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Association of Common Variants of *CYP4A11* and *CYP4F2* with Stroke in the Han Chinese Population

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Abstract

20-Hydroxyeicosatetraenoic acid (20-HETE) has been shown to play an important role in cerebral vascular function. We hypothesized that polymorphisms in genes encoding 20-HETE synthesizing enzymes might confer susceptibility to stroke. To test the hypothesis, haplotype tagging SNPs (htSNPs) and potential functional polymorphisms of CYP4A11 and CYP4F2 genes were genotyped in 558 ischemic stroke patients, 221 hemorrhagic stroke patients and 557 controls. The association analyses were performed at both SNP and haplotype levels. We further verified our findings in an independent cohort of 551 ischemic stroke cases and 48 hemorrhagic stroke subjects and 694 unaffected controls. We identified *CYP4A11* C-296T and *CYP4F2* V433M were associated with significantly increased risk of ischemic stroke (CT+TT vs CC, adjusted odds ratio (OR) 1.50, 95% confidence interval (CI) 1.17–1.93, $P_{combined}$=0.001, $P_{corr=}$0.008; V/M+M/M vs. V/V, OR 1.38, 95% CI, 1.15–1.65, $P_{combined}=5.6\times10^{-4}$, $P_{corr=}$0.005, respectively) Interestingly, the effects of *CYP4F2* V433M on ischemic stroke in our study was only evident in male subjects. Our results suggest that genetic variation in CYP4A11 and CYP4F2 alters susceptibility to stroke in the Han Chinese population.

Keywords

ischemic stroke; Genetics; haplotypes; Han Chinese population

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SUPPLEMENTARY MATERIAL

Supplementary Material is available Online.

Conflict of Interest statement. None

INTRODUCTION

The *CYP4A11* and *CYP4F2* genes encode cytochrome P450 enzymes that are primarily responsible for metabolizing arachidonic acid into 20-HETE, a potent vasoconstrictor[1,2]. 20-HETE constricts cerebral arteries by activating PKC and depolarizing vascular smooth muscle cells through inhibition of the large-conductance Ca^{2+}-sensitive K^+ channel[3–8] and increasing Ca^{2+} influx via L-type Ca^{2+} channels[4]. Previous studies have indicated that nitric oxide (NO) inhibits the formation of 20-HETE and a fall in 20-HETE levels appears to contribute to the vasodilatory response to NO in cerebral arteries[3]. Furthermore, 20-HETE plays an important role in the autoregulation of cerebral blood flow[9], and blockade of the synthesis or vasoconstrictor actions of 20-HETE reduces infarct size in a middle cerebral artery occlusion model of ischemic stroke and also prevents the acute fall in cerebral blood flow after subarachnoid hemorrhage[10–13].

Several polymorphisms in 20-HETE synthesizing enzyme genes have recently been discovered, including two functional variants: F434S of *CYP4A11* (rs1126742) and V433M of *CYP4F2* (rs2108622). Both of these variants produce proteins with a significantly reduced ability to metabolize arachidonic acid and result in reduced 20-HETE production in vitro[14,15]. Moreover, a variety of functional variants and haplotypes of 20-HETE synthesizing enzyme genes have been shown to be associated with hypertension[14,16–18]. On the other hand, cumulative evidence indicates that 20-HETE is involved in endothelial dysfunction[19–23], which is closely linked to cardiovascular events[24,25]. Interestingly, a large urban-based population study conducted in middle-aged Swedes suggested that the V433M polymorphism in *CYP4F2* may increase the risk of ischemic stroke in male subjects only partially through its elevating effect on blood pressure[26]. On the basis of these observations and considering the importance of 20-HETE in cerebral vascular function, polymorphisms in *CYP4A11* and *CYP4F2* are reasonable candidate mutations for altering the risk of stroke. Until now, the systematic association of these genes with stroke had not been examined. Thus, the aim of the present study was to identify putative functional variants in the *CYP4A11* and *CYP4F2* genes, and to determine whether genetic variation in these genes was associated with stroke in the Han Chinese population.

