Human Genome Epidemiology (HuGE) Review

Variants of the Arachidonate 5-Lipoxygenase-Activating Protein (ALOX5AP) Gene and Risk of Stroke: A HuGE Gene-Disease Association Review and Meta-Analysis

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Variants of the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene have been implicated as a risk factor for stroke. However, genetic association studies that have examined the association between ALOX5AP gene variants (HapA haplotype, HapB haplotype, and SG polymorphisms) and stroke have produced conflicting results. Therefore, the authors performed a meta-analysis of all studies with ALOX5AP genotyping (5,194 stroke cases and 4,566 controls). The meta-analysis showed significant heterogeneity among studies ($P = 0.03, I^2 = 63\%)$ and a nonsignificant association between the HapA haplotype ($SG13S25G-SG13S114T-SG13S89G-SG13S32A$) and stroke risk (random-effects (RE) odds ratio (OR) $= 1.13$, 95\% confidence interval (CI): 0.88, 1.45). Regarding the HapB haplotype ($SG13S377A-SG13S114A-SG13S41A-SG13S35G$), there was no association with stroke risk (RE OR $= 1.03$, 95\% CI: 0.77, 1.37). The $SG13S106$, $SG13S377$, and $SG13S42$ polymorphisms were not associated with stroke. The $SG13S106$ and $SG13S377$ polymorphisms revealed evidence of marginal association (RE OR $= 1.23$ (95\% CI: 1.03, 1.46) and RE OR $= 1.25$ (95\% CI: 1.04, 1.50), respectively). However, cumulative meta-analysis for the HapA haplotype showed a downward trend of odds ratios over time, and recursive cumulative meta-analysis indicated insufficient evidence for claiming or denying an association. Tests for bias revealed no evidence of biases. Rigorous genetic association studies investigating gene-gene-environment interactions may generate more conclusive claims about the genetics of stroke.

ALOX5AP; epidemiology; genetics; haplotypes; 5-lipoxygenase-activating protein; meta-analysis; polymorphism, genetic; stroke

Abbreviations: ALOX5AP, arachidonate 5-lipoxygenase-activating protein; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

**Editor’s note:** This article is also available on the Website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm).

GENE

Variants of the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene have been recently shown to be associated with common forms of stroke (1, 2). The ALOX5AP gene is located on chromosome 13q12–13 and is contained in the sequenced region that covers 60 kilobases, including the 5 known exons and introns, the 26-kilobase region 5’ to the first exon, and the 7-kilobase region 3’ to the fifth exon. The ALOX5AP gene encodes 5-lipoxygenase-activating protein. 5-Lipoxygenase-activating protein is a regulator of the leukotriene biosynthetic pathway, which has recently been implicated in the pathogenesis of atherosclerosis (3).

More specifically, biosynthesis of leukotriene is largely confined to leukocytes and can be triggered by a variety of stimuli. In this biosynthetic pathway, unesterified arachidonic acid is converted to leukotriene A4 by the action of 5-lipoxygenase and its activating protein. The unstable epoxide leukotriene A4 is further metabolized to leukotriene
B4 or leukotriene C4 by leukotriene A4 hydrolase or leukotriene C4 synthase, respectively (1, 2). The cysteinyl-containing leukotrienes (leukotriene C4 and its metabolites, leukotrienes D4 and E4) increase vascular permeability in postcapillary venules and are potent vasoconstrictors of brain arteries (3–5). In addition, leukotriene A4 can be exported to neighboring cells that are devoid of 5-lipoxygenase activity and become subject to transcellular leukotriene biosynthesis. The leukotrienes have a variety of proinflammatory effects (3, 4). Leukotriene B4 activates leukocytes, leading to chemotaxis and increased adhesion of leukocytes to vascular endothelium, release of lysosomal enzymes such as myeloperoxidase, and production of superoxide anions (1, 2). Inflammation has been associated with ischemic stroke and its subtypes (6, 7).

GENE VARIANTS

Recent findings have implicated specific polymorphisms of the ALOX5AP gene and 2 at-risk haplotypes (HapA and HapB) in stroke (1, 2). The HapA haplotype is defined by the following single nucleotide polymorphisms (SNPs): SG13S25G (rs17222814, promoter, G/A), SG13S114T (rs10507391, intron 1, T/A), SG13S89G (rs4769874, intron 3, G/A), and SG13S32A (rs9551963, intron 4, C/A). The HapB haplotype is defined by SG13S377A (rs17216473, promoter, G/A), SG13S114A (rs10507391, intron 1, T/A), SG13S41A (rs9315050, intron 5, A/G), and SG13S35G (rs17222842, 3’-untranslated region, G/A). Other relevant variants in ALOX5AP investigated for a possible association with stroke include SG13S106 (rs9579646, intron 1, A/G) and SG13S42 (rs4769060, intron 5, A/G) (8).

Stroke is a polygenic/multifactorial disease, and the genetic basis of the pathophysiologic process of stroke has not yet been deciphered. Thus, single susceptibility gene polymorphisms may have modest effects. ALOX5AP is considered a candidate gene for stroke. However, genetic association studies that have examined whether variants in the ALOX5AP gene are associated with stroke have yielded conflicting or inconclusive results. Although the initial reports (1, 2) claimed an association between ALOX5AP and stroke, subsequent independent efforts failed to replicate the initial findings. The lack of replication might be due to small sample sizes or to the use of different populations, sampling strategies, genotyping procedures, and/or numbers of loci in the studies (9). In order to shed some light on these controversial results and obtain more statistical power to detect smaller effect sizes, a comprehensive meta-analysis of all available studies relating variants of the ALOX5AP gene to the risk of stroke was carried out (9, 10). In addition, heterogeneity between studies and potential bias were explored. Cumulative and recursive cumulative meta-analyses were also performed (9, 11).

