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ORIGINAL ARTICLE

# Ischemic stroke risk in a southeastern Chinese population: Insights from 5-lipoxygenase activating protein and phosphodiesterase 4D single-nucleotide polymorphisms

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*Background/Purpose:* Through a genome-wide linkage scan, an Icelandic genetic research group identified two new genes associated with ischemic stroke: the *5-lipoxygenase activating protein* (*ALOX5AP*) gene and the *phosphodiesterase 4D* (*PDE4D*) gene. Because they regulate arterial inflammation and are closely related to atherosclerosis and plaque instability, these two mutated genes have become a research hotspot. The purpose of this study was to investigate the association between the risk of ischemic stroke and single-nucleotide polymorphisms (SNPs) in the *ALOX5AP* and *PDE4D* genes in a southeastern Chinese population.
*Methods:* A total of 459 patients with stroke and 462 control individuals were recruited in the study. Four ALOX5AP SNPs (SG13S32, SG13S42, SG13S89, and SG13S114), and three PDE4D SNPs (SNP83, SNP87, and SNP45) were studied. SNP genotypes were determined by polymerase chain reaction amplification followed by allele-specific primer extension, with detection by matrix-assisted laser desorption/ionization time-of-flight. Data were coded and entered in SPSS Windows (version 16.0). Odds ratios and 95% confidence intervals were calculated using multivariate logistic regression analysis. Generalized multifactor dimensionality reduction (GMDR) analysis was applied to detect gene–gene interactions.

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*Results:* No statistically significant differences were found in the SNP genotype frequencies between cases and controls for the seven SNPs studied. GMDR analysis revealed no evidence of interactions between these seven polymorphic sites and an increased stroke risk. In addition, no association between different stroke types and the control group was detected. Results showed that only the *ALOX5AP* gene, and specifically the rs9551963 and rs4769060 genotypes, exhibited significantly different distributions between the stroke and control groups in female participants.
*Conclusion:* No association was found between SNPs of ALOX5AP or PDE4D and the risk of overall ischemic stroke in a southeastern Chinese population. Interactions between these two genes were not risk factors for cerebral infarction. In atherothrombotic and small-artery disease subtypes, none of the seven SNPs was associated with any stroke risk; however, the *ALOX5AP* gene might be related to ischemic stroke incidence in females.


## Introduction

Cerebrovascular disease is a significant cause of death and disability in the Chinese population, with the incidence of ischemic stroke accounting for 85—90% of all cerebrovascular diseases.[1,2] Ischemic stroke has multiple etiologies and a varying pathogenesis. Predisposition to ischemic stroke is influenced by various environmental, lifestyle, and genetic factors. It is likely the result of the combined effect of multiple gene—gene and gene—environment interactions. Stroke prevention requires the determination of risk factors and interventions.[3]

Genetic susceptibility to ischemic stroke was first shown in twin pedigree studies in 1992.[4] Jerrard-Dunne et al[5] found that a family history of stroke increased the risks of large-artery atherosclerosis cerebral infarction and small-artery occlusion cerebral infarction by 2.93 and 3.15 times, respectively, in patients ≤65 years of age. This finding suggests a genetic component to stroke risk. Inflammation also plays a key role in atherosclerosis and stroke.[6] Arterial stenosis and atherosclerotic plaque instability are two causes of cerebral infarction. Genetic variations in the components of the inflammatory response have been implicated as stroke risk factors, particularly when combined with conventional proinflammatory risk factors.[7,8]

ALOX5AP encodes 5-lipoxygenase-activating protein, which activates arachidonate 5-lypoxygenase, leading to leukotriene synthesis.[9] Leukotrienes are secreted by various inflammatory cell types that cluster at injured sites in blood vessels and have been implicated in the progression of atherosclerosis.[10] A linkage study identified variations in ALOX5AP to be associated with susceptibility to myocardial infarction.[11] A subsequent study of an Icelandic population found that a four-SNP ALOX5AP haplotype (termed HapA) doubled the risk of coronary heart disease and incidence of stroke.[12]

