MMP9 SNP and MMP SNP–SNP interactions increase the risk for ischemic stroke in the Han Hakka population

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Abstract
Objectives: To investigate the association of eight variants of four matrix metalloproteinase (MMP) genes with ischemic stroke (IS) and whether interactions among these single nucleotide polymorphisms (SNPs) increases the risk of IS.

Methods: Among 547 patients with ischemic stroke and 350 controls, matrix-assisted laser desorption/ionization time of flight mass spectrometry was used to examine eight variants arising from four different genes, including MMP-1 (rs1799750), MMP-2 (rs243865, rs2285053, rs2241145), MMP-9 (rs17576), and MMP-12 (rs660599, rs2276109, and rs652438). Gene–gene interactions were employed using generalized multifactor dimensionality reduction (GMDR) methods.

Results: The frequency of rs17576 was significantly higher in IS patients than in controls (p = .033). Logistic regression analysis revealed the AG and GG genotypes of rs17576 to be associated with a higher risk for IS, with the odds ratio and 95% confidence interval being 2.490 (1.251–4.959) and 2.494 (1.274–4.886), respectively. GMDR analysis showed a significant SNP-SNP interaction between rs17576 and rs660599 (the testing balanced accuracy was 53.70% and cross-validation consistency was 8/10, p = .0107). Logistic regression analysis showed the interaction between rs17576 and rs660599 to be an independent risk factor for IS with an odds ratio of 1.568 and a 95% confidence interval of 1.152–2.135.

Conclusion: An MMP-9 rs17576 polymorphism is associated with increased IS risk in the Han Hakka population and interaction between MMP-9 rs17576 and MMP-12 rs660599 is associated with increased IS risk as well.

KEYWORDS
arteriosclerosis, GMDR, ischemic stroke, MMP-12, MMP-9, polymorphism

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1 | INTRODUCTION

Ischemic stroke (IS) accounts for the majority of all strokes, and its high morbidity, disability, and mortality have seriously threatened human health (Feigin et al., 2018; Zhou et al., 2019). Epidemiological studies have shown that IS has become a major disease in China, making it one of the most important diseases leading to disability (Chao et al., 2021; Wan et al., 2021; Wang et al., 2020). The main mechanism of IS is cerebral vascular obstruction caused by atherosclerosis. Although risk factors are actively controlled, the occurrence of IS is still on the rise. Previous studies have shown that gene polymorphisms play an important role in atherosclerotic IS (Malik et al., 2018). In recent years, people have become increasingly interested in the study of matrix metalloproteinases (MMPs) and their relationship with the pathogenesis of atherosclerotic cerebrovascular disease (Chehaibi et al., 2014; Li et al., 2021; Wang & Khalil, 2018).

MMPs are a multigene family of extracellular zinc- and calcium-dependent endopeptidases, which play an important pathological role in the degradation of extracellular matrix (ECM) (Abilleira et al., 2006; Chang et al., 2016; Hooper, 1994) in IS. The degradation of arterial ECM proteins is a critical step in the development of atherosclerosis (Fujimoto et al., 2008). Moreover, MMPs can digest the components of fibrous plaque caps, which leads to structural damage and accelerates plaque rupture, giving rise to plaque instability (Galis et al., 1994; Ohshima et al., 2010; Schäfers et al., 2010). Furthermore, MMPs mediate many biological and pathological processes during and after cerebral ischemic injury (Su et al., 2005; Yi et al., 2019; Yi et al., 2019). Therefore, matrix metalloproteinases play an important role in atherosclerotic IS. Although there have been many studies on MMP gene polymorphisms in patients with IS, the results have been controversial and ambiguous (Chehaibi et al., 2014; Djurić et al., 2012; Nie et al., 2014; Sheikhvatan et al., 2018; Zhang et al., 2015). Dan Wen et al. (2014) showed that MMP-1-1607 1G/2G and MMP-3-1612 5A/6A were risk factors for IS, while MMP-9-1562C/T was not associated with IS through meta-analysis. Guojian Zhang et al. showed, when a subgroup analysis by ethnicity and Hardy–Weinberg equilibrium (HWE) was performed, that MMP-12-82 A/G gene polymorphisms may be a risk factor for IS in Europe. In Africa, the presence of MMP-1-1607 1G/2G and MMP-12-82 A/G was also correlated with a significant increase in IS (Zhang et al., 2018). Recently, Shubham Misra et al. conducted a meta-analysis of 29 studies suggesting that MMP-9 (−1562C/T) and MMP-12 (−1082 A/G) gene polymorphisms could be risk factors for IS while MMP-1 (−1607 1G/2G), MMP-2 (−1306C/T) & (−735C/T), and MMP-3 (−1612 5A/6A) have no association with the risk of causing IS (Misra et al., 2018). These results are inconsistent, and most scholars currently believe that race and environment are the differentiators. No known studies of MMP gene polymorphisms in the Han Hakka population exist, making this study a necessity.

