Association Between *VEGF* Polymorphisms and Homocysteine Levels in Patients With Ischemic Stroke and Silent Brain Infarction

Ok Joon Kim, MD, PhD; Seung Ho Hong, PhD; Seung Hun Oh, MD; Tae Gon Kim, MD, PhD; Kyung Tae Min, MSc; Doyeun Oh, MD, PhD; Nam Keun Kim, PhD

Background and Purpose—Vascular endothelial growth factor (VEGF) plays a role in atherosclerosis-related diseases such as cerebrovascular or cardiovascular diseases. However, the effect of *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T polymorphisms on the susceptibility to stroke and silent brain infarction has not been reported.

Methods—Using polymerase chain reaction-amplified DNA, *VEGF* polymorphisms were analyzed in 615 patients with ischemic stroke, 376 patients with silent brain infarction, and 494 control subjects.

Results—The AA and CC+CA (*C* allele bearing) genotype frequencies of the -2578C>A polymorphism and the CT+TT (*T* allele-bearing) genotype frequency of the 936C>T polymorphism were significantly different between the stroke and control groups (false discovery rate-adjusted probability values of 0.016, 0.044, and 0.044, respectively). When stratified by the size of the occluded vessel, the *VEGF* polymorphisms were associated with patients with multiple small-artery occlusions. Several haplotypes of the *VEGF* polymorphisms were significantly different between the control and stroke groups. With respect to silent brain infarction, the difference in the frequency of the -634G>C polymorphism between the GC+CC (*C* allele-bearing) genotype and the controls was marginally significant (false discovery rate-adjusted probability value of 0.056). On the other hand, the -634G>C and 936C>T polymorphisms were associated with plasma homocysteine levels of patients with multiple or single small-artery occlusions, respectively.

Conclusions—This study suggests that *VEGF* polymorphisms and haplotypes are possible genetic determinants for the risk of ischemic stroke, particularly in patients with multiple small-artery occlusions. However, *VEGF* polymorphisms had only a weak association with plasma homocysteine levels in the Korean population. **(*Stroke*. 2011;42:2393-2402.)**

Key Words: polymorphism ■ silent brain infarction ■ stroke ■ vascular endothelial growth factor

Stroke is the third most common cause of death in many developed countries. Approximately 80% of strokes are ischemic in origin.[1–3] In South Korea, stroke is the most frequent cause of death after cancer and is more frequent than heart disease.[4] Multiple factors, including hypertension, diabetes, smoking, hyperlipidemia, and hyperhomocysteinemia, are associated with a higher risk of stroke.[3]

Silent brain infarction (SBI) is defined as a cerebral infarction evident by brain imaging but without a clinical syndrome. SBI is characterized by the rapid development of clinical syndromes and signs of focal and, at times, global loss of brain function. SBIs are common with advanced age.[5,6] Although the clinical significance of SBI remains controversial, its presence can predict widespread vascular damage such as a clinically overt stroke.[7–9] Based on several lines of evidence, hyperhomocysteinemia is thought to be an independent risk factor for SBI.[10,11] Other metabolic syndromes, including hypertension and impaired fasting glucose, are also common risk factors for SBI.[5,12–14]

Vascular endothelial growth factor (VEGF) is a major angiogenic factor and a prime regulator of endothelial cell proliferation.[15] The gene that encodes *VEGF* is comprised of a 14-kb coding region with 8 exons and 7 introns located on chromosome 6.[16] *VEGF* undergoes transcriptional and post-transcriptional induction by hypoxia in the vicinity of tumor necrosis and in various models of ischemia.[17,18] Moreover, VEGF couples hypoxia to angiogenesis in diverse tissues, including the brain.[18,19] Because ischemia stimulates *VEGF* expression in the brain, VEGF may be important for the vascular response to cerebral ischemia.[20–22] Several single nucleotide polymorphisms (SNPs) have been described in the *VEGF* gene (National Center for Biotechnology Information, Gene association no: NT 007592). The *VEGF* gene includes at least 4 relatively common polymorphisms, -2578C>A

Received November 8, 2010; final revision received February 23, 2011; accepted March 15, 2011.

From the Department of Neurology (O.J.K., S.O.), The Institute for Clinical Research (K.T.M., D.O., N.K.K.), and the Department of Neurosurgery (T.G.K.), School of Medicine, CHA University, Seongnam, South Korea; and the Department of Science Education (S.H.H.), Teachers College, Jeju National University, Jeju, South Korea.

The online-only Data Supplement is available at http://stroke.ahajournals.org/cgi/content/full/STROKEAHA.110.607739/DC1.

Correspondence to Nam Keun Kim, PhD, Institute for Clinical Research, School of Medicine, CHA University, 351, Yatap-dong, Bundang-gu, Seongnam 463-712, South Korea. E-mail nkkim@cha.ac.kr or namkkim@naver.com

(rs699947), -1154G>A (rs1570360), -634G>C (rs2010963), and 936C>T (rs3025039) SNPs that may influence *VEGF* expression.[23–25] Three of these polymorphisms are located in the promoter region at -2578C>A, -1154G>A, and -634G>C relative to the translation start site. The *-2578A*, *-1154A*, and *-634G* alleles are all associated with decreased *VEGF* expression.[23,24] In addition to promoter region polymorphisms, the *T* allele of the common 936C>T polymorphism in the 3′-untranslated region is associated with significantly decreased serum VEGF levels.[25] These *VEGF* polymorphisms are associated with various diseases, including recurrent abortion, pre-eclampsia, Alzheimer disease, colon cancer, breast cancer, and gastric cancer.[26–32] Recently, Yang et al[33] reported therapeutic effects of different doses of intranasal VEGF on angiogenesis and functional recovery of ischemic brains in adult rats. The *VEGF* -1154G>A (rs1570360) and -634G>C (rs2010963) polymorphisms in patients with ischemic stroke alone have been studied in the Chinese population,[34] although no significant findings were demonstrated. However, to our knowledge, the effects of the *VEGF* -2578C>A (rs699947) and 936C>T (rs3025039) polymorphisms on the risk of stroke and SBI have not been evaluated previously.

Hyperhomocysteinemia has been implicated as a risk factor for a number of vascular diseases, including ischemic stroke and SBI. Increased homocysteine (Hcy) concentrations are found in 40% of patients with coronary, cerebral, or peripheral artery diseases and only in 15% of healthy individuals.[35] Hcy inhibits endothelial cell proliferation and migration resulting in decreased angiogenesis.[36] However, the mechanism by which hyperhomocysteinemia induces the development of vascular lesions is still obscure.[37] The aims of this study were to evaluate the frequencies of *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T polymorphisms in Korean patients with ischemic stroke and SBI and to determine the relationship between *VEGF* polymorphisms and plasma total Hcy (tHcy) levels.

Materials and Methods

Study Population

The study subjects were recruited between 2000 and 2008 from the Seoul and Kyeonggi-do provinces of South Korea. The Institutional Review Board of CHA Bundang Medical Center approved this genetic study in June 2000. We studied 615 consecutive patients with ischemic stroke referred from the Department of Neurology at CHA Bundang Medical Center, CHA University. Ischemic stroke was defined as a stroke (a clinical syndrome characterized by rapidly developing clinical symptoms and signs of focal and/or global loss of brain function) with evidence of a cerebral infarction in clinically relevant areas of the brain according to brain imaging by MRI scan and electrocardiography. Based on the clinical manifestations and neuroimaging data, 2 neurologists classified all ischemic strokes into 4 etiologic subtypes using the criteria from the Trial of Org 10172 in Acute Stroke Treatment (TOAST)[38] as follows: (1) large-artery disease, an infarction lesion ≥15 mm in diameter by MRI, and significant (>50%) stenosis of a major brain artery or a branch cortical artery by cerebral angiography with symptoms associated with that arterial territory; (2) small-artery occlusion (SAO), an infarction lesion <15 mm and ≥5 mm in diameter by MRI, and classic lacunar syndrome without evidence of a cerebral cortical dysfunction or potentially detectable cardiac sources for embolism; (3) cardioembolism, arterial occlusions presumably due to an embolus arising in the heart, as detected by cardiac evaluation; and (4) undetermined etiology, the cause of stroke could not be determined with any degree of confidence or involved >2 etiologies. The frequencies of stroke subtype were 40% (n=247) large-artery disease, 35% (n=214) SAO, 8% (n=47) cardioembolism, and 17% (n=107) undetermined etiology, respectively. These proportions are similar to reported values for the Korean population.[39] Our research focused on patients with small-artery disease. Single and multiple (≥2 lesions) SAOs were distinguished by brain MRI scan. The size and site of cerebral infarction were documented only by MRI.

