CYP metabolic pathway related gene polymorphism increases the risk of embolic and atherothrombotic stroke and vulnerable carotid plaque in southeast China

Minjie Shao,[a] Youbing Mao,[a] Qing Hong,[a] Xinchun Jin,[a] Haibo Cai,[a] and Xingyang Yi,[b]

Objective: To investigate the association of CYP metabolic pathway-related genetic polymorphisms with the susceptibility to ischemic stroke and stability of carotid plaque in southeast China. *Methods:* We consecutively enrolled 294 acute ischemic stroke patients with carotid plaque and 282 controls from Wenling First People's Hospital. The patients were divided into the carotid vulnerable plaque group and stable plaque group according to the results of carotid B-mode ultrasonography. Polymorphisms of *CYP3A5* (G6986A, rs776746), *CYP2C9*2* (C430T, rs1799853), *CYP2C9*3* (A1075C, rs1057910), and *EPHX2* (G860A, rs751141) were determined using polymerase chain reaction and mass spectrometry analysis. *Results: EPHX2* GG may reduce the susceptibility to ischemic stroke (OR = 0.520, 95% CI: 0.288 ~ 0.940, $P = 0.030$) and AA+AG may increase the risk for ischemic stroke (OR = 1.748, 95% CI: 1.001 ~ 3.052, $P = 0.050$). The distribution of *CYP3A5* genotypes showed significant differences between the vulnerable plaque and stable plaque groups (P = 0.026). Multivariate logistic regression analysis found that *CYP3A5* GG could reduce the risk of vulnerable plaques (OR = 0.405, 95% CI: 0.178 ~ 0.920, $P = 0.031$). *Conclusion: EPHX2* G860A polymorphism may reduce the stroke susceptibility, while other SNPs of *CYP* genes are not associated with ischemic stroke in southeast China. Furthermore *CYP3A5* polymorphism was related with carotid plaque instability.

Keywords: CYP—EPHX2—Ischemic stroke—Genetic polymorphism—Carotid plaque

Introduction

Inflammatory responses regulated by genes play important roles in the development of atherosclerosis, plaque instability and plaque rupture, which lead to acute cardiovascular disease and stroke.[1] The mediators of inflammation resolution have been associated with protective effects on cardiovascular events. Numerous trials have investigated the role of the long-chain omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the prevention of cardiovascular events. A meta-analysis has revealed that EPA can reduce the occurrence of myocardial infarction.[2] Anti-inflammatory effect through balanced Omega-6 / Omega-3 ratio may reduce cardiovascular disease.[3] Arachidonic acid (AA) may have a similar effect on vascular disease. AA has three metabolic pathways involving cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP).[4] AA is metabolized by CYP epoxygenases into epoxy eicosatrienoic acids (EETs), which can then be metabolized by soluble epoxide hydrolase (sEH) to generate less biologically active

From the [a]Department of Neurology, Wenling First People's Hospital of Zhejiang Province, Taizhou 317500, Zhejiang, China; and [b]Department of Neurology, People's Hospital of Deyang City, Deyang 618000, Sichuan, China.

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Corresponding author at: Department of Neurology, the People's Hospital of Deyang City, No 173, North Taishan Road, Deyang 618000, Sichuan, China. E-mail: yixingyang64@126.com.

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dihydroxyeicosatrienoic acids (DHETs). Thus, sEH is a key enzyme in metabolic conversion and degradation of EETs, and sEH activity is encoded and regulated by the soluble epoxide hydrolase 2 gene (EPHX2).[5] Variants in *EPHX2* may affect sEH activity and generation of DHET from EET. EET form a unique endothelium derived hyperpolarizing factor (EDHF) that can open Ca^{2+} sensitive potassium channels on the cell membrane, cause membrane hyperpolarization and lower blood pressure through vascular relaxation.[6] Moreover, EDHF has anti-inflammatory effects, inhibits smooth muscle cell proliferation and migration, regulates vascular tone, promotes fibrinolysis, and has anti-atherosclerosis effects.[7] The metabolism of AA is always dynamic, once its balance is destroyed, atherosclerosis and ischemic stroke may occur.

