Introduction

Stroke is a neurological disease caused by vascular, leading to considerably high disability and mortality. The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) showed that stroke and ischemic heart were the top cause of disability over the age of 50. Depending on regional epidemiology, stroke is classified as ischemic stroke (IS) and hemorrhagic stroke, and the most common of which is IS, accounting for 87% of all stroke patients.

In the clinical setting, according to the etiology of ischemic, five subtypes were divided for IS, which was developed for the TOAST study: 1) large-artery atherosclerosis, 2) small-vessel occlusion, 3) cardioembolism, 4) other determined etiology, and 5) undetermined etiology. The most common cause of ischemic is due to arterial occlusion, and the rarer cause is cerebral veins or venous sinuses. There are many causes of stroke, such as hypertension, diabetes, dyslipidemia, smoking, and genetic background. Especially, genetic background accounts for 30–40% of IS.

Background: Inflammation proteins play an important role in stroke occurrence. IL1A, IL1B, PTGS2, MMP2, and MMP9 were the mediators involved in the immune response, and the association of these genetic variations with ischemic stroke (IS) risk was still unclear.

Methods: To investigate the susceptibility of genetic variations of IL1A, IL1B, PTGS2, MMP2, and MMP9 to IS risk, we performed a case–control study involving 299 patients and 300 controls in a Chinese population. Thirteen genetic variations of investigated genes of all participants were genotyped using an improved multiplex ligation detection–reaction technique.

Results: No SNP in all genes showed an association with overall IS. However, in subgroup analysis, PTGS2 rs689466 (dominant model: CT vs TT – ORadjusted= 2.51, 95% CI: 1.22–5.16, p = 0.012; co-dominant model: CT/CC vs TT – ORadjusted= 2.53, 95% CI: 1.26–5.07, p = 0.009; additive model – ORadjusted= 2.26, 95% CI: 1.19–4.28, p = 0.013) and rs5275 (dominant model: GG vs AA – ORadjusted= 0.31, 95% CI: 0.12–0.80, p = 0.016; co-dominant model: GA/GG vs AA – ORadjusted= 0.45, 95% CI: 0.21–0.95, p = 0.036; additive model – ORadjusted= 0.60, 95% CI: 0.39–0.92, p = 0.020) were associated with IS type of small-vessel occlusion.

Conclusion: Our study suggested that PTGS2 rs689466 C and rs5275 A were potentially associated with IS subtype of small-vessel occlusion. Our result should be confirmed with further large sample sized studies.

Keywords: ischemic stroke, genetic variation, IL1A, IL1B, PTGS2, MMP2, MMP9, risk
Inflammation contributes across the spectrum of IS. Firstly, inflammation might not only promote thrombus formation but also inhibit endogenous fibrinolysis to enhance clot stability. Secondly, the inflammatory mediators promote immune cells and solutes into the brain parenchyma and damage the blood–brain barrier further, and contribute the thrombogenesis. Moreover, some inflammatory mediators might be the predictor factors of IS clinical outcome. Multiple inflammation-associated mediators are involved in the process. For example, the interleukin (IL)-1 family, as a highly active proinflammatory cytokine, is a main regulator of inflammation and triggers inflammatory cascade response by binding to the IL1 receptor. IL1α and IL1β are distinct members of IL1 genes and encode IL1α and IL1β, respectively. Experimental studies show that abnormal IL1α and IL1β levels will lead to inflammatory diseases and involve a variety of cellular activities, including adhesion molecule induction and procoagulant activity, which present a higher inflammatory reaction and affect the development of atherosclerosis resulting in IS. Studies showed IL1β had significantly higher blood levels for stroke and contribute to the occurrence of stroke. Prostaglandin-endoperoxide synthase (PTGS2), known as COX2, is an important induced enzyme in vascular endothelial cells, smooth muscle cells, and platelets. It is induced by cytokines and growth factors and is closely associated with the formation of atherosclerosis. Multiple studies reported an association between PTGS2 genetic polymorphisms and IS risk. Matrix metalloproteinases (MMPs), as a category of proteolytic zinc-dependent enzymes, play an important role in the degradation of the extracellular matrix and take part in the progression of atherosclerosis by triggering the migration and proliferation of smooth muscle cells and by causing destabilization of atherosclerotic plaques. Previously, studies have reported that the MMPs protein levels elevate result in blood–brain barrier dysfunction and impact the extent of the infarct, and the polymorphisms of MMPs have been evaluated in IS. The association with IS of MMPs is focused on MMP2 and MMP9, and the high expression level of MMP2 and MMP9 has been reported that was associated with increased risk of IS and major disability.