PDE4D encodes phosphodiesterase 4D, which is a member of a large superfamily of cyclic nucleotide phosphodiesterases. Phosphodiesterase 4D degrades the second messengers cyclic adenosine monophosphate and cyclic guanosine monophosphate, which are the main signal transduction molecules found in many different cell types, including inflammatory, vascular endothelial, and smooth muscle cells.[13,14] Gretarsdottir et al[15] found that certain SNP haplotypes of PDE4D were associated with carotid and cardiogenic strokes, but not with other stroke subtypes. However, thus far, studies relating *ALOX5AP* and *PDE4D* gene polymorphisms with the risk of ischemic stroke in various populations have yielded widely variable conclusions. Most studies have focused on the ALOX5AP HapA and HapB haplotypes and stroke risk in general, with little focus on stroke subtypes or sex comparisons.

Many other genes are being studied for their potential involvement in stroke risk, including *ACE*, *MTHFR*, *NOTCH3*, *β-Fg*, *ApoE*, and *CYP2C9*. However, *ALOX5AP* and *PDE4D* seem to be most closely related to atherosclerosis because they are both involved in the inflammatory process.

The purpose of this study was to evaluate the candidacy of *ALOX5AP* and *PDE4D* as stroke susceptibility genes in a large population of stroke patients and matched controls from southeastern China, using single-marker association tests and a case—control design. Prior results from Chinese populations have not yielded clear findings.[16—18] Moreover, the gene testing methodology was not uniform, making comparisons difficult. The key points for these association studies between the two genes and ischemic stroke depend on sample size, stroke type, control individuals, and which SNPs are tested. We aimed to determine the single-nucleotide polymorphism (SNP) genotypes of ALOX5AP and PDE4D that are associated with increased risks of atherothrombotic (AT) and small-artery disease (SAD) ischemic strokes in a southeastern Chinese population.

## Materials and methods

### Study populations

This study was approved by the Ethical Committee of the Third Affiliated Hospital of Wenzhou Medical College (Zhejiang, China). Informed consent was obtained from each patient prior to study enrollment. The study population comprised 459 stroke patients and 462 controls who were consecutively admitted to the Department of Neurology of the Third Affiliated Hospital of Wenzhou Medical College between March 2010 and November 2012. All participants were Chinese Han in origin and were unrelated. Exclusion criteria included arteritis, infection, tumors, blood diseases, serious cardiopulmonary, liver or kidney diseases, thyroid diseases, and autoimmune diseases.

**Table 1** Amplification and allele-specific extension primers used to genotype ALOX5AP and PDE4D SNPs.

| SNP | Amplification primers | Extension primer |
|---|---|---|
| ALOX5AP | | |
| SG13S32 (rs9551963) | F:5′-ACGTTGGATGAGTTCTTGACCTCACCAACC-3′<br>R:5′-ACGTTGGATGGGGTTCAAGAGAGAAATTCC-3′ | ACTGGGGAAGGATCTCATC |
| SG13S42 (rs4769060) | F:5′-ACGTTGGATGCGTGGTAATGGGTTTTGAGG-3′<br>R:5′-ACGTTGGATGGAAGGGTAGAAGTGTCTCAG-3′ | TGAACTTATTTCAAACCCAAG |
| SG13S89 (rs4769874) | F:5′-ACGTTGGATGTTTCAGGCATGCTCTGCACC-3′<br>R:5′-ACGTTGGATGCACCAGGGAGCAAGCATTAG-3′ | GGATTAGCAATGCATTATCACA |
| SG13S114 (rs10507391) | F:5′-ACGTTGGATGTCCAGATGTATGTCCAAGCC-3′<br>R:5′-ACGTTGGATGCTCTTAAGGTAGGTCTATGG-3′ | GCCTCTCTTTGCAATTCTA |
| PDE4D | | |
| SNP83 (rs966221) | F:5′-ACGTTGGATGGTCCTGCTATTAATAGAAAC-3′<br>R:5′-ACGTTGGATGTTGGAAGGATCTGCTGCTGG-3′ | GCTGCTGGATAAACCAC |
| SNP45 (rs12188950) | F:5′-ACGTTGGATGGTATTGCTGCCATCATTTTAC-3′<br>R:5′-ACGTTGGATGAATAAATGCTTTGGGGACAG-3′ | AGCAGATAGGGCACA |
| SNP87 (rs2910829) | F:5′-ACGTTGGATGCTCTAACCAAGTGCTTGCTG-3′<br>R:5′-ACGTTGGATGTGAGGAAGAATAATGGATGC-3′ | ACATTCATAACATCTACACAA |

