Genetic Factors for Ischemic and Hemorrhagic Stroke in Japanese Individuals

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Background and Purpose—Although genetic epidemiologic studies have implicated several genetic variants as risk factors for ischemic or hemorrhagic stroke, the genetic determinants of these conditions remain largely unknown. We performed an association study to identify gene polymorphisms that confer susceptibility to atherothrombotic cerebral infarction, intracerebral hemorrhage, or subarachnoid hemorrhage.

Methods—The study population comprised 3432 unrelated Japanese individuals: 1362 stroke patients (822 with atherothrombotic cerebral infarction, 333 with intracerebral hemorrhage, and 207 with subarachnoid hemorrhage) and 2070 controls. The genotypes for 50 polymorphisms of 38 candidate genes were determined by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology.

Results—An initial \( \chi^2 \) test (false discovery rate \( P < 0.05 \)) and subsequent multivariable logistic-regression analysis with adjustment for conventional risk factors \( (P < 0.05) \) revealed that the \( -14C \) polymorphism (rs1800977) of \( ABCA1 \), the \( A \) (rs3027898) and \( C \) (Ser532Leu, rs1059703) polymorphisms of \( IRAK1 \), and the \( G \) (Cys2229Ser) polymorphism (rs619203) of \( ROS1 \) were significantly associated with atherothrombotic cerebral infarction; that the \( -428G \) polymorphism (rs710968) of \( LIMK1 \) was significantly associated with intracerebral hemorrhage; and that the \( 13989A \) (Ile118Val) polymorphism (NC_000007.12) of \( CYP3A4 \) was significantly associated with subarachnoid hemorrhage.

Conclusions—Genotypes for \( ABCA1 \), \( IRAK1 \), and \( ROS1 \) may prove useful for assessment of the genetic risk for atherothrombotic cerebral infarction, whereas those for \( LIMK1 \) and \( CYP3A4 \) may be similarly beneficial in assessment of the genetic risk for intracerebral hemorrhage and subarachnoid hemorrhage, respectively. Validation of these findings will require additional studies with independent subject panels. (Stroke. 2008;39:2211-2218.)

Key Words: cerebral infarction ■ cerebral hemorrhage ■ subarachnoid hemorrhage ■ genetic polymorphism ■ genetics

Stroke is a complex multifactorial disorder that is thought to result from an interaction between a person’s genetic background and various environmental factors. It is a common and serious condition, with \( \approx 700,000 \) individuals experiencing a new or recurrent stroke and nearly 150,000 deaths from stroke-related causes in the United States in 2004. The prevalence of stroke in the United States is 5.7 million. Of all such events, 88% are ischemic stroke, 9% are intracerebral hemorrhage (ICH), and 3% are subarachnoid hemorrhage (SAH). In Japan, the prevalence of stroke is 1.4 million (61% ischemic stroke, 25% ICH, and 11% SAH), with nearly 132,000 deaths from this condition each year (Ministry of Health, Labor, and Welfare of Japan, 2005). Despite recent advances in acute stroke therapy, stroke remains the leading cause of severe disability and the third leading cause of death, after heart disease and cancer, in Western countries and Japan. The identification of biomarkers for stroke risk is important both for risk prediction and for intervention to avert future events.
Although genetic epidemiologic studies have implicated several genetic variants as risk factors for ischemic or hemorrhagic stroke, the genetic determinants of these conditions remain largely unknown. We performed an association study for 50 polymorphisms of 38 candidate genes and atherothrombotic cerebral infarction, ICH, or SAH in 3432 Japanese individuals. The purpose of the present study was to identify gene polymorphisms that confer susceptibility to atherothrombotic cerebral infarction, ICH, or SAH and thereby to contribute to the personalized prevention of these conditions.

