

Laboratory Investigation

Polymorphisms of Integrin, Alpha 6 Contribute to the Development and Neurologic Symptoms of Intracerebral Hemorrhage in Korean Population

Hyun-Kyung Park, M.D., Ph.D.,¹ Dae Jean Jo, M.D., Ph.D.²

Departments of Emergency Medicine,¹ Neurosurgery,² Kyung Hee University Hospital at Gangdong, School of Medicine, Kyung Hee University, Seoul, Korea

Objective : The extracellular matrix (ECM) and cell adhesion molecules play crucial roles in angiogenesis, apoptosis, thrombosis, and inflammation, and also contribute to the pathogenesis of stroke. Integrin, alpha 6 (ITGA6) is a member of ECM adhesion receptors. We investigated whether two single nucleotide polymorphisms (SNPs) (rs11895564, Ala380Thr; rs2293649, Asp694Asp) of ITGA6 were associated with the development and clinical phenotypes of intracerebral hemorrhage (ICH) and ischemic stroke (IS).

Methods : We enrolled 199 stroke (78 ICH and 121 IS) and 291 control subjects. Stroke patients were divided into subgroups according to the scores of the National Institutes of Health Stroke Survey (NIHSS, <6 and ≥6) and Modified Barthel Index (MBI, <60 and ≥60). SNPStats, SNPAnalyzer, and Helixtree programs were used to calculate odds ratios, 95% confidence intervals, and *p* values. Multiple logistic regression models were used to analyze genetic data.

Results : A missense SNP rs11895564 was associated with the development of ICH (*p*=0.026 in codominant2, *p*=0.013 in recessive, *p*=0.02 in log-additive models; *p*=0.041 in allele distributions). The A allele frequency of rs11895564 was higher in the ICH group (13.5%) than in the control group (8.1%). In the clinical phenotypes, rs11895564 and rs2293649 showed significant associations in the MBI scores of IS (*p*=0.014 in codominant1 model; *p*=0.02 in allele distributions) and NIHSS scores of ICH (*p*=0.017 in codominant2, *p*=0.035 in recessive, *p*=0.035 in log-additive models), respectively.

Conclusion : These results suggest that ITGA6 may be associated with the development and clinical phenotypes of stroke in Korean population.

Key Words : Intracerebral hemorrhage · Ischemic stroke · Polymorphism · Integrin, alpha 6.

INTRODUCTION

Several lines of evidences support that genetic factors contribute to the risk of stroke^{6,8,10,14}. In very recent, numerous genetic studies have been reported the relationship between stroke and single nucleotide polymorphisms (SNPs) of candidate genes such as methylenetetrahydrofolate reductase [NAD(P)H] (MTHFR)²⁰, tumor protein p53 (TP53)⁵, transforming growth factor, beta 1 (TGFB1)²¹, gap junction protein, alpha 4, 37kDa (GJA4, also known as connexin 37)¹¹, interleukin 10 (IL10)¹⁸, chemokine (C-C motif) ligand 2 (CCL2, also known as MCP1)¹.

The extracellular matrix (ECM) plays a key role in organ and

tissue morphogenesis and in the maintenance of cell and tissue structure. ECM interacts with cell adhesion molecules (CAMs), mainly integrins and also proteoglycans, glycoproteins or immunoglobulin superfamily. These interactions lead to a wide array of physiologic and pathologic processes such as cell adhesion, cell proliferation, angiogenesis, apoptosis, thrombosis, and inflammation. CAMs are also important for the development of neuronal tissue. During the developmental period of the nervous system, synapses develop through formation of cell-cell adhesions. Furthermore cell-cell adhesions serve a crucial role in the brain morphology and functions such as memory and learning. Integrins are transmembrane proteins consisted of an alpha subunit and a beta subunit. Integrin, alpha 6 (ITGA6) interacts beta subunits and then makes integrin heterodimers, α6β1 and α6β4^{15,17}. ITGA1, ITGA6, and ITGB1 are distributed in cerebral microvasculature⁷. It is known that integrins are involved in cerebral microvascular signaling during focal ischemia/perfusion. Wagner et al.²² found that the expression of ITGA6 in ischemic basal ganglia was significantly and abruptly decreased

• Received : April 15, 2011 • Revised : July 18, 2011

• Accepted : October 17, 2011

• Address for reprints : Dae Jean Jo, M.D., Ph.D.

