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*Original Article*

# Relationship between the matrix metalloproteinase-9 gene polymorphisms and ischemic stroke

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**Abstract:** Background: In recent years, with the further research of human genome scanning, the relationship between matrix metalloproteinase-9 (MMP-9) gene polymorphisms and many diseases has aroused increased attention. But there is little research on the relationship between MMP-9 gene polymorphisms and ischemic stroke (IS). This study was conducted to evaluate the relationships between the rs3918242 and rs17577 polymorphisms in the MMP-9 gene and IS in a Chinese population. Methods: 152 cases of IS patients and 152 healthy controls were enrolled in this study. All subjects were genotyped for the MMP-9 rs3918242 and rs17577 polymorphisms by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) and restriction analysis. Results: Rs3918242 showed genotypes TT, TC, and CC, and rs17577 exhibited genotypes AA, AG, and GG. The MMP-9 polymorphisms rs3918242 and rs17577 exhibited complete linkage. Our study found there was no significant difference in genotype and allele between rs3918242 and rs17577 between patients and controls. The MMP-9 gene rs3918242 and rs17577 polymorphisms are not significantly correlated with IS risk. Genetic polymorphisms vary among ethnic and regional populations. Conclusion: Our results suggest that MMP-9 rs3918242 is completely linked to rs17577, while the rs3918242 and rs17577 polymorphisms are not significantly associated with the risk of IS. Genetic polymorphisms vary among ethnic and regional populations.

**Keywords:** Ischemic stroke, matrix metalloproteinases, gene, polymorphism, correlation, regional difference

## Introduction

Matrix metalloproteinases (MMPs) belong to an endogenous $Ca^{2+}$- and $Zn^{2+}$-dependent protease superfamily, which has been found to have 28 family members numbered from MMP-1 to MMP-28 [1]. Ischemic stroke (IS) is closely related to the expression of MMP-9, and serum MMP-9 levels may increase during the acute phase of stroke [2]. Serum MMP-9 levels were significantly increased in patients with acute IS, suggesting that MMP-9 and inflammation are closely related [3] and associated with the severity of IS and infarct volume. Serum MMP-9 levels have important implications for the diagnosis of stroke [4].

The MMP-9 gene contains 13 exons and is located on chromosome 20q12.2-13.1 [5]. Rs3918242 (-1562 C/T), a promoter, is located in the functional region of MMP-9 gene, which has a controlling effect on MMP-9 activity [6-10]. The changes of SNPs in the promoter region may cause changes of the coding activity of the MMP-9 gene, which leading to the occurrence and development of MMP-9 gene-related diseases [8]. A study of Mexican patients found that rs17577 is a promoter of MMP-9 gene, which can alter the structure of MMP-9. Therefore, variant rs17577 is associated with many diseases including asthma, COPD, allergy and cancer [11].

In recent years with the further research of human genome scanning, the relationship between MMP-9 gene polymorphisms and many diseases has aroused increased attention. But there is little research on the relation-

ship between MMP-9 gene polymorphisms and IS. The aim of this study was to investigate the association between rs3918242 and rs17577 MMP-9 gene polymorphisms and the risk of IS.

## Materials and methods

### *Subjects*

This study was a case-control study. The IS group consisted of 152 Chinese adult patients from the Affiliated Hospital of Youjiang Medical University for Nationalities. The patients were unrelated and confirmed to have suffered an IS. The enrollment period was from February 2016 to August 2016. There were 79 males and 73 females. The IS diagnosis was confirmed by a computed tomography (CT) or magnetic resonance imaging (MRI) scan, in line with the WHO diagnostic criteria for IS. Patients were excluded if they had cardiogenic shock, arteritis, trauma, a tumour-induced cerebral infarction, coronary heart disease, or thyroid, liver, kidney, blood, autoimmune disease or other systemic diseases.

In total, 152 outpatients who visited the hospital for health check-ups were randomly selected as control subjects. Subjects in the control group were matched for age and gender with the patients in the IS group. The controls were free of IS and had normal ECG and no clinical signs of trauma or renal, cardiovascular, neurological or inflammatory diseases and no surgery in the near future.

According to the 1964 Declaration of Helsinki, all participants signed a written informed consent form. The research program was approved by the institutional ethics committee of our hospital.

