Interaction Between *CYP4F2* rs2108622 and *CPY4A11* rs9333025 Variants Is Significantly Correlated with Susceptibility to Ischemic Stroke and 20-Hydroxyeicosatetraenoic Acid Level

Duanxiu Liao,¹ Xingyang Yi,¹ Biao Zhang,¹ Qiang Zhou,² and Jing Lin²

Aims: To investigate the association of four variants of two CYP ω -hydroxylase genes and 20-hydroxyeicosatetraenoic acid (HETE) levels with ischemic stroke (IS) and whether gene–gene interactions between these genes increase the risk of IS. **Methods:** Three hundred ninety-six patients with IS and 378 controls were genotyped for rs2269231, rs9333025, rs2108622, and rs3093135. Gene–gene interactions were analyzed using generalized multifactor dimensionality reduction (GMDR) methods. The 20-HETE levels was measured in 218 IS patients and 126 controls. **Results:** The frequency of the GG genotype of rs9333025 was significantly higher in IS patients than in controls ($p < 0.001$). The GMDR analysis showed a significant gene–gene interaction between rs9333025 and rs2108622 ($p = 0.0116$). This gene–gene interaction predicted a significantly higher risk of IS in individuals carrying the genotypes of rs9333025 GG and rs2108622 GG (odds ratio = 1.92, 95% confidence interval = 1.12–4.26, $p = 0.007$). The plasma levels of 20-HETE were significantly higher in IS patients than in controls, and IS patients carrying the genotype combination of rs9333025 GG and rs2108622 GG had higher 20-HETE levels than IS patients with other combinations of the two variants. **Conclusion:** *CYP4A11* rs9333025 GG and *CYP4F2* rs2108622 GG two-loci interaction significantly increases the risk for IS and an elevated 20-HETE level.

Introduction

STROKE HAS EMERGED as a leading cause of mortality worldwide and become a major public health problem (Feigin, 2005; Domingues-Montanari *et al.*, 2008; Jiang *et al.*, 2006). Ischemic stroke (IS) has generally been considered a heterogeneous and multifactorial disorder caused by both conventional environmental risk factors and genetic factors (Dichgans, 2007). Genetic predisposition has been suggested to significantly participate in the pathogenesis of IS (Guo *et al.*, 2010). Therefore, a detailed understanding of genetic factors involved in IS could provide valuable insights into the pathogenesis of potential prevention and treatment strategies for IS (Goldstein *et al.*, 2006).

Many genetic studies related to IS have been conducted in the last decade (Guo *et al.*, 2010; Chehaibi *et al.*, 2014; Das *et al.*, 2014; Jin *et al.*, 2014; Williams *et al.*, 2014). Indeed, IS appears to be a disease that does not follow a simple Mendelian pattern of inheritance involving a single gene, suggesting that single-locus analysis may not be sufficient to investigate the genetic risk factors of IS (Guo *et al.*, 2010). In addition, single-locus analysis may fail to detect significant variants for which

individual effects could be below the threshold of detection but may exert significant influence on the pathogenesis of IS through synergistic interactions with other loci variants (Culverhouse *et al.*, 2002). Thus, the search for gene variants linked to IS risk could be significantly enhanced by thorough investigation of gene–gene interactions using alternative analytical methods, such as the generalized multifactor dimensionality reduction (GMDR) approach (Lou *et al.*, 2007).

Arachidonic acid (AA) is generally metabolized by the cyclooxygenases, lipoxygenases, and/or cytochrome P450 (CYP) epoxygenases (Calder, 2009). AA can also be metabolized by CYP ω -hydroxylase to form 20-hydroxyeicosatetraenoic acid (20-HETE), a potent vasoconstrictor (Powell *et al.*, 1998; Lasker *et al.*, 2000). 20-HETE plays an important role in the autoregulation of cerebral blood flow and systemic blood pressure (BP) (Gebremedhin *et al.*, 2000), and blockade of the synthesis or vasoconstrictor actions of 20-HETE reduces infarct size in a middle cerebral artery occlusion model of IS (Omura *et al.*, 2006). Because the *CYP4A11* and *CYP4F2* genes encode CYP ω -hydroxylase, any genetic variants in these two genes may be associated with the risk of IS. For instance, polymorphisms in *CYP4F2* (Fava *et al.*, 2008; Fu *et al.*, 2008b;

¹Department of Neurology, People's Hospital of Deyang City, Deyang, China.

