Genetic variants in CYP4F2 were significantly correlated with susceptibility to ischemic stroke

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Abstract

Background: Ischemic stroke (IS) is a serious cardiovascular disease and is associated with several single nucleotide polymorphisms (SNPs). However, the role of Cytochrome P450 family 4 subfamily F member 2 (CYP4F2) gene in IS remains unknown. Our study aimed to explore whether CYP4F2 polymorphisms influenced IS risk in the Han Chinese population.

Methods: We selected 477 patients and 495 controls to do a case-control study, and five SNPs in CYP4F2 gene were successfully genotyped. And we evaluated the associations using the Chi-squared test, independent sample t test, and genetic models analyses. Logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs).

Results: In this study, rs12459936 and rs3093144 were associated with IS risk in the overall. After stratified analysis by age (> 61 years), rs3093193 and rs3093144 were related to an increased risk of IS, whereas rs12459936 was related to a decreased risk of IS. In addition, we found that three SNPs (rs3093193, rs3093144 and rs12459936) were associated with the susceptibility to IS in males. We also found five SNPs in the CYP4F2 gene had strong linkage.

Conclusions: Three SNPs (rs3093193, rs3093144 and rs12459936) in the CYP4F2 were associated with IS risk in a Chinese Han population. And, CYP4F2 gene may be involved in the development of IS.

Keywords: Ischemic stroke (IS), Cytochrome P450 family 4 subfamily F member 2 (CYP4F2), Case-control study, Single nucleotide polymorphisms (SNPs)

Background

Stroke is the leading neurological cause of death worldwide [1]. It is also called brain attack, typically characterized by neurological dysfunction due to acute focal injury of the central nervous system [2]. At a given age, the incidence and mortality rates of stroke are higher in men, however, women are more likely to have stroke because of longer life, and stroke events increase rapidly with aging [3]. Moreover, stroke is already becoming a global issue and it is threatening the health of human beings. Additionally, epidemiologic studies reported that the incidence of IS in China is significantly higher than that in the developed countries [4].

Hence, it is urgent to clarify the etiology of stroke. The majority of stroke are ischemic (approximately 80%), whereas 20% is due to primary hemorrhage [5]. Several studies demonstrated that some risk factors are involved in the development of ischemic stroke (IS), such as a family history of cardiovascular disease, older age, sex [6, 7], hypertension [8], diabetes [9]. However, a large proportion of IS remains unclear. Recently, the role of genetic factors in development of IS was determined in large-scale, collaborative, genome-wide association studies (GWAS) [10]. Some susceptibility genes of IS have been found, including PITX2 [11], ABO [12], and HABP2 [13]. It demonstrated that genetic factors may regulate the pathophysiological process of IS.

Cytochrome P450 (CYP450) plays a vital role in the metabolism of exogenous and endogenous compounds and is widely distributed in human [14]. Cytochrome P450 family 4 subfamily F member 2 (CYP4F2) is located on the X chromosome and is involved in xenobiotic metabolism. Some SNPs in CYP4F2 gene were associated with IS risk in a Chinese Han population. This study aimed to investigate whether CYP4F2 polymorphisms influenced IS risk in a Chinese Han population.
P450 family 4 subfamily F member 2 (CYP4F2) as a member of CYP450 superfamily, is located on human chromosome 19p13.11 [15]. Previous studies have demonstrated CYP4F2 gene polymorphisms are associated with the risk of IS. For example, Nakamura et al. [16] conducted a case-control study based on the Japanese population to identify whether single nucleotide polymorphisms (SNPs) in CYP4F2 gene associated with the susceptibility to IS, they found ‘G’ allele of rs2108622 was correlated with increased risk of IS in male patients. A study in Sweden [17] reported the V433 M mutation in the CYP4F2 was correlated with cerebral infarction in male patients. It provided strong evidence on the association of CYP4F2 was correlated with increased risk of IS in male patients. For example, Nakamura et al. [16] conducted a case-control study based on the Japanese population to identify whether single nucleotide polymorphisms (SNPs) in CYP4F2 gene associated with the susceptibility to IS, they found ‘G’ allele of rs2108622 was correlated with increased risk of IS in male patients. A study in Sweden [17] reported the V433 M mutation in the CYP4F2 was correlated with cerebral infarction in male patients. It provided strong evidence on the association of CYP4F2 was correlated with increased risk of IS in male patients. For example, Nakamura et al. [16] conducted a case-control study based on the Japanese population to identify whether single nucleotide polymorphisms (SNPs) in CYP4F2 gene associated with the susceptibility to IS, they found ‘G’ allele of rs2108622 was correlated with increased risk of IS in male patients. A study in Sweden [17] reported the V433 M mutation in the CYP4F2 was correlated with cerebral infarction in male patients. It provided strong evidence on the association of CYP4F2 was correlated with increased risk of IS in male patients. For example, Nakamura et al. [16] conducted a case-control study based on the Japanese population to identify whether single nucleotide polymorphisms (SNPs) in CYP4F2 gene associated with the susceptibility to IS, they found ‘G’ allele of rs2108622 was correlated with increased risk of IS in male patients. A study in Sweden [17] reported the V433 M mutation in the CYP4F2 was correlated with cerebral infarction in male patients. It provided strong evidence on the association of CYP4F2 was correlated with increased risk of IS in male patients.

