The roles of MMP8/MMP10 polymorphisms in ischemic stroke susceptibility

Yong Zhao1 | Qi Zhang2 | Xiaobo Zhang2 | Yu Zhang2 | Ying Lu1 | Xiaojuan Ma3 | Weiping Li2 | Xiaochen Niu2 | Gejuan Zhang1 | Mingze Chang1 | Wenzhen Shi3 | Ye Tian1

1 Department of Neurology, Xi’an Key Laboratory of Cardiovascular and Cerebrovascular Diseases, Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University, Xi’an, Shaanxi, China
2 The College of Life Sciences, Northwest University, Xi’an, Shaanxi, China
3 Medical Research Center, Xi’an Key Laboratory of Cardiovascular and Cerebrovascular Diseases, Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University, Xi’an, Shaanxi, China

Correspondence
Wenzhen Shi, Medical Research Center, Xi’an Key Laboratory of Cardiovascular and Cerebrovascular Diseases, Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University, Xi’an, Shaanxi, China. Email: shiwenzhen736@163.com

Ye Tian, Department of Neurology, Xi’an Key Laboratory of Cardiovascular and Cerebrovascular Diseases, Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University, Xi’an, Shaanxi, China. Email: chhty@sina.com

Abstract

Background: Ischemic stroke (IS), a multifactorial and polygenic disease, is the most common cause of death. This study aimed to determine the roles of MMP8/MMP10 polymorphisms in IS susceptibility in the Chinese Han population.

Methods: MMP8 rs1940475 and rs3765620, and MMP10 rs17860949 from 700 IS patients and 700 controls were genotyped by the MassARRAY iPLEX platform. The impact of polymorphisms on IS risk was evaluated by logistic regression analysis.

Results: Our study indicated that rs17860949 in MMP10 was significantly associated with a reduced risk of IS (OR = 0.632, p = 0.002). Precisely, stratification analysis showed that rs17860949 was related to a decreased susceptibility to IS in patients aged > 55 years (OR = 0.472, p < 0.001), males (OR = 0.632, p = 0.012), nonsmokers (OR = 0.610, p = 0.017), and nondrinkers (OR = 0.559, p = 0.006). All these significant findings were verified by false-positive report probability test. Furthermore, GG genotype and AG genotype in MMP8 rs3765620 polymorphism were related to a reduced triglycerides concentration (p = 0.018).

Conclusion: Our study suggests that rs17860949 in MMP10 may play a protective role in IS in the Chinese Han population.

KEYWORDS genetic polymorphisms, ischemic stroke, MMP8/MMP10, susceptibility

1 INTRODUCTION

Stroke is the most common severe manifestation of cerebrovascular disease and the second leading cause of death in the world with high disability, mortality, and morbidity (Guzik & Bushnell, 2017; Strong et al., 2007). Stroke is divided into ischemic stroke (IS) and hemorrhagic stroke, and the former accounts for about 87% of total strokes. According to the Report on Cardiovascular Health and Diseases in China, the incidence of stroke in China was 246.8/100,000 in 2019, with males (266.4/100,000) higher than females (226.9/100,000), and rural areas (298.2/100,000) significantly higher than urban areas (203.6/100,000) (The Writing Committee of the Annual Report on Cardiovascular Health and Diseases in China, 2021). Risk factors for IS include age, gender, smoking, alcohol consumption, hypertension, diabetes, and so on (Au, 2018). Although stroke has been identified as a cerebrovascular obstruction caused by atherosclerosis (Weber & Noels, 2011), the pathogenesis underlying IS was not fully understood. Furthermore, it is accepted that IS is a polygenic and multifactorial disease caused...
by the combined action of genetic and environmental factors (Wei et al., 2015). Over the past few years, many studies have indicated that genetic polymorphisms exert an important role in IS of atherosclerotic origin (Misra et al., 2018). Numerous genetic susceptibility variants for stroke have been identified in recent studies, such as ACE (Goyal et al., 2021), XPF (Ma et al., 2016), ITGA2 (Jaleel et al., 2021), MMP9 (Wang et al., 2018), MMP2 (Christodoulou et al., 2020), and ESR1 (Fu et al., 2019). Among these variants, matrix metalloproteinases ( MMPs) have a vital role in the occurrence of stroke (Kaplan et al., 2008; Manso et al., 2010; Park et al., 2007).

MMPs are a unique family of extracellular calcium- and zinc-binding endopeptidases, which can accelerate atherosclerosis by degrading extracellular matrix in IS patients (Abilleira et al., 2006; Chang et al., 2016). Previous researches have revealed that MMP genes play an important role in the pathogenesis of stroke (Ohshima et al., 2010; Schäfers et al., 2010; Su et al., 2005). Moreover, polymorphisms of MMP genes, such as MMP1 (Ghilardi et al., 2002), MMP2 (Nie et al., 2014), MMP3 (Ghilardi et al., 2002), MMP9 (Yuan et al., 2013), and MMP12 (Wen et al., 2014), are associated with IS risk. Matrix metalloproteinase-8 (MMP8) is a type of collagenases in the MMPs. Matrix metalloproteinase-10 (MMP10) is a subgroup of stromelysins in the MMPs. The MMP8 and MMP10 genes are also known to be related to the occurrence and development of IS (Lenglet et al., 2013; Navarro-Oviedo et al., 2019; Purroy et al., 2018). MMP8 polymorphisms are risk factors for many human diseases, such as breast cancer (Wang et al., 2018), ankylosing spondylitis (Meng et al., 2018), gastric adenocarcinoma (Lin et al., 2017), and osteoarthritis (Näkki et al., 2016). To our best known, the roles of MMP8 polymorphisms in IS susceptibility are unclear. There have been few studies on the role of MMP10 polymorphisms in IS. Zhu et al. have found that MMP10 polymorphisms (rs17435959 and rs17293607) are not associated with the risk of atherothrombotic cerebral infarction in Jiangsu population (Zhu et al., 2013). However, a latest study by Zhou has indicated that MMP10 polymorphisms are correlated with the susceptibility and formation of carotid atherosclerosis plaques (Wu et al., 2021). In order to obtain more accurate estimation of the polymorphisms of studied genes, we carried out a case-control study. In our present study, we tried to study the influence of MMP8/MMP10 polymorphisms on IS susceptibility in the Shaanxi Han population. The flow chart of this study IS summarized in Figure 1.