We selected 376 patients with SBI (170 men and 206 women; age range, 40 to 88 years) who visited the CHA Bundang Medical Center. The diagnoses were made by MRI examination and by agreement between 2 independent experienced neurologists. All patients underwent a brain MRI scan and electrocardiography. The criteria for SBI were as follows: (1) spotted areas ≥3 mm in diameter in areas supplied by deep perforating arteries showing high intensity in the T2 and fluid-attenuated inversion recovery images and low intensity in the T1 image; (2) the absence of neurological signs and symptoms that could be explained by lesions observed by MRI; and (3) no history of clinical stroke, including transient ischemic attacks. Small punctate hyperintensities (1 to 2 mm in diameter) were likely to represent dilated perivascular spaces and were not considered in the present study. A SBI was excluded when an agreement could not be reached and patients with cerebral hemorrhage were excluded in advance. All examinations were performed according to the methods described previously.[27,40]

We selected 494 control subjects matched for gender and age within 5 years from patients presenting at our hospitals for health examinations, which included biochemistry testing, an electrocardiogram, and brain MRI during the same period and who were free from a recent history of cerebrovascular disease or myocardial infarction. Exclusion criteria were the same as those used in the patient group, as mentioned previously. Hypertension was defined as a systolic pressure >140 mm Hg and/or a diastolic pressure >90 mm Hg on >1 occasion and includes patients currently taking hypertensive medications. Diabetes was defined as a fasting plasma glucose >126 mg/dL (7.0 mmol/L) and includes patients taking diabetic medications. Smoking refers to current smoking. Hyperlipidemia was defined as a high fasting serum total cholesterol level (≥240 mg/dL) or an antihyperlipidemic agent treatment history. Some of the study subjects (n=89 [6%]) were found to have new vascular risk factors such as hypertension or diabetes mellitus at the time of examination. The demographic and laboratory data are summarized in Table 1. Significant differences were detected in patients with hypertension, diabetes mellitus, and hyperlipidemia between the stroke and control groups. Although patients with SBI had a significantly higher prevalence of all conventional vascular risk factors relative to control subjects, patients with SBI also had significantly higher plasma tHcy levels and a greater prevalence of hyperlipidemia than control subjects (Table 1).

Genotype Determination of *VEGF* Polymorphisms

DNA was extracted using the G-DEX blood extraction kit (iNtRON Biotechnology, Inc, Seongnam, South Korea). The 4 best-studied SNPs in the *VEGF* gene were determined by a documentary search, which included 3 5′-untranslated region SNPs (-2578C>A, rs699947; -1154G>A, rs1570360; and -634G>C, rs2010963) and 1 3′-untranslated region SNP (936C>T, rs3025039). All SNP sequences were obtained from the HapMap database (www.hapmap.org). The *VEGF* -2578C>A and 936C>T polymorphisms were analyzed by the polymerase chain reaction–restriction fragment length polymorphism method. Real-time polymerase chain reaction was used to analyze the *VEGF* -1154G>A and -634G>C polymorphisms. The primers and polymerase chain reaction conditions for *VEGF* polymorphism analyses have been previously described.[27,28]

Estimation of Hcy and Folate Levels

Plasma samples were collected to measure the levels of tHcy and folate within 48 hours of the onset of a stroke or SBI. Blood was

Table 1. Baseline Characteristics of Patients With Ischemic Stroke, Silent Brain Infarction (SBI), and Control Subjects

Characteristics	Control, %	Ischemic Stroke, %	*P**	SBI, %	*P*†
No.	494	615		376	
Male (%)	256 (51.8)	348 (56.6)	0.113	170 (45.2)	0.119
Age, y	62.14±11.75	63.47±11.37	0.057	63.12±11.65	0.219
tHcy, μmol/L‡	10.08±4.16 (491)	11.24±5.44 (611)	0.000	11.42±6.27 (372)	0.000
Between-person CV, %	41.3	48.4		54.9	
Folate, nmol/L (no.)‡	9.25±8.41 (363)	7.79±7.42 (525)	0.007	9.11±5.75 (353)	0.787
Between-person CV, %	90.9	95.3		63.1	
Hypertension (%)	231 (46.8)	393 (63.9)	<0.0001	202 (53.7)	0.042
Diabetes mellitus (%)	71 (14.4)	180 (29.3)	<0.0001	60 (16.0)	0.517
Hyperlipidemia (%)	94 (19.0)	215 (35.0)	<0.0001	119 (31.6)	<0.0001
Smoking (%)	111 (22.5)	193 (31.4)	0.001	…	…

tHcy indicates plasma total homocysteine; CV, coefficient of variation; SBI, silent brain infarction.
*Significant difference between patients with ischemic stroke and control subjects.
†Significant difference between patients with SBI and control subjects.
‡Mann-Whitney test of nonparametric test.

collected in a tube containing anticoagulant 12 hours after a patient's previous meal. The tube was centrifuged for 15 minutes at 1000 *g*, and the plasma was separated. The concentration of Hcy in the plasma was measured by fluorescent polarizing immunoassay with IMx (Abbott Laboratories, Chicago, IL). The plasma concentration of folate was determined using a radioassay kit (ACS 180; Bayer, Tarrytown, NY).

Statistical Analysis

The associations among ischemic stroke, SBI, and *VEGF* genotypes were estimated by computing the ORs and 95% CIs from Fisher exact test. The adjusted ORs for *VEGF* polymorphisms were determined from multiple logistic regression analysis using gender, age, diabetes mellitus, hypertension, hyperlipidemia, and smoking. Stratification analysis was used to stroke subgroups according to the size of the occluded vessel. One-way analysis of variance was performed to compare the mean levels of Hcy concentrations among different genotypes. We carried out multiple hypotheses testing using the Benjamini-Hochberg method to control for false discovery rate (FDR) in the unconditional logistic regression analysis.[41] Calculation of the FDR is a way to address the problems associated with multiple comparisons and provides a measure of the expected proportion of false-positives among data. Statistical significance was accepted at the $P<0.05$ level. StatsDirect Statistical Software (Version 2.4.4; StatsDirect Ltd, Altrincham, UK) was used to calculate the adjusted AOR and 95% CI. The linkage disequilibrium between loci was measured using the absolute value of Lewontin D′.[42] Haplotype frequencies for multiple loci were estimated using the expectation-maximization algorithm with SNPAlyze (Version 5.1; DYNACOM Co, Ltd, Yokohama, Japan).

Results

A comparison of genotype frequencies of the *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T polymorphisms between the patients with stroke and those with SBI and control groups is shown in Table 2. Genotype distributions of each polymorphism did not deviate from those expected based on the Hardy-Weinberg equilibrium in the 3 groups. The linkage disequilibrium of the VEGF polymorphisms at loci -2578(rs699947)/-1154(rs1570360)/-634(rs2010963)/936(rs3025039) in patients with ischemic stroke and those with SBI is shown in the Figure. There was strong linkage disequilibrium between loci -1154 and -634 (D′=0.819) and -2578 and -634 (D′=0.807) in patients with ischemic stroke (Figure 1A). Polymorphisms -2578C>A and -634G>C were in strong linkage disequilibrium in the patients with SBI (D′=0.880; Figure 1B).