Department of Neurosurgery, Kyung Hee University Hospital at Gangdong, School of Medicine, Kyung Hee University, 892 Dongnam-ro, Gangdong-gu, Seoul 134-727, Korea

Tel : +82-2-440-6147, Fax : +82-2-440-7494

E-mail : apuzzo@hanmail.net

by 24-hour middle cerebral artery occlusion, compared with non-ischemic zone. Although integrins may be related to stroke susceptibility, the genetic determinants are not fully defined.

In this study, we investigated the association between ITGA6 SNPs and stroke [ischemic stroke (IS) and intracerebral hemorrhage (ICH)], and their clinical phenotypes in Korean population.

MATERIALS AND METHODS

Study population and clinical phenotypes

All participants were enrolled among subjects visiting at the departments of neurosurgery and emergency medicine in our hospital. Control subjects (n=291, 152 males/139 females) were recruited among healthy volunteers to examine the general health check-up program (Table 1). Subjects with neurological diseases, ischemic heart diseases, and other severe diseases were excluded. A total of 199 stroke patients comprised 78 ICH patients (46 males/32 females) and 121 IS patients (68 males/53 females) (Table 1). Subjects with transient ischemic attack, cerebrovascular malformation, brain tumors, congenital brain disorders, and traumatic or iatrogenic stroke were excluded. Stroke patients were diagnosed by computed tomography, magnetic resonance imaging, and angiography. All stroke patients were classified into the clinical phenotypes according to the National Institutes of Health Stroke Survey (NIHSS) score and the Modified Barthel Index (MBI) score. For the neurological functional level of patient, the severity of 13 neurologic symptoms was evaluated by the NIHSS. For the activity of daily living (ADL) of patients, the quality of 10 general life activities was estimated by the MBI. Subjects with inappropriate data were excluded. Protocol in this study was approved by the ethics review committee of Medical Research Institute at our hospital. Written informed consent was obtained from each subject. If stroke patients were incommunicative, it was obtained from close relatives or guardian.

SNP selection

We searched the coding SNPs (cSNPs) of the ITGA6 gene in the SNP database of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/SNP>, BUILD 132). The

cSNPs with heterozygosity below 0.1 or unknown heterozygosity and minor allele frequency (MAF) below 0.1 or unknown MAF were excluded. Out of 12 missense SNPs, SNPs with heterozygosity below 0.1 or unknown heterozygosity were 9 and SNPs with unknown MAF were 2. Among 10 synonymous SNPs, there were 7 SNPs with heterozygosity below 0.1 or unknown heterozygosity, 1 SNPs with unknown MAF, and 1 SNP with MAF 0.1065. Finally, one missense SNP (rs11895564, Ala380Thr) and one synonymous SNP (rs2293649, Asp694Asp) were selected.

SNP genotyping

Peripheral blood samples were collected from all subjects, and genomic deoxyribonucleic acid (DNA) was extracted using QIAamp® DNA mini kit (QIAGEN, Valencia, CA, USA). Genotypes of the two selected SNPs were determined by direct sequencing (MACROGEN, Seoul, Korea). Polymerase chain reactions (PCRs) were performed with the following primers: for rs11895564 (sense, 5'-GGAGCCCCACAGTATTTTGATA-3'; antisense, 5'-TAGTTTCTCCCATGTTGGTCAGG-3'; product size, 348 bp) and for rs2293649 (sense, 5'-GGAAACGTATTGAGGACTTTGC-3'; antisense, 5'-GTTGGCAACACAACCAACTGT-3'; product size, 363 bp). PCR consisted of 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 to extend the reaction. 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).