2 MATERIALS AND METHODS

2.1 Study population

Our study was approved by the ethics committee of Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University (SYXSL-2019-034), and informed written consent was signed and obtained from all individuals before the study began. From January 2019 to November 2021, we recruited 1400 (700 IS patients and 700 healthy volunteers) unrelated Chinese Han population included from Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University. Patients were first diagnosed with IS by two experienced neurologists based on clinical symptoms, cerebral scanner, magnetic resonance imaging (MRI), and/or computed tomography according to the guideline for stroke (Liberman et al., 2016). The patients with a history of stroke, genetic diseases, brain tumor or any types of cancers, autoimmune, cardiogenic, and neurological diseases were excluded. Additionally, all healthy controls with no history of cerebrovascular disease were randomly selected and they underwent physical examination at the same period as cases. The characteristics of all participants (age, gender, smoking, alcohol intake, levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol [LDL-c], and high-density lipoprotein cholesterol [HDL-c]) were obtained from questionnaires or medical records. After obtaining the informed consent of all participants, peripheral blood samples from each individual were harvested at the time of initial diagnosis. A total of 5 ml fasting peripheral venous blood (arm vein) was collected from each patient under aseptic operation in the morning. The blood samples were placed in EDTA-containing vacutainer tubes and stored at −80°C for DNA extraction and genotyping.

2.2 DNA extraction and SNP genotyping

We selected three single nucleotide polymorphisms (SNPs) (rs1940475 and rs3765620 in MMP8, and rs17860949 in MMP10) from the 1000 Genomes Project database with minor allele frequency (MAF) larger than 0.05. Genomic DNA in peripheral venous blood samples was extracted by DNA extraction kit. PCR primers for SNPs genotyping were designed by Agena Bioscience Assay Design software and listed in Table 1. The genotyping of MMP8/MMP10 genetic polymorphisms was identified by the Agena MassARRAY iPLEX platform. Additionally, analysis of the genotyping data was conducted by the Agena Bioscience TYPER 4.0 software.

2.3 Bioinformatics analysis

We used HaploReg v4.1 online software (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) to predict the possible protein functions in these SNPs.

2.4 Statistical analysis

Statistical analysis was processed by SPSS version 20.0, and the significance threshold was set at p < .05. Continuous variables such as age, total cholesterol, triglycerides, HDL-c, and LDL-c were compared by a Student’s t-test. Discrete variables including gender, smoking, alcohol intake, and HWE were tested using the chi-square test. The impacts of MMP8/MMP10 polymorphisms on IS susceptibility were determined by multiple logistic regression models (allele, codominant, dominant, recessive, and log-additive) with adjustments for age, gender, smoking, and alcohol intake. We also detected the association of SNPs with...
risk factors for IS after stratification by age, gender, smoking, and alcohol intake. Besides, a false-positive report probability (FPRP) analysis was used to verify the positive findings in the current study (Deng et al., 2020). Furthermore, we detected the influence of MMP8/MMP10 genetic variants on the risk factors for IS using one-way analysis of variance (ANOVA).

3 | RESULTS

3.1 | Characteristics of study population

This study involved 700 IS patients (459 males and 241 females) and 700 healthy controls (457 males and 243 females). The mean ages of IS patients and healthy controls were 55.02 ± 6.79 years and 55.74 ± 9.14 years, respectively. As shown in Table 2, levels of total cholesterol, HDL-c, and LDL-c in cases were significantly lower than these in controls ($p < .001$, $p = .035$, and $p < .001$, respectively). In terms of age, gender, smoking status, alcohol intake, and triglycerides, there was no statistically significant difference between the case and control groups ($p = .095$, $p = .955$, $p = .957$, $p = .707$, and $p = .510$, respectively).

3.2 | Association of MMP8/MMP10 polymorphisms with IS susceptibility

Three SNPs (included rs1940475 and rs3765620 in MMP8, and rs17860949 in MMP10) were detected in this study. Table 3 presents the details and potential functions of these SNPs. The frequency distributions of rs1940475, rs3765620, and rs17860949 genotypes in
controls the Hardy–Weinberg equilibrium \((p = .487, p = .923, \text{and } p = .837, \text{respectively})\). In addition, five genetic models were applied to investigate the influence of the three studied SNPs on IS risk. As exhibited in Table 4, rs17860949 was significantly associated with a decreased risk of IS in the allele \((OR = 0.632, 95\% CI: 0.469–0.853, p = .002)\), codominant \((OR = 0.641, 95\% CI: 0.467–0.879, p = .006)\), dominant \((OR = 0.627, 95\% CI: 0.458–0.858, p = .004)\), and log-additive models \((OR = 0.627, 95\% CI: 0.463–0.850, p = .003)\).