The CT+TT (*T* allele-bearing) genotype frequency of the 936C>T polymorphism (FDR-adjusted OR, 1.44; 95% CI, 1.09 to 1.90; $P=0.044$) and the AA genotype and CC+CA (*C* allele-bearing) genotype frequencies of the -2578C>A polymorphism (FDR-adjusted OR, 2.13; 95% CI, 1.27 to 3.59; $P=0.016$ and FDR-adjusted OR, 1.93; 95% CI, 1.17 to 3.20; $P=0.044$, respectively; Table 2) were significantly different between the stroke and control groups. The -634 polymorphism in SBI showed a significantly lower frequency of variant genotypes (GC and CC) compared with control groups (adjusted OR, 0.71; 95% CI, 0.52 to 0.97 for GC and adjusted OR, 0.61; 95% CI, 0.40 to 0.94 for CC, and adjusted OR, 0.69; 95% CI, 0.51 to 0.93 for GC+CC compared with the GG genotype; Table 2). However, after measurement by multiple hypothesis testing, the frequency difference for the GC+CC (*C* allele-bearing) genotype of the -634G>C polymorphism was marginally significant between the SBI and control groups (FDR-adjusted $P=0.056$). Interestingly, when the data were stratified by the size of the occluded vessel, patients with SAOs, especially multiple SAOs, were associated with the -2578C>A, -1154G>A, and 936C>T polymorphisms (Table 3). Furthermore, the adjusted OR values of multiple SAOs were much higher than those of SAOs. The -634CC genotype had a lower adjusted OR value (2.27-fold) than the GG genotype in the patients with multiple SAOs. We did not find any significant association between *VEGF* polymorphisms and patients with single SAOs.

We also constructed haplotypes of the -2578C>A, -1154G>A, -634G>C, and 936C>T polymorphisms of the *VEGF* gene (Table 4). Several haplotype frequencies were significantly different between the control subjects and patients with stroke with SAOs, single SAOs, and multiple SAOs. The A-A-G-T, C-A-C-C (-2578/-1154/-634/936), C-A-C (-2578/-1154/-634), and A-C-C (-1154/-634/936) hap-

Table 2. Comparison of Genotype Frequencies and Adjusted OR Values for *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T Polymorphisms in the Patients With Ischemic Stroke, Silent Brain Infarction (SBI), and Control Subjects

		Ischemic Stroke				SBI			
Genotype	Control (%; n=494)	Case (%; n=615)	AOR (95% CI)*	*P*	*P*‡	Case (%; n=376)	AOR (95% CI)†	*P*	*P*‡
VEGF -2578C>A									
CC	262 (53.0)	301 (48.9)	1.00 (Reference)			199 (52.9)	1.00 (Reference)		
CA	203 (41.1)	250 (40.7)	1.13 (0.86–1.49)	0.368	0.491	149 (39.7)	1.00 (0.75–1.33)	0.994	0.994
AA	29 (5.9)	64 (10.4)	2.13 (1.27–3.59)	0.004	0.016	28 (7.4)	1.33 (0.76–2.32)	0.326	0.435
CC versus CA+AA (dominant)			1.25 (0.97–1.62)	0.090	0.180		1.05 (0.79–1.38)	0.754	0.918
CC+CA versus AA (recessive)			1.93 (1.17–3.20)	0.011	0.044		1.39 (0.81–2.40)	0.238	0.317
VEGF -1154G>A									
GG	339 (68.6)	428 (69.6)	1.00 (Reference)			246 (65.4)	1.00 (Reference)		
GA	137 (27.8)	162 (26.3)	0.98 (0.73–1.31)	0.881	0.881	109 (29.0)	1.09 (0.81–1.48)	0.572	0.994
AA	18 (3.6)	25 (4.1)	1.44 (0.72–2.92)	0.306	0.347	21 (5.6)	1.65 (0.85–3.19)	0.141	0.282
GG versus GA+AA (dominant)			1.03 (0.78–1.36)	0.864	0.864		1.16 (0.87–1.55)	0.320	0.640
GG+GA versus AA (recessive)			1.46 (0.73–2.95)	0.288	0.384		1.66 (0.86–3.19)	0.131	0.317
VEGF -634G>C									
GG	135 (27.3)	178 (28.9)	1.00 (Reference)			128 (34.0)	1.00 (Reference)		
GC	270 (54.7)	345 (56.1)	0.86 (0.64–1.17)	0.346	0.491	191 (50.8)	0.71 (0.52–0.97)	0.031	0.124
CC	89 (18.0)	92 (15.0)	0.68 (0.45–1.01)	0.056	0.112	57 (15.2)	0.61 (0.40–0.94)	0.024	0.096
GG versus GC+CC (dominant)			0.82 (0.62–1.10)	0.186	0.248		0.69 (0.51–0.93)	0.014	0.056
GG+GC versus CC (recessive)			0.71 (0.50–1.01)	0.055	0.110		0.77 (0.53–1.12)	0.171	0.317
VEGF 936C>T									
CC	344 (69.6)	381 (62.0)	1.00 (Reference)			261 (69.4)	1.00 (Reference)		
CT	136 (27.6)	214 (34.7)	1.43 (1.08–1.91)	0.014	0.056	106 (28.2)	1.04 (0.76–1.41)	0.823	0.994
TT	14 (2.8)	20 (3.3)	1.46 (0.66–3.24)	0.347	0.347	9 (2.4)	0.81 (0.34–1.94)	0.641	0.641
CC versus CT+TT (dominant)			1.44 (1.09–1.90)	0.011	0.044		1.02 (0.75–1.37)	0.918	0.918
CC+CT versus TT (recessive)			1.33 (0.61–2.89)	0.477	0.477		0.84 (0.35–1.97)	0.682	0.682

VEGF indicates vascular endothelial growth factor; SBI, silent brain infarction.

*The adjusted odds ratio (AOR) on the basis of risk factors such as age, gender, hypertension, hyperlipidemia, diabetes mellitus, and smoking.

†The AOR on the basis of risk factors such as age, gender, hypertension, hyperlipidemia, and diabetes mellitus.

‡False discovery rate-adjusted *P* value for multiple hypothesis testing using the Benjamini-Hochberg method.

lotypes had significant differences in the 3 SAO groups. Several haplotype frequencies were also significantly different between the SBI patient and control groups. The OR values of *VEGF* haplotypes between the patients with stroke and those with SBI and the control subjects are shown in Supplemental Table I (http://stroke.ahajournals.org). The OR values of the *VEGF* haplotypes among the stroke subtypes and control subjects are presented in Supplemental Table II. A comparison of genotype frequency and adjusted OR of VEGF polymorphisms between the stroke subtypes and patients with SBI is shown in Supplemental Table III. The frequency differences for the -2578C>A, -1154G>A, and 936C>T polymorphisms were marginally significant between the multiple SAO subtypes and the patients with SBI.

We sought to determine whether *VEGF* polymorphisms were associated with plasma tHcy levels (Table 5). The -634G>C and 936C>T polymorphisms were associated with tHcy levels in patients with multiple and single SAOs, respectively. However, the *VEGF* polymorphisms did not show any association with tHcy levels in the SBI group. Plasma tHcy levels of the *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T haplotypes among the patients with stroke, patients with SBI, stroke subtypes, and control subjects are shown in Supplemental Table IV. The plasma tHcy levels of the *VEGF* haplotypes between the ischemic stroke subtypes and control subjects are presented in Supplemental Table V. Some haplotypes showed significant differences between the groups.