ALOX5AP = 5-lipoxygenase activating protein; PDE4D = phosphodiesterase 4D; SNP = single-nucleotide polymorphism.

Cerebral infarction for all patients was confirmed by computed tomography and magnetic resonance imaging, and was classified according to a modified version of the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.[19] Two subtypes of cerebral infarction (i.e., AT and SAD) were included. Patients with cardiogenic cerebral embolism, cerebral infarction of unclear etiology, or a previous history of stroke were excluded. Of the 459 patients diagnosed with cerebral infarction, 276 were male and 183 female, with an average age of 68.56 ± 10.97 years.

The controls had no family history of stroke and were unrelated to the stroke patients. A total of 462 healthy volunteers were ascertained, including 276 males and 186 females with an average age of 63.82 ± 9.22 years. Vascular risk factors collected from each individual included age, sex, hypertension, diabetes mellitus (DM), cigarette smoking, alcohol intake, total plasma cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and blood platelets levels. Hypertension was defined as a mean of three independent measures of blood pressure of ≥140/90 mmHg or the use of antihypertensive drugs. DM was diagnosed by a fasting glucose level of >7.8 mmol/L or that of >11.1 mmol/L 2 hours after an oral glucose challenge. Cigarette smoking was defined as having smoked at least one cigarette a day for more than 1 year. Alcohol consumption was defined as drinking alcohol at least 12 times in the past year.[20] Cerebral infarction was ruled out in the control population based on the history, physical examination, and laboratory results described above.

## Marker selection and genotyping

For ALOX5AP, four SNPs were studied: rs9551963A/C, rs4769060A/G, rs4769874A/G, and rs10507391A/T. For PDE4D, three SNPs were studied: rs966221C/T, rs12188950C/T, and rs2910829C/T (http://www.ncbi.nlm.nih.gov/snp/). Previous studies demonstrated significant associations between these SNPs and stroke, and that mutations in these genes may increase the incidence of ischemic stroke.[18,21,22] Additional rationale for studying these SNPs was based on each SNP's minor allele frequency (MAF). The MAF for ALOX5AP SG13S89 (rs4769874) is 0.051, the lowest for all SNPs in the study (http://www.ncbi.nlm.nih.gov/snp/).

Genomic DNA was extracted from peripheral blood using a modified phenol/chloroform method[23] and purified using the UNIQ-10 kit (Sangon Biotech Co., Ltd, Shanghai, China). Amplification of the target sequences was performed in a multiplex reaction containing 5 ng of DNA, 0.95 μL of water, 0.625 μL of polymerase chain reaction (PCR) buffer containing 15 mM $MgCl_2$, 1 μL of 2.5 mM deoxy-ribonucleoside triphosphate (dNTP), 0.325 μL of 25 mM $MgCl_2$, 1 μL of PCR primers (Table 1), and 0.5 U HotStar Taq (Qiagen Co., Ltd, Shanghai, China). The reaction was incubated at 94°C for 15 minutes, followed by 45 cycles at 94°C for 20 seconds, at 56°C for 30 seconds, and at 72°C for 1 minute, and a final incubation at 72°C for 3 minutes. After PCR amplification, remaining dNTPs were dephosphorylated by adding 1.53 μL of water, 0.17 μL of SAP buffer, and 0.3 units of shrimp alkaline phosphatase (Sequenom Inc., San Diego, CA, USA). After incubation at 37°C for 40 minutes, the enzyme was deactivated by incubating at 85°C for 5 minutes.