### 3.3 Associations of MMP8/MMP10 polymorphisms with risk factors for IS

Along with a stratified analysis based on age, gender, smoking, and alcohol intake, we further evaluated the association of MMP8/MMP10 polymorphisms with risk factors for IS. The association stratified by age showed that rs17860949 was associated with a decreased susceptibility to IS in people aged >55 years in the allele \((OR = 0.472, 95\% CI: 0.311–0.715, p < .001)\), codominant \((OR = 0.495, 95\% CI: 0.301–0.814, p = .006)\), dominant \((OR = 0.480, 95\% CI: 0.293–0.787, p = .004)\), and log-additive models \((OR = 0.477, 95\% CI: 0.294–0.774, p = .003)\) (Table 5). After stratification by gender (Table 6), rs17860949 was related to a decreased susceptibility to IS in males (allele: \(OR = 0.632, 95\% CI: 0.441–0.905, p = .012\); codominant: \(OR = 0.647, 95\% CI: 0.438–0.955, p = .028\); dominant: \(OR = 0.636, 95\% CI: 0.433–0.935, p = .021\); and log-additive: \(OR = 0.642, 95\% CI: 0.443–0.929, p = .019\)). As summarized in Table 7, rs17860949 was related to a lower risk of IS in nonsmokers (allele: OR = 0.610, 95% CI = 0.405–0.919, \(p = .017\) and log-additive: OR = 0.636, 95% CI = 0.369–0.847, \(p = .006\); codominant: OR = 0.602, 95% CI = 0.384–0.946, \(p = .028\); dominant: OR = 0.579, 95% CI = 0.370–0.905, \(p = .016\); and log-additive: OR = 0.569, 95% CI = 0.369–0.880, \(p = .011\)).

#### 3.4 FPRP results

The FPRP test was performed to verify positive results, and the threshold was set as 0.2. As shown in Table S1, at the prior probability of 0.25, all the positive findings of the correlation between MMP10 rs17860949 and IS risk remained noteworthy (FPRP < 0.2).

#### 3.5 The impacts of SNPs on clinical indicators of IS patients

As demonstrated in Table 8, GG genotype \((1.760 ± 1.130 \text{ mmol/L})\) and AG genotype \((1.502 ± 0.694 \text{ mmol/L})\) in rs3765620 were related to a reduced triglycerides concentration in IS patients compared with AA genotype \((1.973 ± 1.363 \text{ mmol/L}) (p = .018)\).

### 4 DISCUSSION

Stroke is one of the major causes of mortality, long-term physical and cognitive impairment in China (Tu et al., 2019). Stroke prevention and treatment has been listed as an important campaign of the Healthy China Initiative. The China Stroke Prevention Project Committee (CSPPC) aims to reduce the incidence and mortality of stroke by establishing stroke map, organizing health education and professional training, screening high-risk populations, and conducting follow-up (Chao et al., 2021). Notably, stroke is a polygenic and multifactorial disease. Genetic factors, like gene polymorphisms, play an important role in occurrence of stroke (Syahrul et al., 2018; Worrall et al., 2007), and may also exert a protective effect against stroke. Our study investigated the association of MMP8/MMP10 polymorphisms with the susceptibility to IS in the Shaanxi Han population, suggesting that the rs17860949 polymorphism in MMP10 was associated with a decreased susceptibility to IS.

The rs17860949 polymorphism is located on the second exon of MMP10. In our research, rs17860949 could reduce the risk of IS. However, Zhu has observed that MMP10 rs17435959 and rs17293607 are independent of susceptibility to atherothrombotic cerebral infarction in Zhejiang Han population (Zhu et al., 2013). Wu et al.’s (2021)’s study has indicated that MMP10 rs17435959 is related to the stability and formation of carotid atherosclerosis plaque. Besides, we observed that rs17860949 in MMP10 could have an influence on IS risk in people aged > 55 years, suggesting that there were age differences in the
### TABLE 3  The distribution of allele frequencies of MMP8/MMP10 SNPs

| SNP ID   | Gene | Chromosome position | Alleles (A/B) | MAF | Controls | p<sup>a</sup>-HWE | HaploReg v4.1                                      |
|----------|------|---------------------|-------------|-----|----------|----------------|---------------------------------------------------|
| rs1940475 | MMP8 | chr11: 102722517    | T/C         | 0.103 | 0.097 | 0.487 | Enhancer histone marks, DNase, Motifs changed, GRASP QTL hits, Selected eQTL hits |
| rs3765620 | MMP8 | chr11: 102724761    | G/A         | 0.228 | 0.238 | 0.923 | Enhancer histone marks, DNase, Motifs changed, GRASP QTL hits, Selected eQTL hits |
| rs17860949 | MMP10 | chr11: 102779515 | A/G         | 0.229 | 0.221 | 0.837 | Enhancer histone marks, Motifs changed |

SNP: single nucleotide polymorphisms; A, minor allele; B, major allele; MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium.
The p<sup>a</sup> < .05 are excluded.

### TABLE 4  Association of MMP8/MMP10 polymorphisms with ischemic stroke risk

| SNP ID   | Model | Allele/genotype | Case N | Control N | OR (95% CI) | p     |
|----------|-------|----------------|--------|-----------|-------------|-------|
| rs1940475 | Allele | C             | 873    | 881       | 1           |       |
|          | T     | 521           | 557    | 1.017 (0.872–1.186) | .830 |
|          | Codominant | TC         | 311    | 323       | 0.955 (0.760–1.199) | .690 |
|          | TT    | 105           | 97     | 1.079 (0.782–1.790) | .644 |
|          | CC    | 281           | 279    | 1         |             |       |
|          | Dominant | TC-CC       | 416    | 420       | 0.983 (0.794–1.218) | .878 |
|          | Recessive | CC-TC      | 592    | 602       | 1           |       |
|          | TT    | 105           | 97     | 1.106 (0.820–1.491) | .509 |
|          | Log-additive | –         | –      | –         | 1.018 (0.875–1.184) | .821 |
| rs3765620 | Allele | A             | 891    | 897       | 1           |       |
|          | G     | 507           | 503    | 1.015 (0.870–1.184) | .853 |
|          | Codominant | AG        | 299    | 315       | 0.936 (0.746–1.174) | .565 |
|          | GG    | 104           | 94     | 1.092 (0.791–1.508) | .594 |
|          | AA    | 296           | 291    | 1         |             |       |
|          | Dominant | AG-GG       | 403    | 409       | 0.972 (0.785–1.202) | .790 |
|          | Recessive | AA-AG      | 595    | 606       | 1           |       |
|          | GG    | 104           | 94     | 1.130 (0.836–1.527) | .428 |
|          | Log-additive | –         | –      | –         | 1.016 (0.874–1.182) | .835 |
| rs17860949 | Allele | G             | 1318   | 1283      | 1           |       |
|          | A     | 76            | 117    | 0.632 (0.469–0.853) | .002 |
|          | Codominant | AG       | 74     | 109       | 0.641 (0.467–0.879) | .006 |
|          | AA    | 1             | 4      | 0.246 (0.027–2.212) | .211 |
|          | GG    | 622           | 587    | 1         |             |       |
|          | Dominant | AG-AA     | 75     | 113       | 0.627 (0.458–0.858) | .004 |
|          | Recessive | GG-AG    | 696    | 696       | 1           |       |
|          | AA    | 1             | 4      | 0.261 (0.029–2.349) | .231 |
|          | Log-additive | –         | –      | –         | 0.627 (0.463–0.850) | .003 |