Ischemic stroke is a syndrome with a complex etiology and pathogenesis that accounts for approximately 70% of all cerebrovascular diseases. Vulnerable plaques easily induce and rapidly progresses to thrombosis result from large amounts of lipids, bleeding, ulceration, and hypoechoic performance.[8,9] Recent studies have shown that acute ischemic stroke was closely related to carotid atherosclerotic plaque vulnerability, thus it is has become of widespread interest regardless of whether vulnerable plaques have some genetic susceptibility.[10,11]

The gene promoter region and coding region of the human CYP gene is highly polymorphic and different ethnicities have different allele frequencies. Among these genes, *CYP2C9* has been the most studied in the context of ischemic stroke. The most frequent single nucleotide polymorphisms (SNPs) of functional importance in the *CYP3A5* gene is the A to G transition in intron 3 at position 6986, which is known as *CYP3A5*3*.[12] Kreutz *et al.*,[13] and Naushad *et al.*,[14] supported the opinion that *CYP3A5*3* mutation has a close association with blood pressure. A study from Japanese population also showed that a *CYP3A5*3* polymorphism was associated with salt-sensitive hypertension. However, there were few studies to investigate the relationship between the *CYP3A5* polymorphism and susceptibility to cerebral infarctions.

EET has a clear role in vascular inflammation.[15] Fornage *et al.*,[16] analyzed different SNPs of the *EPHX2* (e.g., K55R, R103C, R287Q, $R402^{I/D}$, E470G) in different races, and suggested that the *EPHX2* may be a risk or protective factor for ischemic stroke. Lee et *al.*,[17] considered that a *EPHX2* (K55R) mutation may be a predisposing factor for coronary heart disease rather than R103C, R287Q, 402InsR, and E470G. Fava *et al.*,[18] also showed that the functional K55R polymorphism of the *EPHX2* confers a higher risk of hypertension and ischemic stroke in male homozygotes.

CYP and EPHX2 gene mutation may result in a variety of biological effects, such as atherosclerosis. According to recent research, *CYP3A5, CYP2C9,* and *EPHX2* are all involved in the metabolism of AA.[19,20] To date, there have been no reports the association between these genetic SNPs and ischemic stroke. In this study, we hypothesized that CYP metabolic pathway related genetic polymorphisms would have a close association with carotid plaque stability and may play a key role in ischemic stroke.

Materials and methods

Ethics statement

This study was approved by the Ethical Committee of Wenling First People's Hospital of Zhejiang Province, all participants provided their written informed consent to participate before enrollment.

Populations

The study population included 462 ischemic stroke patients and 282 controls who were consecutively admitted to the Department of Neurology of Wenling First People's Hospital of Zhejiang Province between November 2020 and March 2022. Ischemic stroke was confirmed using computed tomography (CT) and magnetic resonance imaging (MRI). According to a modified version of the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification,[21] stroke etiology was classified as atherothrombosis (AT), cardioembolic, small vessel disease, other determined etiology, undermined etiology (including multiple causes). Intra-or extracranial carotid arteries were evaluated using CT angiography or MR angiography, as well as color duplex ultrasound in all patients.

Among the 462 patients with ischemic stroke, we selected and enrolled 294 patients with carotid plaque according to carotid artery B-ultrasound, the patients without carotid plaque were excluded. The 282 controls were selected from tension-type headache, Parkinson's disease, Alzheimer's dementia, or peripheral neuropathy with no history of stroke as confirmed by medical history as well as physical and cranial MRI. The controls had no carotid arteriosclerosis and family history of stroke and were genetically unrelated to the ischemic stroke patients. All subjects were Chinese Han in ethnicity and were unrelated. The exclusion criteria included a history of stroke, blood diseases, serious cardiopulmonary, liver, or kidney diseases, thyroid diseases, autoimmune diseases, consumption of any kind of anti-lipemic or anti-platelet aggregation drug in the previous three months, and thrombolytic therapy.

Vascular risk factors were assessed for each individual that included age, sex, hypertension, diabetes mellitus (DM), cigarette smoking, alcohol intake, total plasma cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), blood platelet (PLT), fibrinogen (Fib), homocysteine (Hcy), and D-dimers (DDi) levels.

Carotid artery ultrasound

A GE Vivid7 carotid artery color duplex ultrasound diagnostic apparatus with a 7.5MHz frequency probe was

utilized in this study. Two doctors with good reliability (Kappa = 1) examined proximal 1 cm to the bilateral carotid artery bifurcation, and at the bifurcation and extracranial internal carotid artery segment, and then observed a vertical and horizontal axis real-time two-dimensional image. The carotid plaque was determined as an endoluminal protrusion of at least 1.5 mm or a focal thickening >50% of the intima-media thickness relative to the adjacent wall segment[8,9] A total of the 462 patients with ischemic stroke, 294 patients had carotid plaque. Among the 294 patients with carotid plaque, 69 patients with hypoechoic plaque and mixed echo plaque were divided into the vulnerable plaque group, while 225 patients with hyperechoic plaque were divided into the stable group.