Each *SNP* gene possessed a specific genotype, with two amplification primers and one extension primer (Table 1). The extension primers were then added to the reaction in a buffer containing 0.755 μL of water, 0.2 μL of 10× iPLEX buffer, 0.2 μL of termination mix, 0.041 μL of iPLEX enzyme (Sequenom Inc.), and 0.804 μL of 10 μM extension primer. The single-base extension reaction consisted of an initial denaturation at 94°C for 30 seconds, and then at 94°C for 5 seconds, followed by five cycles at 52°C for 5 seconds and at 80°C for 5 seconds (a total of 40 cycles), and a final extension at 72°C for 3 minutes.

The reaction mix was desalted by adding 6 mg of a cation exchange resin (Sequenom Inc.), mixed, and resuspended in 25 μL of water. At the completion of the primer extension reaction, the completed reactions were spotted onto a 384-well spectroCHIP (Sequenom Inc.) using a MassARRAY

nanodispenser (Sequenom Inc.) and genotyped using a matrix-assisted laser desorption ionization time-of-flight mass spectrometer. Genotype calling was performed in real time using the MassARRAY RT software version 3.0.0.4 and analyzed using the MassARRAY Typer software version 3.4 (Sequenom Inc.).[24]

### Statistical analysis

Allele and genotype frequencies were calculated for each locus. The Hardy–Weinberg equilibrium was tested with Chi-square tests. Continuous variables were expressed as the mean ± standard deviation (SD) and compared by an unpaired Student *t* test, unless otherwise indicated. Categorical variables were assessed by the Chi-square test or Fisher's exact test. Biological and clinical variables were compared between the case and control groups by the Student *t* test and Chi-square test for continuous and categorical variables, respectively.

Clinical characteristics and genotype distribution in both groups were analyzed by a univariate analysis. Multivariate logistic regression analyses were performed with adjustment for certain risk factors, including sex, hypertension, DM, smoking, drinking, and LDL-C, to assess the independent contribution of genotypes and haplotypes to stroke. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the $\beta$ coefficients and standard errors. All statistical tests were performed with the SPSS version 16.0 software package (SPSS Inc., Chicago, IL, USA). A two-tailed $p$ value of $<0.05$ was considered statistically significant. GMDR analysis was applied to detect gene–gene interactions.

## Results

### Clinical characteristics of the participants

Demographic information and clinical characteristics of all study participants are presented in Table 2. A difference was observed in the distribution of age ($p < 0.001$) between cases and controls, but not in that of sex ($p = 0.904$). As expected, stroke patients had a higher prevalence of risk factors for vascular diseases, including increased age, history of hypertension, and DM ($p < 0.001$). Notably, differences in the presence of conventional risk factors, including smoking and alcohol intake, total plasma cholesterol, LDL-C, triglycerides, and blood platelets, were not statistically different between cases and controls ($p > 0.05$).

**Table 2** Clinical characteristics of the participants.

| Characteristic | Cases ($n = 459$) | Controls ($n = 462$) | $p$ |
|---|---|---|---|
| Age (y) | 68.56 ± 10.97 | 63.82 ± 9.22 | <0.001 |
| Male sex | 60.13 | 59.74 | 0.904 |
| Hypertension | 77.56 | 24.19 | <0.001 |
| Diabetes mellitus | 31.81 | 18.61 | <0.001 |
| Cigarette smoking | 44.88 | 42.86 | 0.536 |
| Alcohol intake | 47.28 | 46.75 | 0.874 |
| TC (mmol/L) | 4.73 ± 1.31 | 4.86 ± 1.40 | 0.140 |
| LDL-C (mmol/L) | 2.91 ± 1.12 | 3.03 ± 1.11 | 0.090 |
| TG (mmol/L) | 1.79 ± 1.02 | 1.79 ± 0.97 | 0.993 |
| PLT ($\times 10^9$/L) | 191.78 ± 62.04 | 196.91 ± 45.68 | 0.183 |

Data are presented as % or mean ± SD.
LDL-C = low-density lipoprotein cholesterol; PLT = blood platelet; SD = standard deviation; TC = total plasma cholesterol; TG = triglycerides.