²Department of Neurology, Third Affiliated Hospital of Wenzhou Medical College, Zhejiang, China.

Deng *et al.*, 2010; Munshi *et al.*, 2012) and *CYP4A11* C-296T (Ding *et al.*, 2010) have been reported to be associated with an increased risk of IS.

Despite recent publications focused on the role of AA-metabolizing enzymes in IS pathogenesis, no study on the potential effects of gene–gene interactions of the encoding CYP ω -hydroxylase genes on IS risk has been reported. Because CYP genes encode for enzymes that compensate for or interact with each other in AA metabolism, studying combinations of genetic variants of CYP genes may yield significant discoveries toward elucidating the pathogenesis of IS. The present study aimed to investigate variations in CYP genes encoding CYP ω -hydroxylase and their potential synergistic effects on IS as well as to explore potential gene–gene interactions in relation to IS susceptibility and the 20-HETE level.

Materials and Methods

Study populations

The Ethics Committee of the People's Hospital of Deyang City and the Third Affiliated Hospital of Wenzhou Medical College reviewed and approved this study. All patients or their family members provided written informed consent before enrollment.

The study population was composed of 396 IS patients and 378 controls. Patients who were admitted to the above-mentioned two hospitals suffering from their first IS were consecutively recruited between August 2010 and March 2013. Inclusion criteria were as follows: (1) age ≥ 18 years old; (2) diagnosis of IS based on both clinical findings and the results of a neurological examination using computed tomography or magnetic resonance imaging; and (3) IS-related atherothrombotic ($n = 260$) and small artery disease ($n = 136$) according to the TOAST classification system (Han *et al.*, 2006). The exclusion criteria were as follows: (1) cardiogenic cerebral embolism or any cerebral infarction caused by other factors or unknown causes; (2) family history of apoplexy or a history of strokes; and (3) cerebral hemorrhage.

The control subjects were selected from outpatients with no history or family history (genetically related IS) of stroke, as confirmed by medical history, and physical and laboratory examinations at our medical centers.

The following data regarding vascular risk factors for each individual were collected: age, gender, hypertension, diabetes mellitus (DM), cigarette smoking status, alcohol intake, total plasma cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). Hypertension was

defined as the mean of three independent measures of BP $\geq 140/90$ mmHg or the use of antihypertensive drugs. DM was diagnosed by any one or a combination of fasting glucose level >7.8 mM, >11.1 mM 2 h after oral glucose challenge, and the use of hypoglycemic drugs.

Genotyping

The single-nucleotide polymorphisms (SNPs) of the *CYP4A11* and *CYP4F2* genes encoding CYP ω -hydroxylase were selected from the NCBI database (<http://ncbi.nlm.nih.gov/SNP>), according to the following criteria: (1) the SNPs had been examined in previous studies and shown to have a potential association with stroke (Fava *et al.*, 2008; Fu *et al.*, 2008b; Deng *et al.*, 2010; Ding *et al.*, 2010; Munshi *et al.*, 2012); (2) the SNPs have a minor allele frequency (MAF) >0.05 ; (3) the SNPs lead to amino acid changes; and (4) the SNPs are functional. Four TagSNPs with a MAF ≥ 0.05 were found in the human HapMap project database (<http://hapmap.org>), including *CYP4A11* rs2269231 and rs9333025 and *CYP4F2* rs2108622 and rs3093135.

Whole blood (3 mL) was drawn from an arm vein into a sterile tube containing ethylenediaminetetraacetic acid and stored at -80°C until genotype analysis was performed. Genomic DNA was extracted from peripheral blood using a modified phenol/chloroform method and purified using the UNIQ-10 kit (Sangon Biotech Co., Ltd., Shanghai, China).

Genotypes of four variants were examined using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) methods (Aggarwal *et al.*, 2011; Chi *et al.*, 2014). In brief, each SNP-containing gene possessed a specific genotype, with two amplification primers and one extension primer (Table 1). The reaction mixture was desalted by adding 6 mg of cation exchange resin (Sequenom, Inc., San Diego, CA), mixed, and resuspended in 25 μL pure water. Once the primer extension reaction was completed, the samples were spotted onto a 384-well spectroCHIP (Sequenom, Inc.) using a MassARRAY Nanodispenser (Sequenom, Inc.) and genotyped using a MALDI-TOF mass spectrometer (Sequenom, Inc.). Genotype calling was performed in real time with MassARRAY RT software version 3.0.0.4 and analyzed using the MassARRAY Typer software version 3.4 (Sequenom, Inc.).