CI, confidence interval; OR, odds ratio; SNP: single nucleotide polymorphism; OR, odds ratio, 95% CI; 95% confidence intervals.
The p values were calculated by unconditional logistic regression analysis with adjustments for age, gender, smoking, and drinking.
The p< .05 indicates statistical significance.
Bold values are statistically significant (p< 0.05).
TABLE 5  Associations of MMP8/MMP10 polymorphisms with ischemic stroke risk stratified by age

| SNP ID   | Model   | Genotype | Case >55 years | Control | OR (95% CI) | p       | Case <55 years | Control | OR (95% CI) | p       |
|----------|---------|----------|----------------|---------|-------------|---------|----------------|---------|-------------|---------|
| rs1940475| Allele  | C        | 500            | 374     | 1           | .354    | 373            | 507     | 1           | .271    |
|          |         | T        | 292            | 242     | 0.903 (0.727–1.121) | .354 |
|          | Codominant | TC      | 176            | 144     | 0.872 (0.601–1.266) | .473 |
|          |         | TT       | 58             | 49      | 0.933 (0.557–1.563) | .792 |
|          |         | CC       | 162            | 115     | 1           | .354    | 119            | 164     | 1           | .271    |
|          | Dominant | TC-TT    | 234            | 193     | 0.887 (0.625–1.260) | .503 |
|          | Recessive | CC-TC   | 338            | 259     | 1           | .271    | 254            | 343     | 1           | .271    |
|          | Log-additive | -      | -              | -       | 0.945 (0.740–1.206) | .647 |
| rs3765620| Allele  | A        | 507            | 383     | 1           | .354    | 384            | 514     | 1           | .271    |
|          |         | G        | 287            | 235     | 0.923 (0.742–1.147) | .473 |
|          | Codominant | AG      | 171            | 143     | 0.868 (0.600–1.258) | .473 |
|          |         | GG       | 58             | 46      | 0.998 (0.592–1.681) | .993 |
|          |         | AA       | 168            | 120     | 1           | .354    | 128            | 171     | 1           | .354    |
|          | Dominant | AG-GG    | 229            | 189     | 0.899 (0.635–1.273) | .503 |
|          | Recessive | AA-AH   | 339            | 263     | 1           | .354    | 256            | 343     | 1           | .354    |
|          | Log-additive | -      | -              | -       | 0.966 (0.757–1.234) | .783 |
| rs17860949| Allele  | G        | 755            | 557     | 1           | .354    | 563            | 726     | 1           | .354    |
|          |         | A        | 39             | 61      | 0.472 (0.311–0.715) | <.001 |
|          | Codominant | AG      | 39             | 55      | 0.495 (0.301–0.814) | .006 |
|          |         | GG       | 0              | 3       | /           | /       | 1              | 1       | 1.143 (0.071–18.520) | .925 |
|          | Dominant | AG-AA    | 39             | 58      | 0.480 (0.293–0.787) | .004 |
|          | Recessive | GG-AG   | 397            | 306     | 1           | .354    | 299            | 390     | 1           | .354    |
|          | Log-additive | -      | -              | -       | 0.477 (0.294–0.774) | .003 |

OR, odds ratio; 95% CI, 95% confidence intervals. The p values were calculated by logistic regression with adjustments for gender, smoking, and drinking. The p < .05 indicates statistical significance. Bold values are statistically significant (p < 0.05).
TABLE 6  Associations of MMP8/MMP10 polymorphisms with ischemic stroke risk stratified by gender