Subjects and Methods

Study Population
The study population comprised 3432 unrelated Japanese individuals (1661 men, 1771 women) who either visited outpatient clinics of or were admitted to 1 of the participating hospitals (Gifu Prefectural General Medical Center, Gifu Prefectural Tajimi Hospital, Yokohama General Hospital, Hirokami University Hospital, Reimeikyo Rehabilitation Hospital, and Hiroaki Stroke Center) between October 2002 and March 2007 because of various symptoms or for an annual health checkup or who were recruited to a population-based prospective cohort study of aging and age-related diseases in Guma Prefecture. The 1362 stroke patients included 822 subjects (484 men, 338 women) with atherothrombotic cerebral infarction, 333 subjects (213 men, 120 women) with ICH, and 207 subjects (83 men, 124 women) with SAH. The stroke patients were recruited from consecutive individuals who were admitted to the participating hospitals because of stroke events or visited outpatient clinics regularly. The diagnosis of ischemic or hemorrhagic stroke was based on the occurrence of a new and abrupt focal neurologic deficit, with neurologic symptoms and signs persisting for >24 hours; it was confirmed by positive findings on computed tomography or magnetic resonance imaging (or both) of the head. The type of stroke was determined according to the Classification of Cerebrovascular Diseases III. Individuals with cardiogenic embolic infarction, lacunar infarction alone, transient ischemic attack, or intracranial hemorrhage from cerebrovascular malformations, moyamoya disease, cerebral venous sinus thrombosis, brain tumors, traumatic cerebrovascular diseases, or subdural hematoma were excluded from enrollment in the study, as were those with atrial fibrillation in the absence or presence of valvular heart disease.

The 2070 control subjects (881 men, 1189 women) were recruited from consecutive individuals who visited outpatient clinics of the participating hospitals for an annual health checkup or who were community-dwelling individuals recruited to the prospective cohort study. These subjects either had or did not have conventional risk factors for ischemic or hemorrhagic stroke, including cigarette smoking, hypertension, diabetes mellitus, and hypercholesterolemia. They had no history of ischemic or hemorrhagic stroke or other cerebral diseases; coronary heart disease, peripheral arterial occlusive disease, or other atherosclerotic diseases; or other thrombotic, embolic, or hemorrhagic disorders. The study protocol complied with the Declaration of Helsinki and was approved by the committee on the ethics of human research of Mie University Graduate School on Medicine, Hiroaki University Graduate School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology, and participating hospitals. Written informed consent was obtained from each participant.

Selection and Genotyping of Polymorphisms

Our aim was to identify genes associated with atherothrombotic cerebral infarction, ICH, or SAH in the Japanese population in a case-control association study by examining the relations of 1 to 3 polymorphisms of each candidate gene to these conditions. With the use of public databases, including PubMed (NCBI) and Online Mendelian Inheritance in Man (NCBI), we selected 38 candidate genes that have been characterized and suggested to be associated with atherothrombotic cerebral infarction, ICH, or SAH. On the basis of published studies or by searching PubMed and single nucleotide polymorphism (SNP) databases (dbSNP [NCBI] and Japanese SNP database [JSNP]), we further selected 50 polymorphisms of these genes—most located in the promoter region or exons—that might be expected to result in changes in the function or expression of the encoded protein (supplemental Table I available online at http://stroke.ahajournals.org). Wild-type and variant alleles of the polymorphisms were determined from the original sources. We had not examined relations of these polymorphisms to ischemic or hemorrhagic stroke in our previous study.

Venous blood (7 mL) was collected into tubes containing 50 mmol/L EDTA (disodium salt), and genomic DNA was isolated with a kit (Genomix; Talent, Trieste, Italy). Genotypes of the 50 polymorphisms were determined at G&G Science (Fukuushima, Japan) by a method that combines the polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, Tex). Primers, probes, and other conditions for genotyping of polymorphisms associated with atherothrombotic cerebral infarction, ICH, or SAH are shown in supplemental Table II. Detailed genotyping methodology was described previously.

To confirm the accuracy of genotyping by suspension array technology in the present study, we selected DNA samples from 23 subjects and genotyped 95 polymorphisms by chip-based matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. Only 4 of the total of 2185 genotypes determined by this approach differed from those identified by suspension array technology. We then determined these 4 discrepant genotypes by DNA sequencing with a fluorescence-based automated DNA sequencer (Applied Biosystems, Foster City, Calif) after amplification and cloning of polymorphic regions. All 4 genotypes determined by DNA sequencing were identical to those determined by suspension array technology, demonstrating the accuracy of the latter approach in the present study. The success rate for genotyping all 50 SNPs selected for the present study was ≥99.9%.