Measurement of plasma 20-HETE level

Plasma CYP450 metabolite 20-HETE levels were measured using a stable isotope dilution gas chromatography/

TABLE 1. AMPLIFICATION AND EXTENSION PRIMERS USED IN THIS STUDY

SNPs	Forward primer and reverse primer (5' $\rightarrow$ 3')	Extension primer (5' $\rightarrow$ 3')
<i>CYP4A11</i> (rs2269231)	F:CGTTGGATGGGATAATGAGAGGAAGTTGC R:ACGTTGGATGGTAGATTACATCAGATTCC	AAGGGAGAAAATCGAACTTTGTG
<i>CYP4A11</i> (rs9333025)	F:ACGTTGGATGACACTGATTCCCTCAAGGT R:ACGTTGGATGCTGAAGTAAATGATTCTATG	CATTTCCCTCAAGGTCATAAA
<i>CYP4F2</i> (rs2108622)	F:ATCAACCCGTTCCACCT R:ACATTGTGCTCCCAGACG	CCTAATCAATGAAGCA
<i>CYP4F2</i> (rs3093135)	F:ACGTTGGATGCTGCGGAATTTTGGGATGGG R: ACGTTGGATGACCCACCCTTGGTTTTTCTC	GAGGAGCATTGAGGAC

SNPs, single-nucleotide polymorphisms; F, forward primer; R, reverse primer.

mass spectrometry in 218 IS patients and 126 controls, as described previously (Ward *et al.*, 2011).

Statistical analyses

Based on a suggested sample size requirement for detecting gene–gene interaction (Wang and Zhao, 2003), we expected that our sample size of >360 patients and >360 controls would sufficiently provide 80% power at the 5% significance level calculated using three genetic models: the additive model, the dominant model, and the recessive model.

All statistical analyses were performed using SPSS 16.0 (SPSS, Inc., Chicago, IL). The χ^2 test was used to analyze the deviation of Hardy–Weinberg equilibrium for genotype frequencies. Discrete variables were compared using χ^2 tests, or if expected cell frequencies were small, Fisher's exact tests were conducted. Continuous variables were compared between IS patients and controls using Student's *t*-tests. The 20-HETE levels among the different genotypes of the four variants were compared with analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. The Bonferroni test was applied next for comparisons after ANOVA. A *p* value of <0.05 was considered statistically significant.

Gene–gene interactions were investigated using the statistical software GMDR Beta program version 0.7 (www.healthsystem.virginia.edu/internet/addiction-genomics/Software) and multiple logistic regression. The variables entered into the model included age, gender, cigarette smoking, body–mass index, diabetes, hypertension, hyperlipidemia, rs2269231, rs9333025, rs2108622, rs3093135, and the interactive variable of rs9333025 and rs2108622. The relative risk of genotype and prevalence of cerebral infarction were expressed with odds ratios (ORs) and 95% confidence intervals (CIs).

Results

Clinical characteristics of the subjects

The demographic characteristics of the study subjects, which were also included in our previous study (Chi *et al.*, 2014), are presented in Table 2. The IS patients had a higher prevalence of risk factors for stroke, including a history of hypertension ($p < 0.001$) or diabetes ($p = 0.032$). However, differences in other conventional risk factors, including smoking, alcohol intake, body–mass index, and levels of LDL-C, TC, and TG, did not reach statistical significance between the two groups ($p > 0.05$).

Single-locus analysis

The genotype distributions of the four variants in this study were consistent with the Hardy–Weinberg Equilibrium model ($p > 0.05$). Genotype distributions for IS patients and controls are shown in Table 3. The frequency of the GG genotype for rs9333025 was significantly higher in IS patients than in controls ($p < 0.001$). However, no significant differences were observed in genotype distributions for rs2269231, rs2108622, and rs3093135 between the two groups ($p > 0.05$).

