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Polymorphisms of *IGF1* contribute to the development of ischemic stroke

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Abstract. Insulin-like growth factor 1 (IFG1) is neuroprotective in animal models of focal brain ischemia and correlates with ischemic stroke (IS) outcome in the elderly. In this study, we investigated whether single nucleotide polymorphisms (SNPs) of the *IFG1* gene are associated with the development and clinical features of IS in a Korean population. A total of 119 patients with IS and 289 control subjects were recruited. Stroke patients were classified into subgroups according to the scores of the National Institutes of Health Stroke Survey (NIHSS; <6 and ≥6) and the Modified Barthel Index (MBI; <60 and ≥60). Among the SNPs of the *IFG1* gene, five SNPs were selected and analyzed by direct sequencing: rs2162679 (intron), rs2195239 (intron), rs978458 (intron), rs1520220 (intron) and rs6214 (3' untranslated region; 3'UTR). Multiple logistic regression models were conducted to analyze genetic data. SNPStats, SNPAnalyzer Pro and Helixtree programs were used to calculate odds ratios (ORs), 95% confidence intervals (CIs) and p-values. Two SNPs, rs2162679 and rs6214, were associated with the development of IS. After Bonferroni correction (p^c), the A and G alleles of rs2162679 and rs6214 had significant differences between patients with IS and the controls [rs2162679, OR (95% CI) = 1.64 (1.17-2.31), p=0.004, p^c=0.02; rs6214, OR (95% CI) = 1.52 (1.12-2.07), p=0.007, p^c=0.035], respectively. However, the five selected SNPs were not related to the NIHSS and MBI scores. These results suggest that *IGF1* may be associated with the development of IS.

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Introduction

Stroke is a neurological disease which causes long-term disability and is the third leading cause of death in the United States. Ischemic stroke (IS) accounts for approximately 85% of all strokes. The other 9% are caused by intracerebral hemorrhage and 4-5% by subarachnoid hemorrhage (1,2). Environmental and genetic factors are related to the pathogenesis of IS. Several lines of genetic studies have reported the relationship between IS and single nucleotide polymorphisms (SNPs) of candidate genes, such as the transforming growth factor β1 (TGFB1), transforming growth factor β receptor II (70/80 kDa) (TGFBR2) and tumor necrosis factor (TNF) (3-5).

Insulin-like growth factor 1 (somatomedin C) (IGF1) is similar to insulin in function and plays a crucial role in mammalian growth and development. The IGF1 level declines during the normal aging process, and a low IGF1 level correlates with decreased cognitive abilities (6). *In vitro* studies have shown that IGF1 reduces neuronal cell death in various injury insults (7,8) and IGF1 has a protective effect in ischemic animal models (9,10). Several reports have reviewed the roles of IGF1 in stroke severity and outcome (11,12). They have suggested that IGF levels may be associated with neurological recovery and functional outcome, and have also proposed IGF1 as a predictor of stroke outcome.

In this study, we investigated the association between *IGF1* SNPs and IS in a Korean population. We also assessed the relationship between *IGF1* SNPs and the clinical phenotypes according to the scores of National Institutes of Health Stroke Survey (NIHSS) and the Modified Barthel Index (MBI).

Materials and methods

Study population and clinical phenotypes. IS patients were enrolled among participants visiting the Departments of Neurosurgery and Physical Medicine and Rehabilitation, Kyung Hee Medical Center (Seoul, Republic of Korea). Patients with transient ischemic attack, cerebrovascular malformation, congenital brain disorders and accidental or iatrogenic stroke, were excluded. Stroke patients were diagnosed by computed tomography, magnetic resonance imaging, angiography and