### Associations between ALOX5AP and PDE4D SNP genotypes and risk of overall stroke

Genotyping results for the ALOX5AP and PDE4D SNPs for all study participants are shown in Table 3. Genotype distributions in the control population were consistent with the Hardy–Weinberg equilibrium. Multivariable logistic regression analysis revealed that the genotype frequencies between cases and controls were not significantly different for any of the SNPs tested.

### GMDR analysis

We used GMDR to determine any gene–gene interactions that might increase stroke risk. With covariable adjustments for age, hypertension, and DM, we did not find evidence of any ALOX5AP–PDE4D interaction that might increase stroke risk in this southeastern Chinese population (Table 4).

### Stroke types and sex dependence in ethnic Chinese populations

This study recruited only patients attributed to AT and SAD subtypes based on the TOAST classification. In total, 276 AT and 183 SAD subtypes were identified. Following stratification by stroke type, we found that neither AT nor SAD groups exhibited a different distribution of the seven SNPs compared to the control group (Table 5).

The *ALOX5AP* gene, and specifically the rs9551963 and rs4769060 genotypes, exhibited significantly different distributions between the stroke and control groups in female patients following sex stratification (Table 6; $p < 0.05$). The Rs9551963 C allele showed a significant relationship with female stroke ($p = 0.000$); however, no significant relationship was detected in the rs4769060 A allele ($p = 0.721$). Based on the significant differences in genotypic distributions between groups, an association with cerebral infarction in the female group was indicated.

## Discussion

Stroke is a complex clinical syndrome with various genetic and clinical risk factors including, but not limited to, age, hypertension, DM, and hyperlipidemia. Several recent studies have shown that genetic factors play an important role in stroke. *ALOX5AP*, a gene involved in the leukotriene synthesis pathway, and *PDE4D*, involved in signal transduction by degrading cyclic adenosine

**Table 3** ALOX5AP and PDE4D SNP genotype frequencies in ischemic stroke and control groups.[a]

| Gene | SNP | Genotype | No. of cases (%) | No. of controls (%) | *p* | OR (95% CI) |
|---|---|---|---|---|---|---|
| *ALOX5AP* | SG13S32 (rs9551963) | AC | 230 (0.50) | 214 (0.46) | | |
| | | CC | 69 (0.15) | 80 (0.17) | | |
| | | AA | 160 (0.35) | 168 (0.36) | 0.455 | — |
| | | A alleles | 550 (0.60) | 550 (0.60) | 0.955 | 1.005 (0.834—1.212) |
| | SG13S89 (rs4769874) | AG | 22 (0.048) | 16 (0.035) | | |
| | | GG | 437 (0.952) | 446 (0.965) | 0.244 | 1.470 (0.766—2.821) |
| | | A alleles | 22 (0.024) | 16 (0.017) | 0.322 | 1.387 (0.724—2.659) |
| | SG13S42 (rs4769060) | AA | 172 (0.37) | 197 (0.43) | | |
| | | AG | 225 (0.49) | 209 (0.45) | | |
| | | GG | 62 (0.14) | 56 (0.12) | 0.275 | — |
| | | A alleles | 569 (0.62) | 603 (0.65) | 0.112 | 0.857 (0.709—1.037) |
| | SG13S114 (rs10507391) | AA | 84 (0.18) | 69 (0.15) | | |
| | | TT | 146 (0.32) | 151 (0.33) | | |
| | | AT | 229 (0.50) | 242 (0.52) | 0.386 | — |
| | | A alleles | 397 (0.43) | 380 (0.41) | 0.399 | 1.047 (0.900—1.303) |
| *PDE4D* | SNP83 (rs966221) | CC | 16 (0.03) | 29 (0.06) | | |
| | | TT | 304 (0.66) | 300 (0.65) | | |
| | | CT | 139 (0.30) | 133 (0.29) | 0.142 | — |
| | | C alleles | 171 (0.19) | 191 (0.21) | 0.250 | 0.874 (0.694—1.100) |
| | SNP45 (rs12188950) | CC | 459 (1.00) | 460 (1.00) | | |
| | | CT | 0 (0.00) | 2 (0.00) | 0.499 | 1.004 (0.998—1.010) |
| | | C alleles | 918 (1.00) | 922 (1.00) | 0.500 | 1.002 (0.999—1.005) |
| | SNP87 (rs2910829) | CC | 308 (0.67) | 314 (0.68) | | |
| | | TT | 12 (0.03) | 10 (0.02) | | |
| | | CT | 139 (0.30) | 138 (0.30) | 0.890 | — |
| | | C alleles | 755 (0.82) | 766 (0.83) | 0.564 | 0.931 (0.731—1.186) |