Statistic analysis

SNPAnalyzer Pro (ISTECH, Goyang, Korea), Helixtree (Golden Helix, Bozeman, MT, USA), and SNPStats (<http://bioinfo.iconcologia.net/index.php?module=Snstats>) were performed to obtain ORs, 95% CIs, and *p* values adjusting age and sex as covariables. Hardy-Weinberg equilibrium (HWE) was evaluated by the chi-squared test. Multiple logistic regression models (codominant1, codominant2, dominant, recessive, and log-additive) were used to analyze genetic data. In case of the numbers below 5, *p* value was corrected by the Fisher's exact test.

Haploview version 4.2 (Daly Lab, Cambridge, MA, USA) was also used to determine the linkage disequilibrium (LD) block and haplotypes between two SNPs. The statistical significance level was set at *p*<0.05.

RESULTS

Clinical characteristics of stroke and control subjects

The clinical features of patients with stroke and control subjects are presented in Table 1. The age of ICH, IS, and

Table 1. Clinical characteristics in ICH, IS, and control subjects

	ICH	IS	Control
Total number (n)	78	121	291
Male/Female (n)	46/32	68/53	152/139
Age (mean±SD, years)	56.4±12.5	65.7±12.1	63.0±9.3
NIHSS (score)			
<6	24	56	
≥6	50	57	
MBI (score)			
<60	54	71	
≥60	14	25	

Stroke patients with inappropriate clinical data were excluded. ICH : intracerebral hemorrhage, IS : ischemic stroke, SD : standard deviation, NIHSS : National Institutes of Health Stroke Survey, MBI : Modified Barthel Index

control subjects (mean±SD, years) was 56.4±12.5, 65.7±12.1, and 63.0±9.3 years, respectively. Because the mean age of each group was different ($p<0.05$), age and sex as covariates were adjusted to obtain statistical results in the analysis of genetic data. Stroke patients were divided into two clinical phenotypes in accordance to NIHSS (scores of 13 neurological symptoms; <6 and ≥ 6) and MBI (scores of 10 activities of general life; <60 and ≥ 60). The number of ICH patients with NIHSS scores <6 and ≥ 6 was 24 and 50, respectively. The number of ICH patients with MBI scores <60 and ≥ 60 was 54 and 14, respectively. Out of IS patients, 56 and 57 subjects displayed NIHSS scores of <6 and ≥ 6 , respectively, and 71 and 25 subjects displayed MBI scores of <60 and ≥ 60 , respectively (Table 1).

Genetic analysis

Multiple logistic regression analysis with adjustment for age and sex was performed using the 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).

The genotype and allele frequencies of the two selected SNPs are presented in Table 2, 3, 4. Two cSNPs (rs11895564, Ala380Thr; rs2293649, Asp694Asp) were in HWE in the ICH, IS, and control groups, respectively ($p>0.05$, data not shown). As shown in Table 2, a missense SNP (rs11895564, Ala380Thr) of the ITGA6 gene was associated with the development of ICH ($p=0.026$, OR=16.56, 95% CI=1.58-173.86 in codominant2 model; $p=0.013$, OR=15.21, 95% CI=1.46-158.80 in recessive model;

$p=0.020$, OR=2.00, 95% CI=1.13-3.57 in log-additive model). The A allele frequency of rs11895564 was higher in the ICH group than in the control group (13.5% vs. 8.1%; $p=0.041$, OR=1.77, 95% CI=1.02-3.06). The rs2293649 SNP was not associated with the development of ICH (Table 2). Genotype and allele frequencies of rs11895564 and rs2293649 were no different between IS and controls (Table 3). These results indicate that the rs11895564 SNP of the ITGA6 gene may be associated with ICH, but not IS, and the A allele of rs11895564 could be a risk factor for the development of ICH.