Methods

Subjects

The 477 cases were recruited from the First and Second Affiliated Hospital of Xi’an Jiaotong University between January 2016 and October 2018. In our study, all cases were consistent with the World Health Organization’s diagnostic criteria for IS. IS was confirmed by at least two independent neurologists using computed tomography (CT) scans and/or magnetic resonance imaging (MRI). IS patients with history of transient ischemic attack, coronary artery disease, autoimmune disease, systemic inflammatory disease, malignant tumor and other chronic diseases were excluded from the study. Healthy individuals (495 controls) were identified through the annual health assessment and were randomly recruited from the same hospital physical examination center between April 2016 and October 2018. All members were Han Chinese population living in the Shaanxi Province of China and were unrelated in at least three generations.

SNP selection and genotyping

In total, we successfully selected five variants (rs3093203, rs3093193, rs12459936, rs3093144, and rs3093110) in CYP4F2 and performed a case-control study in Han Chinese population.

Results

Study participants

The basic characteristics of all study participants were shown in Table 2. Four hundred seventy-seven cases

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Table 1: Primers used for this study

| SNP_ID  | 1st-PCR primer                      | 2nd-PCR primer                      | UEP SEQ  |
|---------|-------------------------------------|-------------------------------------|----------|
| rs3093203 | ACGTTGATGATGAAAGGCGCCAAATCCGGCTATG | ACGTTGATGATGACATATGTGACTGTGCCAT | ACGTTGATGATGACATATGTGACTGTGCCAT |
| rs3093193 | ACGTTGATGATGAAAGGCGCCAAATCCGGCTATG | ACGTTGATGATGACATATGTGACTGTGCCAT | ACGTTGATGATGACATATGTGACTGTGCCAT |
| rs12459936 | ACGTTGATGATGAAAGGCGCCAAATCCGGCTATG | ACGTTGATGATGACATATGTGACTGTGCCAT | ACGTTGATGATGACATATGTGACTGTGCCAT |
| rs3093144 | ACGTTGATGATGAAAGGCGCCAAATCCGGCTATG | ACGTTGATGATGACATATGTGACTGTGCCAT | ACGTTGATGATGACATATGTGACTGTGCCAT |
| rs3093110 | ACGTTGATGATGAAAGGCGCCAAATCCGGCTATG | ACGTTGATGATGACATATGTGACTGTGCCAT | ACGTTGATGATGACATATGTGACTGTGCCAT |

SNP single nucleotide polymorphism, UEP SEQ Unextended mini-sequencing primer
Table 2 Basic characteristics of controls and cases

| Characteristics | Cases N (%) | Controls N (%) | p-value |
|-----------------|-------------|----------------|---------|
| **Number**      | 477         | 495            |         |
| **Age(year (mean ± SD)** | 60.05 ± 6.56 | 64.13 ± 10.82 | **0.000**
| > 61            | 274 (57.4%) | 212 (42.8%)    |         |
| ≤61             | 203 (42.6%) | 283 (57.2%)    |         |
| **Gender, no, %** |              |                | **0.898**
| Male            | 316 (66.2%) | 326 (65.9%)    |         |
| Female          | 161 (33.8%) | 169 (34.1%)    |         |

Characteristics: cases, controls; p-values were calculated from Student t tests.