Gene–gene interactions

High-order interactions were investigated for IS using the GMDR method, and significant high-order interactions were detected (Table 4). With covariable adjustments, the best

TABLE 2. COMPARISONS OF CLINICAL CHARACTERISTICS AND 20-HYDROXYEICOSATETRAENOIC ACID LEVEL BETWEEN THE ISCHEMIC STROKE AND CONTROL GROUPS

Characteristics	Stroke patients (n=396)	Controls (n=378)	p value
Men, n (%)	235 (59.34)	222 (58.73)	0.924
Age, years	68.79 ± 11.11	64.98 ± 10.29	<0.001
Hypertension, n (%)	302 (76.26)	99 (26.19)	<0.001
Diabetes mellitus, n (%)	138 (34.85)	97 (25.66)	0.032
Cigarette smoking, n (%)	165 (41.67)	159 (42.06)	0.942
Body–mass index, kg/m ²	24.10 ± 2.33	23.90 ± 2.62	0.221
Alcohol intake, n (%)	184 (46.46)	170 (44.97)	0.694
TG, mM	1.96 ± 1.12	1.83 ± 1.02	0.182
TC, mM	5.54 ± 1.36	5.36 ± 1.21	0.061
LDL, mM	3.15 ± 1.27	2.99 ± 1.19	0.376
20-HETE (pM)	1711 ± 162	1465 ± 123	<0.001

Data shared with our previous study (Chi *et al.*, 2014).

HETE, hydroxyeicosatetraenoic acid; TC, total cholesterol; LDL, low-density lipoprotein; TG, triglycerides.

model for IS included the interaction between rs9333025 and rs2108622, which scored 10/10 for cross-validation consistency and 9 for sign test ($p = 0.0116$). Furthermore, significant interactions among the earlier two-loci models were confirmed by a permutation test ($p = 0.0246$). A one-locus model was also computed for each variant, yielding a minimum $p = 0.9468$, suggesting that their contribution to stroke risk was due to the synergistic action of the two genes and likely not to the additive effects of both loci.

Logistic regression analysis

The relative risks of nine combinations of genotypes of rs9333025 and rs2108622 were considered as interactive variables. Logistic regression analysis indicated that the

TABLE 3. COMPARISON OF GENOTYPES BETWEEN THE ISCHEMIC STROKE AND CONTROL GROUPS (N, %)

Genotype	Stroke patients (n=396)	Controls (n=378)	p value
rs2269231			
AA	78 (19.7)	66 (17.5)	0.362
AT	217 (54.8)	206 (54.5)	
TT	101 (25.5)	106 (28.0)	
rs9333025			
AA	7 (1.8)	17 (4.5)	<0.001
AG	90 (22.7)	113 (29.9)	
GG	299 (75.5)	248 (65.6)	
rs2108622			
GG	209 (52.8)	186 (49.2)	0.293
GA	155 (39.1)	155 (41.0)	
AA	32 (8.1)	37 (9.8)	
rs3093135			
TT	331 (83.6)	314 (83.1)	0.999
AT	58 (14.6)	58 (15.3)	
AA	7 (1.8)	6 (1.6)	

TABLE 4. COMPARISON OF THE BEST MODELS, PREDICTION ACCURACIES, CROSS-VALIDATION CONSISTENCIES, AND *P* VALUES IDENTIFIED BY GENERALIZED MULTIFACTOR DIMENSIONALITY REDUCTION

Best model ^a	Training balanced accuracy	Testing balanced accuracy	Cross-validation consistency	Sign test (p)
1	0.6145	0.4868	9/10	5 (0.9468)
1,2	0.6336	0.5822	10/10	9 (0.0116)
1,2,3	0.5724	0.4736	7/10	2 (0.9326)
1,2,3,4	0.6012	0.4546	6/10	2 (0.9287)

^ars9333025, rs2108622, rs2269231, and rs3093135 are denoted as 1–4, respectively.

gene–gene interaction of rs9333025 GG and rs2108622 GG predicted a significantly higher risk of IS (adjusted for age, hypertension, DM; OR = 1.92, 95% CI = 1.12–4.26, *p* = 0.007; Table 5). There were no statistically significant interactions between the other genetic variant combinations.

20-HETE level and its association with genotype distribution

Plasma 20-HETE levels of IS patients were significantly higher than those of the controls (Table 2). There were no significant associations between the 20-HETE level and any one of the four variant genotype distributions (all *p* > 0.05; Table 6). However, stratified analyses based on different genotype combinations of rs9333025 and rs2108622 revealed that IS patients carrying the genotype combination of rs9333025 GG and rs2108622 GG had a higher 20-HETE level than those carrying other combinations of the two variants (1802 ± 176 vs. 1612 ± 141 pM, *p* < 0.001; Table 6).