Marker selection and genotyping

Among CYP metabolic pathway related genes, four SNPs were selected in this study, including *CYP3A5*3* (rs776746A/G) (Minor allele frequency, MAF=0.3127), *CYP2C9*2* (rs1799853C/T) (MAF=0.0682), *CYP2C9*3* (rs1057910A/C) (MAF=0.0426), and *EPHX2* (rs751141G/A) (MAF=0.1424) (http://www.ncbi.nlm.nih.gov/snp).

Blood samples were collected from subjects after admission and physical examination, and stored in tubes containing 0.5M EDTA (pH 8.0) at -80°C refrigerator. Genomic DNA was extracted from peripheral blood using a modified phenol/chloroform method, and purified using a UNIQ-10 kit. 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 PCR buffer containing 15 mM MgCl2, 1 μl of 2.5 mM dNTP, 0.325 μl of 25 mM MgCl2, 1 μl of PCR primers and 0.5 U Hot Star Taq. The reaction was incubated at 94°C for 15 min followed by 45 cycles at 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min, and a final incubation at 72°C for 3 min. After PCR amplification, remaining dNTPs were dephosphorylated by adding 1.53 μl water, 0.17 μl of SAP buffer, and 0.3 units of shrimp alkaline phosphatase. Following incubation at 37°C for 40 min, the enzyme was deactivated by incubation at 85°C for 5 min.

The extension primers (Table 1) were added to the reaction buffer containing 0.755 μl water, 0.2 μl of 10X iPLEX buffer, 0.2 μl termination mix, 0.041 μl of iPLEX enzyme, and 0.804 μl of 10 uM extension primer. The single base extension reaction consisted of an initial denaturation at 94°C for 30 s, and then 94°C for 5 s, followed by 5 cycles of 52°C for 5 s and 80°C for 5 s: for a total of 40 cycles, and a final extension at 72°C for 3 min.

The reaction mix was desalted by adding 6mg of cation exchange resin (Sequenom Inc., San Diego, CA) that was mixed and re-suspended in 25 μl of water. At the completion of the primer extension reaction, the completed reactions were spotted onto a 384 well spectro CHIP, using a Mass ARRAY Nano dispenser and genotyped using the matrix-assisted laser desorption ionization time-of-flight mass spectrometer (Sequenom Inc., San Diego, CA). Genotype calling was performed in real time with Mass ARRAY RT software version 3.0.0.4 and analyzed using the Mass ARRAY Typer software version 3.4.

Statistical analyses

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

Clinical characteristics and genotype distribution in groups were analyzed using univariate analysis. Multivariate logistic regression analyses were performed to adjust certain risk factors ($P < 0.05$ in univariate analysis) to assess the independent contribution of genotypes and haplotypes to ischemic stroke. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the β coefficients and standard errors. All statistical tests were performed using the SPSS version 23.0 software package

Table 1. *SNPs primers used in this study.*

SNP	Forward primer	Reverse primer	Extension primer
Rs776746	ACGTTGGATGCCATAATC TCTTTAAAGAGC	ACGTTGGATGGATGAAGGG TAATGTGGTCC	CCAAACAGGGAAGAGATA
Rs1799853	ACGTTGGATGCTGCGGA ATTTTGGGATGGG	ACGTTGGATGACCCACCCTT GGTTTTTCTC	GAGGAGCATTGAGGAC
Rs1057910	ACGTTGGATGCTACACAG ATGCTGTGGTGC	ACGTTGGATGTGTCACAGGT CACTGCATGG	CACGAGGTCCAGAGATAC
rs751141	ACGTTGGATGAGCAGATG ACTCTCCATAGC	ACGTTGGATGTTTTCTAGAT CCCTGCTCTG	CCCAGGCAGGTTACC

SNP: single nucleotide polymorphism

(SPSS, IBM, USA). All tests were two sided, and *P* value less than 0.05 was considered statistically significant.

Results

Clinical characteristics

Of the 294 patients who had carotid plaques, 174 were male and 120 were female, with an overall average age of 68.62 ± 11.08 years. Among 282 controls, 160 were males and 122 were females, with an overall average age of 63.91 ± 9.14 years. Compared with the controls, the patients with ischemic stroke were older (*P* = 0.000), had a higher proportion of hypertension (*P* = 0.000) and diabetes (*P* = 0.025). However, there was no significant difference in other factors between the two groups (*P*>0.05) Table 2.