| SNP ID     | Model        | Genotype | Case Male | Control | OR (95% CI) | p    | Case Female | Control | OR (95% CI) | p    |
|------------|--------------|----------|-----------|---------|-------------|------|-------------|---------|-------------|------|
| rs1940475  | Allele       | C        | 574       | 567     | 1           |      | 299         | 314     | 1           |      |
|            |              | T        | 340       | 345     | 0.974 (0.806–1.177) | .781 | 181         | 172     | 1.105 (0.850–1.436) | .455 |
|            | Codominant   | TC       | 206       | 211     | 0.966 (0.725–1.288) | .814 | 105         | 112     | 1.068 (0.714–1.596) | .750 |
|            |              | TT       | 67        | 67      | 1.001 (0.668–1.501) | .996 | 38          | 30      | 1.333 (0.752–2.366) | .325 |
|            |              | CC       | 184       | 178     | 1           |      | 97          | 101     | 1           |      |
|            | Dominant     | TC-TT    | 273       | 278     | 0.975 (0.743–1.278) | .852 | 143         | 142     | 1.127 (0.772–1.644) | .537 |
|            | Recessive    | CC-TC    | 390       | 389     | 1           |      | 202         | 213     | 1           |      |
|            |              | TT       | 67        | 67      | 1.020 (0.701–1.483) | .919 | 38          | 30      | 1.289 (0.756–2.198) | .352 |
|            | Log-additive | -        | -         | -       | 0.992 (0.820–1.201) | .936 | -           | -       | 1.132 (0.866–1.481) | .364 |
| rs3765620  | Allele       | A        | 590       | 580     | 1           |      | 301         | 317     | 1           |      |
|            |              | G        | 328       | 334     | 0.965 (0.798–1.168) | .717 | 179         | 169     | 1.115 (0.858–1.451) | .415 |
|            | Codominant   | AG       | 196       | 202     | 0.961 (0.722–1.280) | .787 | 103         | 113     | 0.984 (0.660–1.467) | .938 |
|            |              | GG       | 66        | 66      | 0.975 (0.651–1.461) | .902 | 38          | 28      | 1.356 (0.759–2.422) | .303 |
|            |              | AA       | 197       | 189     | 1           |      | 99          | 102     | 1           |      |
|            | Dominant     | AG-GG    | 262       | 268     | 0.965 (0.738–1.261) | .793 | 141         | 141     | 1.062 (0.729–1.545) | .755 |
|            | Recessive    | AA-AG    | 393       | 391     | 1           |      | 202         | 215     | 1           |      |
|            |              | GG       | 66        | 66      | 0.995 (0.682–1.450) | .978 | 38          | 28      | 1.367 (0.794–2.353) | .259 |
|            | Log-additive | -        | -         | -       | 0.981 (0.812–1.185) | .842 | -           | -       | 1.114 (0.851–1.457) | .432 |
| rs17860949 | Allele       | G        | 863       | 833     | 1           |      | 455         | 450     | 1           |      |
|            |              | A        | 53        | 81      | 0.632 (0.441–0.905) | 0.122 | 23          | 36      | 0.632 (0.369–1.083) | .093 |
|            | Codominant   | AG       | 51        | 75      | 0.647 (0.438–0.955) | 0.282 | 23          | 34      | 0.710 (0.395–1.276) | .252 |
|            |              | AA       | 1         | 3       | 0.360 (0.037–3.512) | .380 | 0           | 1       | /           | /    |
|            |              | GG       | 406       | 379     | 1           |      | 216         | 208     | 1           |      |
|            | Dominant     | AG-AA    | 52        | 78      | 0.636 (0.433–0.935) | 0.212 | 23          | 35      | 0.689 (0.385–1.235) | .211 |
|            | Recessive    | GG-AG    | 457       | 454     | 1           |      | 239         | 242     | 1           |      |
|            |              | AA       | 1         | 3       | 0.383 (0.039–3.727) | .408 | 0           | 1       | /           | /    |
|            | Log-additive | -        | -         | -       | 0.642 (0.443–0.929) | 0.192 | -           | -       | 0.676 (0.382–1.195) | .178 |

OR, odds ratio; 95% CI; 95% confidence intervals.
The p values were calculated by logistic regression with adjustments for age, smoking, and drinking.
The p < .05 indicates statistical significance.
Bold values are statistically significant (p < 0.05).
| SNP ID     | Model       | Genotype       | Smoking       | Nonsmoking     | Alcohol intake | No alcohol intake |
|------------|-------------|----------------|---------------|----------------|----------------|------------------|
|           |             |                | OR (95% CI)   | p              | OR (95% CI)    | p               | OR (95% CI)      | p               |
| rs1940475  | Allele      | C              | 1             | 1              | 1              | 1               | 1               | 1               |
|           |             | T              | 1.054 (0.846–1.313) | .642          | 0.984 (0.794–1.219) | .882          | 0.983 (0.789–1.224) | .875          | 1.051 (0.848–1.302) | .651          |
|           |             | TC             | 1.015 (0.723–1.426) | .932          | 1.031 (0.747–1.422) | .853          | 0.926 (0.661–1.297) | .653          | 1.146 (0.827–1.589) | .412          |
|           |             | TT             | 1.262 (0.791–2.015) | .330          | 1.041 (0.652–1.660) | .867          | 1.225 (0.763–1.965) | .401          | 1.103 (0.694–1.754) | .679          |
|           |             | CC             | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           | Dominant    | TC-CC          | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           | Recessive   | CC-TT          | 1.073 (0.780–1.476) | .666          | 1.033 (0.763–1.400) | .833          | 0.992 (0.722–1.362) | .959          | 1.136 (0.835–1.545) | .418          |
|           |             | TT             | 1.252 (0.814–1.925) | .306          | 1.024 (0.663–1.583) | .867          | 1.276 (0.824–1.977) | .274          | 1.025 (0.667–1.576) | .909          |
|           | Log-additive| –              | 1.099 (0.880–1.373) | .407          | 1.023 (0.823–1.272) | .838          | 1.061 (0.849–1.327) | .601          | 1.073 (0.863–1.335) | .527          |
| rs3765620  | Allele      | A              | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           |             | G              | 1.083 (0.868–1.351) | .482          | 0.955 (0.770–1.184) | .672          | 1.036 (0.831–1.292) | .753          | 0.996 (0.802–1.236) | .970          |
|           |             | AG             | 1.093 (0.780–1.532) | .607          | 0.912 (0.662–1.257) | .573          | 1.019 (0.729–1.425) | .911          | 0.978 (0.707–1.353) | .893          |
|           |             | GG             | 1.291 (0.809–2.060) | .285          | 1.008 (0.631–1.611) | .974          | 1.254 (0.779–2.020) | .352          | 1.068 (0.674–1.694) | .779          |
|           |             | AA             | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           | Dominant    | AG-GG          | 1.141 (0.832–1.564) | .414          | 0.933 (0.690–1.262) | .654          | 1.072 (0.782–1.469) | .667          | 1.000 (0.738–1.355) | .998          |
|           | Recessive   | AA-AG          | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           |             | GG             | 1.233 (0.799–1.904) | .344          | 1.056 (0.681–1.640) | .807          | 1.242 (0.798–1.932) | .338          | 1.080 (0.701–1.663) | .727          |
|           | Log-additive| –              | 1.126 (0.903–1.404) | .292          | 0.978 (0.787–1.215) | .840          | 1.094 (0.875–1.368) | .430          | 1.019 (0.822–1.264) | .863          |
| rs17860949 | Allele      | G              | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           |             | A              | 0.658 (0.426–1.018) | .059          | 0.610 (0.405–0.919) | .017          | 0.723 (0.470–1.112) | .138          | 0.559 (0.369–0.847) | .006          |
|           |             | AG             | 0.670 (0.417–1.077) | .098          | 0.677 (0.434–1.055) | .085          | 0.740 (0.463–1.183) | .209          | 0.602 (0.384–0.946) | .028          |
|           |             | AA             | 1.485 (0.092–24.000) | .781          | /              | /               | 1.519 (0.094–24.58) | .769          | /              | /              |
|           |             | GG             | 1             | 1              | 1              | 1               | 1               | 1               | 1               |
|           | Dominant    | AG-AA          | 0.683 (0.427–1.091) | .111          | 0.650 (0.419–1.009) | .055          | 0.753 (0.473–1.197) | .230          | 0.579 (0.370–0.905) | .016          |
|           | Recessive   | GG-AG          | 0.657 (0.427–1.091) | .111          | 0.650 (0.419–1.009) | .055          | 0.753 (0.473–1.197) | .230          | 0.579 (0.370–0.905) | .016          |
|           |             | AA             | 1.572 (0.097–25.400) | .750          | /              | /               | 1.584 (0.098–25.62) | .746          | /              | /              |
|           | Log-additive| –              | 0.707 (0.449–1.115) | .136          | 0.636 (0.415–0.975) | .038          | 0.775 (0.494–1.216) | .267          | 0.569 (0.369–0.880) | .011          |