DISEASE

Stroke is a leading cause of mortality and morbidity in the Western world, and ischemic stroke represents the most common subtype, accounting for 80% of all strokes (12). In the current meta-analysis, stroke was defined by the presence of a new focal neurologic deficit, with symptoms and signs persisting for more than 24 hours. Stroke was ascertained from blinded review of medical records, autopsy results, and the judgment of a board-certified neurologist on the basis of clinical reports, computed tomography, or magnetic resonance imaging (13).

The multifactorial etiology of the disease is poorly understood, and the underlying pathogenesis is likely to be mediated by both genetic and environmental risk factors. The process of developing stroke is generally attributed to atherosclerosis with arterial wall inflammation that ultimately leads to plaque rupture, fissure, or erosion (7, 14). This process is known to involve diapedesis of monocytes across the endothelial barrier; activation of neutrophils, macrophages, and platelets; and release of a variety of cytokines and chemokines (14). As we noted above, increased 5-lipoxygenase-activating protein activity could lead to the accumulation of leukotrienes in fatty deposits on the arterial wall. The subsequent breakdown of these deposits by the immune system may then lead to the development of atherosclerosis and an increased risk of stroke (15).

META-ANALYSIS METHODS

Identification and eligibility of relevant studies

We identified all studies published before April 2008 through extended computer-based searches of the PubMed, Web of Science, Scopus, and Google Scholar databases. The following search strategy was used: “5-lipoxygenase-activating protein’’ or “ALOX5AP’’ and “stroke.’’ We then read the retrieved studies in their entirety to assess their appropriateness for inclusion in the meta-analysis. We also reviewed all references cited in the studies to identify additional published work that was not indexed by the searched databases. Case reports, editorials, and review articles were excluded. Non-English articles were also excluded.

Case-control studies that determined the distribution of genetic variants in the ALOX5AP gene or the allelic frequency in stroke cases and controls were eligible for inclusion in the meta-analysis. Only variants that were investigated in at least 2 studies were included. In studies with overlapping cases or controls, the most recent and/or largest study with extractable data was included in the meta-analysis. We considered studies carried out in human subjects that had used standard genotyping methods.

Data extraction

From each study, we extracted the following information: first author, journal, year of publication, ethnicity of the study population, age, gender, type of stroke, diagnosis criteria, matching, genotyping method, and numbers of cases and controls for each genetic variant. In addition, we recorded whether the genotypic data had been read with blinding to disease status.

Meta-analysis

In the meta-analysis, we examined the association between each genetic variant and stroke. For each SNP, the
allele contrast and the recessive and dominant models of the allele at risk were tested (9, 10). The associations were expressed as pooled odds ratios with corresponding 95% confidence intervals. Heterogeneity between studies was tested using the $Q$ statistic (16). If $P$ was less than 0.10, the heterogeneity was considered statistically significant. Heterogeneity was quantified with the $I^2$ metric, which is independent of the number of studies in a meta-analysis. $I^2$ was calculated for more than 2 studies. $I^2$ takes values between 0% and 100%, with higher values denoting a greater degree of heterogeneity (17). The pooled odds ratio was estimated using a random-effects (DerSimonian and Laird) model (18). Random-effects modeling assumes a genuine diversity in the results of various studies, and it incorporates between-study variance into the calculations. When there is a lack of heterogeneity, the random-effects model coincides with the fixed-effects model (19).

Cumulative and recursive cumulative meta-analyses were carried out for the allele contrast of variants investigated in more than 5 studies in order to evaluate the trend in odds ratios over time (11). In the cumulative meta-analysis, studies were chronologically ordered by publication year; then the pooled odds ratio was obtained at the end of each year—that is, at each information step (9, 11). In the recursive cumulative meta-analysis, the relative change in the pooled odds ratio at each information step was calculated. Cumulative and recursive cumulative meta-analyses provide a framework for updating data on a genetic effect from all studies and a measure of how much the genetic effect changes as evidence accumulates (9). Thus, cumulative meta-analysis indicates the trend in the estimated risk effect, and recursive cumulative meta-analysis indicates the stability in the risk effect. We checked for a differential magnitude of effect in large studies versus small studies for haplotypes or allele contrasts (of variants included in the cumulative meta-analysis) using the Egger regression test (20) and the Begg-Mazumdar test, which is based on Kendall’s tau (21). The $z$ statistic was used to assess whether the odds ratio from the first study and the pooled odds ratio from subsequent studies differed beyond chance ($P < 0.05$) (9).

The meta-analysis consisted of the main (overall) analysis, which included all available data; subgroup analyses carried out by ethnicity; and sensitivity analysis, which examines the effect of excluding specific studies (9). The genotype distribution in the control group was tested for Hardy-Weinberg equilibrium using an exact test (22). Studies with controls not in Hardy-Weinberg equilibrium were subjected to a sensitivity analysis (9). Analyses were performed using Meta-Analyist software (Joseph Lau, Tufts-New England Medical Center, Boston, Massachusetts, 1998) (http://www.medepi.net/meta/MetaAnalyist.html) and Compaq Visual Fortran 90 (Compaq Computer Corporation, Houston, Texas) with the IMSL Numerical Libraries (Visual Numerics, Inc., Houston, Texas).