Given the putative role of the above gene involved in the occurrence of IS, to identify the risk of the genetic background to IS risk, we conducted a case–control association study to assess the association between 13 single nucleotide polymorphisms (SNPs) in IL1B, IL1A, PTGS2, MMP9, MMP2, and risk of IS.

Materials and Methods

Study Subjects

A total of 299 patients diagnosed based on clinical symptoms, physical examination, and head computed tomography or magnetic resonance imaging, and 300 healthy controls were enrolled in this case–control study. All patients presented with signs and symptoms lasting more than 24 hours which sudden onset of focal or global neurological deficits. Patients with a history of transient ischemic attacks, hemorrhagic stroke, cerebral trauma, cardiogenic thrombosis, coagulation disorders, autoimmune disease, tumors, or peripheral vascular disease were excluded. The etiology of IS was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) as large-artery atherosclerosis, cardioembolism, small-vessel atherosclerosis, and stroke of other etiology.

Healthy control subjects were recruited during the same period from the Health Medical Center of Nanjing First Hospital. The healthy controls were confirmed according to the routine health examination results. For the controls, the hematologic diseases, tumors, autoimmune diseases, liver ailments, and genetic diseases were excluded. All enrolled participants come from the same geographic region: Nanjing City, Jiangsu, China. The patient information was collected from the hospital information system, and the health control information was from questionnaires. Declaration of Helsinki and all procedures were approved by the Institutional Review Board of Nanjing First Hospital, and all participants were written informed consent.

DNA Extraction and Genotyping

The SNPs of IL1A, IL1B, PTGS2, MMP2, and MMP9 were selected and retrieved from the National Center for Biotechnology Information dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), and then selected potential genes based on the following criteria: 1) positioned in exons, promoter regions, 5’UTRs, 3’UTRs, or splice sites; 2) minor-allele frequency ≥5%; and 3) had been reported to be associated with IS risk. Finally, thirteen genetic variations were selected (see Table S1 for details). The DNA extraction and genotyping were performed as previously described. Genotyping used a method based on an improved multiplex ligase detection reaction technique developed by Genesky Biotech (Shanghai, China). In detail, firstly, genetic-variation loci were amplified by multiplex polymerase chain reaction, and then the amplification products were purified with nuclease and shrimp...
alkaline enzyme. Finally, each locus contained two 5’terminal allele-specific probes and a 3’terminal-specific probe of fluorescent tags, and ligation products were analyzed with an ABI 3730XL finally.

Statistical Analysis
For the distribution of genotypes, a goodness-of-fit Chi-square test was adopted to test the Hardy–Weinberg equilibrium (HWE) in the control group. Differences in the demographic characteristics of the two groups were assessed by t-test or χ² test. Logistic regression was applied to calculate the susceptibility of polymorphisms to IS risk with ORs and 95% CIs based on different genetic models: Dominant model (Rare allele homozygote (RR) or heterozygous (WR) vs wild-type homozygote (WW) genotypes), co-dominant model (RR+WR vs WW), and additive model (WW vs WR vs RR). 31 P < 0.05 was considered statistically significant.

Results
Characteristics of the Study Population
A total of 299 patients with IS and 300 healthy controls were enrolled in this study. Their demographic data and clinical characteristics are summarized in Table S2. There were no significant statistical differences in sex (p=0.312), drinking (p=1.000), or chol (p=0.623), but there were significant differences between the two groups with age (p<0.001), smoking (p<0.001), hypertension (p<0.001). For clinical characteristics, levels of TG (p<0.001), GLU (p<0.001), HYC (p<0.001), and CRP (p<0.001) were significantly higher in patients than in controls. In contrast, levels of HDL in patients were significantly lower than in controls (p=0.004). A total of 56 patients were identified as having small-vessel occlusion, 116 having large-artery, 28 having cardioembolism, and 99 having other etiologies. The HWE result showed that all genotypes were not derived from controls (Table S1).