Table 3 Basic information of candidate SNPs in CYP4F2 and associations with stroke

| SNP-ID | Chr | Gene | Position | Alleles (minor/major) | MAF cases | MAF controls | p²-HWE | OR (95% CI) | p²-value |
|--------|-----|------|----------|-----------------------|-----------|--------------|--------|-------------|----------|
| rs3093203 | 19  | CYP4F2 | 15,878,374 | A/G | 0.235 | 0.227 | 0.608 | 1.04 (0.85–1.29) | 0.691 |
| rs3093193 | 19  | CYP4F2 | 15,881,104 | G/C | 0.324 | 0.287 | 0.584 | 1.19 (0.98–1.44) | 0.081 |
| rs12459936 | 19  | CYP4F2 | 15,882,231 | T/C | 0.412 | 0.462 | 0.470 | 0.82 (0.68–0.98) | 0.028* |
| rs3093144 | 19  | CYP4F2 | 15,891,487 | T/C | 0.199 | 0.164 | 0.621 | 1.27 (1.01–1.60) | 0.043* |
| rs3093110 | 19  | CYP4F2 | 15,896,974 | G/A | 0.127 | 0.126 | 0.839 | 1.01 (0.77–1.32) | 0.930 |

SNP single nucleotide polymorphism, MAF: minor allele frequency, HWE Hardy-Weinberg equilibrium, OR odds ratio, 95% CI: 95% confidence interval

*Bold values indicate statistical significance (p < 0.05)
Table 4 Association of SNPs with risk of stroke based on logistic tests adjusted by gender and age