**RESULTS**

**Eligible studies and study characteristics**

The literature review identified 20 articles in PubMed that met the search criteria. Five articles were reviews (3, 4, 23–25), 1 article was in Chinese and involved animals (26), and 4 articles examined other phenotypes (27–30). In 2 studies, the subjects overlapped (31, 32); however, Zhang et al. (32) provided separate data for males, and data from that study were used in a subgroup analysis of males. Thus, 10 of these articles met the inclusion criteria (1, 2, 5, 6, 8, 31–35). Web of Science identified 23 articles, and Scopus identified 21; of those, 8 and 9 articles met the inclusion criteria, respectively. These articles had already been identified by PubMed. A further sedulous search in Google Scholar identified 1 extra eligible article (36) that had not been traced by the other databases. There were then 11 articles that met the inclusion criteria (1, 2, 5, 6, 8, 31–36). Finally, Kaushal et al. (6) provided data from 2 separate studies of 2 separate ethnic groups: whites and blacks. Thus, data were obtained from 12 studies in total. The articles had been published between 2004 and 2008. Details abstracted from the studies included in the meta-analysis are provided in Table 1.

Five studies dealt with the $HapA$ haplotype (1, 2, 8, 33, 34) and 3 dealt with $HapB$ (2, 4, 5). Five studies dealt with $SG13S114$ (5, 6, 8, 31, 34), 6 with $SG13S89$ (5, 6, 8, 31, 34, 35), 5 with $SG13S25$ (5, 8, 34–36), 5 with $SG13S35$ (8, 34–36), 2 with $SG13S106$ (6, 8), 2 with $SG13S77$ (8, 36), 2 with $SG13S25$ (8, 36), and 2 with $SG13S42$ (8, 36). Two studies provided genotype data for males (8, 32), and 1 study provided data for both genders (34).

The studies were conducted in various populations with different ethnic groups: 9 involved whites (1, 2, 5, 6, 8, 33–36), 1 blacks (6), 2 East Asians (31, 32), and 1 a mixed population (8). For 7 studies, the investigators specified that all cases were patients with ischemic stroke (2, 5, 6, 8, 35, 36). The investigators in 5 studies stated that controls were matched for age and/or gender (6, 8, 31, 34, 36). In 3 studies, the investigators provided results for stroke subtypes (6, 31, 36).

In all studies, standard genotyping methods (polymerase chain reaction followed by restriction enzyme digestion, fluorescent polarization template-directed dye-terminator incorporation, polymerase chain reaction with allele-specific extension products, or the immobilized probe approach) had been used for the determination of genetic polymorphisms. For 3 studies, investigators reported that $ALOX5AP$ gene polymorphisms were in linkage disequilibrium (1, 8, 34). However, in data from the International HapMap Project (http://www.hapmap.org/), there is evidence that the available polymorphisms are in linkage disequilibrium, with the exception of rs10507391 versus rs9551963 and rs9315048 versus rs9315050 (see linkage disequilibrium plot in the Web Figure, which is posted on the Journal’s Web site (http://aje.oxfordjournals.org/)).