Methods

Study population and data collection

This was a multi-center study for the assessment of risk factors for stroke and sponsored by the Ministry of Science and Technology of China. Briefly, a total of 558 ischemic stroke and 221 hemorrhagic stroke subjects were recruited between November 2004 and June 2006 from five hospitals in Wuhan, China. Ischemic stroke patients were further divided into two subtypes - large vessel disease (N=410) and lacunar stroke (N=148). Subjects with subarachnoid hemorrhage, embolic brain infarction, brain tumors and cerebrovascular malformation, and severe systemic diseases such as pulmonary fibrosis, endocrine and metabolic disease (except diabetes mellitus), severe inflammatory diseases, autoimmune disease, tumors and serious chronic diseases (e.g., hepatic cirrhosis, renal failure) were excluded from the study. Subjects with cardioembolic stroke and documented atrial fibrillation were also excluded from the study. Diagnosis of stroke was based on the results of neurological examination, CT or MRI according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [27].

A total of 557 ethnically and geographically matched controls were randomly selected from the population. These controls were normal, community-based residents (89.6%) or inpatients (10.4%) with minor illnesses. All control subjects were free of neurological conditions and followed the same exclusion criteria as cases. They were also asked for a

detailed medical history and received a physical examination with emphasis on the neurological system including evaluation of body mass index.

In order to confirm credibility of results obtained from the first set of populations described above, we introduced the second independent case-control cohort which comprised 551 patients with ischemic stroke (54.5% large vessel disease and 45.6% lacunar stroke), 48 hemorrhagic stroke subjects and 694 unaffected controls recruited simultaneously from Xinhua Hospital, First Wuhan Hospital and Tongji Hospital between August 2007 and May 2009 in Hubei Province, China. The diagnostic criteria for stroke and the recruited criteria for controls were identical to those used in the first study.

All the study protocols were approved by the review board of the Ministry of Public Health, Ministry of Science and Technology of China and the ethics committees at all participating hospitals, and informed written consent was obtained from all participants.

DNA isolation and genotyping

Genomic DNA was isolated from whole blood collected in K_3-EDTA tubes using the QG-Mini80 workflow with a DB-S kit (FUJIFILM Corporation, Tokyo, Japan) as instructed. DNA was quantified and diluted to a final concentration of 10 ng/μl.

All samples were genotyped using the TaqMan™ 7900HT Sequence Detection System according to the manufacturer's instructions. Each assay was carried out using 10ng DNA in a 5μl reaction consisting of TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA, USA), forward and reverse primers and FAM and VIC labelled probes designed by Applied Biosystems (ABI Assays-on-Demand (rs2108622, C_16179493_40; rs9333025, C_29846881_10; rs3093156, C_25925141_10; rs3093135, C_27482167_10) and Assays-on-Design, see Supplementary Table 1). Probe and primer sequences of TaqMan assay for additional unlinked 26 SNPs were designed by ABI Primer Expression 3.0 software and synthesized by Jikang Biotech Co.,Ltd,. Shanghai, China (Supplementary Table 1).

Allelic discrimination was measured automatically using the Sequence Detection Systems 2.1 software (autocaller confidence level 95%). A total of 10% of all genotypes were repeated in independent PCR reactions to check for consistency and to ensure intraplate and interplate genotype quality control. No genotyping discrepancies were detected between the repeated samples. DNA samples for cases and controls were run in the same batches.

Variation screening

CYP4A11 (MIM: 601310) consists of 12 exons spanning 12.3 Kb region on chromosome 1p23 and *CYP4F2* (MIM:604426) consists of 13 exons spanning 20 Kb region on chromosome 19p13, respectively. Common polymorphisms of *CYP4A11* (GenBank Accession: AF525488) and *CYP4F2* (GenBank Accession: AF467894) were identified by direct sequencing of genomic DNA derived from 48 randomly selected controls. We designed PCR arrays to amplify regions up to 1.0 kb upstream from transcription-initiation sites (i.e., the putative proximal promoter region), all exons, and adjacent noncoding regions. A total length of 5.9 kb and 5.5kb were screened for *CYP4A11* and *CYP4F2* genes, respectively. Fluorescent dye-terminator cycle sequencing was performed and products were analyzed with an Applied Biosystems 3730 capillary sequencer. Finally, we used the chromas program to identify SNP candidates that were then confirmed by two independent observers. We further confirmed these SNP positions and individual genotypes by reamplifying and resequencing the SNP site from the opposite strand.