OR, odds ratio; 95% CI, 95% confidence intervals. The p values were calculated by logistic regression with adjustments for age and gender. The p < .05 indicates statistical significance. Bold values are statistically significant (p < 0.05).
Comparisons between clinical indicators and SNP genotypes in ischemic stroke patients

| SNP       | Total cholesterol (mmol/L) | Triglycerides (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) |
|-----------|-----------------------------|------------------------|---------------|----------------|
| rs1940475 | 3.940± 0.893                | 1.812± 1.141           | 1.068± 0.179  | 1.825± 0.662   |
| TT        | 3.948± 1.011                | 1.602± 0.805           | 1.098± 0.214  | 2.021± 0.760   |
| CT        | 3.974± 1.050                | 1.909± 1.386           | 1.111± 0.290  | 1.920± 0.581   |
| CC        | 1.981                       | .186                   | .745          | .368           |
| p         | .981                        | .186                   | .745          | .368           |
| rs3765620 | 3.971± 1.056                | 1.973± 1.363           | 1.101± 0.284  | 1.952± 0.611   |
| AA        | 3.945± 0.994                | 1.502± 0.694           | 1.107± 0.212  | 2.004± 0.755   |
| AG        | 3.970± 0.855                | 1.760± 1.130           | 1.072± 0.176  | 1.885± 0.693   |
| GG        | 0.984                       | 0.018                  | 0.807         | 0.715          |
| p         | 4.056± 1.162                | 1.840± 1.353           | 1.060± 0.233  | 2.088± 0.580   |
| rs17860949| 3.952± 0.987                | 1.729± 1.084           | 1.105± 0.245  | 1.953± 0.696   |
| AA        | .656                        | .666                   | .426          | .393           |
| AG        | 0.994                       | 0.018                  | 0.807         | 0.715          |
| GG        | 1.011                       | 1.602                  | 1.084         | 1.105          |
| p         | 1.011                       | 1.602                  | 1.084         | 1.105          |

The p values were calculated by Kruskal–Wallis H test. The p < .05 indicates statistical significance. Bold values are statistically significant (p< 0.05).

The impact of rs17860949 on the risk of IS. Cai et al. (2020) have reported that rs4646 impacts on stroke susceptibility in people aged > 64 years. Besides, Wu et al. (2020) have showed that genetic polymorphisms are significantly associated with stroke risk in patients aged > 65 years. However, Yang et al. (2020) has found that genetic variants influence stroke risk in patients aged ≤ 64 years. Wang et al. (2019) has reported that gene polymorphism is related to stroke risk in people aged < 60 years. Age is a risk factor for stroke, and the incidence of stroke increases with age, doubling every decade after age 55 (Roger et al., 2012). Taken together, these findings indicate that genetic susceptibility to stroke is influenced by age and highlight the importance of heterogeneity in studies of the association between genetic factors and stroke.

In addition, we observed that the rs17860949 polymorphism was associated with the risk of IS in men but not in women. Similar to our results, Gu et al. (2018) and Titov et al. (2016) have reported that polymorphisms are related to stroke susceptibility in men. On the contrary, Gu et al. (2018) and Xu et al. (2017) have observed that gene polymorphism can have an impact on IS risk in women. These results suggest that the impact of genetic polymorphisms on IS may be dependent on gender. In other words, gender is a nonmodifiable risk factor for stroke. The prevalence of stroke in men is higher than that in women, but the incidence and mortality of stroke in older men are lower than those in older women, suggesting that there are congenital differences in the occurrence of stroke between women and men (Petrea et al., 2009; Wang et al., 2014). In addition, studies have found that gender differences in stroke may be related to factors such as age, sex hormones, genetics, and lifestyle (Appelros & Åsberg, 2020; Samai & Martin-Schild, 2015).