Analysis of clinical phenotypes

All stroke patients were classified into clinical phenotypes according to the NIHSS score (<6 or ≥ 6) and the MBI score (<60 or ≥ 60). In Table 4, the rs2293649 SNP was associated with the NIHSS scores of ICH ($p=0.017$, OR=6.04, 95% CI=1.06-34.46 in codominant model2; $p=0.035$, OR=4.61, 95% CI=0.92-22.94 in recessive model; $p=0.035$, OR=2.22, 95% CI=1.03-4.80 in log-additive model). The G/G genotype frequency of rs2293649 was higher in the NIHSS ≥ 6 group (30.0%) than in the NIHSS <6 group (8.3%). The rs11895564 SNP was not related to the NIHSS scores of ICH. The results suggest that ITGA6 rs2293649 may contribute to the neurological function levels of ICH. In Table 5, the rs11895564 SNP had significant associations between IS patients with MBI <60 and IS patients MBI ≥ 60 ($p=0.014$, OR=3.88, 95% CI=1.32-11.35 in codominant1 model; $p=0.020$, OR=3.24, 95% CI=1.21-8.71 in allele distributions). The A allele frequency of rs11895564 in the MBI ≥ 60 group (18.0%) was about 2.8-fold higher, compared to the MBI <60 group (6.3%). The rs2293649 SNP was not associated with the MBI scores of IS. These data suggest that ITGA6 rs11895564 may be

Table 2. Genotype and allele frequencies of ITGA6 SNPs in controls and ICH

SNP	Type	Control		ICH		Model	OR (95% CI)	<i>p</i>	Fisher's exact <i>p</i>
		n (%)	n (%)	n (%)	n (%)				
rs11895564 Ala380Thr	Genotype	G/G	245 (84.2)	60 (76.9)	Codominant1	1.54 (0.78-3.06)	0.35	0.026*	
		A/G	45 (15.5)	15 (19.2)	Codominant2	16.56 (1.58-173.86)	0.031*		
		A/A	1 (0.3)	3 (3.8)	Dominant	1.84 (0.96-3.51)	0.07		
					Recessive	15.21 (1.46-158.80)	0.013*		
					Overdominant	1.46 (0.74-2.88)	0.28		
					Log-additive	2.00 (1.13-3.57)	0.020*		
	Allele	G	535 (91.9)	135 (86.5)		1			
	A	47 (8.1)	21 (13.5)		1.77 (1.02-3.06)	0.041*			
rs2293649 Asp694Asp	Genotype	A/A	93 (32.0)	23 (29.5)	Codominant1	1.01 (0.55-1.85)	0.75		
		A/G	138 (47.4)	36 (46.1)	Codominant2	1.33 (0.65-2.73)	0.41		
		G/G	60 (20.6)	19 (24.4)	Dominant	1.10 (0.62-1.94)	0.74		
					Recessive	1.33 (0.72-2.45)	0.37		
					Overdominant	0.89 (0.53-1.50)	0.66		
					Log-additive	1.14 (0.79-1.65)	0.47		
	Allele	A	324 (55.7)	82 (52.6)		1			
	G	258 (44.3)	74 (47.4)		1.13 (0.80-1.62)	0.49			

The *p*-values were calculated from logistic regression analysis adjusting age and sex. The Fisher's exact *p*-value was performed, if the number was below 5. *Significant associations. ITGA6: integrin, alpha 6, SNP: single nucleotide polymorphism, ICH: intracerebral hemorrhage, OR: odds ratio, CI: confidence interval