Association Between and Risk of Stroke
Logistic regression analysis revealed that no SNP showed any association with the risk of IS in all genes (Table 1) and subgroup stratified by sex (Table 2). However, subgroup analysis by subtypes of IS showed that PTGS2 rs689466 (dominant model: CT vs TT – ORadjusted=2.51, 95% CI: 1.22–5.16, p=0.012; co-dominant model: CT/CC vs TT – ORadjusted=2.53, 95% CI: 1.26–5.07, p=0.009; additive model – ORadjusted=2.26, 95% CI: 1.19–4.28, p=0.013) was associated with increased risk of IS type of small-vessel occlusion. However, the PTGS2 rs5275 (dominant model: GG vs AA – ORadjusted=0.31, 95% CI: 0.12–0.80, p=0.016; co-dominant model: GA/GG vs AA – ORadjusted=0.45, 95% CI: 0.21–0.95, p=0.036; additive model – ORadjusted=0.60, 95% CI: 0.39–0.92, p=0.020) were associated with decreased risk of IS type of small-vessel occlusion. Additionally, the SNP MPP9 rs3918242 was observed to be susceptible to the cardioembolic subtype of IS with dominant model (CT vs CC – ORadjusted =0.31, 95% CI: 0.11–0.91, p=0.033) but not other genetic models (Table 3). No other genotypes were observed significant differences in stroke risk.

Discussion
The population-based case–control association study had not observed associated SNPs with overall IS. However, PTGS2 rs689466 (C allele) and rs5275 (A allele) were associated with IS subtype of small-vessel occlusion.

Inflammatory proteins play an important role in the pathogenesis of stroke. Inflammatory genes, such as C-reactive protein (CRP), interleukin (IL) 6, transforming growth factor β1 (TGFβ1), were identified to contribute to stroke risk. 11,32–34 It is well known that the elevated levels of inflammatory markers may reflect a high burden of atherosclerosis and thrombosis, which contribute to the occurrence of IS. 13,35

PTGS2, with loci on chromosome 1, is an inducible enzyme that catalyzes arachidonic acid into prostaglandins and plays a vital role in inflammation. 36 The expression of PTGS2 is increased significantly in stroke with inflammatory cells infiltrating, and relevant studies have reported that it is associated with IS. 18 The rs689466 (PTGS2 −1195T>C) is located in the PTGS2 promoter, and the −1195T allele displays a higher PTGS2 level than the −1195C allele. 37 The rs5275 (8473A>G) is located in PTGS2 3’UTR, and the 8273A allele could regulate PTGS2 higher expression by maintaining the stability of PTGS2 mRNA, 38,39 indicating rs5275A allele, associated with increased PTGS2 expression, induce the risk of IS. According to our study, rs689466 (C allele) and rs5275 (A allele) were associated with small-vessel occlusion whether dominant model or additive model, but have no significant difference in all IS. The reason may be that PTGS2 induces thrombosis 17 which causes thrombotic occlusion of small vessels. 37 Chen reported that rs689466 was associated with all IS and the effects were confined to small-vessel occlusion but not large-artery. 37
Table 1 Genotype Distribution of the Polymorphisms in All Participants