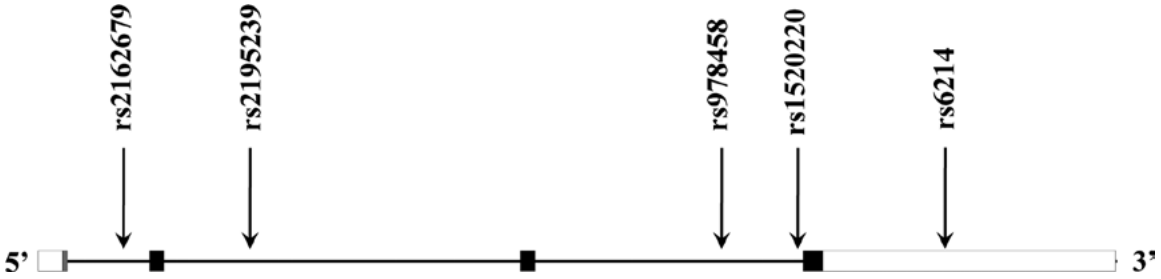

Figure 1. Gene map and single nucleotide polymorphisms in the *IGF* gene on chromosome 12q23.2. Exons are marked with a box. The coding regions are represented by black boxes and untranslation regions by white boxes.

duplex sonography. The control subjects were recruited among healthy volunteers to examine the general health check-up program. Patients with neurological diseases, ischemic heart diseases and other severe diseases, were excluded. All stroke patients were classified into clinical phenotypes according to the NIHSS and MBI scores. For the neurological functional level of IS patient, the severity of 13 neurological symptoms was assessed by the NIHSS score. For the daily living activity of IS patients, the quality of 10 general life activities was evaluated by the MBI score. This study was approved by the Ethics Review Committee of the Medical Research Institute, School of Medicine, Kyung Hee University. Written informed consent was obtained from all patients. If patients were incommunicative, it was obtained from a guardian or close relatives.

SNP selection and genotyping. We searched the coding SNPs (cSNPs) of the *IFG1* gene in the SNP database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP, BUILD 132). The cSNPs with a heterozygosity of <0.05 and/or a minor allele frequency (MAF) of <0.05, were excluded. Out of five missense and two synonymous SNPs, there were no cSNPs with a heterozygosity of >0.05 or a MAF of >0.05. Therefore, we searched the untranslational and intron SNPs of the *IGF1* gene and previous studies (13-15). Finally, five SNPs [rs2162679 (intron), rs2195239 (intron), rs978458 (intron), rs1520220 (intron) and rs6214 (3' untranslated region; 3'UTR)] were selected (Fig. 1). Genotypes of the five selected SNPs were analyzed by direct sequencing (Macrogen, Seoul, Republic of Korea). Polymerase chain reactions (PCRs) were conducted using the forward and reverse primers of each SNP (Table II). PCRs were performed under the following conditions: 35 cycles at 95˚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 PCR products were sequenced by an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA) and genotypes of each SNP were analyzed by SeqManII software (DNASTAR, Madison, WI, USA).

Statistical analysis. SNPStats (http://bioinfo.iconcologia.net/index.php?module=Snpstats), SNPAnalyzer Pro (ISTECH, Goyang, Republic of Korea), and Helixtree (Golden Helix, Bozeman, MT, USA) were used to obtain odds ratios (ORs), 95% confidence intervals (CIs) and p-values. Hardy-Weinberg equilibrium (HWE) was calculated using the Chi-square test. Multiple logistic regression models were conducted using the following models: codominant1 (major allele homozygotes vs. heterozygotes), codominant2 (major allele homozygotes vs. minor allele homozygotes), dominant (major allele homozygotes vs. heterozygotes and minor allele homozygotes), recessive (major allele homozygotes and heterozygotes vs. minor allele homozygotes) and log-additive (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes) (16,17). Bonferroni correction was performed to obtain further statistical significance. Linkage disequilibrium (LD) blocks were estimated using Haploview version 4.2 (Daly Lab, Cambridge, MA, USA). The required case size in each SNP was estimated using the genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html) to obtain the statistical power. The statistical significant level was set at a value of $p<0.05$.

Table I. Clinical characteristics in stroke patients and control subjects.