### *Determination of the MMP-9 genotype*

Venous blood (5 ml) of 152 IS patients and 152 normal healthy people was obtained in the morning after fasting and was collected in EDTA-containing tubes then centrifuged at 3000 r/min for 10 min, and within 30 min, the supernatant fraction was extracted and stored at -80°C until use. TIANamp DNA Blood kits (Tiangen, Beijing, China) were used to isolate genomic DNA from the blood samples. The rs3918242 and rs17577 polymorphisms of the MMP-9 gene were analysed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The following primers were used for the amplification reaction. The forward and reverse primers were 5'-GCCTGGCACATAGTAGGCCC-3' and 5'-CTTCCTAGCCAGCCGGCAT-3' for rs3918242 and 5'-GGCTCAGCACCTGTCTCCTCC-3' and 5'-CTGGGTCCTGGTCTTGGTTGC-3' for rs17577, respectively. The PCR conditions were set as follows. Initial denaturation at 95°C for 2 min followed by 11 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 40 s, and extension at 72°C for 30 s, and 24 cycles of denaturation at 94°C for 20 s, the samples were annealed at 59°C for 30 s and then extended at 72°C for 30 s. The last step is to extend for 2 min at 72°C. Approximately 10 microliters of the PCR products of rs3918242 and rs17577 were digested overnight with 5 U of SphI and StyI restriction endonucleases at 37°C, respectively, and the digested fragments were separated on a 2% agarose gel. The fragment size of the C allele of rs3918242 was 435 bp, while the fragment sizes of the T alleles were 242 and 194 bp. The fragment size of the C allele of rs17577 was 226 bp, while the fragment sizes of the T alleles were 135 and 91 bp. Two samples of each genotype were randomly selected for quality control of genotyping and PCR product sequencing validation such that 100% agreement was achieved between the genotyping assays.

### *Statistical analysis*

Statistical calculations were performed using SPSS version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Gene frequency and allele frequency were calculated by a direct counting method. The rs3918242 and rs17577 genotypes were analysed to determine whether or not they fit the Hardy-Weinberg equilibrium (HWE) using Fisher's exact tests. For baseline characteristics, continuous variables of normal distribution are expressed as the mean ± SD. Pearson's Chi-square test was used to compare the genotype distribution and allele frequency between cases and controls ($x^2$ test). Values of $P < 0.05$ were considered significant.

## Results

### *Verification of the gene sequencing results*

The results of direct sequencing of the PCR products of the MMP-9 polymorphisms rs3918242 and rs17577 were consistent with

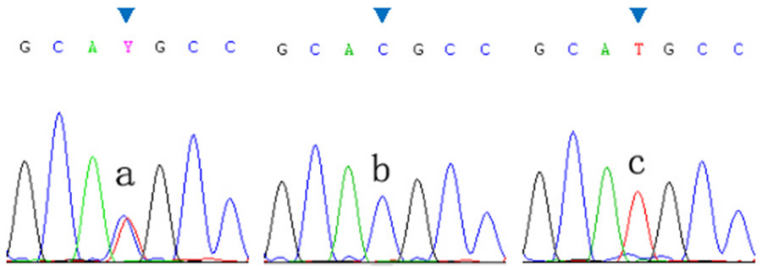


**Figure 1.** Sequencing map of the genotype for the MMP-9 gene rs3918242 polymorphism. The arrows in panels a, b, and c show the T/C, C/C and T/T genotypes, respectively.

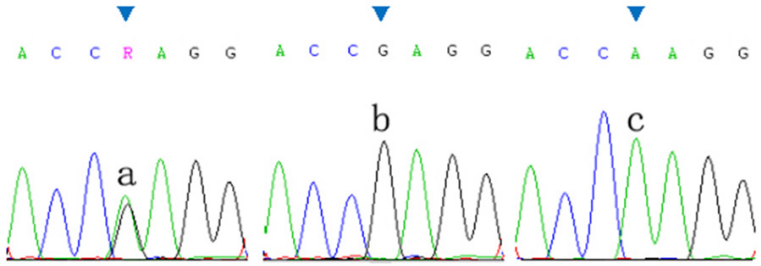


**Figure 2.** Sequencing map of the genotype for the MMP-9 gene rs17577 polymorphism. The arrows in panels a, b, and c show the A/G, G/G and A/A genotypes, respectively.