| Genotype | Patients, n(%) | Controls, n(%) | OR(95% CI)* | P value |
|----------|----------------|----------------|-------------|---------|
| **IL1B** |                |                |             |         |
| rs16944  |                |                |             |         |
| AA       | 77(25.75)      | 89(29.67)      | Reference   |         |
| GA       | 155(51.84)     | 147(49.00)     | 1.10(0.74–1.65) | 0.641   |
| GG       | 67(22.41)      | 64(21.33)      | 1.11(0.68–1.82) | 0.672   |
| GA/GG    | 222(74.25)     | 211(70.33)     | 1.12(0.76–1.63) | 0.571   |
| rs1143627|                |                |             |         |
| GG       | 77(25.75)      | 88(29.33)      | Reference   |         |
| GA       | 151(50.50)     | 145(48.33)     | 1.06(0.71–1.59) | 0.779   |
| AA       | 71(23.75)      | 67(22.33)      | 1.13(0.69–1.83) | 0.632   |
| GA/AA    | 222(74.25)     | 212(70.67)     | 1.09(0.75–1.60) | 0.647   |
| rs1143634|                |                |             |         |
| GG       | 285(95.32)     | 288(96.00)     | Reference   |         |
| GA       | 12(4.01)       | 12(4.00)       | 0.87(0.37–2.05) | 0.750   |
| AA       | 2(0.67)        | 0(0.00)        | –           | 0.988   |
| GA/AA    | 14(4.68)       | 12(4.00)       | 0.96(0.42–2.19) | 0.917   |
| **IL1A** |                |                |             |         |
| rs1800587|                |                |             |         |
| GG       | 239(79.93)     | 252(84.00)     | Reference   |         |
| GA       | 56(18.73)      | 45(15.00)      | 1.33(0.85–2.09) | 0.213   |
| AA       | 4(1.34)        | 3(1.00)        | 1.02(0.21–4.99) | 0.977   |
| GA/AA    | 60(20.07)      | 48(16.00)      | 1.31(0.84–2.03) | 0.230   |
| **PTGS2**|                |                |             |         |
| rs20417  |                |                |             |         |
| CC       | 265(88.63)     | 266(88.67)     | Reference   |         |
| GC       | 34(11.37)      | 34(11.33)      | 1.03(0.60–1.76) | 0.917   |
| GG       | 0(0)           | 0(0)           | –           | –       |
| GC/GG    | 34(11.37)      | 34(11.33)      | 1.03(0.60–1.76) | 0.917   |
| rs689466 |                |                |             |         |
| TT       | 197(65.89)     | 180(60.00)     | Reference   |         |
| CT       | 95(31.77)      | 109(36.33)     | 0.80(0.56–1.15) | 0.222   |
| CC       | 7(2.34)        | 11(3.67)       | 0.52(0.19–1.42) | 0.201   |
| CT/CC    | 102(34.11)     | 120(40.00)     | 0.77(0.54–1.10) | 0.149   |
| rs5275   |                |                |             |         |
| AA       | 90(30.10)      | 98(32.67)      | Reference   |         |
| GA       | 139(46.49)     | 149(49.67)     | 0.98(0.67–1.45) | 0.937   |
| GG       | 70(23.41)      | 53(17.67)      | 1.54(0.93–2.55) | 0.094   |
| GA/GG    | 209(69.9)      | 202(67.33)     | 1.10(0.77–1.59) | 0.597   |
| **MMP9** |                |                |             |         |
| rs17576  |                |                |             |         |
| GG       | 150(50.17)     | 155(51.67)     | Reference   |         |
| GA       | 118(39.46)     | 114(38.00)     | 1.04(0.72–1.49) | 0.843   |
| AA       | 31(10.37)      | 31(10.33)      | 1.03(0.59–1.82) | 0.908   |
| GA/AA    | 149(49.83)     | 145(48.33)     | 1.03(0.74–1.45) | 0.849   |

(Continued)
Shan also showed that rs689466 was associated with small vessel disease, which was consistent with our results. However, Zhao reported that rs68944 has a higher risk in LAA was dissimilar to our study, but its gene–gene interactions of three PTGS genes including rs689466 were an association with small vessel occlusion. For rs5275, another PTGS2 associate gene, few studies have discussed the association between rs5275 and stroke risk to date, but it was reported that was impacted the IS outcome. Our study showed that rs5275 was associated with small-vessel occlusion as well as rs689466, so more large sample studies need to be conducted.