	IS	Control
Total no.	119	289
Male/female (n)	66/53	150/139
Age (mean ± SD, years)	65.8±12.1	62.9±9.3
NIHSS (score)		
<6	55	
≥6	57	
MBI (score)		
<60	71	
≥60	24	

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

Results

Demographic and clinical features of study subjects. Table I shows the demographic and clinical features of the IS patients and the control subjects. The age of the IS patients and control subjects was 65.8±12.1 (mean ± SD) and 62.9±9.3 years, respectively. The number of IS patients and control subjects was 119 (66 male/53 female) and 289 (150 male/139 female), respectively. All IS patients were divided into two clinical subgroups according to the NIHSS (<6 and ≥6) and MBI scores (<60 and ≥60). The numbers of IS patients with a NIHSS score of <6 and ≥6 were 55 (49.1%) and 57 (50.9%), respectively. The

Table II. Primer sequences for each single nucleotide polymorphism (SNP).

SNP	Forward (5'-3')	Reverse (5'-3')	Size (bp)
rs2162679	TCTGCAAGATCAATCACAGGTT	AAAAACCAAAACCCCTGTCTCT	386
rs2195239	CAAATTTCAGCTGCGACTTTATC	GAGCCAAAACCATCTCTACACC	306
rs978458	TCCACTAGAGCCAAAGAAGAGC	GTGAAATGGTGGAGGATGATTT	381
rs1520220	AAAGGATCTAGAGGCCAGAAGG	AGTTCTTGTTTCCTGCACTCCC	390
rs6214	CCAGACATACAGGTTCTGTGGA	TTGGAGAGGATTATGTGTTGGA	304

Table III. Genotype and allele frequencies of *IGF* singe nucleotide polymorphisms in controls and IS patients.

SNP	Type	Control		IS		Model	OR (95% CI)	p-value	p[c]
		n	%	n	%				
rs2162679	A/A	120	41.5	63	53.9	Codominant1	0.68 (0.43-1.07)	0.0900	0.4500
Intron	A/G	129	44.6	48	41.0	Codominant2	0.27 (0.11-0.67)	**0.0050**	**0.0250**
	G/G	40	13.8	6	5.1	Dominant	0.58 (0.37-0.90)	**0.0150**	0.0800
						Recessive	0.32 (0.13-0.79)	**0.0059**	**0.0295**
						Log-additive	0.59 (0.41-0.83)	**0.0020**	**0.0100**
	G	209	36.2	60	25.6		1		
	A	369	63.8	174	74.4		1.64 (1.17-2.31)	**0.0040**	**0.0200**
rs2195239	G/G	93	32.2	43	36.1	Codominant1	0.84 (0.53-1.36)	0.4800	1.0000
Intron	C/G	152	52.6	60	50.4	Codominant2	0.78 (0.39-1.54)	0.4700	1.0000
	C/C	44	15.2	16	13.4	Dominant	0.83 (0.53-1.30)	0.4200	1.0000
						Recessive	0.86 (0.46-1.61)	0.6400	1.0000
						Log-additive	0.87 (0.63-1.21)	0.4100	1.0000
	G	338	58.5	146	61.3		1		
	C	240	41.5	92	38.7		0.89 (0.65-1.21)	0.4500	1.0000
rs978458	G/G	90	31.1	41	34.5	Codominant1	0.92 (0.57-1.48)	0.7200	1.0000
Intron	A/G	144	49.8	60	50.4	Codominant2	0.70 (0.37-1.36)	0.3000	1.0000
	A/A	55	19.0	18	15.1	Dominant	0.86 (0.54-1.35)	0.5100	1.0000
						Recessive	0.74 (0.41-1.34)	0.3200	1.0000
						Log-additive	0.85 (0.62-1.17)	0.3200	1.0000
	G	324	56.1	142	59.7		1		
	A	254	43.9	96	40.3		0.86 (0.64-1.17)	0.3400	1.0000
rs1520220	C/C	90	31.1	41	34.8	Codominant1	0.93 (0.57-1.50)	0.7500	1.0000
Intron	C/G	143	49.5	60	50.9	Codominant2	0.64 (0.33-1.25)	0.2000	1.0000
	G/G	56	19.4	17	14.4	Dominant	0.85 (0.53-1.34)	0.4700	1.0000
						Recessive	0.68 (0.37-1.23)	0.1900	0.9500
						Log-additive	0.83 (0.60-1.13)	0.2300	1.0000
	C	323	55.9	142	60.2		1		
	G	255	44.1	94	39.8		0.84 (0.62-1.14)	0.2600	1.0000
rs6214	G/G	73	25.3	44	37.0	Codominant1	0.66 (0.41-1.08)	0.1000	0.5000
3'UTR	A/G	144	49.8	56	47.1	Codominant2	0.44 (0.23-0.83)	**0.0110**	0.0600
	A/A	72	24.9	19	16.0	Dominant	0.59 (0.37-0.93)	**0.0250**	0.1300
						Recessive	0.57 (0.32-1.00)	**0.0420**	0.2100
						Log-additive	0.66 (0.49-0.90)	**0.0088**	**0.0440**
	A	288	49.8	94	39.5		1		
	G	290	50.2	144	60.5		1.52 (1.12-2.07)	**0.0070**	**0.0350**