Population stratification assessment

Although all the samples were carefully recruited from the same geographically regions, the possible unequal genetic admixture or population subdivision in the control and patient populations could have resulted in a spurious association between a marker and disease. Twenty-six unlinked SNPs were additionally genotyped to test and quantity the levels of background genetic differences[28]. These SNPs (1) are randomly distributed from genome, (2) polymorphic among major ethnic groups (European, Asian and African) and (3) not in linkage disequilibrium with the *CYP4A11* and *CYP4F2* loci and with any other known gene. We applied χ^2 tests to compare allele frequencies of each SNP in patients and controls. The obtained mean χ^2 statistic among the 26 SNPs represents the levels of stratification (μ) between two groups and additionally are used to correct the stratification[29].

Statistical analysis

Statistical analysis was performed with SPSS 13.0 (SPSS Inc, Chicago) for Windows (Microsoft Corp, Redmond, Wash), SAS 9.1 (SAS Institute, Cary NC) and SNPassoc[30] for R package. The distribution of quantitative variables was tested for normality by use of a 1-sample Kolmogorov-Smirnov test. For comparison of the baseline characteristics among groups of subjects, quantitative variables, including age, body mass index, HDL-C, and TC, were compared with 1-way analysis of variance and Dunnett's test. A χ^2 test was used to compare qualitative variables.

Hardy-Weinberg equilibrium was calculated using SNPassoc. Differences of allelic and genotype frequencies between cases and controls of each SNP were determined by the χ^2 test or Fisher's exact test. ORs and 95% CI were used to determine the association between genotypes and the risk of stroke. Allelic frequencies and crude ORs from two case-control populations were combined using Mantel-Haenszel test. Multiple unconditional logistic regression was used to estimate adjusted ORs and 95% CI for each SNP after adjustment for gender, age, body mass index, hypertension, diabetes, hyperlipidemia and smoking status. The combined adjusted ORs for two sample sets were determined by additional adjustment for different populations.

Haplotype frequencies for various SNP combinations were estimated by *haplo.stats* version 1.2.1[31] for the R programming language and double-checked using Haploview 4.0[32]. Both of the aforementioned softwares use an expectation-maximization (EM) method of constructing haplotypes. Two haplotype-based hypothesis tests were conducted. The first (global *p*-values) examines the differences in haplotype frequency profiles between cases and controls using the haplo.stats software. For the second, haplotype-based hypothesis tests of generalized linear models were conducted in which stroke and subtype status were the dependent variables, while haplotypes and other conventional risk factors were the independent and covariate variables.

The present study had 80% power to detect an association with OR>1.45 (assuming a risk effect) or <0.69 (assuming a protective effect) for alleles at 15–40% frequency assuming two-sided level at 0.05 with the use of the QUANTO program in dominant mode[33]. $p<0.05$ was considered statistically significant and all statistical tests were two sided.

Results

Identification and selection of SNPs

Resequencing of the *CYP4A11* and *CYP4F2* genes identified 23 polymorphisms: 4 in promoter regions, 13 in the coding region, 5 in introns, and 1 in a 3′ untranslated region (Supplementary Table 2). In the *CYP4A11* promoter region, one novel polymorphism,