MMP9 locates on chromosome 20q12–q13, is a member of the MMP family, and plays a role in the progression of IS. The MMP9 rs3918242, within the promoter region (−1562 C>T), T allele led to a higher promoter activity of the MMP9. The conclusions of rs3918242 T allele for stroke risk were controversial. A meta-analysis including 14 control studies with 3233 IS patients and 3123 controls showed that MMP9 rs3918242 variants contributed to increasing the risk of IS, and the report showed that T allele carriers of rs3918242 polymorphism probably contributed to increasing in IS than C allele. However, our study shows that CT decreases the risk of cardioembolic stroke than CC. The reason for the contradiction with previous studies may be due to smaller patients included in this study in that only 7 patients with CT genes and no TT genes among the patients with cardioembolic stroke in this study, which may affect the statistical power. Moreover, there was no significant difference under the additive model. For the difference above, our result should be verified by further large sample sized study.

A total of thirteen SNPs were also included in this study. Although these SNPs have been reported to affect the expression of some inflammatory proteins that contribute to the risk

| Genotype | Patients, n(%) | Controls, n(%) | OR(95% CI)* | P value |
|----------|----------------|---------------|-------------|---------|
| rs3918242 CC | 245(81.94) | 254(84.67) | Reference | 0.193 |
| CT | 51(17.06) | 42(14.00) | 1.37(0.86–2.18) | 0.13 |
| TT | 3(1.00) | 4(1.33) | 1.30(0.84–2.08) | 0.230 |
| CT/TT | 54(18.06) | 46(15.33) | | |
| rs9509 TT | 189(63.21) | 184(61.33) | Reference | 0.750 |
| CT | 100(33.44) | 101(33.67) | 0.94(0.66–1.35) | 0.714 |
| CC | 10(3.34) | 15(5.00) | 0.65(0.27–1.54) | 0.325 |
| CT/CC | 110(36.79) | 116(38.67) | 0.91(0.64–1.28) | 0.575 |
| rs7201 AA | 173(57.86) | 178(59.33) | Reference | 0.556 |
| CA | 105(35.12) | 103(34.33) | 0.90(0.62–1.29) | 0.556 |
| CC | 21(7.02) | 19(6.33) | 1.13(0.56–2.29) | 0.714 |
| CA/CC | 126(42.14) | 122(40.67) | 0.93(0.66–1.32) | 0.694 |
| rs2285053 CC | 171(57.19) | 176(58.67) | Reference | 0.511 |
| CT | 102(34.11) | 103(34.33) | 1.03(0.71–1.48) | 0.895 |
| TT | 26(8.70) | 21(7.00) | 0.96(0.73–2.64) | 0.643 |
| CT/TT | 128(42.81) | 124(41.33) | 1.08(0.77–1.53) | 0.621 |
| rs243864 TT | 238(79.60) | 238(79.33) | Reference | 0.583 |
| CT | 56(18.73) | 58(19.33) | 0.89(0.57–1.37) | 0.583 |
| CC | 5(1.67) | 4(1.33) | 2.45(0.60–9.91) | 0.210 |
| CT/CC | 61(20.40) | 62(20.67) | 0.95(0.63–1.45) | 0.821 |

Note: *Adjusted for age, sex, smoking, and drinking.
Table 2 Genotype Distribution of Polymorphisms in All Participants Stratified by Sex