Statistical Analysis

Quantitative data were compared between subjects with atherothrombotic cerebral infarction, ICH, or SAH and controls by the unpaired Student t test. Categorical data were compared by the χ² test. Allele frequencies were estimated by the gene counting method, and the χ² test was used to identify departure from Hardy-Weinberg equilibrium. In the initial screen, the genotype distribution of each autosomal polymorphism was compared by the χ² test (3 × 2) between subjects with atherothrombotic cerebral infarction, ICH, or SAH and controls. For polymorphisms of genes located on the X chromosome, allele frequencies were compared by the χ² test (2 × 2).

Given the multiple comparisons of genotypes with each type of stroke, the false discovery rate (FDR) was calculated from the distribution of probability values for the 50 polymorphisms. Polymorphisms with an FDR <0.05 were further examined by multivariable logistic-regression analysis with adjustment for covariates that differed significantly between subjects with each type of stroke and controls. Multivariable logistic-regression analysis was thus performed with atherothrombotic cerebral infarction as a dependent variable and independent variables including sex (0 = woman, 1 = man), the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia (0 = no history of these conditions, 1 = positive history), and genotype of each polymorphism; with ICH as a dependent variable and independent variables including age, sex, body mass index (BMI), the prevalence of hypertension and diabetes mellitus, and genotype of each polymorphism; or with SAH as a dependent variable and independent variables including age, the prevalence of hypertension and diabetes mellitus, and genotype of each polymorphism. Each genotype was assessed according to the wild-type homozygote, 1 = heterozygote = variant homozygote, recessive (0 = wild-type homozygote = heterozygote, 1 = variant homozygote), and additive (0,0) = wild-type homozygote, (1,0) = heterozygote, (0,1) = variant homozygote] genetic models,
Table 1. Characteristics of the 3432 Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Atherothrombotic Cerebral Infarction</th>
<th>ICH</th>
<th>SAH</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>282</td>
<td>333</td>
<td>207</td>
<td>2070</td>
</tr>
<tr>
<td>Age, y</td>
<td>67.9±10.6</td>
<td>62.0±11.3*</td>
<td>56.6±12.0*</td>
<td>67.5±10.0</td>
</tr>
<tr>
<td>Sex, male/female, %</td>
<td>58.3/41.7*</td>
<td>64.0/36.0*</td>
<td>40.1/59.9</td>
<td>42.6/57.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4±3.3</td>
<td>22.8±3.7*</td>
<td>23.0±3.2</td>
<td>23.5±3.2</td>
</tr>
<tr>
<td>Current or former smoker, %</td>
<td>24.1</td>
<td>28.5</td>
<td>30.2</td>
<td>24.7</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>69.0*</td>
<td>70.0*</td>
<td>51.2*</td>
<td>29.8</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>37.4*</td>
<td>25.9*</td>
<td>18.4*</td>
<td>7.9</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>33.2*</td>
<td>23.1</td>
<td>24.9</td>
<td>25.5</td>
</tr>
</tbody>
</table>

Age and BMI values are mean±SD. Smoker indicates smoking ≥10 cigarettes daily; hypertension, systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg (or both), or taking antihypertensive medication; diabetes mellitus, fasting blood glucose ≥6.93 mmol/L or glycosylated hemoglobin ≥6.3% (or both), or taking antidiabetes medication; hypercholesterolemia, serum total cholesterol ≥5.72 mmol/L or taking lipid-lowering medication.