C-296T, was identified. Besides the three previously reported non-synonymous polymorphisms (*CYP4A11* F434S, rs1126742; *CYP4F2* V433M, rs2108622; *CYP4F2* G12W, rs3093105), two additional polymorphisms giving rise to amino acid changes were identified in the *CYP4A11* gene (*CYP4A11* K277T, rs41300339; *CYP4A11* S353G, rs3899049). However, rs41300339 significantly deviated from Hardy-Weinberg equilibrium (HWE) and rs3899049 was rare in the Chinese Han population. We resequenced rs41300339 in our sample again and successfully genotyped (Taqman-assay) this polymorphism in additional 200 randomly selected control individuals and found that it remained did not conform to Hardy-Weinberg equilibrium, indicating it is not a sequencing error. Three identified polymorphisms (rs3093090, rs3093091 and rs3093092) were adjacent to one another in the *CYP4F2* promoter and this resulted in technical difficulties with Taqman-MGB genotyping assays. Thus, we eliminated these three polymorphisms from the genotyping effort.

In order to obtain maximum haplotype information for each of the genes and intergenic regions that are likely to harbor regulatory elements, we selected haplotype tagging SNPs (htSNPs) from common variants (minor-allele frequency [MAF] > 0.05) spanning the *CYP4A11* and *CYP4F2* genes (from 1 kb upstream of transcription-initiation sites to 1 kb downstream of stop codes for each gene) in the Han Chinese population(HCB) of the HapMap project (the Phase II database) using Haploview 4.0 software (pairwise tagging only, r^2>0.75). The initial genotyped SNPs captured 82% variants for CYP4a11 and 83% variants for CYP4F2 in HapMap database. In addition, polymorphisms were selected for genotyping on the basis of their known or likely functional significance and frequency (>5%) within the Chinese Han population. Ultimately, we chose nine polymorphisms spanning the *CYP4A11* and *CYP4F2* genes for genotyping in our study.

Single locus analysis

The demographic details for the two case-control studies are shown in Tables 1 and 2. Supplementary Tables 3 and 4 summarize the *CYP4A11* and *CYP4F2* allele frequencies for individuals with ischemic stroke, subtypes of ischemic stroke, and hemorrhagic stroke in two independent population sets. The results of association analyses for each SNP are also shown as crude and adjusted odds ratios. All SNPs conformed to HWE except rs3093168 (SNP in intron of *CYP4F2*), which significantly deviated from HWE in controls and cases. Thus, eight SNPs were selected for further analysis.

As shown in Table 3, several $P_{dominant}$ values of two SNPs, *CYP 4A11* C-296T and *CYP4F2* V433M were statistical significant (p<0.05) in initial study (population set1) and replicated in the second independent cohort (population set 2). Combined analysis from data set 1 and set 2 showed an even stronger association between ischemic stroke with *CYP 4A11* C-296T (OR 1.50, 95% CI 1.17–1.93; $P_{combined}$=0.001) as well as *CYP4F2* V433M (OR 1.38, 95% CI 1.15–1.65; $P_{combined}=5.6\times10^{-4}$) with adjustment for clinical covariates and different population sets (Table 3), which even remained significant after stringent Bonferroni correction (P_{corr}= 0.008 for *CYP 4A11* C-296T and P_{corr}= 0.005 for *CYP4F2* V433M, respectively). To exclude the possible effect of subtypes of ischemic stroke on the detection of association, we then analyzed the data based on large vessel disease and lacunar infraction and found that the effects of aforementioned two SNPs in our study only evident in large vessel disease (*CYP 4A11* C-296T, OR 1.50, 95% CI 1.17–1.93, $P_{combined}$ =0.001, P_{corr}= 0.008; *CYP4F2* V433M (OR 1.38, 95% CI 1.04–2.02; $P_{combined}$ =0.003, P_{corr}= 0.024). Interestingly, we further found that the effects of *CYP4F2* V433M on ischemic stroke and large vessel disease in our study was only evident in male subjects (for both, OR 1.48, 95% CI 1.13–1.93; $P_{combined}$ =0.004, P_{corr}= 0.032)(Table 4).