| SNP-ID       | Model       | Genotype | No. (frequency) | Adjusted<sup>a</sup> | p<sup>b</sup>-value |
|--------------|-------------|----------|-----------------|-----------------------|---------------------|
|              |             |          | Case            | Control               | OR (95% CI)         |                     |
| rs3093203    | codominant  | G/G      | 275 (57.7%)     | 292 (59.2%)           | 1.00                | –                   |
|              |             | A/G      | 180 (37.7%)     | 178 (36.1%)           | 1.07 (0.82–1.41)    | 0.614               |
|              |             | A/A      | 22 (4.6%)       | 23 (4.7%)             | 0.90 (0.48–1.69)    | 0.737               |
|              | dominant    | G/G      | 275 (57.7%)     | 292 (59.2%)           | 1.00                | –                   |
|              |             | A/G-A/A  | 202 (42.3%)     | 201 (40.8%)           | 1.02 (0.82–1.27)    | 0.868               |
|              | recessive   | G/G-A/G  | 455 (95.4%)     | 470 (95.3%)           | 1.00                | –                   |
|              |             | A/A      | 22 (4.6%)       | 23 (4.7%)             | 1.05 (0.81–1.37)    | 0.706               |
|              | log-additive| –        | –               | –                     | 0.87 (0.47–1.63)    | 0.670               |
| rs3093193    | codominant  | C/C      | 216 (45.3%)     | 248 (50.2%)           | 1.00                | –                   |
|              |             | G/C      | 213 (44.7%)     | 208 (42.1%)           | 1.14 (0.87–1.50)    | 0.342               |
|              |             | G/G      | 48 (10.1%)      | 38 (7.7%)             | 1.39 (0.86–2.23)    | 0.178               |
|              | dominant    | C/C      | 216 (45.3%)     | 248 (50.2%)           | 1.00                | –                   |
|              |             | G/C-G/G  | 261 (54.7%)     | 246 (49.8%)           | 1.16 (0.95–1.42)    | 0.143               |
|              | recessive   | C/C-G/C  | 429 (89.9%)     | 456 (92.3%)           | 1.00                | –                   |
|              |             | G/G      | 48 (10.1%)      | 38 (7.7%)             | 1.18 (0.91–1.53)    | 0.214               |
|              | log-additive| –        | –               | –                     | 1.30 (0.82–2.05)    | 0.259               |
| rs12459936   | codominant  | C/C      | 158 (33.5%)     | 139 (28.1%)           | 1.00                | –                   |
|              |             | T/C      | 238 (50.5%)     | 254 (51.4%)           | 0.89 (0.66–1.19)    | 0.422               |
|              |             | T/T      | 75 (16.0%)      | 101 (20.5%)           | 0.71 (0.48–1.05)    | 0.082               |
|              | dominant    | C/C      | 158 (33.5%)     | 139 (28.1%)           | 1.00                | –                   |
|              |             | T/C-T/T  | 313 (66.5%)     | 355 (61.9%)           | 0.85 (0.70–1.03)    | 0.089               |
|              | recessive   | C/C-T/C  | 396 (84.0%)     | 393 (79.5%)           | 1.00                | –                   |
|              |             | T/T      | 75 (16.0%)      | 101 (20.5%)           | 0.84 (0.63–1.11)    | 0.212               |
|              | log-additive| –        | –               | –                     | 0.77 (0.55–1.08)    | 0.123               |
| rs3093144    | codominant  | C/C      | 304 (64.0%)     | 344 (69.5%)           | 1.00                | –                   |
|              |             | T/C      | 153 (32.2%)     | 140 (28.3%)           | 1.18 (0.88–1.56)    | 0.266               |
|              |             | T/T      | 18 (3.8%)       | 11 (2.2%)             | 1.95 (0.89–4.28)    | 0.095               |
|              | dominant    | C/C      | 304 (64.0%)     | 344 (69.5%)           | 1.00                | –                   |
|              |             | T/C-T/T  | 171 (36.0%)     | 151 (30.5%)           | 1.24 (0.98–1.58)    | 0.075               |
|              | recessive   | C/C-T/C  | 457 (96.2%)     | 484 (97.8%)           | 1.00                | –                   |
|              |             | T/T      | 18 (3.8%)       | 11 (2.2%)             | 1.23 (0.93–1.62)    | 0.142               |
|              | log-additive| –        | –               | –                     | 1.86 (0.85–4.05)    | 0.120               |
| rs3093110    | codominant  | A/A      | 364 (76.3%)     | 378 (76.5%)           | 1.00                | –                   |
|              |             | G/A      | 105 (22.0%)     | 108 (21.9%)           | 1.01 (0.74–1.37)    | 0.977               |
|              |             | G/G      | 8 (1.7%)        | 8 (1.6%)              | 1.00 (0.37–2.75)    | 0.994               |
|              | dominant    | A/A      | 304 (64.0%)     | 344 (69.5%)           | 1.00                | –                   |
|              |             | G/A-G/G  | 171 (36.0%)     | 151 (30.5%)           | 1.00 (0.76–1.32)    | 0.978               |
|              | recessive   | A/A-G/A  | 469 (98.3%)     | 480 (98.4%)           | 1.00                | –                   |
|              |             | G/G      | 8 (1.7%)        | 8 (1.6%)              | 1.01 (0.74–1.36)    | 0.977               |
|              | log-additive| –        | –               | –                     | 1.00 (0.37–2.74)    | 0.996               |

<sup>a</sup> Adjusted for age and sex in a logistic regression model

<sup>b</sup> p values were calculated from wald test

p values indicate statistical significance (p < 0.05)
Table 5: Stratification analysis of the association of CYP4F2 polymorphisms with stroke under genetic models