| Genotype | Male OR(95% CI)* | Male P-valuea | Female OR(95% CI)* | Female P-valuea |
|----------|-----------------|--------------|--------------------|-----------------|
| **IL1B** |                 |              |                    |                 |
| rs16944  |                 |              |                    |                 |
| AA       | Reference        |              | Reference          |                 |
| GA       | 1.35(0.80,2.29)  | 0.264        | 0.75(0.37,1.52)    | 0.424           |
| GG       | 1.37(0.72,2.61)  | 0.335        | 0.88(0.38,2.07)    | 0.759           |
| GA/GG    | 1.36(0.83,2.23)  | 0.223        | 0.81(0.42,1.55)    | 0.517           |
| Additive model | 1.18(0.86,1.61) | 0.302 | 0.90(0.59,1.39)    | 0.636           |
| rs1143627 |                 |              |                    |                 |
| GG       | Reference        |              | Reference          |                 |
| GA       | 1.26(0.74,2.14)  | 0.402        | 0.76(0.38,1.55)    | 0.454           |
| AA       | 1.44(0.77,2.70)  | 0.259        | 0.83(0.36,1.94)    | 0.671           |
| GA/AA    | 1.32(0.80,2.17)  | 0.276        | 0.81(0.42,1.55)    | 0.517           |
| Additive model | 1.20(0.88,1.63) | 0.249 | 0.89(0.60,1.36)    | 0.581           |
| rs1143634 |                 |              |                    |                 |
| GG       | Reference        |              | Reference          |                 |
| GA       | 0.60(0.19,1.86)  | 0.371        | 1.43(0.33,6.27)    | 0.634           |
| AA       | –               | –            | –                  | –               |
| GA/AA    | 0.70(0.24,2.03)  | 0.506        | 1.43(0.33,6.27)    | 0.634           |
| Additive model | 0.82(0.32,2.10) | 0.687 | 1.43(0.33,6.27)    | 0.634           |
| **IL1A** |                 |              |                    |                 |
| rs1800587 |                 |              |                    |                 |
| GG       | Reference        |              | Reference          |                 |
| GA       | 1.20(0.68,2.14)  | 0.531        | 1.50(0.68,3.33)    | 0.317           |
| AA       | 1.40(0.08,24.45) | 0.819        | 0.60(0.08,4.49)    | 0.622           |
| GA/AA    | 1.21(0.68,2.13)  | 0.515        | 1.35(0.64,2.88)    | 0.432           |
| Additive model | 1.20(0.70,2.06) | 0.512 | 1.18(0.62,2.27)    | 0.615           |
| **PTGS2**|                 |              |                    |                 |
| rs20417  |                 |              |                    |                 |
| CC       | Reference        |              | Reference          |                 |
| GC       | 0.89(0.41,1.92)  | 0.769        | 1.11(0.48,2.52)    | 0.804           |
| GG       | –               | –            | –                  | –               |
| GC/GG    | 0.89(0.41,1.92)  | 0.769        | 1.11(0.49,2.52)    | 0.804           |
| Additive model | 0.89(0.41,1.92) | 0.769 | 1.10(0.49,2.52)    | 0.804           |
| rs689466 |                 |              |                    |                 |
| TT       | Reference        |              | Reference          |                 |
| CT       | 0.73(0.45,1.18)  | 0.197        | 0.81(0.44,1.47)    | 0.483           |
| CC       | 0.47(0.11,2.08)  | 0.325        | 0.46(0.11,1.95)    | 0.293           |
| CT/CC    | 0.71(0.44,1.13)  | 0.151        | 0.76(0.42,1.36)    | 0.357           |
| Additive model | 0.72(0.48,1.10) | 0.130 | 0.75(0.46,1.24)    | 0.266           |
| rs5275   |                 |              |                    |                 |
| AA       | Reference        |              | Reference          |                 |
| GA       | 0.77(0.46,1.28)  | 0.310        | 1.24(0.64,2.41)    | 0.517           |
| GG       | 1.77(0.93,3.39)  | 0.085        | 1.16(0.48,2.79)    | 0.744           |
| GA/GG    | 0.97(0.61,1.55)  | 0.899        | 1.21(0.65,2.27)    | 0.552           |
| Additive model | 1.20(0.88,1.64) | 0.254 | 1.08(0.71,1.64)    | 0.713           |

(Continued)
of IS, no statistically significant association was observed; thus, large-sample studies should be confirmed in the Chinese population. Admittedly, there were some limitations of this study, such as, the genes enrolled in this study may be affected by many SNPs, here we selected some of them, therefore our study was not comprehensive enough; moreover, the study failed to assess the protein expression in patients, which protein plays a decisive role; finally, the sample size of this study was relatively small, which may affect the statistical power.