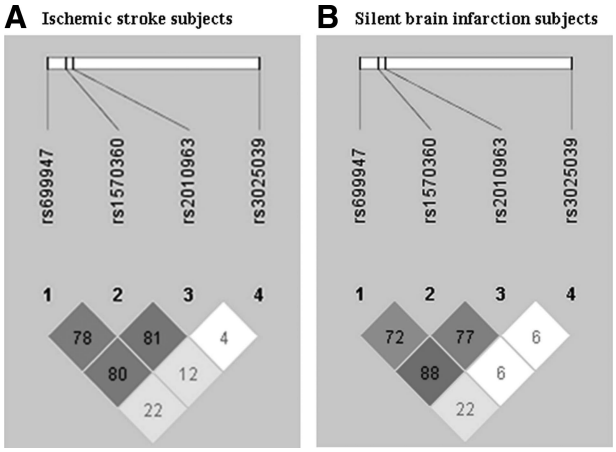

Figure. Linkage disequilibrium (LD) patterns of *VEGF* SNPs. Values in squares are LD between single markers. **A**, There were strong LDs between loci -1154G>A (rs1570360) and -634G>C (rs2010963; D′=0.819), and -2578C>A (rs699947) and -634G>C (rs2010963; D′=0.807) in ischemic stroke subjects. **B**, There was strong LD between loci -2578C>A and -634G>C (D′=0.880) in patients with silent brain infarction subjects. Dark squares indicate high r^2 and bright squares indicate low r^2 values.

A total of 1485 individuals (615 patients with stroke, 376 patients with SBI, and 494 control subjects) from 2 different case–control samples, Sample 1 and Sample 2, were analyzed according to recruitment duration (Supplemental Table VI). The genotype frequencies of the *VEGF* polymorphisms were significantly different between the control, ischemic stroke, and SBI groups in Samples 1 and 2 (Supplemental Tables VII to XI). These results suggest that *VEGF* is also a candidate susceptibility gene of ischemic stroke, although the association of *VEGF* -2578C>A, 936C>T, and ischemic stroke was not replicated in subjects from Sample 1 and Sample 2 by multivariable logistic regression analysis.

Discussion

Angiogenesis is critical to the progression of atherogenesis, collateral vessel development in ischemia, and plaque instability.[43,44] VEGF is believed to be important for initiating angiogenesis and is a major mediator of the progression of atherothrombotic vascular disease, including ischemic stroke. The effects of VEGF on the risk of stroke have been suggested in a number of biological and pathological studies. For example, it has been suggested that the VEGF/VEGF receptor system, which is induced by hypoxia, leads to growth of new vessels after cerebral ischemia. Exogenous support of this natural protective mechanism might lead to enhanced survival after stroke.[45] Sun et al[46] have suggested that, in the ischemic brain, VEGF exerts an acute neuroprotective effect as well as longer-lasting effects on the survival of new neurons and on angiogenesis and that these effects may operate independently. Verheul et al[47] reported that VEGF-stimulated human umbilical endothelial cells promote the adhesion and activation of platelets. They also found that activated platelets are present in the microvessels of VEGF-producing soft tissue sarcomas.[48] There is strong evidence to support a close relationship between VEGF and ischemic stroke. Intranasal administration of VEGF may induce angiogenesis in the ischemic boundary and improve behavioral recovery after cerebral ischemia in rats.[49] Astrocytes, which morphologically resemble injury-induced VEGF-positive cells, also react to injury by increasing VEGF expression, indicating that VEGF might participate in the central nervous system response to injury.[50] Therefore, VEGF may improve the histological and functional outcomes of stroke through multiple mechanisms.

SBI is a kind of cerebral infarction event. Despite the functional studies of VEGF described here, the effect of *VEGF* polymorphisms on the risk of stroke and SBI has not been reported. Recently, several investigators performed genomewide association studies and meta-analysis of the genetic susceptibility to stroke in Asian populations[51–54]; however, they did not find an association with *VEGF* in Asian populations. Based on the known biological and pathological significance of VEGF, it is reasonable to hypothesize that VEGF is a good candidate for determining the risk of developing a stroke and SBI. In the present study, although only the *VEGF* -2578C>A and 936C>T polymorphisms were associated with the risk of ischemic stroke, the -2578C>A, -1154G>A, and 936C>T polymorphisms were associated with SAOs when the data were stratified by the size of the occluded vessel. Moreover, when patients with SAOs were divided into single and multiple SAOs by brain MRI, variant alleles of the -2578C>A, -1154G>A, and 936C>T polymorphisms were only significantly different in patients with multiple SAOs compared with control subjects. Although we do not know the exact causes of stroke, research on the etiologic heterogeneity and subtypes of stroke has been performed.[55–57] Some evidence suggests that there are different pathophysiological mechanisms for single and multiple SAOs.[58,59] Therefore, despite the heterogeneity of ischemic strokes, our data suggest that *VEGF* polymorphisms are an independent risk factor for multiple SAOs. Several articles have shown that the *MTHFR* 677C>T polymorphisms are associated with multiple infarctions.[60–62]

Haplotype analysis in this study revealed that the frequencies of several haplotypes were significantly different between the control subjects and patients with subtypes of stroke (SAOs, single SAOs, and multiple SAOs) and SBI. Thus, the haplotypes of *VEGF* polymorphisms provide data for susceptibility to stroke and SBI.

SBI shares a close similarity with pathophysiological aspects of single SAOs. Thus, we can expect that their genetic compositions are also similar. As shown in Supplemental Table III, there were no significant differences in the -2578C>A, -1154G>A, -634G>C, and 936C>T polymorphisms between SBI and SAOs and single SAO subtypes. However, the genotype frequencies of *VEGF* polymorphisms, except for -634G>C in SBI, were only marginally different from ones in multiple SAOs, suggesting a difference in the pathophysiological mechanisms of SBI and multiple SAOs.

Angiogenesis is regulated by a balance of various cytokines and biological molecules. The final outcome does not occur through the independent actions of these factors, but rather depends on the relative input of each factor. Hcy and VEGF have been implicated in angiogenesis and in the development and progression of atherothrombotic vascular

Table 3. Comparison of Genotype Frequencies and Adjusted OR for *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T Polymorphisms Between the Ischemic Stroke Subtypes With Small-Artery Occlusion, Single and Multiple Small-Artery Occlusion, and Control Subjects