ALOX5AP = 5-lipoxygenase activating protein; CI = confidence interval; OR = odds ratio; PDE4D = phosphodiesterase 4D; SNP = single-nucleotide polymorphism.

[a] Statistical analysis was performed by Chi-square test between controls and patients with ischemic stroke.

monophosphate and cyclic guanosine monophosphate, were identified through linkage analysis to confer risk of stroke in an Icelandic population.[12,15] In this study, we attempted to determine whether certain SNPs within these two genes, *ALOX5AP* and *PDE4D*, were associated with an increased risk of overall stroke in a southeast Chinese population. The SNPs chosen for this study have appeared in several non-European studies of stroke risk and all have an MAF of over 5%. We also thought that there might be some measurable gene—gene interaction between *ALOX5AP* and *PDE4D* and risk of stroke, because both genes are involved in inflammation. However, in contrast to the European studies, we found neither any association between overall stroke susceptibility and the seven SNPs studied, nor any evidence of interactions between the two genes influencing stroke risk.

In addition to the SNPs comprising the HapA and HapB risk haplotypes identified in the European studies, other ALOX5AP SNPs have been associated with ischemic stroke in various populations.[25—27] Contrasting results reporting no

**Table 4** Generalized multifactor dimensionality reduction analysis showing no evidence of interactions between ALOX5AP and PDE4D SNP genotypes and risk of ischemic stroke.

| Best model | Training balanced accuracy | Testing balanced accuracy | Sign test (*p*) | Cross-validation consistency |
|---|---|---|---|---|
| 1 | 0.5325 | 0.4887 | 4 (0.8281) | 6/10 |
| 2, 3 | 0.5678 | 0.5216 | 7 (0.1719) | 7/10 |
| 2, 3, 4 | 0.5907 | 0.5002 | 5 (0.6230) | 5/10 |
| 1, 2, 3, 5 | 0.6368 | 0.5693 | 8 (0.0547) | 10/10 |
| 1, 2, 3, 4, 5 | 0.6610 | 0.5565 | 8 (0.0547) | 10/10 |
| 1, 2, 3, 4, 5, 6 | 0.6666 | 0.5585 | 8 (0.0547) | 10/10 |
| 1, 2, 3, 4, 5, 6, 7 | 0.6666 | 0.5585 | 8 (0.0547) | 10/10 |

Model components: 1, ALOX5AP SG13S42; 2, ALOX5AP SG13S114; 3, PDE4D SNP83; 4, ALOX5AP SG13S32; 5, PDE4D SNP87; 6, ALOX5AP SG13S89; and 7, PDE4D SNP45.

ALOX5AP = 5-lipoxygenase activating protein; PDE4D = phosphodiesterase 4D; SNP = single-nucleotide polymorphism.