| Genotype     | Male                                | Female                              |
|--------------|-------------------------------------|-------------------------------------|
|              | OR(95% CI)^[a] | P-value^[a] | OR(95% CI)^[a] | P-value^[a] |
| **MMP9**     |                      |                      |                      |                      |
| rs17576      |                      |                      |                      |                      |
| GG           | Reference            |                      | Reference            |                      |
| GA           | 0.93(0.58,1.49)      | 0.768                 | 1.23(0.66,2.30)      | 0.518                 |
| AA           | 1.01(0.48,2.12)      | 0.987                 | 1.17(0.47,2.90)      | 0.742                 |
| GA/AA        | 0.95(0.61,1.47)      | 0.801                 | 1.12(0.68,2.16)      | 0.513                 |
| Additive model | 0.97(0.70–1.36)   | 0.889                 | 1.12(0.74–1.70)      | 0.592                 |
| rs3918242    |                      |                      |                      |                      |
| CC           | Reference            |                      | Reference            |                      |
| CT           | 1.13(0.62,2.04)      | 0.689                 | 1.96(0.82,4.69)      | 0.132                 |
| TT           | 2.52(0.35,18.32)     | 0.363                 | --                  | --                   |
| CT/TT        | 1.19(0.67,2.12)      | 0.552                 | 1.53(0.67,3.48)      | 0.315                 |
| Additive model | 1.23(0.73–2.06) | 0.442                 | 1.18(0.56–2.48)      | 0.665                 |
| rs9509       |                      |                      |                      |                      |
| TT           | Reference            |                      | Reference            |                      |
| CT           | 0.96(0.60,1.54)      | 0.867                 | 0.76(0.41,1.41)      | 0.389                 |
| CC           | 0.70(0.23,2.14)      | 0.531                 | 0.47(0.10,2.25)      | 0.346                 |
| CT/CC        | 0.93(0.59–1.46)      | 0.742                 | 0.73(0.40–1.32)      | 0.295                 |
| Additive model | 0.91(0.62,1.34)   | 0.641                 | 0.73(0.44,1.23)      | 0.236                 |
| **MMP2**     |                      |                      |                      |                      |
| rs7201       |                      |                      |                      |                      |
| AA           | Reference            |                      | Reference            |                      |
| CA           | 1.08(0.67,1.74)      | 0.757                 | 0.79(0.42,1.50)      | 0.471                 |
| CC           | 1.18(0.48,2.90)      | 0.713                 | 1.10(0.30,4.05)      | 0.890                 |
| CA/CC        | 1.10(0.70,1.73)      | 0.683                 | 0.86(0.47,1.55)      | 0.611                 |
| Additive model | 1.09(0.77–1.55)   | 0.635                 | 0.93(0.57–1.53)      | 0.785                 |
| rs2285053    |                      |                      |                      |                      |
| CC           | Reference            |                      | Reference            |                      |
| CT           | 0.81(0.51,1.31)      | 0.394                 | 1.40(0.74,2.63)      | 0.304                 |
| TT           | 1.44(0.62,3.32)      | 0.394                 | 1.15(0.39,3.46)      | 0.797                 |
| CT/TT        | 0.90(0.58,1.41)      | 0.650                 | 1.34(0.74,2.43)      | 0.327                 |
| Additive model | 1.01(0.72–1.44)   | 0.917                 | 1.20(0.76–1.90)      | 0.438                 |
| rs243864     |                      |                      |                      |                      |
| TT           | Reference            |                      | Reference            |                      |
| CT           | 0.84(0.48–1.49)      | 0.550                 | 1.16(0.55–2.45)      | 0.699                 |
| CC           | 9.40(0.88–100.44)    | 0.064                 | 0.89(0.13–6.14)      | 0.909                 |
| CT/CC        | 0.96(0.56–1.68)      | 0.897                 | 1.11(0.55–2.26)      | 0.770                 |
| Additive model | 1.10(0.66–1.84) | 0.705                 | 1.05(0.58–1.92)      | 0.864                 |

Note: *Adjusted for age, sex, smoking, and drinking.
In summary, our study suggested that PTGS2 rs689466 C and rs5275 A were potentially associated with IS subtype of small-vessel occlusion risk. Our result should be confirmed with further large sample sized studies.

Ethical Statement and Consent
The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the Nanjing First Hospital, and all participants were written informed consent.

Author Contributions
All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure
The authors report no conflicts of interest in this work.