| SNP       | Model  | Genotype | Male (OR95%CI) | p-value | Female (OR95%CI) | p-value |
|-----------|--------|----------|----------------|----------|------------------|----------|
| rs3093193 | allele | G        | 1.34 (1.02–1.77) | **0.038** | 1.04 (0.79–1.37) | 0.791   |
|           | co-dominant | CC      | 1.00            | 1.00     | 1.00             | 1.00     |
|           | GC     | 1.56 (1.02–2.39) | 0.101 | 1.09 (0.74–1.61) | 0.657 | 1.10 (0.79–1.54) | 0.567 | 1.21 (0.76–1.92) | 0.421 |
|           | GG     | 1.86 (0.89–3.92) | **0.040** | 1.27 (0.64–2.50) | 0.494 | **2.29 (1.21–4.32)** | **0.011** | 0.64 (0.29–1.41) | 0.267 |
|           | dominant | C/C     | 1.00            | 1.00     | 1.00             | 1.00     |
|           | G/C-G/G | 1.61 (1.07–2.42) | **0.022** | 1.12 (0.77–1.63) | 0.551 | 1.24 (0.90–1.71) | 0.195 | 1.08 (0.70–1.68) | 0.731 |
|           | recessive | C/C/C   | 1.00            | 1.00     | 1.00             | 1.00     |
|           | G/G    | 1.50 (0.73–3.05) | 0.268 | 1.21 (0.63–2.33) | 0.560 | **2.19 (1.18–4.05)** | **0.013** | 0.58 (0.27–1.23) | 0.157 |
|           | log-additive | –      | 1.44 (1.05–1.98) | **0.024** | 1.11 (0.83–1.49) | 0.472 | **1.31 (1.02–1.69)** | **0.036** | 0.93 (0.67–1.31) | 0.693 |
| rs12459936 | allele | T        | 0.73 (0.56–0.94) | **0.016** | 0.96 (0.74–1.23) | 0.727 | **0.78 (0.63–0.98)** | **0.031** | 0.89 (0.65–1.21) | 0.449 |
|           | co-dominant | CC      | 1.00            | 1.00     | 1.00             | 1.00     |
|           | TC     | 0.70 (0.44–1.10) | 0.117 | 1.13 (0.73–1.75) | 0.582 | 0.94 (0.64–1.36) | 0.729 | 0.80 (0.49–1.31) | 0.383 |
|           | TT     | 0.48 (0.26–0.88) | **0.018** | 0.79 (0.45–1.38) | 0.406 | 0.65 (0.40–1.05) | 0.079 | 0.86 (0.45–1.62) | 0.637 |
|           | dominant | C/C     | 1.00            | 1.00     | 1.00             | 1.00     |
|           | T/C-T/T | 0.63 (0.41–0.97) | **0.036** | 1.03 (0.68–1.56) | 0.898 | 0.85 (0.60–1.22) | 0.380 | 0.82 (0.52–1.30) | 0.393 |
|           | recessive | C/C-T/C  | 1.00            | 1.00     | 1.00             | 1.00     |
|           | T/T    | 0.59 (0.34–1.03) | 0.060 | 0.73 (0.45–1.17) | 0.189 | 0.68 (0.44–1.03) | 0.068 | 0.97 (0.55–1.72) | 0.922 |
|           | log-additive | –      | 0.69 (0.52–0.93) | **0.015** | 0.91 (0.69–1.19) | 0.493 | 0.82 (0.65–1.04) | 0.103 | 0.91 (0.66–1.24) | 0.529 |
| rs3093144 | allele | T        | 1.53 (1.09–2.14) | **0.013** | 1.06 (0.76–1.48) | 0.741 | **1.36 (1.01–1.82)** | **0.040** | 1.13 (0.77–1.66) | 0.520 |
|           | co-dominant | CC      | 1.00            | 1.00     | 1.00             | 1.00     |
|           | TC     | 1.54 (0.98–2.41) | 0.059 | 1.03 (0.68–1.55) | 0.890 | 1.15 (0.81–1.64) | 0.438 | 1.24 (0.77–2.00) | 0.383 |
|           | TT     | 2.57 (0.80–8.25) | 0.114 | 1.75 (0.58–5.31) | 0.324 | **4.5 (1.22–16.64)** | **0.024** | 0.93 (0.31–2.81) | 0.902 |
|           | dominant | C/C     | 1.00            | 1.00     | 1.00             | 1.00     |
|           | T/C-T/T | 1.62 (1.05–2.49) | **0.029** | 1.08 (0.73–1.60) | 0.569 | 1.25 (0.89–1.77) | 0.198 | 1.20 (0.76–1.89) | 0.445 |
|           | recessive | C/C-T/C  | 1.00            | 1.00     | 1.00             | 1.00     |
|           | T/T    | 2.25 (0.70–7.17) | 0.171 | 1.73 (0.58–5.22) | 0.328 | **4.32 (1.17–15.89)** | **0.028** | 0.87 (0.29–2.60) | 0.807 |
|           | log-additive | –      | 1.56 (1.07–2.27) | **0.020** | 1.12 (0.79–1.58) | 0.522 | 1.34 (0.98–1.82) | 0.067 | 1.11 (0.76–1.64) | 0.579 |

*p values were calculated from Wald test
**Bold values indicate statistical significance (p < 0.05)

95% CI = 1.17–15.89, p = 0.028) and log-additive model (rs3093193, OR = 1.31, 95% CI = 1.02–1.69, p = 0.036). Conversely, rs12459936 was associated with decreased risk of IS in allele model (T vs. C, OR = 0.78, 95% CI = 0.63–0.98, p = 0.031).