		Small-Artery Occlusion				Single Small-Artery Occlusion				Multiple Small-Artery Occlusion			
Genotype	Control (%; n=494)	Case (%; n=214)	AOR (95% CI)*	*P*	*P*†	Case (%; n=118)	AOR (95% CI)*	*P*	*P*†	Case (%; n=96)	AOR (95% CI)*	*P*	*P*†
VEGF -2578C>A													
CC	262 (53.0)	96 (44.9)	1.00 (Reference)			58 (49.2)	1.00 (Reference)			38 (39.6)	1.00 (Reference)		
CA	203 (41.1)	99 (46.2)	1.37 (0.96–1.97)	0.085	0.116	52 (44.0)	1.16 (0.75–1.81)	0.502	0.657	47 (48.9)	1.84 (1.11–3.04)	0.019	0.038
AA	29 (5.9)	19 (8.9)	1.98 (1.00–3.90)	0.049	0.156	8 (6.8)	1.41 (0.58–3.41)	0.444	0.687	11 (11.5)	3.19 (1.37–7.44)	0.007	0.028
CC versus CA+AA (dominant)			1.46 (1.03–2.07)	0.033	0.048		1.20 (0.78–1.84)	0.400	0.715		2.03 (1.25–3.29)	0.004	0.008
CC+CA versus AA (recessive)			1.70 (0.88–3.27)	0.116	0.155		1.25 (0.53–2.95)	0.611	0.815		2.42 (1.08–5.43)	0.032	0.087
VEGF -1154G>A													
GG	339 (68.6)	132 (61.7)	1.00 (Reference)			80 (67.8)	1.00 (Reference)			52 (54.2)	1.00 (Reference)		
GA	137 (27.8)	70 (32.7)	1.39 (0.95–2.04)	0.087	0.116	33 (28.0)	1.13 (0.70–1.82)	0.621	0.657	37 (38.5)	1.97 (1.19–3.26)	0.008	0.032
AA	18 (3.6)	12 (5.6)	2.13 (0.92–4.93)	0.078	0.156	5 (4.2)	1.44 (0.48–4.33)	0.515	0.687	7 (7.3)	3.01 (1.10–8.29)	0.033	0.061
GG versus GA+AA (dominant)			1.47 (1.03–2.12)	0.036	0.048		1.16 (0.73–1.82)	0.536	0.715		2.11 (1.31–3.40)	0.002	0.008
GG+GA versus AA (recessive)			1.99 (0.86–4.59)	0.108	0.155		1.39 (0.47–4.15)	0.552	0.815		2.57 (0.95–6.96)	0.065	0.087
VEGF -634G>C													
GG	135 (27.3)	58 (27.1)	1.00 (Reference)			30 (25.4)	1.00 (Reference)			28 (29.2)	1.00 (Reference)		
GC	270 (54.7)	128 (59.8)	1.04 (0.69–1.56)	0.864	0.864	71 (60.2)	1.12 (0.68–1.86)	0.657	0.657	57 (59.4)	0.92 (0.53–1.59)	0.767	0.767
CC	89 (18.0)	28 (13.1)	0.65 (0.37–1.14)	0.135	0.180	17 (14.4)	0.79 (0.39–1.60)	0.512	0.687	11 (11.4)	0.44 (0.20–0.98)	0.046	0.061
GG versus GC+CC (dominant)			0.94 (0.64–1.39)	0.763	0.763		1.05 (0.65–1.70)	0.850	0.850		0.80 (0.48–1.34)	0.398	0.398
GG+GC versus CC (recessive)			0.64 (0.39–1.04)	0.072	0.155		0.72 (0.40–1.30)	0.277	0.875		0.49 (0.24–0.98)	0.045	0.087
VEGF 936C>T													
CC	344 (69.6)	130 (60.7)	1.00 (Reference)			76 (64.4)	1.00 (Reference)			54 (56.3)	1.00 (Reference)		
CT	136 (27.6)	79 (37.0)	1.51 (1.04–2.20)	0.030	0.116	40 (33.9)	1.34 (0.84–2.13)	0.218	0.657	39 (40.6)	1.71 (1.04–2.81)	0.034	0.045
TT	14 (2.8)	5 (2.3)	1.44 (0.46–4.49)	0.532	0.532	2 (1.7)	1.02 (0.21–4.94)	0.982	0.982	3 (3.1)	1.98 (0.47–8.38)	0.353	0.353
CC versus CT+TT (dominant)			1.50 (1.05–2.17)	0.028	0.048		1.31 (0.83–2.06)	0.245	0.715		1.73 (1.07–2.81)	0.026	0.035
CC+CT versus TT (recessive)			1.21 (0.40–3.67)	0.740	0.740		0.86 (0.18–4.06)	0.846	0.846		1.64 (0.41–6.54)	0.480	0.480

VEGF indicates vascular endothelial growth factor.

*The adjusted odds ratio (AOR) on the basis of risk factors such as age, gender, hypertension, hyperlipidemia, diabetes mellitus, and smoking.

†False discovery rate-adjusted *P* value using the Benjamini-Hochberg method.

disease.[63,64] Ischemic stroke is a multifactorial disorder in which genetic and environmental factors, including defects in Hcy metabolism, play a major role. Hcy inhibits angiogenesis in vitro and in vivo.[65] Hcy increases the expression of *VEGF* through a mechanism involving endoplasmic reticulum stress and the transcription factor ATF4[66] and in differentiated THP-1 macrophages.[67] Shastry et al[68] have reported that Hcy inhibits angiogenesis, partly by decreasing VEGF, as shown in an experiment using mouse brain microvascular endothelial cells. Furthermore, Atta et al[69] reported that lowering Hcy levels with vitamin B and folic acid results in a substantial reduction of VEGF plasma levels in patients with peripheral arterial disease or diabetes mellitus. Thus, it is possible that VEGF genotypes are associated with circulating tHcy levels.

In this study, we found that the *VEGF* -634G>C and 936C>T genotypes were only associated with tHcy levels in patients with multiple and single SAOs, respectively, suggesting that *VEGF* polymorphisms may weakly influence tHcy levels. However, the -2578C>A and -1154G>A polymorphisms were not associated with tHcy levels. Therefore, *VEGF* polymorphisms showed a weak association with tHcy levels in Koreans. However, there have been no prior reports of an association between *VEGF* polymorphisms and tHcy levels in any population. One possible explanation for this is that differences in tHcy levels and the prevalence of ischemic stroke may exist among various ethnic populations due to environmental factors such as dietary habits, daily folate intake, and lifestyle. Thus, we cannot exclude strong associations between *VEGF* polymorphisms and tHcy levels in other ethnic populations.

Hypoxia is a potent stimulus for VEGF expression in vivo and in vitro. Hypoxia-induced proteins bind to the 3′-untranslated region of the *VEGF* mRNA, resulting in a significantly increased half-life of the mRNA.[66] One explanation for our results is that the 936C>T polymorphism, located in the 3′-untranslated region, leads to the loss of a potential binding site for AP-4.[31,70] Posttranscriptional regulation could also affect not only the *VEGF* gene, but also

Table 4. The Haplotype Analysis of *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T Polymorphisms Among the Patients With Ischemic Stroke, Silent Brain Infarction (SBI), and Control Subjects

		Patients With Ischemic Stroke				
Haplotype	Control	Total	SAO	Single SAO	Multiple SAO	SBI
VEGF -2578/-1154/-634/936						
C-G-C-C	0.3448	0.3204	0.3430	0.3608	0.3213	0.3293
C-G-G-C	0.2719	0.2429	0.2275*	0.2501	0.1988†	0.2623
A-A-G-C	0.1054	0.1010	0.1138	0.0899	0.1233	0.1247
A-G-G-C	0.0679	0.0926*	0.0736	0.0866	0.0638	0.0722
C-G-C-T	0.0670	0.0814	0.0640	0.0720	0.0539	0.0613
A-A-G-T	0.0378	0.0469	0.0765†	0.0662†	0.1071†	0.0332
A-G-G-T	0.0339	0.0391	0.0348	0.0334	0.0358	0.0348
C-G-G-T	0.0202	0.0269	0.0222	0.0148	0.0314	0.0315
C-A-C-C	0.0183	0.0000†	0.0000†	0.0000†	0.0069*	0.0073*
A-G-C-C	0.0162	0.0243	0.0125	0.0000†	0.0232	0.0077*
C-A-G-C	0.0095	0.0123	0.0217	0.0140	0.0283†	0.0316†
C-A-C-T	0.0041	0.0006	0.0015	0.0000	0.0000*	0.0000*
A-G-C-T	0.0030	0.0001	0.0028	0.0000	0.0061	0.0000
C-A-G-T	0.0000	0.0083†	0.0000	0.0000	0.0000	0.0040*
A-A-C-C	0.0000	0.0000	0.0000	0.0120†	0.0000	0.0000
A-A-C-T	0.0000	0.0033	0.0061*	0.0000	0.0000	0.0000
Overall‡		<0.0001	<0.0001	<0.0001	<0.0001	0.0001
VEGF -2578/-1154/-634						
C-G-G	0.2916	0.2713	0.2500*	0.2662	0.2295†	0.2939
A-A-G	0.1431	0.1480	0.1910†	0.1553	0.2302†	0.1576
A-G-G	0.1027	0.1302*	0.1084	0.1198	0.1002	0.1069
C-A-C	0.0228	0.0006†	0.0026†	0.0000†	0.0068†	0.0072†
A-G-C	0.0183	0.0258	0.0155	0.0000†	0.0290	0.0081*
C-A-G	0.0091	0.0205*	0.0208	0.0139	0.0287†	0.0360†
A-A-C	0.0000	0.0034	0.0052*	0.0131†	0.0000	0.0000
VEGF -2578/-1154/936						
C-G-C	0.6146	0.5643*	0.5730*	0.6157	0.5192†	0.5919
A-G-C	0.0847	0.1163*	0.0851	0.0836	0.0872	0.0803
A-A-T	0.0354	0.0507	0.0856†	0.0679†	0.1064†	0.0342
C-A-C	0.0274	0.0126†	0.0243	0.0145*	0.0367	0.0399
C-A-T	0.0050	0.0088	0.0000*	0.0000*	0.0000	0.0037
VEGF -2578/-634/936						
C-C-C	0.3633	0.3226*	0.3450	0.3582	0.3228*	0.3370
C-G-C	0.2810	0.2565	0.2479	0.2644	0.2279†	0.2939
A-G-T	0.0715	0.0930	0.1162	0.0937	0.1382†	0.0684
C-G-T	0.0201	0.0348*	0.0230	0.0150	0.0336	0.0357*
VEGF -1154/-634/936						
G-G-C	0.3363	0.3417	0.3039	0.3436	0.2582†	0.3380
A-G-C	0.1156	0.1133	0.1425	0.1241	0.1708†	0.1549†
G-C-T	0.0648	0.0876*	0.0756	0.0882*	0.0668	0.0634
G-G-T	0.0612	0.0598	0.0552	0.0401*	0.0727	0.0641
A-G-T	0.0335	0.0551*	0.0685†	0.0472	0.0869†	0.0373
A-C-C	0.0195	0.0000†	0.0000†	0.0000†	0.0000†	0.0085*
A-C-T	0.0065	0.0040	0.0086	0.0109	0.0080	0.0000**