**Summary statistics, studies’ associations, and Hardy-Weinberg equilibrium**

Overall, the studies provided 2,727 cases and 3,038 controls for analysis of $HapA$, 1,411 cases and 1,593 controls for $HapB$, 3,394 cases and 3,270 controls for $SG13S114$, 4,268 cases and 4,175 controls for $SG13S89$, 3,015 cases and 2,518 controls for $SG13S25$, 3,122 cases and 2,532...
<table>
<thead>
<tr>
<th>First Author and Year (Ref. No.)</th>
<th>Study Area and Ethnic Group</th>
<th>Genetic Variant Included in Meta-Analysis</th>
<th>Significant Results Under Any Genetic Contrast?</th>
<th>Frequency of Haplotype or Risk Allele, %</th>
<th>Single Nucleotide Polymorphism in Hardy-Weinberg Equilibrium?</th>
<th>Selection and Characteristics of Cases With Stroke</th>
<th>Selection and Characteristics of Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helgadottir, 2004 (1)</td>
<td>Iceland, whites</td>
<td>HapA</td>
<td>Yes</td>
<td>15.0</td>
<td>Yes</td>
<td>702 patients with all forms of stroke; M/F ratio: 373/339; free of myocardial infarction; diagnosis based on signs, symptoms, electrocardiograms, cardiac enzymes, and necropsy findings</td>
<td>624 population-based controls; males and females aged 20–90 years; unknown medical history</td>
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<td>HapB</td>
<td>No</td>
<td>7.0</td>
<td>Yes</td>
<td>450 patients with ischemic stroke; M/F ratio: 261/189; mean age = 66.8 years (SD, 0.6); 55% with hypertension; diagnosis based on CT</td>
<td>710 controls with no history of stroke or transient ischemic attack; M/F ratio: 362/347; mean age = 67.2 years (SD, 0.4); 24% with hypertension</td>
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<td>Helgadottir, 2005 (2)</td>
<td>Scotland, whites</td>
<td>HapA</td>
<td>No</td>
<td>18.4</td>
<td>Yes</td>
<td>639 patients with stroke (601 ischemic and 38 hemorrhagic); M/F ratio: 403/236; mean age = 65 years (SD, 1.2); diagnosis based on CT, MRI, echocardiography, and magnetic resonance angiography</td>
<td>736 population-based controls; M/F ratio: 447/289; mean age = 62 years (SD, 11.7); matched by age and gender</td>
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<td>HapB</td>
<td>No</td>
<td>6.9</td>
<td>Yes</td>
<td>357 patients with ischemic stroke; 252 whites (M/F ratio: 229/128; 45% with hypertension) and 105 blacks (M/F ratio: 41/64; 82% with hypertension); diagnosis based on medical records</td>
<td>303 population-based controls; 232 whites (M/F ratio: 112/120; 47% with hypertension) and 71 blacks (M/F ratio: 31/40; 67% with hypertension); matched by age, ethnicity, and gender</td>
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<tr>
<td>Löhmussaar, 2005 (34)</td>
<td>Germany, whites</td>
<td>HapA</td>
<td>Yes</td>
<td>7.6</td>
<td>Yes</td>
<td>259 patients with ischemic stroke; mean age = 61 years (SD, 0.3); all males; 47% with hypertension; diagnosis based on clinical reports, CT, and MRI</td>
<td>259 controls matched by age; mean age = 60.8 years (SD, 0.3); 29% with hypertension; controls were matched by age, smoking history, and length of follow-up</td>
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<td>SG13S25</td>
<td>No</td>
<td>89.4</td>
<td>Yes</td>
<td>3,993 patients with stroke (1,172 were genotyped); mean age = 60.4 years (SD, 4.5); M/F ratio: 1,200/993; 63% with hypertension; diagnosis based on CT and MRI</td>
<td>1,889 controls (1,713 were genotyped); mean age = 59.6 years (SD, 8.5); M/F ratio: 1,085/806; 26% with hypertension; matched by age</td>
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<td>SG13S114</td>
<td>No</td>
<td>68.5</td>
<td>Yes</td>
<td>377 patients with ischemic stroke; M/F ratio: 202/175; mean age = 64.8 years (SD, 1.5); 69% with hypertension; diagnosis based on WHO criteria, CT, and MRI</td>
<td>263 controls; M/F ratio: 100/165; mean age = 60 years (SD, 14.7); 39% with hypertension</td>
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<td>Kaushal, 2007 (6)</td>
<td>United States (Kentucky), whites and blacks</td>
<td>SG13S106</td>
<td>Yes (whites)</td>
<td>72.6</td>
<td>Yes</td>
<td>259 patients with ischemic stroke; mean age = 61 years (SD, 0.3); all males; 47% with hypertension; diagnosis based on clinical reports, CT, and MRI</td>
<td>259 controls matched by age; mean age = 60.8 years (SD, 0.3); 29% with hypertension; controls were matched by age, smoking history, and length of follow-up</td>
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<td>SG13S114</td>
<td>No (whites)</td>
<td>26.2</td>
<td>Yes</td>
<td>303 population-based controls; 232 whites (M/F ratio: 112/120; 47% with hypertension) and 71 blacks (M/F ratio: 31/40; 67% with hypertension); matched by age, ethnicity, and gender</td>
<td>303 population-based controls; 232 whites (M/F ratio: 112/120; 47% with hypertension) and 71 blacks (M/F ratio: 31/40; 67% with hypertension); matched by age, ethnicity, and gender</td>
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<td></td>
<td>SG13S89</td>
<td>No (whites)</td>
<td>94.7</td>
<td>Yes</td>
<td>259 patients with ischemic stroke; mean age = 61 years (SD, 0.3); all males; 47% with hypertension; diagnosis based on clinical reports, CT, and MRI</td>
<td>259 controls matched by age; mean age = 60.8 years (SD, 0.3); 29% with hypertension; controls were matched by age, smoking history, and length of follow-up</td>
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<td>SG13S32</td>
<td>No (whites)</td>
<td>46.9</td>
<td>Yes</td>
<td>303 population-based controls; 232 whites (M/F ratio: 112/120; 47% with hypertension) and 71 blacks (M/F ratio: 31/40; 67% with hypertension); matched by age, ethnicity, and gender</td>
<td>303 population-based controls; 232 whites (M/F ratio: 112/120; 47% with hypertension) and 71 blacks (M/F ratio: 31/40; 67% with hypertension); matched by age, ethnicity, and gender</td>
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<td>Zee, 2006 (8)</td>
<td>United States, whites</td>
<td>HapA</td>
<td>Yes</td>
<td>5.6</td>
<td>Yes</td>
<td>1,893 patients with stroke (1,172 were genotyped); mean age = 60.4 years (SD, 4.5); M/F ratio: 1,200/993; 63% with hypertension; diagnosis based on CT and MRI</td>
<td>1,891 controls (1,713 were genotyped); mean age = 59.6 years (SD, 8.5); M/F ratio: 1,085/806; 26% with hypertension; matched by age</td>
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<td>HapB</td>
<td>No</td>
<td>6.9</td>
<td>Yes</td>
<td>377 patients with ischemic stroke; M/F ratio: 202/175; mean age = 64.8 years (SD, 1.5); 69% with hypertension; diagnosis based on WHO criteria, CT, and MRI</td>
<td>263 controls; M/F ratio: 100/165; mean age = 60 years (SD, 14.7); 39% with hypertension</td>
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<td>Shen, 2007 (31)</td>
<td>China, East Asians</td>
<td>SG13S114</td>
<td>No</td>
<td>70.6</td>
<td>Yes</td>
<td>1,893 patients with stroke (1,172 were genotyped); mean age = 60.4 years (SD, 4.5); M/F ratio: 1,200/993; 63% with hypertension; diagnosis based on CT and MRI</td>
<td>1,891 controls (1,713 were genotyped); mean age = 59.6 years (SD, 8.5); M/F ratio: 1,085/806; 26% with hypertension; matched by age</td>
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<td>SG13S89</td>
<td>No</td>
<td>5.0</td>
<td>Yes</td>
<td>377 patients with ischemic stroke; M/F ratio: 202/175; mean age = 64.8 years (SD, 1.5); 69% with hypertension; diagnosis based on WHO criteria, CT, and MRI</td>
<td>263 controls; M/F ratio: 100/165; mean age = 60 years (SD, 14.7); 39% with hypertension</td>
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<td>Meschia, 2005 (5)</td>
<td>United States, mixed (74% whites)</td>
<td>SG13S25</td>
<td>No</td>
<td>91.2</td>
<td>Yes</td>
<td>1,893 patients with stroke (1,172 were genotyped); mean age = 60.4 years (SD, 4.5); M/F ratio: 1,200/993; 63% with hypertension; diagnosis based on CT and MRI</td>
<td>1,891 controls (1,713 were genotyped); mean age = 59.6 years (SD, 8.5); M/F ratio: 1,085/806; 26% with hypertension; matched by age</td>
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<td>SG13S114</td>
<td>No</td>
<td>57.8</td>
<td>Yes</td>
<td>377 patients with ischemic stroke; M/F ratio: 202/175; mean age = 64.8 years (SD, 1.5); 69% with hypertension; diagnosis based on WHO criteria, CT, and MRI</td>
<td>263 controls; M/F ratio: 100/165; mean age = 60 years (SD, 14.7); 39% with hypertension</td>
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<td>SG13S32</td>
<td>No</td>
<td>50.8</td>
<td>Yes</td>
<td>377 patients with ischemic stroke; M/F ratio: 202/175; mean age = 64.8 years (SD, 1.5); 69% with hypertension; diagnosis based on WHO criteria, CT, and MRI</td>
<td>263 controls; M/F ratio: 100/165; mean age = 60 years (SD, 14.7); 39% with hypertension</td>
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<td>SG13S89</td>
<td>Yes</td>
<td>12.6</td>
<td>Yes</td>
<td>1,893 patients with stroke (1,172 were genotyped); mean age = 60.4 years (SD, 4.5); M/F ratio: 1,200/993; 63% with hypertension; diagnosis based on CT and MRI</td>
<td>1,891 controls (1,713 were genotyped); mean age = 59.6 years (SD, 8.5); M/F ratio: 1,085/806; 26% with hypertension; matched by age</td>
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controls for \( SG13S32 \), 617 cases and 562 controls for \( SG13S32 \), 975 cases and 1,025 controls for \( SG13S377 \), 1,017 cases and 1,070 controls for \( SG13S35 \), and 1,094 cases and 1,153 controls for \( SG13S42 \). Investigators in 4 studies (1, 35, 36) provided data on allele frequencies only. The prevalences of \( HapA \) and \( HapB \) haplotypes in cases/controls were 15%/14% and 7%/7%, respectively. The prevalences of alleles \( SG13S114T \), \( SG13S89G \), \( SG13S25G \), \( SG13S32A \), \( SG13S106A \), \( SG13S77G \), \( SG13S35G \), and \( SG13S42A \) in cases/controls were 66%/65%, 37%/39%, 90%/90%, 49%/49%, 49%/45%, 88%/85%, 92%/91%, and 56%/57%, respectively.