Distribution of genotypes between patients and controls

There were no mutations in *CYP2C9*2* and a low mutation rate for *CYP2C9*3* in southern Chinese populations (Table 3). Consistent with published findings from other countries,[22,23,24] the *CYP2C9*2* and *CYP2C9*3* SNPs were not significantly correlated with ischemic stroke (Table 3). Meanwhile, There were no significant differences in genotype distributions of *CYP3A5*3* (rs776746) and *EPHX2* (rs751141) between the patients and controls (Table 3).

Logistic regression analysis risk factors for ischemic stroke

Multivariate logistic regression was used to evaluate the risk factors for ischemic stroke, and the results showed age, hypertension, diabetes mellitus, and SNPs of *EPHX2* rs751141 were independently associated with the risk for ischemic stroke (Table 4). *EPHX2* rs751141 GG might reduce the stroke susceptibility (OR = 0.520, 95% CI: 0.288 ~ 0.940, *P* = 0.030) and *EPHX2* rs751141 AA+AG genotype might increase the risk for ischemic stroke (OR =1.748, 95% CI: 1.001 ~ 3.052, P = 0.050) (Table 4).

Table 3. *SNP frequencies in the patient and control groups (n, %).*

SNP	Stroke group (n=294)	Control group (n=282)	P value
CYP3A5*3(rs776746)			
AA	31 (10.6)	29 (10.3)	0.437[a]
AG	113 (38.4)	100 (35.5)	
GG	150 (51.0)	153 (54.2)	0.726[b]
CYP2C9*2(rs1799853)			
CC	294 (1.000)	282 (1.000)	—
CYP2C9*3(rs1057910)			
CC	1 (0.3)	0 (0.000)	0.451[a]
CA	26 (8.8)	21 (7.5)	
AA	267 (91.1)	261 (92.5)	0.420 [b] (Fisher)
EPHX2(rs751141)			
AA	12 (4.1)	18 (6.4)	0.830[a]
GA	97 (33.0)	89 (31.6)	
GG	185 (62.9)	175 (62.0)	0.456[b]

SNP: single nucleotide polymorphism; three "a" means AA+AG and GG, CC+CA, and AA, AA+GA, and GG compared between the two groups, respectively. "b" means a distribution comparison between the three groups.

Clinical characteristic of vulnerable plaque group and stable plaque group

Of the 294 patients with carotid plaque, 66 were vulnerable plaque, and 225 were stable plaque. Fibrinogen level was higher in the patients with vulnerable plaque than those with stable plaque (*P* = 0.03, Table 5). There was no significant difference in other factors between the two groups (*P*>0.05).

SNPs distributions in the vulnerable plaque and stable plaque groups

The *CYP3A5* rs776746 genotype distributions were different between the vulnerable plaque and stable plaque groups, the AA+AG genotype was significantly more

Table 2. *Clinical characteristics of the patient and control groups.*

Items	Stroke group (n=294)	Control group (n=282)	P value
Sex (M/F)	174/120	160/122	0.552
Age (years)	68.62±11.08	64.06±9.12	0.000
HT (n, %)	236 (80.3)	75 (26.6)	0.000
DM (n, %)	98 (33.3)	70 (24.8)	0.025
Smoking (n, %)	130 (44.2)	115 (40.8)	0.384
Drinking (n, %)	136 (46.3)	125 (44.3)	0.589
TG (mmol/L)	1.73±0.99	1.83±1.03	0.200
TC (mmol/L)	4.64±1.33	4.61±1.04	0.756
LDL-C (mmol/L)	2.85±1.16	2.82±1.12	0.773
HDL-C (mmol/L)	1.20±0.76	1.29±0.59	0.149
PLT (10*9/L)	181.90±68.64	186.97±40.49	0.521

HT: hypertension; DM: diabetes mellitus; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PLT: blood platelet.