To identify the variants are indeed genuinely associated with ischemic stroke, we additionally genotyped 26 unlinked SNPs markers to test and quantity the levels of background genetic differences (population set 1, 557 controls and 558 ischemic stroke patients; population set 2, 694 controls and 551 ischemic stroke patients). None of the SNPs deviated from HWE in both control subjects and ischemic stroke patients. The mean χ^2 statistic among the 26 SNPs for the comparison of allele frequencies between the control and case groups (Supplementary table 5), which represents the levels of stratification (μ), were 0.972 (p=0.324) for population set 1 and 0.954 (p=0.329) for population set 2, respectively, indicating that the two groups in two population sets were not significantly stratified and no correction was necessary.

Haplotype analysis

We used the *haplo.stats* program to determine whether the combined effects of common SNPs in the *CYP4A11* and *CYP4F2* genes were associated with ischemic or hemorrhagic stroke risk. Given the possibility of false positive findings related to low frequency haplotypes, only haplotypes with a frequency greater or equal to 3% were considered in the association analysis. Although some risk haplotypes were found to significantly alter the risk of ischemic stroke or hemorrhagic stroke in the first discovery study, none of them reproducible in the second replication population (see Supplementary Tables 6 to 13).

Discussion

The purpose of this study was to examine potential associations between polymorphisms in the *CYP4A11* and *CYP4F2* genes and stroke in two independent the Chinese stroke cohorts. Results showed that a novel SNPs *CYP4A11* C-296T and the functional variant *CYP4F2* V433M were independently associated with ischemic stroke and large vessel disease in two population sets. These results support our hypothesis that variation in *CYP4A11* and *CYP4F2* genes are associated with stroke risk in the Chinese Han population.

Similar to the Korean population, the MAFs of polymorphisms in *CYP4A11* and *CYP4F2* in the Chinese Han population were different than in Europeans. For example, three synonymous polymorphisms in *CYP4A11* (H323H, H352H, I458I) were reported to be common in Korean and Chinese Han population, but were nonexistent in Europeans[34]. Gainer et al. reported two common polymorphisms in the promoter region of *CYP4A11* in Caucasians[14], while minimal variability was detected in this region in the Chinese Han population. However, we did identify a novel common polymorphism (−296C>T) in this region in the Chinese Han population. The results from our study suggest genetic diversity in different ethnic populations and highlight the necessity for population-specific polymorphism screening.

In single SNP analysis, the novel −296C>T polymorphism was significantly associated with large vessel disease in a dominant model. To determine whether the −296C>T polymorphism in the promoter region of *CYP4A11* would influence transcription of this gene, we constructed two plasmids with *CYP4A11* genomic fragments containing the two alleles upstream of a luciferase reporter. Results from this experiment showed that the effect of the −296C>T single base pair substitution on baseline transcriptional activity was not significant (data not shown). These results suggest that the −296C>T polymorphism of *CYP4A11* may not be the causal variant, but instead may be in linkage disequilibrium with the causal variant. Regardless, this novel SNP may serve as a genetic marker for large vessel disease stroke risk in this population.

Kinetic analysis shows that the capacity of CYP4F2 to convert AA to 20-HETE in the human kidney is nearly 10-fold greater than that of CYP4A11[2]. Immunoprecipitation studies

also reveal that treatment with anti-CYP4F2 inhibited 20-HETE formation in renal microsomes nearly two times more than treatment with anti-CYP4A11[2]. Ward et al. also showed that a single polymorphism in the *CYP4F2* gene, but not the *CYP4A11* gene, was associated with increased 20-HETE secretion and blood pressure[18]. Based on these results, it is possible that mutations in CYP4F2 may contribute to altered 20-HETE production which could ultimately lead to altered risk for stroke. In agreement with this suggestion, we found that the functional variant of *CYP4F2*, V433M (rs2108622), but not the functional variant of CYP4A11, F434S (rs1126742), was significantly associated with ischemic stroke independent of conventional cardiovascular risk factors in the Chinese Han population. These results are consistent with those reported by Fava et al., which showed that the V433M variant of *CYP4F2* is associated with ischemic stroke independently from its effects on blood pressure[26]. Future studies are necessary in order to determine the effect of the V433M variant of *CYP4F2* on production and circulating levels of 20-HETE in human subjects.