*P<0.01 vs controls.

and the probability value, odds ratio (OR), and 95% CI were calculated. Additive models included the additive 1 model (homozygotes vs wild-type homozygotes) and the additive 2 model (variant homozygotes vs wild-type homozygotes), which were analyzed simultaneously with a single statistical model. With the exception of the initial screen by the \( \chi^2 \) test (FDR<0.05), a value of \( P<0.05 \) was considered statistically significant. Statistical significance was examined by 2-sided tests performed with JMP version 5.1 software (SAS Institute, Cary, NC). Examinations of Hardy-Weinberg equilibrium, linkage disequilibrium, and association of haplotypes with each type of stroke were performed with SNPAlyze version 6 software (Dynacom, Yokohama, Japan).

Results

The characteristics of the 3432 study subjects are shown in Table 1. The frequency of men and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia were greater in subjects with atherothrombotic cerebral infarction than in controls. Age and BMI were smaller, whereas the frequency of men and the prevalence of hypertension and diabetes mellitus were higher in subjects with ICH than in controls. Age was younger and the prevalence of hypertension and diabetes mellitus was higher in subjects with SAH than in controls.

Evaluation of genotype distributions or allele frequencies by the \( \chi^2 \) test revealed that the −14C→T polymorphism (rs1800977) of ATP-binding cassette, subfamily A, member 1 (ABCA1), the A→C (rs3027898) and C→T (Ser532Leu, rs1059703) polymorphisms of interleukin-1 receptor–associated kinase-1 (IRAK1), and the G→C (Cys229Ser) polymorphism (rs619203) of v-Ros avian UR2 sarcoma virus oncogene homolog-1 (ROSI) were significantly (FDR<0.05) associated with the prevalence of atherothrombotic cerebral infarction; the −428G→A polymorphism (rs710968) of LIMK1 with ICH; and the 13989A→G (Ile118Val) polymorphism (NC_000007.12) of cytochrome P450, subfamily IIIA, polypeptide 4 (CYP3A4) with SAH (Table 2). These polymorphisms were further analyzed for their relations to each disorder.

Multivariable logistic-regression analysis with adjustment for sex and the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia revealed that the −14C→T polymorphism of ABCA1 (dominant, recessive, and additive 2 models), the A→C (dominant and additive 2 models) and C→T (Ser532Leu; recessive and additive 2 models) polymorphisms of IRAK1, and the G→C (Cys229Ser) polymorphism of ROSI (dominant and additive 1 and 2 models) were significantly (\( P<0.05 \)) associated with atherothrombotic cerebral infarction (Table 3). Given that IRAK1 is located on the X chromosome, we examined the relations of the polymorphisms of this gene to atherothrombotic cerebral infarction in men and women separately. For men, a single genetic model (0= wild-type allele, 1= variant allele) was used. Multivariable logistic-regression analysis with adjustment for the prevalence of hypertension, diabetes mellitus, and hypercholesterolemia revealed that the A→C (\( P=0.0141; \ OR=1.49; \ 95\% \ CI, 1.09–2.07 \)) and C→T (Ser532Leu) (\( P=0.0124; \ OR=0.67; \ 95\% \ CI, 0.48–0.91 \)) polymorphisms of IRAK1 were significantly associated with atherothrombotic cerebral infarction in men, whereas the A→C (\( P=0.7543, 