**Table 5** Subtype-dependent ALOX5AP and PDE4D SNP genotypic frequencies in ischemic stroke.

| Gene | SNP | Genotype | AT (%) | SAD (%) | *p* | OR (95% CI) |
|---|---|---|---|---|---|---|
| *ALOX5AP* | SG13S32 (rs9551963) | AC | 133 (0.482) | 103 (0.563) | | |
| | | CC | 35 (0.127) | 24 (0.131) | | |
| | | AA | 108 (0.391) | 56 (0.306) | 0.160 | |
| | | C alleles | 203 (0.368) | 151 (0.413) | 0.172 | 0.828 (0.632—1.086) |
| | SG13S89 (rs4769874) | AG | 9 (0.033) | 11 (0.060) | | |
| | | GG | 267 (0.967) | 172 (0.940) | 0.158 | 0.527 (0.214—1.298) |
| | | A alleles | 9 (0.016) | 11 (0.030) | 0.162 | 0.535 (0.219—1.304) |
| | SG13S42 (rs4769060) | AA | 113 (0.450) | 60 (0.328) | | |
| | | AG | 129 (0.467) | 95 (0.519) | | |
| | | GG | 34 (0.123) | 28 (0.153) | 0.195 | |
| | | A alleles | 355 (0.643) | 215 (0.587) | 0.089 | 1.266 (0.965—1.660) |
| | SG13S114 (rs10507391) | AA | 55 (0.199) | 26 (0.142) | | |
| | | TT | 92 (0.333) | 53 (0.290) | | |
| | | AT | 129 (0.468) | 104 (0.568) | 0.086 | |
| | | A alleles | 239 (0.433) | 156 (0.426) | 0.858 | 1.025 (0.785—1.338) |
| *PDE4D* | SNP83 (rs966221) | CC | 10 (0.036) | 7 (0.038) | | |
| | | TT | 190 (0.689) | 108 (0.590) | | |
| | | CT | 76 (0.275) | 68 (0.372) | 0.086 | |
| | | C alleles | 96 (0.174) | 82 (0.224) | 0.060 | 0.729 (0.524—1.014) |
| | SNP45 (rs12188950) | CC | 276 (1.000) | 183 (1.000) | | |
| | | CT | 0 (0.000) | 0 (0.000) | — | — |
| | | C alleles | 552 (1.000) | 366 (0.100) | — | — |
| | SNP87 (rs2910829) | CC | 195 (0.707) | 114 (0.623) | | |
| | | TT | 9 (0.032) | 8 (0.044) | | |
| | | CT | 72 (0.261) | 61 (0.333) | 0.174 | |
| | | C alleles | 462 (0.837) | 289 (0.790) | 0.069 | 1.368 (0.976—1.918) |

ALOX5AP = 5-lipoxygenase activating protein; AT = atherothrombotic; CI = confidence interval; OR = odds ratio; PDE4D = phosphodiesterase 4D; SAD = small-artery disease; SNP = single-nucleotide polymorphism.