Two-sided χ^2 test, each haplotype compared with all other haplotypes.

VEGF indicates vascular endothelial growth factor; SAO, small-artery occlusion.

*$P<0.05$.

†$P<0.01$.

‡P value was calculated using the omnibus χ^2 test.

Table 5. Plasma Homocysteine Levels and Variability in Plasma Homocysteine of *VEGF* -2578C>A, -1154G>A, -634G>C, and 936C>T Genotypes Among Ischemic Stroke, Ischemic Stroke Subtypes, Silent Brain Infarction (SBI), and Control Subjects

Group	Mean±SD (no.)	CV, %	Mean±SD (no.)	CV, %	Mean±SD (no.)	CV, %	*P**
VEGF -2578C>A	CC		CA		AA		
Control subjects	9.88±3.93 (261)	39.8	10.38±4.50 (201)	43.4	9.83±3.68 (29)	37.4	0.554
Ischemic stroke	11.10±5.86 (298)	52.8	11.40±5.08 (249)	44.6	11.21±4.71 (64)	42.0	0.199
Small-artery occlusion (SAO)	11.07±5.48 (96)	49.5	11.69±4.73 (99)	40.5	11.58±4.75 (19)	41.0	0.277
Single SAO	11.11±5.69 (58)	51.2	10.61±4.11 (52)	38.7	12.46±5.18 (8)	41.6	0.611
Multiple SAO	11.00±5.22 (38)	47.5	12.89±5.12 (47)	39.7	10.95±4.57 (11)	41.7	0.091
SBI	11.12±5.06 (196)	45.5	11.95±7.84 (148)	65.6	10.74±4.32 (28)	40.2	0.615
VEGF -1154G>A	GG		GA		AA		
Control subjects	9.97±4.19 (337)	42.0	10.22±4.15 (136)	41.0	11.24±3.60 (18)	32.0	0.149
Ischemic stroke	11.18±5.73 (425)	51.3	11.20±4.49 (161)	40.1	12.50±5.88 (25)	47.0	0.284
SAO	11.16±5.27 (132)	47.2	11.95±4.73 (70)	39.6	10.88±4.89 (12)	44.9	0.283
Single SAO	10.87±5.26 (80)	48.4	11.13±4.27 (33)	38.4	11.70±6.01 (5)	51.4	0.715
Multiple SAO	11.61±5.30 (52)	45.7	12.67±5.06 (37)	39.9	10.30±4.25 (7)	41.3	0.271
SBI	11.35±5.33 (243)	47.0	11.56±8.40 (108)	72.7	11.53±2.98 (21)	25.8	0.438
VEGF -634G>C	GG		GC		CC		
Control subjects	10.16±4.61 (135)	45.4	9.87±3.48 (268)	35.3	10.60±5.21 (88)	49.2	0.959
Ischemic stroke	10.60±4.45 (178)	42.0	11.67±5.76 (341)	49.4	10.86±5.85 (92)	53.9	0.109
SAO	10.56±4.57 (58)	43.3	11.86±5.29 (128)	44.6	11.03±4.93 (28)	44.7	0.231
Single SAO	11.26±5.48 (30)	48.7	10.94±4.68 (71)	42.8	10.63±5.66 (17)	53.2	0.730
Multiple SAO	9.82±3.27 (28)	33.3	13.01±5.81 (57)	44.7	11.64±3.69 (11)	31.7	0.043
SBI	11.60±5.51 (124)	47.5	11.66±7.64 (168)	65.5	10.63±3.69 (80)	34.7	0.504
VEGF 936C>T	CC		CT		TT		
Control subjects	10.00±3.89 (343)	38.9	9.98±4.39 (245)	44.1	10.50±5.24 (56)	49.9	0.683
Ischemic stroke	10.87±4.99 (378)	45.9	11.65±5.58 (214)	47.9	13.92±9.96 (19)	71.6	0.072
SAO	10.96±4.79 (130)	43.7	12.29±5.43 (79)	44.2	8.76±4.84 (5)	55.3	0.079
Single SAO	10.63±4.46 (76)	42.0	11.97±5.75 (40)	48.0	4.26±1.05 (2)	24.6	0.041
Multiple SAO	11.43±5.22 (54)	45.7	12.62±5.14 (39)	40.7	11.77±3.53 (3)	30.0	0.268
SBI	11.45±7.09 (259)	61.9	11.46±3.68 (104)	32.1	10.20±5.29 (9)	51.6	0.118

VEGF indicates vascular endothelial growth factor; CV, between-person coefficient of variation; SAO, small-artery occlusion; SBI, silent brain infarction.

*Kruskal-Wallis test of non-parametric test with plasma homocysteine levels in genotypes.

other hypoxia-inducible genes such as erythropoietin or tyrosine hydroxylase.[71]

There are several limitations of the present study: (1) as noted, it is not yet clear which genetic polymorphisms predict the phenotypes associated with ischemic stroke and SBI. The study population comprised of only Korean individuals, and our findings will need to be validated in other ethnic groups; (2) this was a hospital-based case–control study, which had a relatively small sample size of individual stroke subtypes. However, we think that the recruitment of >1000 individuals from an ethnically homogeneous population (Koreans have a low degree of interracial marriage) is enough to give reliable data; (3) control subjects were not recruited from a completely "healthy" population, because some of them were seeking medical attention. Those who agreed to diagnostic evaluation differed from those who did not agree. Therefore, it would not be easy to identify the casual effects of vascular risk factors in these subjects. Our results may underestimate the true impact of individual risk factors based on a selection bias. However, in our experience, recruitment of healthy participants markedly reduces recruitment rates because of the refusal of laboratory and imaging studies. Only interview-dependent risk factor assessment without laboratory and imaging studies may fail to find covert risk factors and asymptomatic lesions (for example, SBI), leading to another potential for bias. In the present study, approximately 6% of the study subjects were found to have a new vascular risk factor through laboratory tests at the time of examination. All of our study subjects had brain imaging and were confirmed by negative symptomatic brain lesion, thereby enhancing the diagnostic accuracy and exact case–control grouping. Although a population-based study may be optimal to reduce the referral or selection bias, it is difficult to obtain sufficient numbers of stroke incidents among the cohort because the estimated annual incidence of stroke is known to be low in the general population; and (4) our results cannot be extrapolated

to other races because interethnic variability in frequencies of stroke subtypes and genotypes may produce different results.