Associations between haplotype analyses and IS risk
The LD and haplotype analyses of the CYP4F2 polymorphisms in the cases and controls were further studied. All SNPs in CYP4F2 gene were found to exist in LD block (Fig. 1). The distributions of different haplotypes in two groups are presented in Table 6. Although the five SNPs in the CYP4F2 gene had strong linkage, haplotype analysis did not show the significant association (p > 0.05).

In silico analysis
In silico analysis, HaploReg v4.1 was used to assess the function of the selected variants in CYP4F2 gene (Additional file 1: Table S1). The results suggested rs3093203, rs3093193, and rs12459936 were associated with Motifs changed, selected eQTL hits. And
rs3093144 was associated with enhancer histone marks, motifs changed, selected eQTL hits. Rs3093110 was related to promoter histone marks, motifs changed, selected eQTL, GRASP QTL hits.

**Discussion**

In the present study, allele, genotype and haplotype frequencies of five SNPs in the *CYP4F2* gene between IS patients and healthy controls were compared and stratified analyses by age and gender were conducted. We found that three SNPs (rs3093193, rs3093144 and rs12459936) in the *CYP4F2* were associated with IS risk in a Chinese Han population. Rs3093144 and rs12459936 were associated with IS risk in the overall. For the individuals older than 61 years old, rs3093193 and rs3093144 were related to the increased risk of IS, whereas rs12459936 was related to the decreased risk of IS. In addition, we observed that three SNPs (rs3093193, rs3093144 and rs12459936) were associated with the susceptibility to IS in the male. And five SNPs in the *CYP4F2* gene showed strong linkage. These findings suggested that genetic polymorphisms in *CYP4F2* may play an important role in the etiology of IS.

Genetic variation is the molecular basis of human genetic diversity. IS is a complex polygenic disease resulting from the genetic factors and environmental risk factors.

**Table 6** Haplotype frequencies of *CYP4F2* SNPs and the association with stroke

| Block | Haplotype                        | Frequency | OR (95% CI) | p-value |
|-------|----------------------------------|-----------|-------------|---------|
| Block 1 | rs3093203|rs3093193|rs12459936|rs3093144|rs3093110 |            |            |         |
|       | GGCGG  | 418 (87.7%) | 436 (88%) | 0.99 (0.75–1.31) | 0.944 |
|       | GGCTA  | 94 (19.8%)  | 80 (16.1%) | 1.25 (0.99–1.60) | 0.065 |
|       | GCTCA  | 280 (58.8%) | 268 (54.1%) | 1.17 (0.97–1.41) | 0.109 |
|       | ACCCA  | 111 (23.2%) | 111 (22.4%) | 1.02 (0.82–1.28) | 0.837 |
|       | GCCCA  | 465 (97.4%) | 483 (97.5%) | 1.01 (0.57–1.81) | 0.970 |

OR: odds ratio; 95%CIs: 95% confidence intervals

*p* values were calculated by logistic regression with adjustment for age and gender