Table 4. *Logistic regression analysis for the relationships between genes frequencies and clinical characteristics in the patient and control groups.*

Items	Wald	Exp(B)	95%CI	P value
Age	17.522	1.054	1.029¶1.081	0.000
HT	87.726	15.625	8.790¶27.775	0.000
DM	3.817	0.554	0.306¶1.002	0.051
EPHX2 GG	4.693	0.520	0.288¶0.940	0.030
Constant	3.540	0.099	—	0.060
Items	Wald	Exp(B)	95%CI	P value
Age	18.192	1.055	1.030¶1.082	0.000
HT	87.990	15.374	8.686¶27.212	0.000
DM	3.928	0.550	0.305¶0.993	0.047
EPHX2 AA+AG	3.849	1.748	1.001¶3.052	0.050
Constant	13.784	0.014	—	0.000

HT: hypertension; DM: diabetes mellitus; EPHX2 shows three different polymorphism models, which includes GG/AA/AG.

common in the vulnerable plaque group and the GG genotype was significantly more common in the stable plaque group. However, the genotype distributions of *CYP2C9*2*, *CYP2C9*3*, and *EPHX2* rs751141 were not different between the two groups (Table 6).

Logistic regression analysis for vulnerable plaques of ischemic stroke patients

The logistic regression analysis revealed that *CYP3A5* rs776746 GG and fibrinogen levels were independently associated with vulnerable plaque after adjusting for other risk factors, *CYP3A5* rs776746 GG might reduce the risk for vulnerable plaque (OR=0.405, 95%CI: 0.178¶0.920, P = 0.031), and fibrinogen levels might increase the risk for vulnerable plaque (OR = 1.59, 95%CI: 1.028¶2.444, P = 0.037) (Table 7).

Discussion

We found that *CYP3A5* polymorphism (GG genotype) might reduce the risk for vulnerable plaque. Furthermore, a novel finding was that *EPHX2* GG might be a protective factor for ischemic stroke, and *EPHX2* AA+AG might increase the risk of stroke. We did not detect any mutations in *CYP2C9*2* and low mutation rates for *CYP2C9*3* in southeast Chinese patients.

The *CYP3A5* gene is located in 7q21.1-22.1, and encodes and controls CYP enzyme active, which metabolize AA to EETS. When the *CYP* gene undergoes mutation, CYP enzyme activity and EETs production decrease, the balance of AA metabolites are broken. EETs are metabolized by sEH to generate DHETs in many nerve cells, including oligodendrocytes, astrocytes, arterial smooth muscle, and the choroid plexus.[25] A study performed on animals

Table 5. *Clinical characteristic of the two plaque group.*

Items	Vulnerable plaque group (n=69)	Stable plaque group (n=225)	OR(95%CI) or 95%CI	P value
Sex (M/F)	43/26	131/94	1.19 (0.68¶2.07)	0.55
Age (years)	70.71±1.29	71.19±0.75	-1.34¶4.66	0.28
HT (n, %)	56 (81.2)	180 (80.0)	1.08 (0.54¶2.14)	0.83
DM (n, %)	23 (33.3)	75 (33.3)	1.00 (0.56¶1.77)	1.00
Smoking (n, %)	30 (43.5)	100 (44.6)	0.95 (0.55¶1.64)	0.87
Drinking (n, %)	34 (49.3)	102 (45.7)	1.15 (0.67¶1.98)	0.61
TG (mmol/L)	1.77±0.95	1.71±1.00	-0.21¶0.32	0.68
TC (mmol/L)	4.88±1.14	4.57±1.38	-0.04¶0.68	0.09
LDL-C (mmol/L)	3.01±1.02	2.81±1.20	-0.11¶0.52	0.21
HDL-C (mmol/L)	1.14±0.34	1.22±0.85	-0.29¶0.12	0.42
PLT (10*9/L)	182.60±52.64	181.70±72.87	-17.95¶19.76	0.93
Fib (g/L)	3.91±1.20	3.55±1.22	0.04¶0.70	0.03
Hcy (umol/L)	13.63±5.54	15.11±8.64	-4.16¶1.20	0.28
DDi (mg/L)	0.82±1.29	0.93±1.52	-0.53¶0.30	0.59

HT: hypertension; DM: diabetes mellitus; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; PLT: blood platelet; Fib: fibrinogen; Hcy: homocysteine; DDi: D-dimer.