Individual studies produced significant results for the \( HapA \) haplotype (1), the \( SG13S106 \) allele contrast (6), the \( SG13S89 \) allele contrast (5, 6), and the \( SG13S89 \)-dominant model (5) (Table 1). For \( SG13S89 \), the distribution of genotypes in the control group deviated from Hardy-Weinberg equilibrium in 2 analyses (mixed ethnicities and whites) by Meschia et al. (5) (Table 1). Since lack of Hardy-Weinberg equilibrium indicates possible genotyping error and/or population stratification, a sensitivity analysis was carried out excluding the study by Meschia et al. (5).

Meta-analysis main results and subgroup and sensitivity analyses

Figure 1 and Table 2 show results for the association between the different genetic variants and the risk of stroke.

The meta-analysis investigating the association between the \( HapA \) haplotype and stroke revealed significant heterogeneity among studies (\( P_Q = 0.03, I^2 = 63% \)) and a non-significant association between the \( HapA \) haplotype and risk of stroke on the basis of the current evidence (odds ratio (OR) = 1.13, 95% confidence interval (CI): 0.88, 1.45). For the \( HapB \) haplotype, there was no association with risk of stroke (OR = 1.03, 95% CI: 0.77, 1.37; \( P_Q = 0.67, I^2 = 0% \)).