Table 3. Genotype and allele frequencies of ITGA6 SNPs in controls and IS

SNP	Type	Control		IS		Model	OR (95% CI)	p	Fisher's exact p
		n (%)	n (%)	n (%)	n (%)				
rs11895564 Ala380Thr	Genotype	G/G	245 (84.2)	96 (79.3)	Codominant1	1.34 (0.77-2.34)	0.27	0.48	
		A/G	45 (15.5)	24 (19.8)	Codominant2	2.43 (0.15-40.35)	0.51		
		A/A	1 (0.3)	1 (0.8)	Dominant	1.37 (0.79-2.36)	0.27		
					Recessive	2.29 (0.14-37.95)	0.57		
					Overdominant	1.33 (0.77-2.32)	0.31		
	Allele		535 (91.9)	216 (89.3)		1			
			47 (8.1)	26 (10.7)		1.37 (0.83-2.27)	0.22		
rs2293649 Asp694Asp	Genotype	A/A	93 (32.0)	34 (28.1)	Codominant1	1.29 (0.78-2.12)	0.41		
		A/G	138 (47.4)	62 (51.2)	Codominant2	1.12 (0.61-2.07)	0.67		
		G/G	60 (20.6)	25 (20.7)	Dominant	1.23 (0.77-1.98)	0.38		
					Recessive	0.96 (0.57-1.63)	0.88		
					Overdominant	1.23 (0.80-1.89)	0.35		
	Allele	A	324 (55.7)	130 (53.7)		1			
		G	258 (44.3)	112 (46.3)		1.08 (0.80-1.46)	0.61		

The p-values were calculated from logistic regression analysis adjusting age and sex. The Fisher's exact p-value was performed, if the number was below 5. ITGA6 : integrin, alpha 6, SNP: single nucleotide polymorphism, IS : ischemic stroke, OR : odds ratio, CI : confidence interval

Table 4. Genotype and allele frequencies of ITGA6 SNPs in ICH subgroups according to the NIHSS scores

SNP	Type	NIHSS (<6)		NIHSS (≥6)		Model	OR (95% CI)	p	Fisher's exact p
		n (%)	n (%)	n (%)	n (%)				
rs11895564 Ala380Thr	Genotype	G/G	18 (75.0)	40 (80.0)	Codominant1	0.97 (0.24-3.91)	0.99	1.00	
		A/G	4 (16.7)	9 (18.0)	Codominant2	0.11 (0.01-1.46)	0.24		
		A/A	2 (8.3)	1 (2.0)	Dominant	0.63 (0.18-2.15)	0.46		
					Recessive	0.11 (0.01-1.45)	0.08		
					Overdominant	1.08 (0.28-4.22)	0.91		
	Allele	A	8 (16.7)	11 (11.0)		1			
		G	40 (83.3)	89 (89.0)		1.62 (0.61-4.33)	0.34		
rs2293649 Asp694Asp	Genotype	A/A	11 (45.8)	11 (22.0)	Codominant1	1.59 (0.49-5.14)	0.16	0.017*	
		A/G	11 (45.8)	24 (48.0)	Codominant2	6.04 (1.06-34.46)	0.020*		
		G/G	2 (8.3)	15 (30.0)	Dominant	2.28 (0.76-6.89)	0.15		
					Recessive	4.61 (0.92-22.94)	0.035*		
					Overdominant	0.83 (0.29-2.37)	0.73		
	Allele	A	33 (68.8)	54 (54.0)		1			
		G	15 (31.3)	46 (46.0)		1.87 (0.91-3.87)	0.09		

The p-values were calculated from logistic regression analysis adjusting age and sex. The Fisher's exact p-value was performed, if the number was below 5. *Significant associations. ITGA6 : integrin, alpha 6, SNP: single nucleotide polymorphism, NIHSS : National Institutes of Health Stroke Survey, ICH : intracerebral hemorrhage, OR : odds ratio, CI : confidence interval

involved in the ADL of IS. However, rs2293649 was not contributed to the MBI scores of IS (Table 5). In addition, rs11895564 showed borderline significances in the NIHSS scores of IS (p=0.057 in codominant1 model; p=0.070 in allele distributions) (data not shown).

haplotypes between rs11895564 and rs2293649 in the coding region of the ITGA6 gene. The LD block between two SNPs in the control groups was not made (D'=0.29 and r²=0.009). Therefore, we did not analyze the haplotypes.