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Table 3 Association Between Genetic Variations and Types of Ischemic Stroke Risk

| Genotype | Small-Vessel Occlusion | Large-Artery | Cardioembolism | Other Type |
|----------|------------------------|--------------|---------------|-----------|
|          | OR (95% CI)* | P value | OR (95% CI)* | P value | OR (95% CI)* | P value | OR (95% CI)* | P value |
| rs1800587|                       |          |              |          |             |        |              |        |
| GG       | Reference             | 0.59(0.29–1.21) | 0.151 | 0.93(0.50–1.74) | 0.828 | 0.89(0.31–2.57) | 0.822 | 0.76(0.40–1.44) | 0.399 |
| GA       | –                     | –         | 0.65(0.09–4.50) | 0.658 | –          | –         | 0.63(0.09–4.26) | 0.632 |
| AA/GA    | 0.63(0.31–1.28)      | 0.202   | 0.91(0.50–1.66) | 0.751 | 0.97(0.34–2.78) | 0.958 | 0.75(0.41–1.38) | 0.354 |
| Additive model | 0.70(0.36–1.37) | 0.299   | 0.89(0.53–1.54) | 0.694 | 1.06(0.40–2.82) | 0.906 | 0.77(0.45–1.33) | 0.346 |
| rs689466 |                       |          |              |          |             |        |              |        |
| TT       | Reference             | 2.51(1.22–5.16) | 0.012 | 0.86(0.54–1.38) | 0.535 | 1.45(0.58–3.63) | 0.432 | 1.49(0.88–2.53) | 0.138 |
| CC       | 3.27(0.40–26.84)      | 0.270   | 1.83(0.37–9.08) | 0.461 | 0.91(0.15–5.39) | 0.914 | 2.33(0.49–11.16) | 0.290 |
| CT/CC    | 2.53(1.26–5.07)      | 0.009   | 0.91(0.57–1.45) | 0.686 | 1.35(0.57–2.23) | 0.499 | 1.56(0.93–2.61) | 0.094 |
| Additive model | 2.26(1.19–4.28) | 0.013   | 0.99(0.65–1.49) | 0.941 | 1.18(0.58–2.45) | 0.641 | 1.51(0.96–2.40) | 0.078 |
| rs3918242|                       |          |              |          |             |        |              |        |
| TT       | Reference             | 1.25(0.49–3.19) | 0.641 | 0.87(0.46–1.61) | 0.648 | 0.31(0.11–0.91) | 0.033 | 0.62(0.33–1.17) | 0.137 |
| CC       | 0.27(0.05–1.58)      | 0.146   | 2.23(0.29–25.14) | 0.517 | –          | –         | –          | –         |
| CT/TT    | 1.01(0.44–2.32)      | 0.987   | 0.92(0.50–1.69) | 0.790 | 0.39(0.14–1.09) | 0.072 | 0.69(0.37–1.29) | 0.239 |
| Additive model | 0.86(0.43–1.71) | 0.661   | 0.98(0.57–1.71) | 0.954 | 0.55(0.23–1.35) | 0.192 | 0.80(0.45–0.42) | 0.435 |
| rs5275   |                       |          |              |          |             |        |              |        |
| AA       | Reference             | 0.49(0.22–1.07) | 0.072 | 1.28(0.76–2.15) | 0.350 | 1.47(0.55–3.97) | 0.443 | 1.18(0.67–2.07) | 0.574 |
| GG       | 0.31(0.12–0.80)      | 0.016   | 0.97(0.50–1.88) | 0.927 | 0.83(0.24–2.95) | 0.775 | 0.54(0.27–1.00) | 0.089 |
| GA/GG    | 0.45(0.21–0.95)      | 0.036   | 1.20(0.74–1.93) | 0.462 | 1.20(0.49–2.96) | 0.686 | 0.96(0.57–1.61) | 0.874 |
| Additive model | 0.60(0.39–0.92) | 0.020   | 1.05(0.75–1.46) | 0.782 | 0.94(0.50–1.78) | 0.855 | 0.81(0.57–1.14) | 0.217 |

Note: *Adjusted for age, sex, smoking, and drinking; the results with a significant difference are in bold.
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