Discussion

The present study showed that the variation of rs9333025, but not rs2269231, rs2108622, and rs3093135, differed significantly between IS patients and controls with a single-locus analytical approach. Our results were consistent with those of previous studies (Fu *et al.*, 2008a; Ding *et al.*, 2010; Liang *et al.*, 2014), but contradicted the findings of some other studies (Fava *et al.*, 2008; Fu *et al.*, 2008b; Ward *et al.*, 2008; Deng *et al.*, 2010; Munshi *et al.*, 2012). For instance, Ward *et al.* (2008) reported that a single polymorphism in the *CYP4F2* gene, but not the *CYP4A11* gene, is associated with increased 20-HETE secretion and BP. A population study suggested that the V433M polymorphism in *CYP4F2* may

TABLE 6. ASSOCIATION OF 20-HYDROXYEICOSATETRAENOIC ACID LEVELS WITH GENOTYPE DISTRIBUTION IN PATIENTS

	20-HETE (pM)	p value
rs2269231		
AA (n = 43)	1673 ± 156	
AT (n = 119)	1686 ± 161	
TT (n = 56)	1701 ± 178	0.768
rs9333025		
AA (n = 4)	1694 ± 159	
AG (n = 49)	1725 ± 172	
GG (n = 165)	1708 ± 168	0.694
rs2108622		
GG (n = 115)	1726 ± 182	
GA (n = 85)	1697 ± 167	
AA (n = 18)	1711 ± 172	0.465
rs3093135		
TT (n = 182)	1679 ± 146	
AT (n = 31)	1687 ± 164	
AA (n = 5)	1693 ± 156	0.823
Genetic combination of rs9333025 GG and rs2108622 GG		
Yes (n = 94)	1802 ± 176	<0.001
No (n = 124)	1612 ± 141	

increase the risk of IS in male subjects (Fava *et al.*, 2008), and the G allele of rs2108622 is associated with IS in Japanese men (Fu *et al.*, 2008b). One study in south India also found an association between 1347 G/A polymorphism and stroke (Munshi *et al.*, 2012). There are a number of potential explanations for the differences between our results and those of others. First, racial differences exist among the investigations. Second, IS etiology can be complicated, and it is highly likely that the progression to IS requires several variations, each with minor and or potentially undetectable individual effects (Schork *et al.*, 2009). Therefore, a linkage analysis, which is used to investigate single-gene disorders, seems unsuitable for genetic studies on IS.

A GMDR approach has been used to identify genetic IS risk factors (Liu *et al.*, 2009; Yi *et al.*, 2013) and also was used in the present study. GMDR analysis can reveal interesting synergistic effects of gene variant–gene variant interaction. Specifically, the risk for IS was noted to be increased by 1.92-fold in individuals carrying both the rs9333025 GG and rs2108622 GG genotypes, indicating that the interaction between rs9333025 and rs2108622 variants plays a key role in the genetic predisposition to IS. Our findings add to the evidence that interactions between genes with individually marginal effects may significantly increase the risk of common and complex diseases, such as IS.

However, the nature of the two-gene variant interaction between rs9333025 and rs2108622 remains unclear. One possible explanation is that these two genes participate in AA metabolism. The *CYP4A11* and *CYP4F2* genes encode CYP ω-hydroxylase, which is primarily responsible for metabolizing AA into 20-HETE, a potent vasoconstrictor (Powell *et al.*, 1998; Lasker *et al.*, 2000). In the current study, we found that the 20-HETE level of IS patients was significantly higher compared with that of the controls, and IS patients carrying the genotype combination of rs9333025 GG and rs2108622 GG had a higher 20-HETE level than those with other combinations of the two variants. The results indicated

TABLE 5. MULTIPLE REGRESSION ANALYSIS OF THE MAJOR RISK FACTORS FOR CEREBRAL INFARCTION

Risk factor	Wald	OR	95% CI	p value
Age	5.23	1.46	1.01–1.99	0.026
Hypertension	13.32	6.12	2.32–10.68	0.000
Diabetes mellitus	1.46	0.98	0.54–1.42	0.236
rs9333025 GG	2.66	1.26	0.92–1.88	0.062
Interactive variable ^a	7.88	1.92	1.12–4.26	0.007

^ars9333025 GG and rs2108622 GG.