DISCUSSION

Linkage disequilibrium and haplotypes

Haploview version 4.2 was used to evaluate the LD block and

Brain damage after stroke is related to the multiple complex

Table 5. Genotype and allele frequencies of ITGA6 SNPs in IS subgroups according to the MBI scores

SNP	Type		MBI (<60)	MBI (≥60)	Model	OR (95% CI)	<i>p</i>	Fisher's exact <i>p</i>
			n (%)	n (%)				
rs11895564 Ala380Thr	Genotype	G/G	62 (87.3)	16 (64.0)	Codominant1	3.88 (1.32-11.35)	0.014*	
		A/G	9 (12.7)	9 (36.0)	Codominant2	0.00 (0.00-NA)		
		A/A	0 (0.0)	0 (0.0)	Dominant	0.00 (0.00-NA)		
	Allele	G	133 (93.7)	41 (82.0)		1	0.020*	
		A	9 (6.3)	9 (18.0)		3.24 (1.21-8.71)		
rs2293649 Asp694Asp	Genotype	A/A	19 (26.8)	10 (40.0)	Codominant1	0.51 (0.17-1.52)	0.30	
		A/G	36 (50.7)	11 (44.0)	Codominant2	0.44 (0.11-1.84)		
		G/G	16 (22.5)	4 (16.0)	Dominant	0.49 (0.17-1.37)		
	Allele	G	68 (47.9)	19 (38.0)		1	0.23	
		A	74 (52.1)	31 (62.0)		1.50 (0.78-2.90)		

The *p*-values were calculated from logistic regression analysis adjusting age and sex. The Fisher's exact *p*-value was performed, if the number was below 5. *Significant associations. ITGA6 : integrin, alpha 6, SNP: single nucleotide polymorphism, MBI : Modified Barthel Index, IS : ischemic stroke, OR : odds ratio, CI : confidence interval, NA : not applicable

interactions such as excitotoxicity, acidotoxicity, ionic imbalance, oxidative stress, inflammation, and apoptosis⁴). Milner et al.¹⁶ found that focal cerebral ischemia induced loss of ITGA1 and ITGA6, and oxygen-glucose deprivation produced a significant decreasing both ITGA1 and ITGA6 by murine astrocytes. The integrin $\alpha_6\beta_4$ and ITGB4 were identified between the myointima and the astrocyte compartments in cerebral microvessels. Middle cerebral artery occlusion produced a significant reduction of the $\alpha_6\beta_4$ at the astrocyte-matrix interface in microvessels, suggesting that the expression of $\alpha_6\beta_4$ is extraordinarily sensitive to ischemia²²). Variants in ITGA2 have been reported to be associated with an increased risk for ischemic stroke^{3,13,19}). Our data revealed that a missense SNP rs11895564 (Ala380Thr) of ITGA6 was associated with the development of ICH, but not IS (Table 2, 3). Although we cannot exactly explain this discrepancy, it seems to be different mechanisms for the etiology of ICH or IS might be involved. However, the biological roles of rs11895564 on stroke development will be need in future. Loughlin et al.¹² reported that microsatellite of ITGA6 was associated with hip osteoarthritis in female. Johnatty et al.⁹ showed the association between ITGA6 rs13027811 intron SNP (iSNP) and ovarian cancer. The rs12621278 iSNP of ITGA6 was associated with the progression of prostate cancer²). We found that the synonymous SNP rs2293649 (Asp694Asp) of ITGA6 was associated with the NIHSS scores of ICH (Table 4), suggesting rs2293649 may be contribute to the neurological functional levels of ICH. We also found that the missense SNP rs11895564 was associated with the MBI scores of IS (Table 5), suggesting rs11895564 may be contribute to the ADL of IS. Based on previous and present studies, we speculate that ITGA6 may be involved in the develop-

ment and clinical characteristics of stroke.