*p* values indicate statistical significance (*p* < 0.05)
[23]. Approximately 80% of stroke is IS, and the incidence of IS increases with aging. As a member of the CYP450 superfamily, CYP4F2 plays a pivotal role in the metabolism of exogenous and endogenous compounds [24]. It is expressed in the liver, heart, lungs, and kidneys, and is involved in leukotriene B4 (LTB4) and 20-hydroxy eicosanoid arachidonic acid (20-HETE) metabolism [25]. 20-HETE is involved in leukotriene B4 (LTB4) and 20-hydroxy eicosanoid arachidonic acid metabolism [25]. 20-HETE in vivo can depolarize vascular smooth muscle by blocking Ca2+ activation and K+ channels, resulting in strong vasoconstriction [26]. In hypertensive rat models, increased 20-HETE can lead to oxidative stress and endothelial cell damage, thus increasing the incidence of IS [27]. CYP4F2 is the main synthetase that catalyzes the generation of 20-HETE from arachidonic acid. Recent studies revealed that CYP4F2 was associated with the IS risk. H.-Q. Yan et al. [28] conducted a case-control study to identify the association between the selected SNPs in CYP4F2 gene and the risk of IS. The results showed that the ‘GG’ genotype of CYP4F2 rs2108622 was correlated with an increased risk of IS. In addition, Colás-Campás L et al. [29] found rs2108622 ‘AA’ genotype in CYP4F2 gene was significantly associated with a risk of early IS in non-valvular atrial fibrillation patients under vitamin K antagonists treatment. However, study based on this CYP4F2 gene is rare. Hence, our study discussed the relationship between CYP4F2 and IS risk. We found that three SNPs (rs3093193, rs3093144 and rs12459936) in the CYP4F2 were associated with IS risk in a Chinese Han population, suggesting CYP4F2 gene may play an important function in affecting IS.

In addition, it is worth noting that in our results, there were two SNPs (rs3093193 and rs3093144) increased the IS risk and one (rs12459936) decreased the IS risk. However, the haplotype analysis among the three SNPs mentioned above found it was significantly associated with an increased risk of IS (Table 7). For rs12459936, the minor allele ‘T’ when compared to the wild-type allele ‘C’ was protective factor. In turn, the wild-type allele ‘C’ was associated with an increased risk of stroke when compared with the minor allele ‘T’ as reference allele. Thus, based on the positive loci, the haplotype “GCT” was related to elevating risk of IS.

Furthermore, we have compared results with other stroke studies performed in the same population. Lee et al. [30] conducted that a genome-wide association study links small-vessel ischemic stroke to autophagy. And their study focused on Han Chinese in Taiwan. In addition, the study was replicated in an independent Han Chinese population. Imputation analysis also supported the association between three SNPs (rs2594966, rs2594973, rs4684776) in ATG7 gene and stroke-small-vessel occlusion (SVO). When compared to their study, our sample size was not large enough because of strict recruitment criteria. And the study failed to replicate the results within an independent sample. So that’s a part of what we’re going to do next.

Our study also has some potential limitations. The ethnicity of study subjects was limited to the Han Chinese population. Hence, whether our results could apply to other ethnicities is unclear. Furthermore, our current research is fundamental, functional studies are required to understand function of genetic variants and mechanisms underlying this association.

**Conclusions**
To sum up, we firstly provide new evidence for the association between CYP4F2 variants and IS risk in Han Chinese population, which may support for screening of IS in Han Chinese population and shed light on the mechanism of IS.

**Supplementary information**
Supplementary information accompanies this paper at (https://doi.org/10.1186/s12881-019-0888-6).

### Additional file 1: Table S1.
Functional prediction results of selected loci in the database. (DOCX 13 kb)

**Abbreviations**
20-HETE: 20-hydroxy eicosanoid arachidonic acid; CI: Confidence interval; CT: Computed tomography scan; CYP4F2: Cytochrome P450 family 4 subfamily F member 2; EDTA: Ethylenediamine tetraacetic acid; GWAS: Genome-wide association studies; HWE: Hardy–Weinberg equilibrium; IS: Ischemic stroke; LD: Linkage disequilibrium; MAF: Minor allele frequency; MRI: Magnetic resonance imaging; OR: Odds ratio; SNP: Single nucleotide polymorphism
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Authors’ contributions
Conceived and designed the experiments: M Z, Y W. Performed the experiments: JZ Z, Y Z. Analyzed the data: TJ H. Contributed reagents/materials/analysis tools: XD M. Wrote the paper: HG P. Revised M Z. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its additional file.

Ethics approval and consent to participate
This study was conducted in accordance with the ethical standards of the Declaration of Helsinki and approved by the ethical committee of the Second Affiliated Hospital of Xi’an Jiaotong University. Written informed consents were obtained from all subjects before this study. The procedures were in accordance with the institutional guidelines.

Consent for publication
All subjects whose data is described in this manuscript signed informed consent in written giving permission for their data to be published anonymously.

Competing interests
The authors declare that they have no competing interests.

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