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ORIGINAL RESEARCH

Genetic Variation of Inflammatory Genes to Ischemic Stroke Risk in a Chinese Han Population

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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 ligase 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 – $OR_{adjusted}$= 2.51, 95% CI: 1.22–5.16, p = 0.012; co-dominant model: CT/CC vs TT – $OR_{adjusted}$= 2.53, 95% CI: 1.26–5.07, p = 0.009; additive model – $OR_{adjusted}$= 2.26, 95% CI: 1.19–4.28, p = 0.013) and rs5275 (dominant model: GG vs AA – $OR_{adjusted}$= 0.31, 95% CI: 0.12–0.80, p = 0.016; co-dominant model: GA/GG vs AA – $OR_{adjusted}$= 0.45, 95% CI: 0.21–0.95, p = 0.036; additive model – $OR_{adjusted}$= 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

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.[1] 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:[2] 1) large-artery atherosclerosis, 2) small-vessel occlusion, 3) cardioembolism, 4) other determined etiology, and 5) undermined etiology. The most common cause of ischemic is due to arterial occlusion, and the rarer cause is cerebral veins or venous sinuses.[3] There are many causes of stroke, such as hypertension, diabetes, dyslipidemia, smoking, and genetic background. Especially, genetic background accounts for 30–40% of IS.[4]

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Inflammation contributes across the spectrum of IS.[5] Firstly, inflammation might not only promote thrombus formation but also inhibit endogenous fibrinolysis to enhance clot stability.[6] Secondly, the inflammatory mediators promote immune cells and solutes into the brain parenchyma and damage the blood–brain barrier further, and contribute the thrombogenesis.[7,8] Moreover, some inflammatory mediators might be the predictor factors of IS clinical outcome.[9,10] Multiple inflammation-associated mediators are involved in the process.[11] 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.[12] *IL1A* and *IL1B* 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[12] 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.[13] Studies showed IL1β had significantly higher blood levels for stroke[14] and contribute to the occurrence of stroke.[15] 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[16] and is closely associated with the formation of atherosclerosis.[17] Multiple studies reported an association between *PTGS2* genetic polymorphisms and IS risk.[18,19] 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.[20] Previously, studies have reported that the MMPs protein levels elevate result in blood–brain barrier dysfunction and impact the extent of the infarct,[21–23] and the polymorphisms of *MMP*s have been evaluated in IS.[24,25] The association with IS of *MMPs* is focused on *MMP2* and *MMP9*[26], and the high expression level of MMP2 and MMP9 has been reported that was associated with increased risk of IS and major disability.[15,27–29]

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 (SNP*s*) 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.[30] 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.[30] 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 χ^2 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 – $OR_{adjusted}$=2.51, 95% CI: 1.22–5.16, p=0.012; co-dominant model: CT/CC vs TT – $OR_{adjusted}$=2.53, 95% CI: 1.26–5.07, p=0.009; additive model – $OR_{adjusted}$=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 – $OR_{adjusted}$=0.31, 95% CI: 0.12–0.80, p=0.016; co-dominant model: GA/GG vs AA – $OR_{adjusted}$=0.45, 95% CI: 0.21–0.95, p=0.036; additive model – $OR_{adjusted}$=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 *MMP9* rs3918242 was observed to be susceptible to the cardioembolic subtype of IS with dominant model (CT vs CC – $OR_{adjusted}$ =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 (*TGFB1*), 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)[a]	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*)

Table 1 (Continued).

Genotype	Patients, n(%)	Controls, n(%)	OR(95% CI)[a]	P value
rs3918242				
CC	245(81.94)	254(84.67)	Reference	
CT	51(17.06)	42(14.00)	1.37(0.86–2.18)	0.193
TT	3(1.00)	4(1.33)	0.86(0.17–4.36)	0.852
CT/TT	54(18.06)	46(15.33)	1.32(0.84–2.08)	0.230
rs9509				
TT	189(63.21)	184(61.33)	Reference	
CT	100(33.44)	101(33.67)	0.94(0.66–1.35)	0.750
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
MMP2				
rs7201				
AA	173(57.86)	178(59.33)	Reference	
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)	0.741
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	
CT	102(34.11)	103(34.33)	1.03(0.71–1.48)	0.895
TT	26(8.70)	21(7.00)	1.39(0.73–2.64)	0.311
CT/TT	128(42.81)	124(41.33)	1.08(0.77–1.53)	0.643
rs243864				
TT	238(79.60)	238(79.33)	Reference	
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: [a]Adjusted for age, sex, smoking, and drinking.

Shan also showed that rs689466 was associated with small vessel disease,[40] which was consistent with our results. However, Zhao reported that rs68944 has a higher risk in LAA was dissimilar to our study,[41] 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.[42] 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.[43] The *MMP9* rs3918242, within the promoter region (−1562 C>T), T allele led to a higher promoter activity of the *MMP9*.[44–46] The conclusions of rs3918242 T allele for stroke risk were controversial.[47–49] 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,[49] 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

Table 2 Genotype Distribution of Polymorphisms in All Participants Stratified by Sex

Genotype	Male		Female	
	OR(95% CI)[a]	P-value[a]	OR(95% CI)[a]	P-value[a]
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*)

Table 2 (Continued).

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: [a]Adjusted for age, sex, smoking, and drinking.

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.

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

Genotype	Small-Vessel Occlusion		Large-Artery		Cardioembolism		Other Type	
	OR (95% CI)[a]	*P* value	OR (95% CI)[a]	*P* value	OR (95% CI)[a]	*P* value	OR (95% CI)[a]	*P* value
rs1800587								
GG	Reference		Reference		Reference		Reference	
GA	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
AA	–	–	0.65(0.09–4.50)	0.658	–	–	0.63(0.09–4.26)	0.632
GA/AA	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		Reference		Reference		Reference	
CT	**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(0.57–3.23)	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								
CC	Reference		Reference		Reference		Reference	
CT	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
TT	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		Reference		Reference		Reference	
GA	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(0.27–1.10)	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: [a]Adjusted for age, sex, smoking, and drinking; the results with a significant difference are in bold.

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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