For the \( SG13S114 \) polymorphism and its association with stroke, the allele contrast showed a lack of significant heterogeneity among studies (\( P_Q = 0.39, I^2 = 4% \)), and the pooled odds ratio was nonsignificant (OR = 1.03, 95% CI: 0.95, 1.11). In subgroup analysis, results for whites and males were also nonsignificant (OR = 1.06 (95% CI: 0.91, 1.22) and OR = 1.02 (95% CI: 0.86, 1.21), respectively). The recessive and dominant models for the effect of allele \( T \) produced patterns similar to those of the allele contrast, overall and for whites.

However, regarding the association between the \( SG13S89 \) polymorphism and stroke, the allele contrast showed heterogeneity among studies (\( P_Q < 0.01, I^2 = 86% \)). The analysis showed nonsignificant association overall (OR = 1.25, 95% CI: 0.85, 1.86) and in whites (OR = 1.27, 95% CI: 0.69, 2.33), though the effect size was relatively large. The recessive and dominant models also showed a lack of significant association. The sensitivity analysis (excluding the study with controls not in Hardy-Weinberg equilibrium (5)) did not alter the pattern of results.

The \( SG13S25 \) and \( SG13S32 \) polymorphisms were not associated with stroke overall or in whites, for the contrasts
under investigation. In addition, there was a lack of significant heterogeneity among studies \((P_Q > 0.50, I^2 = 0\%).

For the \textit{SG13S106} polymorphism, the heterogeneity among studies was nonsignificant \((P_Q = 0.43, I^2 = 0\%)\), and there was evidence of marginal association overall \((OR = 1.23, 95\% CI: 1.03, 1.46)\) and in whites \((OR = 1.25, 95\% CI: 1.00, 1.56)\); however, the findings were based on only 3 studies. In addition, the \textit{SG13S377} polymorphism showed a marginal association \((OR = 1.25, 95\% CI: 1.04, 1.50)\). The \textit{SG13S35} and \textit{SG13S42} polymorphisms produced nonsignificant associations.

### Bias diagnostics

For 2 studies, investigators reported that genotyping was blinded to clinical status \((5, 8)\). A sensitivity analysis for these studies did not alter the pattern of results for any variant \((\textit{HapA}: OR = 1.20, 95\% CI: 0.93, 1.56; \textit{HapB}: OR = 1.08, 95\% CI: 0.78, 1.48; \textit{GS13S114}: OR = 1.04, 95\% CI: 0.93, 1.17; \textit{SG13S89}: OR = 1.28, 95\% CI: 0.75, 2.17; \textit{SG13S25}: OR = 0.96, 95\% CI: 0.83, 1.11; \textit{SG13S2A}: OR = 0.97, 95\% CI: 0.88, 1.06)\).

Cumulative meta-analysis for the \textit{HapA} haplotype showed a downward trend in odds ratios for the period 2004–2007: The odds ratios declined monotonically from 1.68 in 2004 \((\text{first study (1)})\) to 1.13 in 2007 \((\text{Figure 2})\). In recursive cumulative meta-analysis, the relative change in odds ratios did not stabilize in a specific odds ratio \((0.77 \text{ in 2005/2004, 0.91 in 2006/2005, 0.97 in 2007/2006})\), indicating that there was no sufficient evidence for claiming or denying an association. Only the first study \((1)\) produced a significant association; the rest of the studies did not replicate this finding. There was a significant difference between the odds ratio from the first study \((OR = 1.68, 95\% CI: 1.19, 2.41)\) and the pooled odds ratio from the subsequent studies \((OR = 1.03, 95\% CI: 0.84, 1.27)\) \((P = 0.04)\). The Egger test and the Beg-Mazumdar test indicated that there was no differential magnitude of effect in large studies versus small studies \((P = 0.70 \text{ and } P = 0.82, \text{ respectively})\).

For the \textit{SG13S114} polymorphism, none of the individual studies produced a significant association. Cumulative and recursive cumulative meta-analyses showed a decline in odds ratios over the period 2005–2007 \((\text{from 1.07 in 2005 to 1.03 in 2007})\), and the relative change in the odds ratio was 0.96, indicating a lack of association for this period based on the current evidence. On the contrary, for the \textit{SG13S89} polymorphism, in cumulative meta-analysis the odds ratio increased from 1.05 in 2005 to 1.40 in 2007 and then declined to 1.25 in 2008; however, the relative change in the odds ratio was not stable \((0.98 \text{ in 2006/2005, 1.36 in 2007/2006, and 0.89 in 2008/2007})\), indicating the need for more evidence to support an association. For both polymorphisms, the Egger test and the Beg-Mazumdar test indicated that there was no differential magnitude of effect in large studies versus small studies \((P > 0.34 \text{ and } P > 0.24, \text{ respectively})\). For the \textit{SG13S32} polymorphism, cumulative meta-analysis showed that the odds ratio remained stable \((\text{ranging from 0.96 to 0.98})\) during the period 2005–2008. The Egger test and the Beg-Mazumdar test showed nonsignificant results \((P = 0.38 \text{ and } P = 0.82, \text{ respectively})\).

### DISCUSSION

In the present meta-analysis, we examined polymorphisms and haplotypes in the \textit{ALOX5AP} gene and their relation with the risk of stroke. The strength of the present analysis was the accumulation of published data, providing more information with which to detect significant differences. In the main analysis and in whites, gene polymorphisms and haplotypes produced nonsignificant results, and heterogeneity ranged from none to high. The pooled odds ratio for the \textit{HapA} haplotype showed that if there is an effect, it is unlikely to be larger than 45%. Only the \textit{SG13S106} polymorphism indicated a marginal association, but the result was based on only 3 studies. On the basis of currently available data and the meta-analysis results, genetic testing for...
ALOX5AP variations cannot be applied in the general population in order to predict the risk of stroke.