The p-values were calculated from logistic regression analysis adjusting age and gender. Bold numbers indicate significant associations. IS, ischemic stroke; SNP, singe nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table IV. Genotype frequencies of *IGF* singe nucleotide polymorphisms in stroke subgroups according to the NIHSS and MBI scores.

SNP	Type	Subgroups				Model	OR (95% CI)	p-value
		n	%	n	%			
NHISS		(<6)		(≥6)				
rs2162679	A/A	27	49.1	32	58.2	Codominant1	0.63 (0.29-1.38)	0.25
Intron	A/G	26	47.3	20	36.4	Codominant2	1.26 (0.19-8.23)	0.81
	G/G	2	3.6	3	5.5	Dominant	0.67 (0.31-1.45)	0.31
						Recessive	1.56 (0.25-9.79)	0.63
						Log-additive	0.79 (0.41-1.53)	0.49
rs2195239	G/G	23	41.8	16	28.1	Codominant1	2.00 (0.87-4.59)	0.10
Intron	C/G	24	43.6	33	57.9	Codominant2	1.46 (0.45-4.73)	0.53
	C/C	8	14.6	8	14.0	Dominant	1.87 (0.84-4.12)	0.12
						Recessive	0.97 (0.34-2.80)	0.95
						Log-additive	1.36 (0.77-2.38)	0.28
rs978458	G/G	22	40.0	15	26.3	Codominant1	1.89 (0.82-4.39)	0.14
Intron	A/G	25	45.5	32	56.1	Codominant2	1.87 (0.60-5.87)	0.28
	A/A	8	14.6	10	17.5	Dominant	1.89 (0.85-4.21)	0.12
						Recessive	1.27 (0.46-3.51)	0.65
						Log-additive	1.46 (0.83-2.54)	0.18
rs1520220	C/C	22	40.0	15	26.8	Codominant1	1.77 (0.76-4.10)	0.18
Intron	C/G	26	47.3	31	55.4	Codominant2	2.12 (0.66-6.86)	0.21
	G/G	7	12.7	10	17.9	Dominant	1.84 (0.83-4.12)	0.13
						Recessive	1.50 (0.52-4.28)	0.45
						Log-additive	1.52 (0.86-2.67)	0.14
rs6214	G/G	20	36.4	21	36.8	Codominant1	0.96 (0.42-2.20)	0.92
3'UTR	A/G	27	49.1	26	45.6	Codominant2	1.22 (0.40-3.73)	0.73
	A/A	8	14.6	10	17.5	Dominant	1.02 (0.47-2.23)	0.96
						Recessive	1.25 (0.45-3.45)	0.67
						Log-additive	1.07 (0.63-1.84)	0.79
MBI		(<60)		(≥60)				
rs2162679	A/A	37	53.6	15	62.5	Codominant1	0.96 (0.35-2.65)	0.94
Intron	A/G	29	42.0	9	37.5	Codominant2	0.00 (0.00-NA)	
	G/G	3	4.3	0	0.0	Dominant	0.91 (0.33-2.50)	0.85
						Recessive	0.00 (0.00-NA)	0.32
						Log-additive	0.83 (0.32-2.15)	0.70
rs2195239	G/G	25	35.2	8	33.3	Codominant1	1.06 (0.36-3.12)	0.91
Intron	C/G	36	50.7	13	54.2	Codominant2	0.86 (0.17-4.33)	0.86
	C/C	10	14.1	3	12.5	Dominant	1.02 (0.36-2.88)	0.97
						Recessive	0.83 (0.19-3.61)	0.80
						Log-additive	0.96 (0.46-2.03)	0.92
rs978458	G/G	24	33.8	7	29.2	Codominant1	1.40 (0.46-4.25)	0.55
Intron	A/G	35	49.3	14	58.3	Codominant2	0.83 (0.17-4.15)	0.82
	A/A	12	16.9	3	12.5	Dominant	1.26 (0.43-3.66)	0.67
						Recessive	0.67 (0.16-2.82)	0.58
						Log-additive	1.00 (0.48-2.07)	1.00
rs1520220	C/C	24	34.3	7	29.2	Codominant1	1.44 (0.47-4.36)	0.52
Intron	C/G	35	50.0	14	58.3	Codominant2	0.90 (0.18-4.57)	0.90
	G/G	11	15.7	3	12.5	Dominant	1.31 (0.45-3.81)	0.62
						Recessive	0.72 (0.17-3.06)	0.65
						Log-additive	1.04 (0.50-2.17)	0.92