Interesting, the effects of *CYP4F2* V433M on ischemic stroke and large vessel disease in our study were only evident in male subjects. This results was consistent with those reported by Fava et al., which showed that the *CYP4F2* V433M is associated with ischemic stroke independently from its effects in male subjects[26]. These finding further support the interaction of *CYPs* and gender, whereby the sexual hormone exert their different effects on ischemic stroke through *CYPs* remained to be elucidated. We did not find the same kind of interaction for *CYP4A11* C-296T, because of relative low MAF.result in less statistical power.

In order to control false-positive findings, several approaches were considered. First, we carefully recruited control subjects and cases from the same geographically regions. Our population stratification assessment revealed no significantly population stratification between different groups in two population sets in these data. Second, these two candidate genes selected have a substantial priori probability of involvement in cerebral vascular function. Third, we used conservative Bonferroni correction to control the false-positive findings potentially because of the multiple testing. Finally, the successful replication of association signals in two independent cohorts improves the plausibility of our study. Therefore, the observed positive association in this study may not be spurious.

There are some remained important limitations to this study that must be acknowledged. 20-HETE levels were not measured and so correlations between *CYP4A11* and *CYP4F2* polymorphisms, potential alterations in 20-HETE levels and stroke risk could not be definitively established in this study population. Additionally, only functional polymorphisms and tagSNPs selected from Hapmap were examined for their association with stroke. Other polymorphisms in these genes, such as those located in large intronic regions or distal promoter regions not identified in the present study, might also be associated with stroke. In order to describe the complete haplotype structure of *CYP4A11* and *CYP4F2* genes, it might be necessary to screen for additional intronic and distal promoter polymorphisms in future studies. Finally, the number of hemorrhagic stroke patients examined in the present study was relatively small and certainly weakened our statistical power in the single locus analysis.

In conclusion, our results implicate variation in the *CYP4A11* and *CYP4F2* genes with altered susceptibility to stroke in the Chinese Han population. However, further studies in other populations, as well as functional analysis *in vivo*, will be required to further define the role of genetic variation in *CYP4A11* and *CYP4F2* in stroke pathogenesis. Such studies may lead to innovative therapies, preventive measures and insight into the pharmacogenetics of such intervention strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline Characteristics of First Study Population

Characteristics	Controls (n=557)	Ischemic Stroke			Hemorrhage (n=221)
		Total (n=558)	LVD (n=410)	Lacunar (n=148)	
Age, y	62.2 ± 9.3	61.1 ± 9.5	60.0 ± 9.7*	64.2 ± 8.3*	57.7 ± 11.2*
Men, %	62.1	64.7	65.4	62.8	67.4
BMI, kg/m^2	23.7 ± 3.2	24.5 ± 3.7*	24.4 ± 3.4*	24.7 ± 4.4*	23.7 ± 3.5
SBP, mm Hg	131.3 ± 20.8	146.6 ± 23.3*	147.1 ± 24.1*	145.0 ± 21.0*	153.5 ± 25.3*
DBP, mm Hg	78.8 ± 11.1	86.4 ± 13.9*	87.0 ± 14.2*	84.9 ± 12.9*	93.3 ± 16.9*
HDL-C, mmol/L	1.33 ± 0.36	1.04 ± 0.54*	0.99 ± 0.30*	1.16 ± 0.91*	1.05 ± 0.40*
TC, mmol/L	4.56 ± 1.70	4.57 ± 1.13*	4.57 ± 1.13*	4.57 ± 1.12*	4.34 ± 1.07*
Hypertension, %	19.2	69.7*	69.0*	71.6*	65.2*
Diabetes, %	3.2	17.9*	19.8*	12.8*	4.1
Hyperlipidemia, %	21.0	35.0*	35.4*	33.8*	11.3*
Smokers, %	37.3	46.9*	45.8*	50.0*	47.1*

Mean ± standard deviation; BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol.

* $p<0.01$ vs control.