The genotype and allele frequencies of the *VEGF* polymorphisms may vary among different populations. For example, Park et al[29] reported a comparison of *VEGF* polymorphism data in healthy populations obtained from various studies, finding that the frequency of the *VEGF -2578A* allele was 0.378 to 0.504 in whites and 0.276 to 0.280 in Asians. For the *VEGF -1154A* allele, the frequencies were 0.30 to 0.32 in whites and 0.18 in Koreans,[26] suggesting that there is a racial difference in the allele frequencies of the -2578C>A and -1154G>A polymorphisms. Therefore, additional studies involving different racial or ethnic groups or samples of populations of homogeneous origin are needed to confirm our results.

In conclusion, the *VEGF* polymorphisms were associated with the risk of ischemic stroke, particularly in patients with multiple SAOs. The adjusted OR values of the *-2578A*, *-1154A*, *-634G,* and *936T* alleles, which are related to abnormal VEGF expression levels, were much higher in multiple SAO patients than in the single SAO group. The *VEGF* polymorphisms were significantly different between patients with SBI and multiple SAOs. These findings suggest that *VEGF* polymorphisms are a genetic determinant for the risk of multiple SAOs in the Korean population. Further studies of other racial or ethnic populations and of the biological functions of VEGF are needed to fully understand the role of *VEGF* polymorphisms in the risk of multiple SAOs in patients with ischemic stroke and patients with SBI.

Sources of Funding

This work was partly supported by National Research Foundation of Korea Grant funded by the Korean Government (2009-0070341) and partly supported by Priority Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093821).

Disclosures

None.

References

1. World Health Organization. The World Health Report 2002: reducing risks, promoting healthy life. Geneva: WHO; 2002. Available at: www.who.int/whr/2002/en/. Accessed October 4, 2003.
2. Warlow CP. Epidemiology of stroke. *Lancet.* 1998;352:SIII1–SIII4.
3. Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, et al. Primary prevention of ischemic stroke: a statement for healthcare professionals from the Stroke Council of the American Heart Association. *Stroke*. 2001;32:280–299.
4. Korea National Statistical Office. Change in leading causes of death (1999–2009). 2009. Available at: kosis.kr/ups3/service/ch_file_down.jsp?PUBCODE=YD&FILE_NAME=/ups3/upload/101/YD/VD0005.xls&SEQ=8. Accessed June 26, 2011.
5. Vermeer SE, Koudstaal PJ, Oudkerk M, Hofman MM. Prevalence and risk factors of silent brain infarcts in the population-based Rotterdam Scan Study. *Stroke*. 2002;33:21–25.
6. Kase CS, Wolf PA, Chodosh EH, Zacker HB, Kelly-Hayes M, Kannel WB, et al. Prevalence of silent stroke in patients presenting with initial stroke: the Framingham Study. *Stroke*. 1989;20:850–852.
7. Malinow MR. Homocyst(e)ine and arterial occlusive disease. *J Intern Med.* 1994;236:603–617.
8. Durand P, Lussier-Cacan S, Blache D. Acute methionine load-induced hyperhomocysteinemia enhances platelet aggregation, thromboxane biosynthesis, and macrophage-derived tissue factor activity in rats. *FASEB*. 1997;11:1157–1168.
9. Duell PB, Malinow MR. Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease. *Curr Opin Lipidol.* 1997;8:28–34.
10. Matsui T, Arai H, Yuzuriha T, Yao H, Miura H, Hashimoto S, et al. Elevated plasma homocysteine levels and risk of silent brain infarction in elderly people. *Stroke*. 2001;32:1116–1119.
11. Vermeer SE, van Dijk EJ, Koudstaal PJ, Oudkerk M, Hofman A, Clarke R, et al. Homocysteine, silent brain infarcts, and white matter lesions: the Rotterdam Scan Study. *Ann Neurol*. 2002;51:285–289.
12. Howard G, Wagenknecht LE, Cai J, Cooper L, Kraut MA, Toole JF. Cigarette smoking and other risk factors for silent cerebral infarction in the general population. *Stroke*. 1998;29:913–917.
13. Kobayashi S, Okada K, Koide H, Bokura H, Yamaguchi S. Subcortical silent brain infarction as a risk factor for clinical stroke. *Stroke*. 1997;28:1932–1939.
14. Kwon HM, Kim BI, Lee SH, Choi SH, Oh BH, Yoon BW. Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke*. 2006;37:466–470.
15. La Rosa S, Uccella S, Finzi G, Albarello L, Sessa F, Capella C. Localization of vascular endothelial growth factor and its receptors in digestive endocrine tumors: correlation with microvessel density and clinicopathologic features. *Hum Pathol*. 2003;34:18–27.
16. Vincenti V, Cassano C, Rocchi M, Persico G. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. *Circulation.* 1996;93:1493–1495.
17. Leung DW, Cachianes G. Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*. 1989;246:1306–1309.
18. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*. 1992;359:843–845.
19. Ogunshola OO, Stewart WB, Mihalcik V, Solli T, Madri JA, Ment LR. Neuronal VEGF expression correlates with angiogenesis in postnatal developing rat brain. *Brain Res*. 2000;119:139–153.
20. Kovacs Z, Ikezaki K, Samoto K, Inamura T, Fukui M. VEGF and Flt. Expression time kinetics in rat brain infarct. *Stroke*. 1996;27:1865–1872.
21. Hayashi T, Abe K, Suzuki H, Itoyama Y. Rapid induction of vascular endothelial growth factor gene expression after transient middle cerebral artery occlusion in rats. *Stroke*. 1997;28:2039–2044.
22. Lennmyr F, Ata KA, Funa K, Olsson Y, Terent A. Expression of vascular endothelial growth factor (VEGF) and its receptors (Flt-1 and Flk-1) following permanent and transient occlusion of the middle cerebral artery in the rat. *J Neuropathol Exp Neurol*. 1998;57:874–882.
23. Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5′UTR regions of the human vascular endothelial growth factor gene. *Hum Immunol*. 1999;60:1245–1249.
24. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine*. 2000;12:1232–1235.
25. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J Vasc Res*. 2000;37:443–448.
26. Lee HH, Hong SH, Shin SJ, Ko JJ, Oh D, Kim NK. Association study of vascular endothelial growth factor polymorphisms with the risk of recurrent spontaneous abortion. *Fertil Steril*. 2010;93:1244–1247.
27. Papazoglou D, Galazios G, Koukourakis MI, Panagopoulos I, Kontomanolis EN, Papatheodorou K, et al. Vascular endothelial growth factor gene polymorphisms and pre-eclampsia. *Mol Hum Reprod*. 2004;10:321–324.
28. Del Bo R, Scarlato M, Ghezzi S, Martinelli F, Boneschi M, Fenoglio C, et al. Vascular endothelial growth factor gene variability is associated with increased risk for AD. *Ann Neurol*. 2005;57:373–380.
29. Park HM, Hong SH, Kim JW, Oh D, Hwang SG, An HJ, et al. Gender-specific association of the VEGF -2578C>A polymorphism in Korean patients with colon cancer. *Anticancer Res*. 2007;27:2535–2539.
30. Jin Q, Hemminki K, Enquist K, Lenner P, Grzybowska E, Klaes R, et al. Vascular endothelial growth factor polymorphisms in relation to breast cancer development and prognosis. *Clin Cancer Res*. 2005;11:3647–3653.
31. Bae SJ, Kim JW, Kang H, Hwang SG, Oh D, Kim NK. Gender-specific association between polymorphism of vascular endothelial growth factor (VEGF 936C>T) gene and colon cancer in Korea. *Anticancer Res*. 2008;28:1271–1276.