We further investigated the relationship of polymorphisms with IS risk after stratified by modifiable risk factors, such as smoking and alcohol consumption. Our analysis showed that rs17860949 has a protective role in IS in nonsmokers and nondrinkers. Özcelik has revealed that variants could increase the susceptibility to IS in smokers (Türkanoğlu Özcelik et al., 2017; Türkanoğlu Özcelik et al., 2018). Lin and Kamdee have reported that gene polymorphisms significantly increase the susceptibility to IS in drinkers (Kamdee et al., 2021; Lin et al., 2021). These results indicated that the impact of gene polymorphisms on IS risk may be linked to smoking and alcohol consumption.

Triglyceride is an important component of blood lipids and triglyceride is a component of dyslipidemia (Dron & Hegele, 2017). Triglyceride levels are risk factors for IS (Iso et al., 2014; Tanne et al., 2001). Dziedzic et al. (2004) have found that lower triglyceride concentrations are related to an increased severity of stroke. We discovered that GG genotype and AG genotype in MMP8 rs3765620 were related to a reduced triglyceride level, which means this mutation may accelerate the occurrence of IS.

Our study has some limitations. First, the association between genetic polymorphism and MMP8/MMP10 gene expression is not evaluated in the present study, and it will be investigated in next studies. Second, risk factors for IS, such as hypertension, diabetes, and hyperlipidemia, were not obtained, which limited our ability to assess the effect of interactions between gene and environment on IS risk. Third, we did not explore the molecular mechanism of MMP8/MMP10 polymorphisms in IS.

5 | CONCLUSION

MMP10 rs17860949 can impact the risk of IS in the Shaanxi Han population. More interestingly, GG genotype and AG genotype in MMP8
rs3765620 are related to a reduced triglyceride level in patients with IS.

ACKNOWLEDGMENT
The authors thank all participants and volunteers in this study.

FUNDING
This work was supported by Natural Science Foundation of China (No. 82104155-Wenzhen Shi), Key Research and Development Program of Shaanxi (2020ZDLSF04-03-Ye Tian and 2021SF-096-Wenzhen Shi), and Xi’an Science and Technology Planning Project (21YXY0038-Wenzhen Shi and 21XYJ0004-Mingze Chang).

CONFLICT OF INTEREST
All authors declared that they have no conflict of interests.

DATA AVAILABILITY STATEMENT
Participant informed consent statements did not seek consent for data to be made publicly available; however, data will be made available to individual researchers upon reasonable request.

ETHICAL STATEMENT
All procedures involving human participants performed in this study were in accordance with the ethical standards of Xi’an No. 3 Hospital, the Affiliated Hospital of Northwest University and the Helsinki’s Declaration. Informed consent was obtained from all individual participants.