ITGA6 protein (UniProt ID, P23229) consists of 1,130 amino acids (AAs) with the signal peptide the signal peptide (from 1 to 23 AAs), integrin alpha-6 heavy chain (from 24 to 938 AAs), and integrin alpha-6 light chain (from 942 to 1,130 AAs). ITGA6 also composed of several regions : the extracellular topological domain, from 24 to 1,050 AAs; helical transmembrane, from 1,051 to 1,076 AAs; cytoplasmic topological domain, from 1,077 to 1,130 AAs; interaction with HPS5, from 1,077 to 1,083 AAs; GFFKR motif, from 1,079 to 1,083 AAs) (<http://www.uniprot.org/uniprot>). Two SNPs (rs11895564, Ala380Thr; rs2293649, Asp694Asp) are located at both the integrin alpha-6 heavy chain and extracellular topological domain. In the SNP database (<http://www.ncbi.nlm.nih.gov/SNP>, BUILD 132), the G and A allele frequencies in the rs11895564 SNP have been reported to be 0.704 and 0.296 in European, 0.624 and 0.376 in Sub-Saharan African, 0.878 and 0.122 in Japanese, and 0.884 and 0.116 in Chinese, respectively. In our control group, the G and A allele frequencies were 0.919 and 0.081, which are similar to those seen in Asian populations (Table 2). The G and A allele frequencies in the rs2293649 SNP have been reported to be 0.819 and 0.181 in European, 0.841 and 0.159 in Sub-Saharan African, 0.395 and 0.605 in Japanese, and 0.453 and 0.547 in Chinese, respectively. In our control group, the G and A allele frequencies were 0.443 and 0.557, which are similar to those seen in Chinese population (Table 2). There is ethnic difference in the allele distributions of rs2293649. The incidence of ICH is higher in Asians than in other populations.

To our knowledge, this is the first study that whether ITGA6 SNPs are associated with the development and clinical phenotypes

of stroke. Our results revealed that a missense SNP rs11895564 was associated with the development of ICH ($p=0.026$ in codominant2, $p=0.013$ in recessive, $p=0.02$ in log-additive models; $p=0.041$ in allele distributions) and the MBI scores of IS ($p=0.014$ in codominant1 model; $p=0.02$ in allele distributions). The A allele frequency of rs11895564 in the ICH group was about 2.8-fold (13.5%), compared to that in the control group (8.1%). Moreover, the A/A genotype frequency of rs11895564 was much different between ICH patients and control subjects (3.8% vs. 0.3%). These data strongly suggest that the A allele of rs11895564 may be a risk factor for the development of ICH. The synonymous SNP rs2293649 was significantly difference between ICH patients with NIHSS <6 and ICH patients with NIHSS ≥ 6 ($p=0.017$ in codominant2, $p=0.035$ in recessive, $p=0.035$ in log-additive models). Present study has limitations to make our conclusion clear. Because we evaluated small cases, additional studies with larger number of cases or other populations should be conducted in the future.

CONCLUSION

In conclusion, we report that ITGA6 may be associated with the development and clinical characteristics of stroke in Korean population.

• Acknowledgements

This work was supported by the Program of Kyung Hee University for the Young Researcher of Medical Science in 2010 (KHU-20100760).