Table IV. Continued.

SNP	Type	Subgroups				Model	OR (95% CI)	p-value
		n	%	n	%			
rs6214	G/G	26	36.6	10	41.7	Codominant1	0.41 (0.13-1.32)	0.14
3'UTR	A/G	37	52.1	8	33.3	Codominant2	1.56 (0.40-6.13)	0.53
	A/A	8	11.3	6	25.0	Dominant	0.62 (0.22-1.75)	0.37
						Recessive	2.50 (0.73-8.61)	0.15
						Log-additive	1.06 (0.52-2.17)	0.86

The p-values were evaluated from logistic regression analysis adjusting age and gender. Subjects with an undetected genotype were excluded. SNP, singe nucleotide polymorphism; NIHSS, National Institutes of Health Stroke Survey; MBI, Modified Barthel Index; OR, odds ratio; CI, confidence interval.

numbers of IS patients with a MBI score of <60 and ≥60 were 71 (74.7%) and 24 (25.3%), respectively. In two clinical subgroups, patients with inappropriate or insufficient clinical data, were excluded (Table I).

Genetic analysis of IGF1 SNPs. Table III represents the genotype and allele frequencies of the five examined SNPs (rs2162679, rs2195239, rs978458, rs1520220 and rs6214) in the IS patients and the control subjects. The HWE of the five SNPs showed no difference in the control group (rs2162679, p=0.61; rs2195239, p=0.18; rs978458, p=0.91; rs1520220, p=1.00; rs6214, p=1.00). Multiple logistic regression analysis adjusting for age and gender was performed. An intron SNP (rs2162679) was associated with the development of IS [p=0.0050, OR = 0.27, 95% CI = 0.11-0.67 in the codominant2 model (A/A vs. G/G); p=0.0150, OR = 0.58, 95% CI = 0.37-0.90 in the dominant model (A/A vs. A/G and G/G); p=0.0059, OR = 0.32, 95% CI = 0.13-0.79 in the recessive model (A/A and A/G vs. G/G); and p=0.0020, OR = 0.59, 95% CI = 0.41-0.83 in the log-additive model (A/A vs. A/G vs. G/G)]. A 3'UTR SNP (rs6214) was also associated with the development of IS [p=0.0110, OR = 0.44, 95% CI = 0.23-0.83 in the codominant2 model (G/G vs. A/A); p=0.0250, OR = 0.59, 95% CI = 0.37-0.93 in the dominant model (G/G vs. A/G and A/A); p=0.0420, OR = 0.57, 95% CI = 0.32-1.00 in the recessive model (G/G and A/G vs. A/A); and p=0.0088, OR = 0.66, 95% CI = 0.49-0.90 in the log-additive model (G/G vs. A/G vs. A/A)]. The A allele frequency of rs2162679 was higher in the IS group (74.4%) than in the control group (63.8%) (p=0.004, OR = 1.64, 95% CI = 1.17-2.31). The G allele frequency of rs6214 was higher in the IS group (60.5%) than in the control group (50.2%) (p=0.007, OR = 1.52, 95% CI = 1.12-2.07). After Bonferroni correction (p^c), the allele frequencies of rs2162679 and rs6214 showed significant differences between IS and the controls (rs2162679, p^c=0.004; rs6214, p^c=0.007). The other SNPs (rs2195239, rs978458 and rs1520220) had no differences between IS and the controls (Table III). The LD block was estimated using Haploview version 4.2. One LD block was strongly made between rs1520220 and rs978458 (D'=1.0 and r^2=0.993 in the control group). However, the haplotypes of these two SNPs were not associated with the development of IS (data not shown). IS patients were classified into two clinical subgroups according to the NIHSS (<6 and ≥6) and MBI (<60 and ≥60) scores. As shown in Table IV, the five tested SNPs were not associated with the NIHSS and MBI scores.