ORCID
Ye Tian https://orcid.org/0000-0001-7653-9764

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/brb3.2797

REFERENCES
Abilleira, S., Bevan, S., & Markus, H. S. (2006). The role of genetic variants of matrix metalloproteinases in coronary and carotid atherosclerosis. Journal of Medical Genetics, 43(12), 897–901.
Appelros, P., & Åsberg, S. (2020). Sex differences in stroke. Handbook of Clinical Neurology, 175, 299–312.
Au, A. (2018). Metabolomics and lipidomics of ischemic stroke. Advances in Clinical Chemistry, 85, 31–69.
Cai, Q., Zheng, J., Bai, M., He, X., Wang, L., He, Y., Yuan, D., Huang, T., Zhao, J., Wu, Y., Ma, X., Zhang, M., Jin, T., & Gao, G. (2020). Genetic variations of CYP19A1 gene and stroke susceptibility: A case-control study in the Chinese Han population. Journal of Cardiovascular Pharmacology, 75(4), 344–350.
Chang, J. J., Stanfill, A., & Pourmotabbed, T. (2016). The role of matrix metalloproteinase polymorphisms in ischemic stroke. International Journal of Molecular Sciences, 17(8).
Chao, B. H., Yan, F., Hua, Y., Liu, J. M., Yang, Y., Ji, X. M., Peng, B., Zhao, G. G., Wang, Y. J., Kang, D. Z., Wang, Y. L., Zeng, J. S., Chu, L., Li, T. X., Xu, Y. M., Liu, M., He, L., Xu, Y. W., J., . . . Wang, L. D. (2021). Stroke prevention and control system in China: CSPPC-Stroke program. International Journal of Stroke: Official Journal of the International Stroke Society, 16(3), 265–272.
Christodoulou, A., Bagli, E., Gazouli, M., Moschos, M. M., & Kitsos, G. (2020). Association of MMP2-1306C/T polymorphism with ischemic retinal vein occlusion. Archives of Medical Research, 51(7), 710–713.
Deng, Y., Zhou, L., Yao, J., Liu, Y., Zheng, Y., Yang, S., Wu, Y., Li, N., Xu, P., Lu, L., Zhang, D., Lu, Y., & Dai, Z. (2020). Associations of IncRNA H19 polymorphisms at MicroRNA binding sites with glioma susceptibility and prognosis. Molecular Therapy. Nucleic Acids, 20, 86–96.
Dron, J. S., & Hegele, R. A. (2017). Genetics of triglycerides and the risk of atherosclerosis. Current Atherosclerosis Reports, 19(7), 31.
Dziedzic, T., Slowik, A., Grynz, E. A., & Szczdulk, A. (2004). Lower serum triglyceride level is associated with increased stroke severity. Stroke: A Journal of Cerebral Circulation, 35(4), e151–e152.
Fu, R., Shen, Y., & Zheng, J. (2019). Association between common genetic variants in ESR1 and stroke risk: A systematic review and meta-analysis. Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association, 28(11), 104355.
Ghilardi, G., Biondi, M. L., DeMonti, M., Turri, O., Guagnellini, E., & Scorza, R. (2002). Matrix metalloproteinase-1 and matrix metalloproteinase-3 gene promoter polymorphisms are associated with carotid artery stenosis. Stroke: A Journal of Cerebral Circulation, 33(10), 2408–2412.
Goyal, A., Saluja, A., Saraswathy, K. N., Bansal, P., & Dhamija, R. K. (2021). Role of ACE polymorphism in acute ischemic stroke. Neurology India, 69(5), 1217–1221.
Gu, L., Huang, J., Li, J., Huang, S., Li, M., Gong, L., Li, T., & Su, L. (2018). Association of CALM1 rs3179089 polymorphism with ischemic stroke in Chinese Han population. NeuroMolecular Medicine, 20(2), 271–279.
Gu, L., Huang, J., Liang, B., Chen, Q., Xia, J., Yang, J., Yan, Y., & Tang, Q. (2018). TLR4 polymorphisms affect stroke risk and inflammatory response in Chinese ischemic stroke patients. Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology, 39(1), 127–133.
Guzik, A., & Bushnell, C. (2017). Stroke epidemiology and risk factor management. Continuum (Minneapolis, Minn.), 23(1), 15–39.
The Writing Committee of the Annual Report on Cardiovascular Health and Diseases in China. (2021). Interpretation of annual report on cardiovascular health and diseases in China 2019. Cardiology Discovery, 1(4), 269–284.
Iso, H., Imano, H., Yamagishi, K., Ohira, T., Cui, R., Noda, H., Sato, S., Kiyama, M., Okada, T., Hitsumoto, S., Tanigawa, T., & Kitamura, A., CIRCS Investigators. (2014). Fasting and non-fasting triglycerides and risk of ischemic cardiovascular disease in Japanese men and women: The circulatory risk in communities study (CIRCS). Atherosclerosis, 237(1), 361–368.
Jalel, A., Midani, F., Fredj, S. H., Messaoud, T., Bentati, F., & Soualhim, H. (2021). Association of BglII polymorphism in ITG2 and (894G/T and -786T/C) polymorphisms in eNOS gene with stroke susceptibility in Tunisian patients a2 gene polymorphism in a2p1 integrin and eNOS gene variants and stroke. Biological Research for Nursing, 23(3), 408–417.
Kamdee, K., Panadasko, N., Mueangson, O., Nuinoon, M., Janwan, P., Poonsawat, W., Pongpanitanont, P., Kitkumthorn, N., Thongjroy, J., & Chunglork, W. (2021). Promoter polymorphism of TNF-α (rs1800629) is associated with ischemic stroke susceptibility in a southern Thai population. Biomedical Reports, 15(3), 78.
Kaplan, R. C., Smith, N. L., Zucker, S., Heckbert, S. R., Rice, K., & Psaty, B. M. (2008). Matrix metalloproteinase-3 (MMP3) and MMP9 genes and risk of myocardial infarction, ischemic stroke, and hemorrhagic stroke. Atherosclerosis, 201(1), 130–137.
Lenglet, S., Mach, F., & Montecucco, F. (2013). Role of matrix metalloproteinase-8 in atherosclerosis. Mediators of Inflammation, 2013, 659282.
Liberman, A. L., Kamel, H., Mullen, M. T., & Messé, S. R. (2016). International classification of diseases, ninth revision (ICD-9) diagnosis codes can identify cerebral venous thrombosis in hospitalized adults. The Neurohospitalist, 6(4), 147–150.
Lin, C. H., Nfor, O. N., Ho, C. C., Hsu, S. Y., Tantoh, D. M., Liaw, Y. C., Daria, M. R., Chen, C. H., & Liaw, Y. P. (2021). Association of ADH1B
VNTR polymorphism in ischemic stroke: Analysis in 3 populations. Stroke: A Journal of Cerebral Circulation, 38(4), 1189–1196.

Wu, G., Cai, H., Li, G., Meng, S., Huang, J., Xu, H., Chen, M., Hu, M., Yang, W., Wang, C., Wu, Z., & Cai, Y. (2020). Influence of the matrix metalloproteinase 9 geners3918242 polymorphism on development of ischemic stroke: A meta-analysis. World Neurosurgery, 133, e31–e61.

Wu, L. N., Wang, W. F., Wang, X. W., Li, W. L., Luo, S., Ni, H., Zheng, H. B., Hong, W. J., Jiang, Y. Q., & Zhu, F. (2021). MMP-10 rs17435959 polymorphism is associated with the formation and stability of carotid atherosclerosis plaque: A case-control study. Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association, 30(10), 106045.

Xu, Z., Li, Y., Huang, X., Shen, W., Bai, J., Shen, C., & Zhao, Y. (2017). ESR2 genetic variants and combined oral contraceptive use associated with the risk of stroke. Archives of Medical Research, 48(2), 203–211.

Yang, W., Ma, F., Wang, L., He, X., Zhang, H., Zheng, J., Wang, Y., Jin, T., Yuan, D., & He, Y. (2020). The association analysis between CYP24A1 genetic polymorphisms and the risk of ischemic stroke in Chinese Han population. Brain and Behavior, 10(2), e01503.

Yuan, M., Zhan, Q., Duan, X., Song, B., Zeng, S., Chen, X., Yang, Q., & Xia, J. (2013). A functional polymorphism at miR-491-5p binding site in the 3'-UTR of MMP-9 gene confers increased risk for atherosclerotic cerebral infarction in a Chinese population. Atherosclerosis, 226(2), 447–452.

Zhu, F., Jin, X. P., Zhu, M., Zhang, L. L., Wang, F., Wang, W. F., Hu, X. F., Li, W. L., Li, C., & Zheng, Z. (2013). Matrix metalloproteinase 10 gene polymorphism and atherothrombotic cerebral infarction risk in a Han Chinese population. International Journal of Clinical and Experimental Medicine, 6(7), 567–575.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhao, Y., Zhang, Q., Zhang, X., Zhang, Y., Lu, Y., Ma, X., Li, W., Niu, X., Zhang, G., Chang, M., Shi, W., & Tian, Y. (2022). The roles of MMP8/MMP10 polymorphisms in ischemic stroke susceptibility. Brain and Behavior, 12, e2797. https://doi.org/10.1002/brb3.2797