In this meta-analysis, the tests for bias that were performed revealed no evidence of any biases; however, these tests may have been underpowered, since they were based on a small number of studies. The results from such a meta-analysis should therefore be treated with caution. However, independent studies produced very different results, and hypothesis-generating findings were not replicated across several studies. The meta-analysis showed large heterogeneity between studies, which might be due to genuine differences in the studied populations and to differences in study design and conduct. When heterogeneity between studies exists, the results should be interpreted in the context of cumulative and recursive cumulative meta-analysis (9, 11). In investigating the HapA haplotype, the cumulative meta-analysis showed a downward trend in association and instability in the relative change of the odds ratio. Thus, it is evident that existing research cannot establish an association and more evidence is required. In addition, the design and conduct of individual studies should be as homogeneous as possible (9).

The overall lack of association between the variants in the ALOX5AP gene and risk of stroke and the discrepancy between results on various ethnic groups might be due to other, unidentified functional mutations in the ALOX5AP gene that affect susceptibility to stroke and to frequency differences in minor alleles and haplotypes. In addition, other polymorphisms involved in the leukotriene pathway may affect the risk of developing stroke. However, the individual polymorphisms are in linkage disequilibrium, and interaction of the polymorphisms within haplotypes could be a bigger determinant of disease susceptibility than the individual polymorphism (9, 37). Thus, individual SNPs might not be reliable markers for stroke risk.

### Table 2. Random-Effects Odds Ratios for Stroke Risk and Heterogeneity Results ($I^2$ and $P_Q$ Values) for Genetic Contrasts of Variants in the Arachidonate 5-Lipoxigenase-Activating Protein (ALOX5AP) Gene

<table>
<thead>
<tr>
<th>Marker (Risk Allele)</th>
<th>Population (Risk Allele)</th>
<th>No. of Studies</th>
<th>OR</th>
<th>95% CI</th>
<th>$I^2$ %</th>
<th>$P_Q$ Value</th>
<th>No. of Studies</th>
<th>OR</th>
<th>95% CI</th>
<th>$I^2$ %</th>
<th>$P_Q$ Value</th>
<th>No. of Studies</th>
<th>OR</th>
<th>95% CI</th>
<th>$I^2$ %</th>
<th>$P_Q$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS13S114 (T)</td>
<td>All</td>
<td>6</td>
<td>1.03</td>
<td>0.95, 1.11</td>
<td>4</td>
<td>0.39</td>
<td>3</td>
<td>1.03</td>
<td>0.91, 1.15</td>
<td>0</td>
<td>0.64</td>
<td>3</td>
<td>0.96</td>
<td>0.89, 1.05</td>
<td>0</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>4</td>
<td>1.06</td>
<td>0.91, 1.22</td>
<td>36</td>
<td>0.20</td>
<td>2</td>
<td>1.02</td>
<td>0.81, 1.30</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>0.94</td>
<td>0.80, 1.09</td>
<td>15</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>3</td>
<td>1.02</td>
<td>0.86, 1.21</td>
<td>48</td>
<td>0.15</td>
<td>2</td>
<td>0.94</td>
<td>0.80, 1.09</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>All in Hardy-Weinberg equilibrium</td>
<td>6</td>
<td>1.22</td>
<td>0.78, 1.93</td>
<td>87</td>
<td>0.01</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
<td>2</td>
<td>1.11</td>
<td>0.52, 2.32</td>
<td>87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SG13S89 (G)</td>
<td>All</td>
<td>7</td>
<td>1.25</td>
<td>0.85, 1.86</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
<td>2</td>
<td>1.11</td>
<td>0.52, 2.32</td>
<td>87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>5</td>
<td>1.07</td>
<td>0.86, 1.32</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.02</td>
<td>0.81, 1.30</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>0.94</td>
<td>0.80, 1.09</td>
<td>15</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>3</td>
<td>1.02</td>
<td>0.86, 1.21</td>
<td>48</td>
<td>0.15</td>
<td>2</td>
<td>0.94</td>
<td>0.80, 1.09</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Alleles in Hardy-Weinberg equilibrium</td>
<td>6</td>
<td>1.22</td>
<td>0.78, 1.93</td>
<td>87</td>
<td>0.01</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
<td>2</td>
<td>1.11</td>
<td>0.52, 2.32</td>
<td>87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SG13S32 (A)</td>
<td>All</td>
<td>5</td>
<td>0.98</td>
<td>0.91, 1.06</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.00</td>
<td>0.73, 1.36</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>0.96</td>
<td>0.71, 1.36</td>
<td>87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>5</td>
<td>0.98</td>
<td>0.91, 1.06</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.00</td>
<td>0.73, 1.36</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>0.96</td>
<td>0.71, 1.36</td>
<td>87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>3</td>
<td>1.02</td>
<td>0.86, 1.21</td>
<td>48</td>
<td>0.15</td>
<td>2</td>
<td>0.94</td>
<td>0.80, 1.09</td>
<td>NA</td>
<td>0.99</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Alleles in Hardy-Weinberg equilibrium</td>
<td>6</td>
<td>1.22</td>
<td>0.78, 1.93</td>
<td>87</td>
<td>0.01</td>
<td>2</td>
<td>1.20</td>
<td>0.60, 2.41</td>
<td>15</td>
<td>0.31</td>
<td>2</td>
<td>1.11</td>
<td>0.52, 2.32</td>
<td>87</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SG13S106 (A)</td>
<td>All</td>
<td>3</td>
<td>1.23</td>
<td>1.03, 1.46</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.25</td>
<td>1.04, 1.50</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Whites</td>
<td>2</td>
<td>1.25</td>
<td>1.04, 1.50</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SG13S357 (G)</td>
<td>Whites</td>
<td>2</td>
<td>1.25</td>
<td>1.04, 1.50</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SG13S35 (G)</td>
<td>Whites</td>
<td>2</td>
<td>1.25</td>
<td>1.04, 1.50</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SG13S42 (A)</td>
<td>Whites</td>
<td>2</td>
<td>1.25</td>
<td>1.04, 1.50</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
<td>2</td>
<td>1.15</td>
<td>0.88, 1.49</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio.