0.7965, 0.8069, \) and 0.7342 in dominant, recessive, additive 1, and additive 2 models, respectively) and C→T (\( P=0.7493, 0.9734, 0.7270, \) and 0.9691 in dominant, recessive, additive 1, and additive 2 models, respectively) polymorphisms were not related to this condition in women. Multivariable logistic-regression analysis with adjustment for age, sex, BMI, and the prevalence of hypertension and diabetes mellitus revealed that the −428G→A polymorphism of LIMK1 (dominant and additive 1 models) was significantly associated with ICH (Table 3). Similar analysis with adjustment for age and the prevalence of hypertension and diabetes mellitus revealed that the 13989A→G (Ile118Val) polymorphism of CYP3A4 (dominant and additive 1 models) was significantly associated with SAH (Table 3). The genotype distributions of these various polymorphisms among controls and subjects with each type of stroke are shown in Table 4. Those of polymorphisms of ABCA1 (Hardy-Weinberg \( P=0.6782 \), \( \text{ROSI} \) (\( P=0.3536 \), LIMK1 (\( P=0.0787 \), and CYP3A4 (\( P=0.1011 \)) among controls were in Hardy-Weinberg equilibrium. Given that IRAK1 is located on the X chromosome, the genotype distributions of the A→C (\( P=0.2373 \)) and C→T (\( P=0.2891 \)) polymorphisms of
The atherothrombotic cerebral infarction is the most common type of stroke and, in most patients, is caused by atherosclerosis. Several genetic determinants contribute to the risk of atherothrombotic cerebral infarction. ABCA1 mediates transport of intracellular cholesterol and phospholipids across the plasma membrane of cells, most prominently in macrophages. These lipid molecules are then removed from the cells by apolipoprotein AI and other apolipoproteins of nascent HDL-cholesterol in a process that contributes to the initial step of reverse cholesterol transport. This process plays an important role in maintaining cellular cholesterol homeostasis and exerts a protective effect against atherosclerosis. Common polymorphisms of ABCA1 have been associated with susceptibility to coronary heart disease or with the severity of atherosclerosis without an effect on plasma lipid levels. The −14C→T polymorphism in the promoter of ABCA1 was previously shown to be associated with the plasma concentration of HDL-cholesterol. However, we did not detect a relation between this polymorphism and the serum concentration of HDL-cholesterol in the Japanese individuals of the present study (CC, 1.47±0.40 mmol/L; CT, 1.50±0.40 mmol/L; TT, 1.39±0.38 mmol/L, P=0.14). This suggests that ABCA1 may not be a major contributor to the variation in HDL-cholesterol levels in the general population. However, it is possible that other genetic factors, such as other polymorphisms in the ABCA1 gene, may influence HDL-cholesterol levels in specific subpopulations. Further studies are needed to investigate the role of ABCA1 polymorphisms in the variation of HDL-cholesterol levels in different populations.
1.50±0.41 mmol/L; TT, 1.47±0.39 mmol/L; P=0.2512 by ANOVA). We have now shown that the −14C→T polymorphism of ABCA1 was associated with atherothrombotic cerebral infarction, with the T allele representing a risk factor for this condition, although the mechanism responsible for this association remains to be elucidated.

