-1) and reactive oxygen species. FASEB J 17:503-505.
14. Spudich A, Kilic E, Xing H, Kilic U, Rentsch KM, Wunderli-Allenspach H, Bassetti CL, Hermann DM (2006) Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. Nat Neurosci 9:487-488.
15. Lazarowski A, Caltana I, Merelli A, Rubio MD, Ramos Al, Brusco A (2007) Neuronal mdr-1 gene expression after experimental focal hypoxia: a new obstacle for neuroprotection? J Neurol Sci 258:84-92.
16. Bochud M, Bovet P, Burnier M, Eap CB (2009) CYP3A5 and ABCB1 genes and hypertension. Pharmacogenomics 10:477-487.
17. Tilley BC, Marler J, Geller NL, Lu M, Legler J, Brott T, Lyden P, Grotta J (1996) Use of a global test for multiple outcomes in stroke trials with application to the National Institute of Neurological Disorders and Stroke t-PA Stroke Trial. Stroke 27:2136-2142.
18. Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Ebeler R, Hertzberg V (1989) Measurements of acute cerebral infarction: a clinical examination scale. Stroke 20:864-870.
19. Collin C, Wade DT, Davies S, Horne V (1988) The Barthel ADL Index: a reliability study. Int Disabil Stud 10:61-63.
20. Saver JL, Johnston KC, Homer D, Wityk R, Koroshetz W, Truskowski LL, Haley EC (1999) Infarct volume as a surrogate or auxiliary outcome measure in ischemic stroke clinical trials. The RANNTAS Investigators. Stroke 30:293-298.
21. Fujii T, Ota M, Hori H, Sasayama D, Hattori K, Teraishi T, Yamamoto N, Hashikura M, Tatsumi H, Higuchi T, Kunugi H (2012) Association between the functional polymorphism (C3435T) of the gene encoding P-glycoprotein (ABCB1) and major depressive disorder in the Japanese population. J Psychiatr Res 46:555-559.
22. Urayama KY, Wiencke JK, Buffler PA, Chokkalingam AP, Metayer C, Wiemels JL (2007) MDR1 gene variants, indoor insecticide exposure, and the risk of childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev 16:1172-1177.
23. Shaulian E, Karin M (2002) AP-1 as a regulator of cell life and death. Nat Cell Biol 4:E131-E136.
24. Whitfield J, Neame SJ, Paquet L, Bernard O, Ham J (2001) Dominant-negative c-Jun promotes neuronal survival by reducing BIM expression and inhibiting mitochondrial cytochrome c release. Neuron 29:629-643.
25. Sanyal S, Sandstrom DJ, Hoeffer CA, Ramaswami M (2002) AP-1 functions upstream of CREB to control synaptic plasticity in Drosophila. Nature 416:870-874.
26. Boreas AM, Lodder J, Kessels F, de Leeuw PW, Troost J (2001) Predictors of poststroke blood pressure level and course. J Stroke Cerebrovasc Dis 10:85-91.
27. Zolk O, Jacobi J, Pahl A, Fromm MF, Schmieder RE (2007) MDRI genotype-dependent regulation of the aldosterone system in humans. Pharmacogenet Genomics 17:137-144.
28. Adu D, Turney J, Michael J, McMaster P (1983) Hyperkalemia in cyclosporin-treated renal allograft recipients. Lancet 2:370-372.
29. Lemmens R, Buysschaert I, Geelen V, Fernandez-Cadenas I, Montaner J, Schmidt H, Schmidt R, Attia J, Maguire J, Levi C, Jood K, Blomstrand C, Jern C, Wnuk M, Slowik A, Lambrechts D, Thijs V; International Stroke Genetics Consortium (2010) The association of the 4q25 susceptibility variant for atrial fibrillation with stroke is limited to stroke of cardioembolic etiology. Stroke 41:1850-1857.

30. Ikram MA, Seshadri S, Bis JC, Fornage M, DeStefano AL, Aulchenko YS, Debette S, Lumsley T, Folsom AR, van den Herik EG, Bos MJ, Beiser A, Cushman M, Launer LJ, Shahar E, Struchalin M, Du Y, Glazer NL, Rosamond WD, Rivadeneira F, Kelly-Hayes M, Lopez OL, Coresh J, Hofman A, DeCarli C, Heckbert SR, Koudstaal PJ, Yang Q, Smith NL, Kase CS, Rice K, Haritunians T, Roks G, de Kort PL, Taylor KD, de Lau LM, Oostra BA, Uitterlinden AG, Rotter JL, Boerwinkle E, Psaty BM, Mosley TH, van Duijn CM, Breteler MM, Longstreth WT Jr, Wolf PA (2009) Genomewide association studies of stroke. N Engl J Med 360:1718-1728.

31. International Stroke Genetics Consortium (ISGC); Wellcome Trust Case Control Consortium 2 (WTCCC2), Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, Burgess AI, Pirinen M, Jackson CA, Traylor M, Strange A, Su Z, Band G, Syme PD, Malik R, Pera J, Norrving B, Lemmens R, Freeman C, Schanz R, James T, Poole D, Murphy L, Segal H, Cortellini L, Cheng YC, Woo D, Nalls MA, Müller-Myhsok B, Meisinger C, Seedorf U, Ross-Adams H, Boonen S, Wloch-Kopec D, Valant V, Slark J, Furie K, Delavaran H, Langford C, Deloukas P, Edkins S, Hunt S, Gray E, Dronov S, Peltonen L, Gretarsdottir S, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boncoraglio GB, Parati EA, Attia J, Hollliday E, Levi C, Franzosi MG, Goel A, Helgadottir A, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Jankowski J, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawyer SJ, Trembath RC, Viswanathan AC, Wood NW, Worrall BB, Kittner SJ, Mitchell BD, Kissela B, Meschia JF, Thijs V, Lindgren A, Macleod MJ, Slowik A, Walters M, Rosand J, Sharma P, Farrall M, Sudlow CL, Rothwell PM, Dichgans M, Donnelly P, Markus HS (2012) Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. Nat Genet 44:328-333.