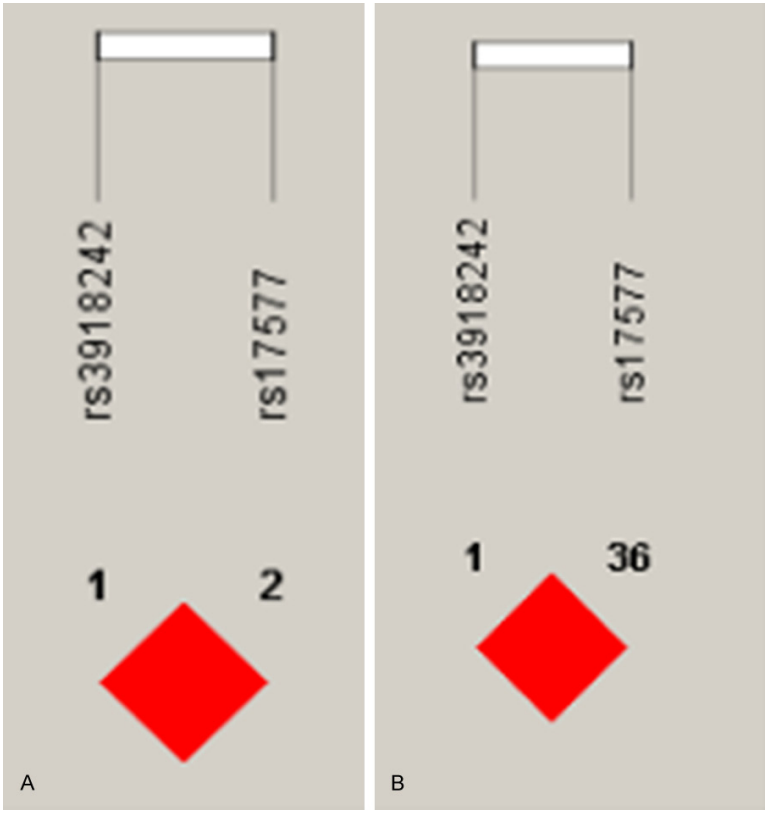


**Figure 3.** The rs3918242 and rs17577 polymorphisms exhibit complete linkage. A: MMP-9 gene (this study data). B: MMP-9 gene (1000 Genomes reference data).

those of the restriction enzyme digestion. There were TT, TC, CC genotypes in rs3918242, and AA, AG and GG genotypes in rs17577. Gene sequencing confirmed our test results (**Figures 1** and **2**).

*Rs3918242 and rs17577 exhibit complete linkage*

This study found that MMP-9 rs3918242 and rs17577 exhibit complete linkage, which is consistent with the data presented in the National Center for Biotechnology Information (NCBI) database (**Figure 3**).

*Comparison of genotype and allele frequencies of rs3918242 and rs17577 in the case and control groups*

*Demographic characteristics of IS group and control group:* One hundred and fifty-two IS patients in the case group and 152 matched non-IS subjects in the control group were genotyped for rs3918242 and rs17577 polymorphisms of MMP-9. The demographic characteristics of all studied subjects are shown in **Table 1**. The mean age of the patients with IS was 60.49 ± 10.22 years, and that of the control subjects was 58.10 ± 10.16 years. The IS group consisted of 79 females and 73 males, while the healthy control group consisted of 80 females and 72 males. There were no significant differences in age or gender between the case and control groups ($P$ = 0.93, $P$ = 0.05 and $P$ = 0.91, respectively).

*The prevalence of genotype and allele frequencies of MMP-9 rs3918242 and rs17577 in patients and controls:* The prevalence of genotype and allele frequencies of

**Table 1.** Demographic characteristics of control group and IS group

| Variables | Controls (n = 152) | % | Patients (n = 152) | % | $t/\chi^2$ test | *P* value |
|---|---|---|---|---|---|---|
| Age, years | | | | | | |
| Mean ± SD | 58.10 ± 10.16 | | 60.49 ± 10.22 | | 0.09 | 0.93 |
| ≤ 60 | 86 | 56.6 | 69 | 45.4 | | |
| > 60 | 66 | 43.4 | 83 | 54.6 | 3.80 | 0.05 |
| Sex | | | | | | |
| Females | 72 | 47.4 | 73 | 48.0 | | |
| Males | 80 | 52.6 | 79 | 52.0 | 0.01 | 0.91 |