OR, odds ratio; CI, confidence interval.

that rs9333025 GG and rs2108622 GG two-loci interaction can increase capability to metabolize AA, resulting in increased 20-HETE production. 20-HETE constricts cerebral arteries and regulates cerebral vascular tone (Lange *et al.*, 1997; Gebremedhin *et al.*, 2000) by activating intracellular signaling pathways, including protein kinase C (PKC), which are involved in apoptosis and cell death (Sun *et al.*, 1999; Randriamboavonjy *et al.*, 2003). 20-HETE also depolarizes vascular smooth muscle cells through inhibition of the large-conductance Ca^{2+} -sensitive K^{+} channel and Na^{+} , K^{+} -ATPase activity together with an increase in Ca^{2+} influx through L-type Ca^{2+} channels (Gladden *et al.*, 1998; Cheng *et al.*, 2008). Finally, 20-HETE promotes the formation of oxygen radicals (Guo *et al.*, 2007). Accumulating evidence indicates that 20-HETE is involved in endothelial dysfunction (Singh *et al.*, 2007), which is closely linked to cardiovascular events (Corrado *et al.*, 2008). Inhibition of the synthesis of 20-HETE has been shown to reverse the decrease in cerebral blood flow following subarachnoid hemorrhage and to reduce infarct size following transient cerebral ischemia (Takeuchi *et al.*, 2005; Tanaka *et al.*, 2007). All of these previous observations suggest a potential molecular mechanism by which a two-factor interaction influences the development of IS.

Limitations

The present study, however, focused only on four variants of two CYP ω -hydroxylase genes and the interactions among them in a small localized population; our results cannot represent those for a full spectrum of the Chinese population. Thus, our findings need to be confirmed in large sample size and multicenter studies. Our results indicated that IS patients carrying the combination of rs9333025 GG and rs2108622 GG had a higher 20-HETE level than those with combinations of one of the two variants and others. However, we did not obtain direct experimental evidence linking the elevated 20-HETE level and rs9333025 GG/rs2108622 GG genetic combination of CYP4A and CYP4F genes. Thus, the present results will need to be validated in further studies. Although this study examined the role of several known important CYP450 ω -hydroxylase genes, other known and unknown genes were not captured. Thus, future studies involving a larger set of genetic variants should be conducted to elucidate the full extent of gene–gene interaction effects on IS pathogenesis.

Summary

To the best of our knowledge, the present study is the first to identify an interaction between rs9333025 and rs2108622 gene variants in IS patients. Specifically, we discovered that this gene–gene interaction of rs9333025 GG and rs2108622 GG two loci confers a significant increase in the risk for IS and 20-HETE level in the Chinese population. Such double-loci variant analytical approaches may provide further insight into the complex pathogenesis of IS. However, our conclusion may need to be confirmed in multicenter studies with a larger sample size, as well as in animal studies, before it is specific and efficiently used for predicting the risk of IS in humans.

Acknowledgments

This study was supported by the Scientific Research Foundation of Sichuan Provincial Health Department (Grant

No. 140025) and Deyang City Science and Technology Research Foundation (Grant No. 2014SZ035).

Author Contributions

D.L. contributed to the study design and literature. X.Y. contributed to data interpretation and writing. B.Z. contributed to data analysis and data collection. Q.Z. contributed to data collection and figures. J.L. contributed to data collection. All authors reviewed and approved the article.

Author Disclosure Statement

No competing financial interests exist.