References

- Buraczynska K, Luchowski P, Wojczal J, Ksiazek A, Stelmasiak Z : Monocyte chemoattractant protein (MCP-1) A-2518G gene polymorphism in stroke patients with different comorbidities. *Clin Biochem* 43 : 1421-1426, 2010
- Cheng I, Plummer SJ, Neslund-Dudas C, Klein EA, Casey G, Rybicki BA, et al. : Prostate cancer susceptibility variants confer increased risk of disease progression. *Cancer Epidemiol Biomarkers Prev* 19 : 2124-2132, 2010
- Cole VJ, Staton JM, Eikelboom JW, Hankey GJ, Yi Q, Shen Y, et al. : Collagen platelet receptor polymorphisms integrin alpha2beta1 C807T and GPVI Q317L and risk of ischemic stroke. *J Thromb Haemost* 1 : 963-970, 2003
- Doyle KP, Simon RP, Stenzel-Poore MP : Mechanisms of ischemic brain damage. *Neuropharmacology* 55 : 310-318, 2008
- Gomez-Sanchez JC, Delgado-Esteban M, Rodriguez-Hernandez I, Sobrino T, Perez de la Ossa N, Reverte S, et al. : The human Tp53 Arg-72Pro polymorphism explains different functional prognosis in stroke. *J Exp Med* 208 : 429-437, 2011
- Grysiwicz RA, Thomas K, Pandey DK : Epidemiology of ischemic and hemorrhagic stroke : incidence, prevalence, mortality, and risk factors. *Neurol Clin* 26 : 871-895, vii, 2008
- Haring HP, Akamine BS, Habermann R, Koziol JA, Del Zoppo GJ : Distribution of integrin-like immunoreactivity on primate brain microvasculature. *J Neuropathol Exp Neurol* 55 : 236-245, 1996
- Ikram MA, Seshadri S, Bis JC, Fornage M, DeStefano AL, Aulchenko YS, et al. : Genomewide association studies of stroke. *N Engl J Med* 360 : 1718-1728, 2009
- Johnatty SE, Beesley J, Chen X, Macgregor S, Duffy DL, Spurdle AB, et al. : Evaluation of candidate stromal epithelial cross-talk genes identifies association between risk of serous ovarian cancer and TERT, a cancer susceptibility "hot-spot". *PLoS Genet* 6 : e1001016, 2010
- Lanktree MB, Dichgans M, Hegele RA : Advances in genomic analysis of stroke : what have we learned and where are we headed? *Stroke* 41 : 825-832, 2010
- Leu HB, Chung CM, Chuang SY, Bai CH, Chen JR, Chen JW, et al. : Genetic variants of connexin37 are associated with carotid intima-medial thickness and future onset of ischemic stroke. *Atherosclerosis* 214 : 101-106, 2011
- Loughlin J, Dowling B, Chapman K, Marcelline L, Mustafa Z, Southam L, et al. : Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. *Proc Natl Acad Sci U S A* 101 : 9757-9762, 2004
- Matarin M, Brown WM, Hardy JA, Rich SS, Singleton AB, Brown RD Jr, et al. : Association of integrin alpha2 gene variants with ischemic stroke. *J Cereb Blood Flow Metab* 28 : 81-89, 2008
- Matarin M, Brown WM, Scholz S, Simón-Sánchez J, Fung HC, Hernandez D, et al. : A genome-wide genotyping study in patients with ischaemic stroke : initial analysis and data release. *Lancet Neurol* 6 : 414-420, 2007
- McCarty JH : Cell adhesion and signaling networks in brain neurovascular units. *Curr Opin Hematol* 16 : 209-214, 2009
- Milner R, Hung S, Wang X, Berg GI, Spatz M, del Zoppo GJ : Responses of endothelial cell and astrocyte matrix-integrin receptors to ischemia mimic those observed in the neurovascular unit. *Stroke* 39 : 191-197, 2008
- Mousa SA : Adhesion molecules : potential therapeutic and diagnostic implications. *Methods Mol Biol* 663 : 261-276, 2010
- Munshi A, Rajeshwar K, Kaul S, Al-Hazzani A, Alshatwi AA, Sai Babu M, et al. : Interleukin-10-1082 promoter polymorphism and ischemic stroke risk in a South Indian population. *Cytokine* 52 : 221-224, 2010
- Nikolopoulos GK, Tsantes AE, Bagos PG, Travlou A, Vaiopoulos G : Integrin, alpha 2 gene C807T polymorphism and risk of ischemic stroke : a meta-analysis. *Thromb Res* 119 : 501-510, 2007
- Somarajan BI, Kalita J, Mittal B, Misra UK : Evaluation of MTHFR C677T polymorphism in ischemic and hemorrhagic stroke patients. A case-control study in a Northern Indian population. *J Neurol Sci* 304 : 67-70, 2011
- Tao HM, Chen GZ, Lu XD, Chen GP, Shao B : TGF- β 1 869T/C polymorphism and ischemic stroke : sex difference in Chinese. *Can J Neurol Sci* 37 : 803-807, 2010
- Wagner S, Tagaya M, Koziol JA, Quaranta V, del Zoppo GJ : Rapid disruption of an astrocyte interaction with the extracellular matrix mediated by integrin alpha 6 beta 4 during focal cerebral ischemia/reperfusion. *Stroke* 28 : 858-865, 1997