MMP-9 rs3918242 and rs17577 in both the case and control groups are listed in **Tables 2** and **3**. The genotype distribution between the IS patients and controls were in line with the HWE (*P* = 0.28 and *P* = 0.72, respectively). The $x^2$ test showed that there were no significant differences in genotype frequency and T or C allele frequency of MMP-9 rs3918242 between the IS group and the control group ($x^2$ = 1.25, *P* = 0.54 > 0.05 and $x^2$ < 0.001, *P* = 1.0 > 0.05, respectively). Also, the genotype frequency and A or G allele frequency of MMP-9 gene rs17577 showed no significant differences ($x^2$ = 1.25, *P* = 0.54 > 0.05 and $x^2$ < 0.001, *P* = 1.0 > 0.05, respectively).

The relationship between MMP-9 rs3918242 and rs17577 genotype frequencies and allele frequencies in patients with cerebral infarction: Genotype distributions of MMP-9 rs3918242 and rs17577 were shown in **Table 4**. The genotype frequencies were not significantly different in frequencies of the MMP-9 rs3918242 between the patients and controls (TC vs. TT: OR = 3.36, 95% CI = 0.33~33.93, P = 0.30; CC vs. TT: OR = 2.95, 95% CI = 0.30~28.76, P = 0.35; TT vs. [TC+CC]: OR = 3.04, 95% CI = 0.31~29.56, P = 0.34; TC vs. [CC+TT]: OR = 0.86, 95% CI = 0.51~1.47, P = 0.59). The genotype frequencies of MMP-9 rs17577 were not significantly different between the patients and controls (AG vs. AA: OR = 3.36, 95% CI = 0.33~33.93, P = 0.30; GG vs. AA: OR = 2.95, 95% CI = 0.30~28.76, P = 0.35; AA vs. [AG+GG]: OR = 3.04, 95% CI = 0.31~29.56, P = 0.34; AG vs. [GG+AA]: OR = 0.86, 95% CI = 0.51~1.47, P = 0.59).

Comparison of MMP-9 rs3918242 and rs17577 genotype frequencies in Guangxi population in China with other ethnic and regional populations: We compared the frequency of MMP-9 rs3918242 and rs17577 genotype distribution in Guangxi population with those of other ethnic and regional populations (**Tables 5**, **6**). There is no MMP-9 rs3918242 genotype data at International Human Genome HapMap Project, therefore, we compared the data with related articles. The results showed that the MMP-9 rs3918242 genotype in Guangxi population was significantly different from the Chinese Shanghai population and the Henan population (P < 0.001). However, there is no statistically significant difference between the Chinese Guangdong population and the Polish population (P > 0.05). We compared the MMP-9 rs17577 genotype in Guangxi population data with International Human Genome HapMap Project data. The results showed that the MMP-9 rs17577 genotype in the Guangxi population was significantly different from the HapMap-JPT population (P < 0.05). There was no significant difference between the HapMap-CEU, HapMap-YRI and HapMap-HCB populations (P > 0.05).

## Discussion

MMP-9 has a physiological function in cleavage of structural proteins and regulation of protease activities [12, 13]. Previous studies have reported the relationship between MMP9 rs3918242 and the development of ischemic stroke, however, the results are not consistent [14-18]. Some research reported that MMP-9 rs3918242 was associated with IS in the population of Shaanxi Province and Henan Province of China [14, 18]. However, some studies had different subjects and suggested that rs3918242 was not directly associated with cerebral infarction. For example, Manso's research object was Portuguese; Montaner's research object was Spanish; Szczudlik's research object was Poles [15-17]. These results showed that genetic polymorphisms were vary among different ethnic and regional populations. Whether there is a relationship between rs3918242 and IS in Chinese Guangxi population is not answered by any reports. Meanwhile, rs17577, like rs3918242, is also a promoter of MMP-9