Table 6. *SNP distributions in the two plaque groups (n, %).*

SNP	Vulnerable plaque group (n=69)	Stable plaque group (n=225)	P value
Rs776746			
AA	5 (7.2)	26 (11.6)	0.047[a]
AG	36 (52.2)	77 (34.2)	0.026[b]
GG	28 (40.6)	122 (54.2)	
Rs1799853			
CC	69(1.000)	225(1.000)	—
Rs1057910			
CC	0(0.000)	1(0.4)	0.873[a]
CA	6(7.4)	20(8.9)	
AA	63(92.6)	204(90.7)	0.764[b] (Fisher)
Rs751141			
AA	3(4.4)	9(4.0)	0.652[a]
GA	21(30.4)	76(33.8)	
GG	45(65.2)	140(62.2)	0.873[b](Fisher)

SNP: single nucleotide polymorphism; Three "a" means AA+AG and GG, CC+CA, and AA, AA+GA, and GG compared between the two groups, respectively. "b" means a distribution comparison between the three groups.

showed sEH gene knockout or sEH inhibitors can increase EET content and protect against stroke-induced brain injury.[26] The *CYP3A5*3* allele gene mutation frequency is the highest in various ethnic populations and is the main *CYP3A5* gene polymorphism. Several studies assessing the relationship between *CYP* mutations and cerebral infarction susceptibility have recently been published. In this study, the frequencies of *CYP3A5*3* and other genes were not statistically different between the patients and controls, and is similar to the results published by Li *et al.*,[27] who also found no significant association was identified between *CYP3A5*3* and the 1-year outcome in patients with ischemic stroke. Yi *et al.*,[28] showed no significant differences in the *ALOX5AP* and *CYP3A5* genotype distributions between ischemic stroke patients and controls by single-gene variant analysis, but gene-gene interactions among *ALOX5AP* and *CYP3A5* could increase susceptibility to ischemic stroke. The above conclusions share similarities with our research findings. However, they did not conduct in-depth research on the vulnerability of carotid plaques in patients with cerebral infarction.

The *CYP2C9* gene is located at 10q24 and the frequencies of *CYP2C9*2* and **3* are significantly higher in Caucasians than in other ethnic groups. The frequency of *CYP2C9*2* and **3* is 22.7% and 13.4% in Caucasians, respectively.[29] In contrast, East Asian populations, including Chinese, Japanese, and Koreans, have no *CYP2C9*2* mutations, the *CYP2C9*3* allele frequency is 1.1% in Koreans,[30] 2.2% in Japanese,[31] and 3.3% in Chinese.[32] In addition, *CYP2C9*2* and *CYP2C9*3* mutant alleles are not found in Canadian Aboriginal populations.[33] Our study was consistent with the Canadian population.

The *EPHX2* gene is located on chromosome 8p12-p21 and its encoding leads to sEH hydrolysis of EET in ischemic cells. Gene deletions of sEH and sEH inhibitors can increase the levels of EET and protect against brain injury following stroke.[34] EET can prevent ischemic cell death in animal model.[35] Polymorphisms of human *EPHX2* increase the risk of coronary heart disease, and also lead to increase sEH activity and thereby increase incidence of ischemic stroke.[36] To date, this relationship remains unclear. In this study, *EPHX2* polymorphisms were

Table 7. *Logistic regression analyses in the two plaque groups.*

Items	Wald	Exp(B)	95%CI	P value
Fib	4.340	1.585	1.028¶2.444	0.037
CYP3A5 GG	4.659	0.405	0.178¶0.920	0.031
Constant	3.468	0.014	-	0.063
Items	Wald	Exp(B)	95%CI	P value
Fib	4.366	1.560	1.028¶2.367	0.037
CYP3A5 AA+AG	2.747	1.917	0.888¶4.138	0.097
Constant	6.181	0.005	-	0.013

Fib: fibrinogen; CYP3A5 shows three different polymorphism models, which includes GG/AA/AG.

related to ischemic stroke susceptibility, but not related to plaque stability.

The summary is as follows: EETs, the metabolite of CYP, have many effects, such as regulating vascular tension, anticoagulation, inhibiting proliferation of smooth muscle cells and anti-inflammation, while the mutation of *EPHX2* causes EET to be hydrolyzed, which is disadvantageous to ischemic cells, and increase the risk for ischemic stroke. CYP3A5 is involved in the metabolism of blood lipids related to endogenous substances, and there is a certain correlation between *CYP3A5* and carotid plaque instability in patients with ischemic stroke.

Although there are many important functional mutation sites, this study only detected a small number of SNPs of *CYP* and *EPHX2* genes. This is because the sample was small and restricted to only people from the southeast region of China. It is suggested that future studies expand on the sample used in this study and include more subjects from different ethnic groups, increase the corresponding gene loci, and gene-gene interaction should be investigated. Therefore, further research should be performed to assess the significance of correlations between other genetic markers and cerebral infarction and arterial plaque stability.

Declaration of Competing Interest

All authors disclosed no relevant relationships.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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