**Table 6** Sex-dependent ALOX5AP and PDE4D SNP genotypic frequencies in ischemic stroke and control groups.

| Gene | SNP | Genotype | Cases—F (%) | Controls—F (%) | *p* | OR (95% CI) |
|---|---|---|---|---|---|---|
| *ALOX5AP* | SG13S32 (rs9551963) | AC | 106 (0.579) | 76 (0.409) | | |
| | | CC | 59 (0.323) | 46 (0.247) | | |
| | | AA | 18 (0.098) | 64 (0.344) | 0.000 | |
| | | C alleles | 224 (0.612) | 168 (0.452) | 0.000 | 1.915 (1.429—2.568) |
| | SG13S89 (rs4769874) | AG | 14 (0.076) | 11 (0.059) | | |
| | | GG | 169 (0.924) | 175 (0.941) | 0.440 | |
| | | A alleles | 14 (0.038) | 11 (0.030) | 0.437 | 0.859 (0.586—1.260) |
| | SG13S42 (rs4769060) | AA | 59 (0.323) | 68 (0.366) | | |
| | | AG | 111 (0.606) | 92 (0.494) | | |
| | | GG | 13 (0.071) | 26 (0.140) | 0.035 | |
| | | A alleles | 229 (0.626) | 228 (0.613) | 0.721 | 1.056 (0.784—1.421) |
| | SG13S114 (rs10507391) | AA | 36 (0.197) | 35 (0.188) | | |
| | | TT | 62 (0.339) | 57 (0.307) | | |
| | | AT | 85 (0.464) | 94 (0.505) | 0.722 | |
| | | A alleles | 157 (0.429) | 164 (0.441) | 0.744 | 0.953 (0.712—1.275) |
| *PDE4D* | SNP83 (rs966221) | CC | 8 (0.044) | 18 (0.097) | | |
| | | TT | 141 (0.770) | 134 (0.720) | | |
| | | CT | 34 (0.186) | 34 (0.183) | 0.135 | |
| | | C alleles | 50 (0.137) | 70 (0.188) | 0.058 | 0.683 (0.460—1.014) |
| | SNP45 (rs12188950) | CC | 183 (1.000) | 184 (0.995) | | |
| | | CT | 0 (0.00) | 2 (0.005) | 0.485 | |
| | | C alleles | 366 (1.000) | 370 (0.995) | 0.486 | 0.503 (0.468—0.540) |
| | SNP87 (rs2910829) | CC | 122 (0.667) | 115 (0.618) | | |
| | | TT | 3 (0.016) | 5 (0.027) | | |
| | | CT | 58 (0.317) | 66 (0.355) | 0.549 | |
| | | C alleles | 302 (0.825) | 296 (0.796) | 0.308 | 1.212 (0.838—1.753) |

association are equally abundant in the literature.[28–30] Our results, like many others, show no evidence of a link between ALOX5AP SNP genotypes and risk of stroke in a southeastern Chinese population. The association between PDE4D SNPs and stroke is similarly debated in the literature.[15,27,31–35] Our analysis of three PDE4D SNPs also did not yield any evidence of an association with stroke risk.

Although the link between *ALOX5AP* gene mutations and stroke risk is still debated, the mechanism by which it may affect cerebral infarction is through its role in leukotriene formation. Inflammation may alter the vascular permeability, promoting cerebral atherosclerosis. *PDE4D* also mediates inflammation by promoting the proliferation of smooth muscle cells and the inflammatory response through regulation of intracellular second messenger activity. Because both these two genes are involved in inflammation, an interaction between *ALOX5AP* and *PDE4D* may increase stroke susceptibility by promoting atherosclerosis and/or regulating plaque stability. Although this hypothesis is attractive, our GMDR analysis did not provide any evidence supporting this relationship. This is a novel finding; we found no other reports in the literature studying the interactions between these two genes and influence of stroke risk.

A study conducted in an East Indian population found that SNP 83 was significantly associated with these stroke subtypes, but the association with other subtypes was determined to be insignificant.[36] The Taiwan population provides preliminary evidence suggesting that the *ALOX5AP* gene polymorphisms were associated with AT stroke.[37] However, a Swedish study indicated that SNP45 had no effect on ischemic stroke, results being derived from either pooled multicenter data or a meta-analysis. In addition, significant associations were not found when evaluating patients from the three different TOAST subgroups separately.[38] A study in Han Chinese of eastern China showed a negative association between the ALOX5AP variants and ischemic stroke risk. In addition, no relationship between different TOAST subtypes was detected, and a sex stratification analysis showed an absence of increased sex incidence.[18] Given these differences, additional studies attempted to determine whether the seven SNPs exhibited a relationship with alternative TOAST classifications. Meanwhile, our study concluded that the risks associated with the *ALOX5AP* gene were sex dependent in ethnic Chinese populations. The *ALOX5AP* gene, which included rs9551963 and rs4769060 genotypes, might add to ischemic stroke incidence in females.

Published accounts of associations between stroke risk and *ALOX5AP* or *PDE4D* gene polymorphisms offer widely variable conclusions. However, it is difficult to compare these studies directly, due to differences in population, race, and gene target(s). In this study, we attempted to minimize bias due to population structure by recruiting controls and patients from the same geographical region of China. Our results should be validated in a larger, multicenter study.

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