References

- Aggarwal S, Ali S, Chopra R, *et al.* (2011) Genetic variations and interactions in anti-inflammatory cytokine pathway genes in the outcome of leprosy: a study conducted on a MassARRAY platform. *J Infect Dis* 204:1264–1273.
- Calder PC (2009) Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie* 91:791–795.
- Chehaibi K, Hrira MY, Nouira S, *et al.* (2014) Matrix metalloproteinase-1 and matrix metalloproteinase-12 gene polymorphisms and the risk of ischemic stroke in a Tunisian population. *J Neurol Sci* 342:107–113.
- Cheng J, Ou JS, Singh H, *et al.* (2008) 20-Hydroxyeicosatetraenoic acid causes endothelial dysfunction via eNOS uncoupling. *Am J Physiol Heart Circ Physiol* 294:H1018–H1026.
- Chi LF, Yi XY, Shao MJ, *et al.* (2014) Interaction between ALOX5AP and CYP3A5 gene variants significantly increases the risk for cerebral infarctions in Chinese. *Neuroreport* 25:452–457.
- Corrado E, Rizzo M, Coppola G, *et al.* (2008) Endothelial dysfunction and carotid lesions are strong predictors of clinical events in patients with early stages of atherosclerosis: a 24-month follow-up study. *Coron Artery Dis* 19:139–144.
- Culverhouse R, Suarez BK, Lin J, *et al.* (2002) A perspective on epistasis: limits of models displaying no main effect. *Am J Hum Genet* 70:461–471.
- Das S, Roy S, Kaul S, *et al.* (2014) CRP gene (1059G>C) polymorphism and its plasma levels in ischemic stroke and hemorrhagic stroke in a South Indian population. *Inflammation* 37:1683–1688.
- Deng S, Zhu G, Liu F, *et al.* (2010) CYP4F2 gene V433M polymorphism is associated with ischemic stroke in the male Northern Chinese Han population. *Prog Neuropsychopharmacol Biol Psychiatry* 34:664–668.
- Dichgans M (2007) Genetics of ischaemic stroke. *Lancet Neurol* 6:149–161.
- Ding H, Cui G, Zhang L, *et al.* (2010) Association of common variants of CYP4A11 and CYP4F2 with stroke in the Han Chinese population. *Pharmacogenet Genomics* 20:187–194.
- Domingues-Montanari S, Mendioroz M, del Rio-Espinola A, *et al.* (2008) Genetics of stroke: a review of recent advances. *Expert Rev Mol Diagn* 8:495–513.
- Fava C, Montagnana M, Almgren P, *et al.* (2008) The V433M variant of the CYP4F2 is associated with ischemic stroke in male Swedes beyond its effect on blood pressure. *Hypertension* 52:373–380.
- Feigin VL (2005) Stroke epidemiology in the developing world. *Lancet* 365:2160–2161.
- Fu Z, Nakayama T, Sato N, *et al.* (2008a) Haplotype-based case study of human CYP4A11 gene and cerebral infarction in Japanese subject. *Endocrine* 33:215–222.