**Table 2.** The polymorphism distribution of MMP-9 gene rs3918242 in control group and IS group

| | N | Genotype % | | | HWE | $\chi^2$ test | *P* value | Allele (N) | Allele % | | $\chi^2$ test | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TT | TC | CC | | | | | T | C | | |
| Controls | 152 | 3 (1.9) | 33 (21.7) | 116 (76.3) | 0.72 | | | 304 | 39 (12.8) | 265 (87.2) | | |
| Patients | 152 | 1 (0.7) | 37 (24.3) | 114 (75.0) | 0.28 | 1.25 | 0.54 | 304 | 39 (12.8) | 265 (87.2) | < 0.001 | 1.00 |

**Table 3.** The polymorphism distribution of MMP-9 gene rs17577 in control group and IS group

| | N | Genotype % | | | HWE | $\chi^2$ test | *P* value | Allele (N) | Allele % | | $\chi^2$ test | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | AG | GG | | | | | A | G | | |
| Controls | 152 | 3 (1.9) | 33 (21.7) | 116 (76.3) | 0.72 | | | 304 | 39 (12.8) | 265 (87.2) | | |
| Patients | 152 | 1 (0.7) | 37 (24.3) | 114 (75.0) | 0.28 | 1.25 | 0.54 | 304 | 39 (12.8) | 265 (87.2) | < 0.001 | 1.00 |

**Table 4.** Correlation between MMP-9 rs3918242 and rs17577 gene polymorphisms and ischemic stroke pathogenesis

| SNPs | Controls N = 152 (%) | Patients N = 152 (%) | OR (95% CI) | *P* value |
|---|---|---|---|---|
| rs3918242 | | | | |
| TT | 3 (2.0) | 1 (0.7) | 1.00 (Ref) | |
| TC | 33 (21.7) | 37 (24.3) | 3.36 (0.33~33.93) | 0.30 |
| CC | 116 (76.3) | 114 (75.0) | 2.95 (0.30~28.76) | 0.35 |
| TT vs. [TC+CC] | | | 3.04 (0.31~29.56) | 0.34 |
| TC vs. [CC+TT] | | | 0.86 (0.51~1.47) | 0.59 |
| rs17577 | | | | |
| AA | 3 (2.0) | 1 (0.7) | 1.00 (Ref) | |
| AG | 33 (21.7) | 37 (24.3) | 3.36 (0.33~33.93) | 0.30 |
| GG | 116 (76.3) | 114 (75.0) | 2.95 (0.30~28.76) | 0.35 |
| AA vs. [AG+GG] | | | 3.04 (0.31~29.56) | 0.34 |
| AG vs. [GG+AA] | | | 0.86 (0.51~1.47) | 0.59 |

gene, which plays an important role in the regulation of protein synthesis [8, 11]. However, currently, there are few articles about the association of MMP-9 gene rs17577 with ischemic stroke. Therefore, we tried to study the relationship between rs3918242 and rs17577 MMP-9 gene polymorphisms and the risk of IS.

In this study, we analysed the association of MMP-9 rs3918242 and rs17577 polymorphisms with IS in a Chinese Guangxi population of patients. This study revealed that MMP-9 rs3918242 and rs17577 exhibit complete linkage (**Figure 3**), suggesting that the occurrence of IS may simultaneously accept the regulation of these two loci. When the rs17577 gene mutation occurs, the rs3918242 gene mutation also occurs at the corresponding site. When multiple gene loci simultaneously regulate a human disease, the loci control the genetic stability with a higher degree of accuracy, thus ensuring the stability of race genetics. Genetic polymorphisms are common in human genes. The most common polymorphisms are single nucleotide polymorphisms (SNPs). SNPs were found to be closely related to the occurrence and prevalence of human immunodeficiency virus, and two characteristics were stable and relatively variable in the experiments by Mah et al [19].

Some studies found that MMP-9, which is known to be involved in the blood-brain barrier disruption after stroke, was significantly increased in blood plasma [20, 21]. Lin et al [22] found that when cerebral ischemia/reperfusion injury occurs, inhibition of MMP-9 expression can reduce the extent of damage to the blood-brain barrier in the ischemic penumbra and reduce the degree of neuronal damage. Hao et al [23] reported that in patients with the CC genotype and CC + TC genotypes, the risk of IS is significantly increased.