Sample power. We estimated the sample power using a genetic power calculator to obtain the required sample size. The sample powers (α=0.05, genotype relative risk 2-fold, number of case for 70% power) of each SNP in the IS group were 0.757 for rs2162679 (n=104), 0.800 for rs2195239 (n=93), 0.801 for rs978458 (n=93), 0.801 for rs1520220 (n=93) and 0.801 for rs6214 (n=93). Therefore, the results of the five examined SNPs in the *IGF1* gene had statistical confidence.

Discussion

IGF1 is an endogenous survival factor for neurons, glial cells and endothelial cells. IGF1 plays an important role in tissue repair and cell proliferation. IGF1 induces the synthesis of elastin and prevents apoptosis of vascular smooth muscle cells. Therefore, low levels of IGF1 may be a risk factor of stroke. As shown in previous studies, the expression of IGF1 increased after hypoxic injury in regions with neuronal loss (18) and IGF1 reduced infarct volume and improved neurological function after ischemia in an animal study (19).

There are several reports that *IGF1* polymorphisms are associated with certain diseases, such as adenocarcinoma (EAC) and colorectal cancer (15,20). McElholm *et al* observed that *IGF1* SNP rs6214 was associated with Barrett's esophagus (BE) in EAC (15). Using GG genotype as reference, OR for BE in AA (wild-type) was 0.43 (95% CI 0.24-0.75). Feik *et al* also suggested that rs6214 could have an impact on developing colorectal cancer and colorectal polyps with villous elements (20). Based on previous studies, we believed that the SNPs of *IGF1* were associated with the development of IS and clinical features according to the NIHSS and MBI scores in Korean stroke patients. In the present study, we found a significant association between *IGF1* SNPs and IS. The G allele frequency of rs6214 in the *IGF1* gene was higher in the IS group (60.5%) than in the control group (50.2%). The A allele of rs2162679 was also higher in the IS group (74.4%) than in the control group (74.4%). Thus, our results suggest that *IGF1* may be a risk factor of IS development. However, all five SNPs (rs2162679, rs2195239, rs978458, rs1520220 and rs6214) of *IGF1* did not contribute to the NIHSS and MBI scores of

IS. To our knowledge, this is the first study on whether *IGF1* SNPs are associated with the development of IS in a Korean population. Additional studies with a larger number of cases or different populations are required in order to confirm our results.

In conclusion, we suggest that an intron SNP (rs2162679) and 3'UTR SNP (rs6214) of the *IGF1* gene may be associated with the development of IS.

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