IRAK1 is a key intracellular signaling protein that is activated by ligands of Toll-like receptors. IRAK1 activation by interleukin-6 results in phosphorylation and activation of the transcription factor STAT3 and consequent transcriptional activation of the gene for C-reactive protein in Hep3B cells.20 cDNA microarray analysis revealed that IRAK1 is expressed at high levels in human coronary arteries,21 and IRAK1 has been shown to be constitutively activated in blood mononuclear cells isolated from individuals with atherosclerosis.22 In addition, IRAK1 is implicated in regulation of the 3’ untranslated region of the anti-inflammatory cytokine interleukin-10.22 These observations suggest that activation of the innate immune system in general, and of IRAK1 in particular, may contribute to the increased levels of inflammatory proteins associated with atherosclerosis. Genetic variants of IRAK1 were found to be associated with the plasma concentration of C-reactive protein in white women.23 We have now shown that the A→C and C→T (Ser532Leu) polymorphisms of IRAK1 were associated with atherothrombotic cerebral infarction in men. Haplotypes of these 2 plus 1 other polymorphism of this gene were also associated with this condition. These associations may be attributable to effects of the polymorphisms on vascular inflammation, although the underlying molecular mechanism remains to be determined.

ROS1 is a type I integral membrane protein with tyrosine kinase activity that may function as a receptor for a growth or differentiation factor and thereby play an important role in the regulation of cell proliferation, differentiation, migration, metabolism, or apoptosis.24,25 Human ROS1 is the closest homolog of the v-Ros oncogene of the avian sarcoma UR2 retrovirus, a replication-defective virus that was isolated from a spontaneous chicken tumor.26 In an association study of 11,053 polymorphisms in 6891 genes and myocardial infarction, the A→G (Asn2213Asp) and G→C (Cys2229Ser) polymorphisms of ROS1, which were in linkage disequilibrium, were associated with the prevalence of this condition.27 Another study replicated the association of the G→C (Cys2229Ser) polymorphism of ROS1 with the risk of myocardial infarction.28 The thiol groups of 2 cysteine residues have the potential to form a disulfide bond that can either link 2 peptide chains together, as in insulin, or cause a single peptide chain to fold back on itself in a loop. By affecting such an intramolecular loop, the Cys2229Ser substitution of ROS1 may alter the structural stability, substrate binding affinity, or catalytic function of the protein.28 Our present results show that the G→C (Cys2229Ser) polymorphism of ROS1 was associated with atherothrombotic cerebral infarction, with the C allele protecting against this condition. The reason for the difference in the effect of the C allele between the previous studies in which it was a risk factor for myocardial infarction27,28 and our study, in which it was a protective factor for atherothrombotic cerebral infarction, remains unclear. The functional consequence of this polymorphism also remains to be elucidated.

Although hypertension is the most important risk factor for ICH, familial aggregation of cases was demonstrated in a prospective study, which found that 10% of affected individuals had a family history of ICH.29 A functional haplotype spanning ELN and Lim domain kinase-1 (LIMK1) confers susceptibility to intracranial aneurysm.7 A systematic analysis of 166 polymorphisms and corresponding haplotypes that reside within the chromosome 7q11 linkage peak thus identified a highly significant association between intracranial aneurysm and a distinct linkage disequilibrium block containing the 3’ untranslated region of ELN and the promoter region of LIMK1.7 The strongest association was found with the +695G→C tag SNP of ELN for a risk haplotype composed of the +502A insertion of ELN and the −187C→T polymorphism of LIMK1. Functional studies revealed that the...
+502A insertion of ELN reduces the rate of ELN transcription, whereas the −187C→T substitution in LIMK1 reduces promoter activity.7 LIMK1 is an important regulator of the actin cytoskeleton. It requires phosphorylation by Rho kinase for its activation, and it regulates the organization of actin stress fibers indirectly by phosphorylating cofilin, rendering it unable to bind to actin and to mediate actin depolymerization.8 We have now shown that the −428G→A polymorphism of LIMK1, which was also previously related to intracranial aneurysm,7 was associated with ICH, with the A allele being protective against this condition, although the underlying molecular mechanism of this association remains to be characterized.

Intracranial aneurysm, which accounts for most of the incidence of SAH, has a genetic component.31 First-degree relatives of patients with aneurysmal SAH have a risk of experiencing a ruptured intracranial aneurysm that is 14.93 times that of the general population.32 CYP3A4 is expressed in the prostate, breast, gut, colon, and small intestine, but it is most abundant in the liver, where it accounts for 30% of the total CYP protein content.33–35 It exhibits broad substrate specificity and is responsible for oxidation of many therapeutic drugs and a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. In liver microsomes, it contributes to an NADP-dependent electron transport pathway. CYP3A4 plays an important role in the oxidation of both testosterone (2β-, 6β-, or 15β-hydroxylation) and estrogen (4-α- and 16α-hydroxylation).36,37 CYP3A4 is highly polymorphic, with at least 78 genetic variations having been identified.38 Such variation in CYP3A4 may lead to a reduced potential for oxidation of testosterone, increasing the amount of this hormone available for metabolism to the biologically active form of dihydrotestosterone, the principal androgenic hormone responsible for regulation of prostate growth. The 13989A→G

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Overall Frequency Controls</th>
<th>Atherothrombotic Cerebral Infarction</th>
<th>χ² P Value</th>
<th>Permutation P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-G</td>
<td>0.8464</td>
<td>0.8339</td>
<td>0.8869</td>
<td>0.0018</td>
</tr>
<tr>
<td>A-A</td>
<td>0.0985</td>
<td>0.1030</td>
<td>0.0707</td>
<td>0.0095</td>
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<tr>
<td>A-G</td>
<td>0.0544</td>
<td>0.0569</td>
<td>0.0393</td>
<td>0.0636</td>
</tr>
<tr>
<td>G-A</td>
<td>0.0007</td>
<td>0.0003</td>
<td>0.0031</td>
<td>0.0074</td>
</tr>
</tbody>
</table>

Haplotypes consist of the −428G→A (rs710968) and −916G→A (rs6460071) polymorphisms of LIMK1.