Other studies, however, have reported different experimental conclusions [15]. Szczudlik and Borratyńska [17] assessed the significance of MMP-9 polymorphisms in a Polish population as a risk factor for cerebrovascular diseases. They studied 418 IS patients. The statistical analysis showed no significant differences in the incidence of CC, CT, or TT genotypes or in C

**Table 5.** Comparison of the frequency of MMP-9 rs3918242 genotypes among populations in different ethnicities and regions in Guangxi, China

| SNPs | Research population | n | Genotype (%) | | | HWE | $\chi^2$ test | *P* value |
|---|---|---|---|---|---|---|---|---|
| | | | TT | TC | CC | | | |
| rs3918242 | Guangxi population | 152 | 3 (2.0) | 33 (21.7) | 116 (76.3) | 0.72 | - | - |
| | Hao et al | 317 | 242 (76.4) | 66 (20.8) | 9 (2.8) | 0.09 | 316.915 | < 0.001* |
| | Li et al | 1258 | 15 (1.2) | 202 (16.0) | 1041 (82.8) | 0.15 | 3.933 | 0.14 |
| | Kinga et al | 410 | 8 (2.0) | 119 (29.0) | 283 (69.0) | 0.26 | 3.024 | 0.22 |
| | Zhao et al | 335 | 254 (75.8) | 71 (21.2) | 10 (3.0) | 0.08 | 325.378 | < 0.001* |

*P < 0.05.

**Table 6.** Comparison of the frequency of MMP-9 rs17577 genotypes among populations in different ethnicities and regions in Guangxi, China

| SNPs | Research population | n | Genotype (%) | | | HWE | $\chi^2$ test | *P* value |
|---|---|---|---|---|---|---|---|---|
| | | | AA | AG | GG | | | |
| rs17577 | Guangxi population | 152 | 3 (2.0) | 33 (21.7) | 116 (76.3) | 0.72 | - | - |
| | HapMap-CEU | 120 | 8 (6.7) | 30 (25.0) | 82 (68.3) | 0.03 | 4.552 | 0.103 |
| | HapMap-YRI | 120 | 8 (6.7) | 30 (25.0) | 82 (68.3) | 0.03 | 4.552 | 0.103 |
| | HapMap-HCB | 90 | 0 (0) | 18 (20.0) | 72 (80.0) | 0.29 | 1.954 | 0.377 |
| | HapMap-JPT | 90 | 6 (6.7) | 30 (33.3) | 54 (60.0) | 0.52 | 8.423 | 0.015* |

*P < 0.05.

alleles or T alleles compared with control subjects. The results showed that the MMP-9 rs3918242 polymorphism had no relation with IS. Kaplan et al [24] studied 367 patients with IS, and the MMP-9 haplotype and SNP were found to be independent from myocardial infarction or stroke.

In the present study, there was no significant difference in genotype and allele between rs3918242 and rs17577 between patients and controls (**Tables 2** and **3**). Also, there was no significant correlation between MMP-9 rs3918242 or rs17577 polymorphisms and the risk of IS (**Table 4**). These data are consistent with the previous studies [15, 17, 24].

Regarding the controversial conclusions of MMP-9 gene SNPs and genetic susceptibility to different diseases, the author believes that there are several possibilities: the control group was artificially identified as having no history of cerebrovascular disease. However, without brain imaging studies, tests to show neurological deficits or laboratory tests, some subjects in the control group might have been affected by a silent stroke. This can result in errors in the statistical analysis or reduce the accuracy of the statistics. Another limitation is that MMP-9 interacts with other candidate genes in different subjects, and these interactions may be somewhat different and are often overlooked. Also we had small sample size in our experiments. If we can carry out large-sample, prospective studies in future experiments, the results will be more valuable. Last but not the least, genetic polymorphisms vary among different ethnic and regional populations, as shown in **Tables 5** and **6**. By comparison, we found that the rs3918242 and rs17577 genotype showed differences in different races, and even different ethnic groups of the same race. Therefore, even in the same gene locus, people with different genetic backgrounds have a different genetic susceptibility to disease.

In conclusion, for the first time, our results suggest that MMP-9 rs3918242 and rs17577 polymorphisms exhibit complete linkage. There was no significant difference in genotype and allele between rs3918242 and rs17577 between patients and controls. The MMP-9 gene rs3918242 and rs17577 polymorphisms were not significantly correlated with IS risk. Genetic polymorphisms vary among ethnic and regional populations. Therefore, future studies with larger sample size and multi-regional studies may contribute to the results.

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## Disclosure of conflict of interest

None.

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