Table 2

Baseline Characteristics of Second Study Population

Characteristics	Controls (n=694)	Ischemic Stroke			Hemorrhage (n=48)
		Total (n=551)	LVD (n=300)	Lacunar (n=251)	
Age, y	61.3±8.7	63.4±10.2*	62.3±10.8 *	64.6±9.4*	54.6±10.1 *
Men, %	45.5	67.6*	68.5*	66.5*	60.4*
BMI, kg/m^2	23.9±3.7	24.1±2.7	24.1±2.7	24.1±2.58	23.9±3.6
SBP, mm Hg	130±21.6	147.3±23.4*	148.4±24.1*	146.1±22.5*	156.9±21.2*
DBP, mm Hg	81.3±12.0	85.2±13.3*	86.0±13.9*	84.3±12.4*	91.3±15.5*
HDL-C, mmol/L	1.40±0.37	1.15±0.34*	1.16±0.34*	1.12±0.33*	1.20±0.30*
TC, mmol/L	4.03±1.90	4.50±0.34*	4.49±1.11*	4.50±1.14*	4.59±1.12*
Hypertension, %	47.4	68.2*	69.2*	66.9*	75.0*
Diabetes, %	11.5	18.6*	18.2*	19.1*	10.4
Hyperlipidemia, %	35.6	22.2*	21.2*	23.5*	8.3*
Smokers, %	26.4	57.9*	63.9*	50.6*	35.4*

Mean ± standard deviation; BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol.

* $p<0.01$ vs control.

Table 3

Polymorphism Association with Ischemic Stroke and Subtypes of Ischemic Stroke Among two independent Chinese Han Population

Population	Subject Group	MAF	P_{allele}	Crude ORs (95% CI)	Adjusted ORs (95% CI)	$P_{dominant}$
***CYP4A11* promoter (C>T)**						
1	Control	0.056		1.0	CT+TT vs CC (ref)	
	Ischemic	0.086	0.005	1.66(1.18–2.34)	1.40(0.93, 2.12)	0.106
	LVD	0.091	0.002	1.79(1.24–2.57)	1.57(1.01, 2.44)	0.046
	Lacunar	0.071	0.320	1.32(0.78–2.25)	1.19(0.64, 2.21)	0.587
2	Control	0.071		1.0	CT+TT vs CC (ref)	
	Ischemic	0.097	0.022	1.44(1.07–1.95)	1.48(1.05–2.07)	0.024
	LVD	0.103	0.018	1.55(1.09–2.21)	1.55(1.09–2.21)	0.015
	Lacunar	0.090	0.185	1.31(0.89–1.93)	1.38(0.90–2.10)	0.141
1+2	Control	0.064		1.0	CT+TT vs CC (ref)	
	Ischemic	0.083	0.014	1.39(1.11–1.73)	1.50(1.17–1.93)	0.001
	LVD	0.096	0.034	1.66(1.29–2.14)	1.50(1.17–1.93)	0.001
	Lacunar	0.083	0.127	1.32(0.82–1.96)	1.36(0.97–1.9)	0.077
***CYP4F2* V433M (C>T)**						
1	Control	0.219		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.239	0.256	1.16 (0.91–1.47)	1.36 (1.02–1.82)	0.039
	LVD	0.254	0.075	1.26 (0.97–1.63)	1.53 (1.11–2.11)	0.010
	Lacunar	0.199	0.463	0.91 (0.62–1.32)	0.94 (0.61–1.46)	0.794
2	Control	0.223		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.278	0.002	1.41(1.08–1.69)	1.52(1.17–1.97)	0.001
	LVD	0.269	0.025	1.38(1.05–1.82)	1.38(1.05–1.82)	0.020
	Lacunar	0.287	0.004	1.44(1.08–1.93)	1.57(1.14–2.16)	0.006
1+2	Control	0.221		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.258	0.003	1.06(0.90–1.24)	1.38(1.15–1.65)	5.6×10^{-4}
	LVD	0.261	0.005	1.07(0.89–1.28)	1.38(0.94–2.02)	0.003

Population	Subject Group	MAF	P_{allele}	Crude ORs (95% CI)	Adjusted ORs (95% CI)	$P_{dominant}$
	Lacunar	0.254	0.068	1.03(0.83–1.30)	1.29(1.00–1.65)	0.047

MAF: minor allele frequency; LVD: large vessel disease.