**Figure 2.** Random-effects pooled odds ratio at the end of each year-information step in a cumulative meta-analysis of the relation between the HapA haplotype of the arachidonate 5-lipoxigenase-activating protein (ALOX5AP) gene and risk of stroke. Bars, 95% confidence interval.
Although the authors of the first published study (1) reported linkage at the locus of the ALOX5AP gene and a significant association between the HapA haplotype and stroke, subsequent studies failed to replicate the initial findings. In meta-analysis, frequently the results of a first study do not correlate with the results of subsequent studies, and usually the first study tends to overestimate the magnitude of an association (9). This can be due to bias or to diversity of the populations involved in the studies (38). The first study that showed an association was carried out in Iceland, which has an isolated population; therefore, the ALOX5AP gene may play a lesser role in other white populations because of different population linkage disequilibrium structures (8). Discrepant results could also be due to other loci that may affect genetic susceptibility to stroke which are also in linkage with the examined variants in the ALOX5AP gene. Moreover, other complex mechanisms and environmental interactions may play a role in the pathogenesis of stroke (3, 39). Many environmental factors, such as age, obesity, hypertension, alcohol, smoking, hyperlipidemia, etc., may be related to increased risk of stroke (31, 40). Despite difficulties in study design and assessment of exposures, such parameters should be incorporated into future studies and meta-analyses (38).

The association between ALOX5AP variants and the risk of myocardial infarction is also being investigated; however, results have been contradictory (1, 8, 30, 42), and more studies will be required in order to draw safe conclusions. In particular, for the HapA haplotype, 1 study claimed an association (1) whereas others did not (8, 30, 42). For the HapB haplotype, 2 studies provided evidence of an association (1, 30) and 2 studies showed a lack of association (8, 42).

In the present meta-analysis, 1 study was excluded because it was published in Chinese (26); however, that study involved animals. In conducting a meta-analysis, the investigator should make an effort to include all published studies and to search for unpublished work. When non-English, nonindexed, and nonpublished studies are not reviewed, bias is introduced (42, 43). Usually, articles with significant results are more likely to get published, especially in English-language indexed journals, whereas articles with negative findings are more likely to be published in local journals, which are often nonindexed (43). It has been reported, at least for controlled trials, that exclusion of non-English studies may not have an effect on pooled estimates (44, 45) and that non-English studies are of lower methodological quality (42).

The search for susceptibility loci has probably been complicated by the increased number of contributing loci and susceptibility alleles (8). Furthermore, the published studies reported on different sets of SNPs and haplotypes, making direct comparisons across studies difficult in the context of the meta-analysis. However, elucidating the pathogenesis of stroke would demand investigation of association for many genetic variants of genes which constitute distinct pathophysiologic pathways (7, 39).

Elucidation of the genetics of stroke largely relies on the designing and undertaking of rigorous genetic association studies. So far, differences in study design may have led to the conflicting results in the literature (6). Future studies should be planned with the idea of the results’ being incorporated with those of other, similar studies into a meta-analysis. Meta-analysis also offers the opportunity to place each study in the context of all others and to examine why investigators in different studies reach different conclusions (38, 46, 47).

In addition to candidate gene approaches, genomic expression analyses can assist in the selection of candidate variants by identifying the most active genes involved in the disease’s pathophysiology (48, 49). Then, a possible “genomic convergence” of data sourcing from different research venues may provide further insights into the molecular mechanisms involved in stroke (50).

For most meta-analyses, sample sizes of individual studies tend to be small. Low statistical power and epistatic interactions are expected to produce a high false-discovery rate. Synthesis of data from many studies would be expected to improve power and reduce the false-discovery rate in all circumstances, and the gain could be considerable, unless there were very large genuine between-study heterogeneity (51). However, power calculations are usually considered inappropriate in meta-analysis, since these data are already assembled (9).

In summary, the accumulated evidence indicates a lack of association between variants in the ALOX5AP gene and risk of stroke. The results of the present meta-analysis were based on relatively small numbers of studies; consequently, their interpretation should be cautious. Therefore, the relation between ALOX5AP and stroke remains an unresolved issue. The results of long-term prospective and case-control studies (52, 53), designed for the investigation of gene-gene and gene-environment interactions and utilizing the amount of data being generated by genomic studies, might produce more conclusive claims about the genetics of stroke.

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