32. Bae SJ, Ahn DH, Hong SP, Kang H, Hwang SG, Oh D, et al. Gender-specific association between polymorphism of vascular endothelial growth factor (VEGF 936C>T) gene and patients with stomach cancer. *Yonsei Med J*. 2008;49:783–791.
33. Yang JP, Liu HJ, Wang ZL, Cheng SM, Cheng X, Xu GL, et al. The dose-effectiveness of intranasal VEGF in treatment of experimental stroke. *Neurosci Lett*. 2009;461:212–216.
34. Zhang W, Sun K, Zhen Y, Wang D. Wang Y, Chen J, et al. VEGF receptor-2 variants are associated with susceptibility to stroke and recurrence. *Stroke*. 2009;40:2720–2726.
35. Welch GN, Upchurch GJR, Loscalzo J. Hyperhomocyst(e)inemia and atherothrombosis. *Ann NY Acad Sci.* 1997;811:48–58.
36. Nagai Y, Tasaki H, Takatsu H, Nihei S, Yamashita K, Nakashima Y. Homocysteine inhibits angiogenesis in *vitro* and *in vivo. Biochem Biophys Res Commun*. 2001;281:726–731.
37. Guerzoni AR, Biselli PM, Godoy MF, Souza DR, Haddad R, Eberlin MN, et al. Homocysteine and MTHFR and VEGF gene polymorphisms: impact on coronary artery disease. *Arq Bras Cardiol*. 2009;92:249–254.
38. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 1993;24:35–41.
39. Lee BC, Roh JK. International experience in stroke registries: Korean Stroke Registry. *Am J Prev Med*. 2006;31:S243–S245.
40. Kim NK, Choi BO, Jung WS, Choi YJ, Choi KG. Hyperhomocysteinemia as an independent risk factor for silent brain infarction. *Neurology*. 2003;61:1595–1599.
41. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Stat Soc Ser B*. 1995;57:289–300.
42. Hedrick PW. Gametic disequilibrium measures: proceed with caution. *Genetics*. 1987;117:331–341.
43. Simmons M. Angiogenesis. Where do we stand now? *Circulation*. 2005; 111:1556–1566.
44. Losordo DW, Dimmeler S. Therapeutic angiogenesis and vasculogenesis for ischemic disease part I: angiogenic cytokines. *Circulation*. 2004;109: 2487–2491.
45. Marti HJ, Bernaudin M, Bellail A, Schoch H, Euler M, Petit E, et al. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. *Am J Pathol*. 2000;156: 965–976.
46. Sun Y, Jin K, Xie L, Childs J, Mao XO, Mogvinova A, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest*. 2003;111:1843–1851.
47. Verheul HMW, Jorna AS, Hoekman K, Broxterman HJ, Gebbink MF, Pinedo HM. Vascular endothelial growth factor-stimulated endothelial cells promote adhesion and activation of platelets. *Blood*. 2000;96: 4216–4221.
48. Verheul HMW, Hoekman K, Lupu F, Broxterman HJ, Kakkar AK, Pinedo HM. High VEGF concentration and activation of coagulation pathway, including platelets, in aspirated fluids of soft tissue sarcomas. *Clin Cancer Res*. 2000;6:166–171.
49. Yang JP, Liu HJ, Liu XF. VEGF promotes angiogenesis and functional recovery in stroke rats. *J Invest Surg*. 2010;23:149–155.
50. Papavassiliou E, Gegate N, Proescholdt M, Heiss JD, Walbridge S, Edwards NA, et al. Vascular endothelial growth factor (vascular permeability factor) expression in injured rat brain. *J Neurosci Res*. 1997;49: 451–460.
51. Kubo M. Genetic risk factors of ischemic stroke identified by a genome-wide association study *Brain Nerve.* 2008;60:1339–1344.
52. Yamada Y, Fuku N, Tanaka M, Aoyagi Y, Sawabe M, Metoki N, et al. Identification of CELSR1 as a susceptibility gene for ischemic stroke in Japanese individuals by a genome-wide association study. *Atherosclerosis*. 2009;207:144–149.
53. Ding H, Wu B, Wang H, Lu Z, Yan J, Wang X, et al. A novel loss-of-function DDAH1 promoter polymorphism is associated with increased susceptibility to thrombosis stroke and coronary heart disease. *Stroke*. 2010;41:177–180.
54. Banerjee I, Gupta V, Ganesh S. Association of gene polymorphism with genetic susceptibility to stroke in Asian populations: a meta-analysis. *J Hum Genet*. 2007;52:205–219.
55. Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI. Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. *Stroke.* 2003;31:1069–1075.
56. Markus HS, Ali S, Swaminathan R, Sankaralingam A, Mollo J, Powell J. A common polymorphism in the methylenetetrahydrofolate reductase gene, homocysteine, and ischemic cerebrovascular disease. *Stroke*. 1997; 28:1739–1743.
57. Szolnoki Z, Somogyvari F, Kondacs A, Szabo M, Fodor L. Evaluation of the interactions of common genetic mutations in stroke subtypes. *J Neurol*. 2002;249:1391–1397.
58. Roman GC, Erkinjuntti T, Wallin A, Oantoni L, Chui HC. Subcortical ischemic vascular dementia. *Lancet Neurol*. 2002;1:426–436.
59. O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Saewada T, Pantoni L, et al. Vascular cognitive impairment. *Lancet Neurol*. 2003;2:89–98.
60. Choi BO, Kim NK, Kim SH, Kang MS, Lee S, Ahn JY, et al. Homozygous C677T mutation in the MTHFR gene as an independent risk factor for multiple small-artery occlusions. *Thromb Res*. 2003;111: 39–44.
61. Morita H, Kurihara H, Tsubaki S, Sugiyama T, Hamada C, Kurihara Y, et al. Methylene-tetrahydrofolate reductase gene polymorphism and ischemic stroke in Japanese. *Arterioscler Thromb Vasc Biol*. 1998;18: 1465–1469.
62. Yoo JH, Choi GD, Kang SS. Pathogenicity of thermolabile methylene-tetrahydrofolate reductase for vascular dementia. *Arterioscler Thromb Vasc Biol*. 2000;20:1921–1925.
63. Makin AJ, Chung NAY, Silverman SH, Lip GY. Vascular endothelial growth factor and tissue factor in patients with established peripheral artery disease: a link between angiogenesis and thrombogenesis? *Clin Sci*. 2003;104:397–404.
64. Inoue M, Itoh H, Ueda M, Naruko T, Kojima A, Komatsu R, et al. Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions: possible pathophysiological significance of VEGF in progression of atherosclerosis. *Circulation*. 1998;98: 2108–2116.
65. Roybal CN, Yang S, Sun CW, Hurtado D, Jagt DLV, Townes TM, et al. Homocysteine increases the expression of VEGF by a mechanism involving endoplasmic reticulum stress and transcription factor ATF4. *J Biol Chem*. 2004;279:14844–14852.
66. Maeda M, Yamamoto I, Fujio Y, Azuma J. Homocysteine induces vascular endothelial growth factor expression in differentiated THP-1 macrophages. *Biochim Biophys Acta*. 2003;1623:41–46.
67. Mazure NM, Chen EY, Yeh P, Laderoute KR, Giaccia AJ. Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. *Cancer Res*. 1996;56:3436–3440.
68. Shastry S, Tyagi N, Hayden MR, Tyagi SC. Proteomic analysis of homocysteine inhibition of microvascular endothelial cell angiogenesis. *Cell Mol Biol*. 2004;50:932–937.
69. Atta HM, El-Rehani MA, Raheim SA, Galal AMF. Lowering homocysteine decreases levels and expression of $VEGF_{165}$ and endostatin. *J Surg Res*. 2008;146:202–210.
70. Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wascher TC, Paulweber B, et al. A common 936C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. *Int J Cancer*. 2003;106:468–471.
71. Scandurro AB, Beckman BS. Common proteins bind mRNAs encoding erythropoietin, tyrosine hydroxylase, and vascular endothelial growth factor. *Biochem Biophys Res Commun*. 1998;246:436–440.