- Fu Z, Nakayama T, Sato N, *et al.* (2008b) A haplotype of the CYP4F2 gene is associated with cerebral infarction in Japanese men. *Am J Hypertens* 21:1216–1223.
- Gebremedhin D, Lange AR, Lowry TF, *et al.* (2000) Production of 20-HETE and its role in autoregulation of cerebral blood flow. *Circ Res* 87:60–65.
- Gladden MH, Jankowska E, Czarkowska-Bauch J (1998) New observations on coupling between group II muscle afferents and feline gamma-motoneurons. *J Physiol* 512:507–520.
- Goldstein LB, Adams R, Alberts MJ, *et al.* (2006) Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: the American Academy of Neurology affirms the value of this guideline. *Stroke* 37:1583–1633.
- Guo AM, Arbab AS, Falck JR, *et al.* (2007) Activation of vascular endothelial growth factor through reactive oxygen species mediates 20-hydroxyeicosatetraenoic acid-induced endothelial cell proliferation. *J Pharmacol Exp Ther* 321:18–27.
- Guo JM, Liu AJ, Su DF (2010) Genetics of stroke. *Acta Pharmacol Sin* 31:1055–1064.
- Han SW, Kim SH, Lee JY, *et al.* (2006) A new subtype classification of ischemic stroke based on treatment and etiologic mechanism. *Eur Neurol* 57:96–102.
- Jin J, Li W, Peng L, *et al.* (2014) Relationship between interleukin-10-1082A/G polymorphism and risk of ischemic stroke: a meta-analysis. *PLoS One* 9:e94631.
- Lange A, Gebremedhin D, Narayanan J, *et al.* (1997) 20-Hydroxyeicosatetraenoic acid-induced vasoconstriction and inhibition of potassium current in cerebral vascular smooth muscle is dependent on activation of protein kinase C. *J Biol Chem* 272:27345–27352.
- Lasker JM, Chen WB, Wolf I, *et al.* (2000) Formation of 20-hydroxyeicosatetraenoic acid, a vasoactive and natriuretic eicosanoid, in human kidney. Role of Cyp4F2 and Cyp4A11. *J Biol Chem* 275:4118–4126.
- Liang JQ, Yan MR, Yang L, *et al.* (2014) Association of a CYP4A11 polymorphism and hypertension in the Mongolian and Han populations of China. *Genet Mol Res* 13:508–517.
- Liu J, Sun K, Bai Y, *et al.* (2009) Association of three-gene interaction among MTHFR, ALOX5AP and NOTCH3 with thrombotic stroke: a multicenter case-control study. *Hum Genet* 125:649–656.
- Lou XY, Chen GB, Yan L, *et al.* (2007) A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. *Am J Hum Genet* 80:1125–1137.
- Munshi A, Sharma V, Kaul S, *et al.* (2012) Association of 1347 G/A cytochrome P450 4F2 (CYP4F2) gene variant with hypertension and stroke. *Mol Biol Rep* 39:1677–1682.
- Omura T, Tanaka Y, Miyata N, *et al.* (2006) Effect of a new inhibitor of the synthesis of 20-HETE on cerebral ischemia reperfusion injury. *Stroke* 37:1307–1313.
- Jiang B, Wang WZ, Chen H, *et al.* (2006) Incidence and trends of stroke and its subtypes in China: results from three large cities. *Stroke* 37:63–68.
- Powell PK, Wolf I, Jin R, *et al.* (1998) Metabolism of arachidonic acid to 20-hydroxy-5,8,11, 14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. *J Pharmacol Exp Ther* 285:1327–1336.
- Randriamboavonjy V, Busse R, Fleming I (2003) 20-HETE-induced contraction of small coronary arteries depends on the activation of Rho-kinase. *Hypertension* 41:801–806.
- Schork NJ, Murray SS, Frazer KA, *et al.* (2009) Common vs. rare allele hypotheses for complex diseases. *Curr Opin Genet Dev* 19:212–219.
- Singh H, Cheng J, Deng H, *et al.* (2007) Vascular cytochrome P450 4A expression and 20-hydroxyeicosatetraenoic acid synthesis contribute to endothelial dysfunction in androgen-induced hypertension. *Hypertension* 50:123–129.
- Sun CW, Falck JR, Harder DR, *et al.* (1999) Role of tyrosine kinase and PKC in the vasoconstrictor response to 20-HETE in renal arterioles. *Hypertension* 33:414–418.
- Takeuchi K, Renic M, Bohman QC, *et al.* (2005) Reversal of delayed vasospasm by an inhibitor of the synthesis of 20-HETE. *Am J Physiol Heart Circ Physiol* 289:H2203–H2211.
- Tanaka Y, Omura T, Fukasawa M, *et al.* (2007) Continuous inhibition of 20-HETE synthesis by TS-011 improves neurological and functional outcomes after transient focal cerebral ischemia in rats. *Neurosci Res* 59:475–480.
- Wang S, Zhao H (2003) Sample size needed to detect gene-gene interactions using association designs. *Am J Epidemiol* 158:899–914.
- Ward NC, Croft KD, Blacker D, *et al.* (2011) Cytochrome P450 metabolites of arachidonic acid are elevated in stroke patients compared with healthy controls. *Clin Sci* 121:501–507.
- Ward NC, Tsai IJ, Barden A, *et al.* (2008) A single nucleotide polymorphism in the CYP4F2 but not CYP4A11 gene is associated with increased 20-HETE excretion and blood pressure. *Hypertension* 51:1393–1398.
- Williams SR, Yang Q, Chen F, *et al.* (2014) Genome-wide meta-analysis of homocysteine and methionine metabolism identifies five one carbon metabolism loci and a novel association of ALDH1L1 with ischemic stroke. *PLoS Genet* 10:e1004214.
- Yi XY, Zhou Q, Lin J, *et al.* (2013) Interaction between ALOX5AP-SG13S114A/T and COX-2-765G/C increases susceptibility to cerebral infarction in a Chinese population. *Genet Mol Res* 12:1660–1669.

Address correspondence to:

Xingyang Yi, MD

Department of Neurology

People's Hospital of Deyang City

Deyang, Sichuan 618000

China

E-mail: yixingyang64@126.com