P_{allele}: significance of minor allele frequency differences determined by χ^2 tests, cases vs. controls; ORs: odds ratios with 95% confidence intervals (CI); P_{allele} and crude ORs from two case-control populations were combined using Mantel-Haenszel test.

$P_{dominant}$: significance of adjusted OR computed with multivariate unconditional logistic regression analysis, adjusting for gender, age, body mass index, hypertension, diabetes, hyperlipidemia and smoking status; Combined adjusted ORs were determined by additional adjustment for different populations.

Table 4

Assessment of Association between *CYP4F2* V433M with Ischemic Stroke and Subtypes of Ischemic Stroke After stratification for Sex

Population	Subject Group[a]	MAF	P_{allele}	Crude ORs (95% CI)	Adjusted ORs (95% CI)	$P_{dominant}$
Men						
1	Control	0.212		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.224	0.587	1.10(0.81–1.49)	1.34(0.93–1.92)	0.112
	LVD	0.239	0.271	1.21(0.88–1.61)	1.56(1.05–2.32)	0.027
	Lacunar	0.183	0.375	0.82(0.51–1.33)	0.86(0.50–1.48)	0.578
2	Control	0.242		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.269	0.243	1.36(1.01–1.84)	1.43(1.06–1.94)	0.017
	LVD	0.280	0.166	1.35(0.95–1.92)	1.49(1.01–2.20)	0.042
	Lacunar	0.274	0.257	1.37(0.94–2.00)	1.48(0.98–2.22)	0.059
1+2	Control	0.227		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.258	0.141	1.26(1.02–1.57)	1.49(1.16–1.91)	0.004
	LVD	0.257	0.081	1.32(1.04–1.68)	1.48(1.13–1.93)	0.004
	Lacunar	0.242	0.715	1.16(0.87–1.56)	1.22(0.89–1.98)	0.215
Women						
1	Control	0.230		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.267	0.226	1.28(0.86–1.89)	1.32(0.80–2.32)	0.248
	LVD	0.282	0.119	1.37(0.89–2.10)	1.46(0.82–2.62)	0.198
	Lacunar	0.227	0.220	1.06(0.58–1.93)	1.20(0.54–2.70)	0.652
2	Control	0.208		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.277	0.011	1.28(0.89–1.84)	1.32(0.87–2.00)	0.193
	LVD	0.274	0.050	1.37(0.87–2.17)	1.49(0.87–2.54)	0.148
	Lacunar	0.310	0.004	1.51(0.94–2.43)	1.68(1.00–2.84)	0.052
1+2	Control	0.216		1.0	V/M+M/M vs V/V (ref)	
	Ischemic	0.272	0.005	1.28(0.98–1.67)	1.29(0.96–1.75)	0.095
	LVD	0.268	0.023	1.24(0.91–1.70)	1.30(0.89–1.88)	0.175

Population	Subject Group[a]	MAF	P_{allele}	Crude ORs (95% CI)	Adjusted ORs (95% CI)	$P_{dominant}$
	Lacunar	0.277	0.029	1.32(0.91–1.91)	1.41(0.93–2.15)	0.106

M: major allele; m: minor allele; MAF: minor allele frequency; LVD: large vessel disease.

P_{allele}: significance of minor allele frequency differences determined by χ^2 tests, cases vs. controls; ORs: odds ratios with 95% confidence intervals (CI); P_{allele} and crude ORs from two case-control populations were combined using Mantel-Haenszel test.

$P_{dominant}$: significance of adjusted OR computed with multivariate unconditional logistic regression analysis, adjusting for age, body mass index, hypertension, diabetes, hyperlipidemia and smoking status; Combined adjusted ORs were determined by additional adjustment for different populations.