Table 4. Genotype Distributions of Polymorphisms Associated With Atherothrombotic Cerebral Infarction, ICH, or SAH Among Controls and Subjects With These Conditions

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Atherothrombotic Cerebral Infarction</th>
<th>ICH</th>
<th>SAH</th>
<th>Controls</th>
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<tbody>
<tr>
<td>ABCA1</td>
<td>−14C→T</td>
<td>CC</td>
<td>54.2</td>
<td>52.4</td>
<td>57.8</td>
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<tr>
<td></td>
<td></td>
<td>CT</td>
<td>37.1</td>
<td>41.0</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>8.8</td>
<td>6.6</td>
<td>4.4</td>
</tr>
<tr>
<td>IRAK1</td>
<td>A→C</td>
<td>AA</td>
<td>12.9</td>
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<tr>
<td></td>
<td></td>
<td>AC</td>
<td>14.4</td>
<td>13.6</td>
<td>21.4</td>
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<tr>
<td></td>
<td></td>
<td>CC</td>
<td>72.7</td>
<td>69.3</td>
<td>67.5</td>
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<tr>
<td>IRAK1</td>
<td>C→T (Ser532Leu)</td>
<td>CC</td>
<td>72.2</td>
<td>69.3</td>
<td>67.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>14.3</td>
<td>13.0</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>13.5</td>
<td>17.8</td>
<td>10.7</td>
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<tr>
<td>ROS1</td>
<td>G→C (Cys2229Ser)</td>
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<td>5.1</td>
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<td></td>
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<td>GC</td>
<td>21.1</td>
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<td>CC</td>
<td>75.0</td>
<td>73.1</td>
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<td>LIMK1</td>
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<tr>
<td>CYP3A4</td>
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<td>2.7</td>
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<td></td>
<td>GG</td>
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</table>

Data are expressed as percentages.
(Ile118Val) polymorphism of CYP3A4 has been associated with the activity of CYP3A4, with the activity in heterozygotes (AG genotype) being lower than that in wild-type homozygotes (AA genotype). We have now shown that the 13989A→G (Ile118Val) polymorphism of CYP3A4 was associated with SAH, with the G allele representing a risk factor for this condition. SAH is 1.6 times more common in women than in men. Hormonal factors likely explain this sex-specific risk, given that it is higher in postmenopausal women than in premenopausal women. The association of the 13989A→G (Ile118Val) polymorphism of CYP3A4 with SAH might thus be attributable to the effect of this polymorphism on the metabolism of sex hormones, although the underlying molecular mechanism remains to be elucidated.

If we set statistical power to 0.001 ($P=0.05/50$) and the difference to detect to 1% in the initial screen by the $\chi^2$ test, the total number of samples required is 827. Given that 2892, 2403, and 2277 samples were analyzed for atherothrombotic cerebral infarction, ICH, and SAH, respectively, there was sufficient statistical power in our study. The stroke patients were recruited from individuals who were admitted to the hospitals or visited outpatient clinics, whereas the control subjects were recruited from individuals who visited outpatient clinics or from community-dwelling individuals. Given that subjects were recruited by different methods, selection bias could not be excluded completely in the present study. It is possible that 1 or more of the polymorphisms associated with each type of stroke in the present study is in linkage disequilibrium with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of these conditions. In addition, the functional relevance of the identified polymorphisms to gene transcription or to protein structure or function was not determined in the present study. Despite these limitations, our present results suggest that ABCA1, IRAK1, and ROS1 are susceptibility loci for atherothrombotic cerebral infarction, and that LIMK1 and CYP3A4 constitute susceptibility loci for ICH and SAH, respectively. Validation of our findings will require their replication with independent subject panels.

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### Disclosures
None.

### References
and plasma lipids in males from three ethnic populations in Singapore. 


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