Previously, many studies on gene polymorphisms in IS have mainly focused on single gene polymorphisms, and only a few studies on gene–gene interactions are to be found. Russian scholars have shown that MMPs can be used as regulatory targets of various genes, such as rs4322086 of RASEF, rs11556924 of ZC3HC1, rs899997 of SLCO1B1, and rs12449964 of PEMT. A synergistic effect between certain genes can increase the occurrence of cerebral infarction (Polonikov et al., 2019). Khouloud Chehaibi et al. advises to be aware of joint effects or haplotypes of MMP polymorphisms as they are stronger than the individual effect of each polymorphism (Chehaibi et al., 2014). Furthermore, Yi Xingyang et al. showed the interaction of MMP-9 gene polymorphisms plays an important role in the damage of the blood brain barrier (BBB) in cerebral infarction (Yi et al., 2019). Therefore, it is believed that gene–gene interactions may play an important role in the occurrence and development of IS. Currently, only a few studies on MMP SNP–SNP interactions exist and there are no relevant studies of the Han Hakka population. Therefore, it is necessary to analyze MMP gene polymorphism interactions in the Han Hakka population.

This is a case-control study investigating whether eight SNPs of four different MMP genes influence the risk of IS in the Han Hakka population in Western Fujian, China.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the ethics committee of the Longyan First Affiliated Hospital of Fujian Medical University (No:2017-013), in compliance with the Declaration of Helsinki. Each of the participants provided written, informed consent before participating in this study.

2.2 | Study populations

The study population was comprised of 547 patients with IS and 350 healthy controls in the Hakka population in Western Fujian, China. According to the trial of ORG 10172 in the acute stroke treatment classification system (Adams et al., 1993), patients with atherothrombosis were enrolled. From December 2018 to September 2020, data were consecutively collected on 547 patients that were hospitalized in Longyan First Hospital for IS. The patients with IS were all admitted to the hospital within 72 h after the onset of symptoms, had focal neurological deficit symptoms, symptoms persisting for more than 24 h, and were confirmed by brain computed tomography (CT) as well as magnetic resonance imaging (MRI). All enrolled patients were Hakka people residing in Western Fujian.

Exclusion criteria were: (1) patients with other types of IS; (2) patients with familial IS; (3) patients with transient ischemic attacks or intracranial hemorrhage; (4) patients who were unwilling to participate in the trial; (5) patients with tumors, thyroid diseases, blood system diseases, arthritis, immune related diseases, infection, severe heart disease, severe kidney disease, and severe liver disease.

A total of 350 Hakka participants undergoing physical examination were selected as the control group during the same period in our hospital. All controls had no previous family history of stroke as confirmed by CT, MRI, and medical history. The participants enrolled in the study as
controls were free of tumors, thyroid diseases, blood system diseases, immune related diseases, infection, severe heart disease, kidney disease, and liver disease.

The demographic data and risk factors were recorded in detail, including age, gender, smoking status, drinking habits, hypertension, diabetes, total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), and homocysteine (HCY) levels. All data were registered and kept confidentially by two doctors in the department (Dongping Chen and Yinjuan Chen, Department of Neurology, Longyan First Affiliated Hospital of Fujian Medical University).

### 2.3 Genotyping

#### 2.3.1 Genome and SNPs selection

SNPs were selected according to the following criteria: (1) the SNP had been assessed in previous research; (2) the Human SNP of each gene was newly registered in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/snp); (3) the SNP was logged in the human Hap Map database to locate tagged SNPs (http://www.hapmap.org/), or SNPs that identify particular haplotypes; (4) the minimum allele frequency (MAF) was greater than 0.05 in the HapMap. With these criteria, eight variants were selected, including MMP-1 (rs1799750), MMP-2 (rs243865, rs2285053, and rs2241145), MMP-9 (rs17576), and MMP-12 (rs660599, rs2276109, and rs652438).

#### 2.4 Primer synthesis

Multiple polymerase chain reaction primers were designed for the qualified SNPs of MMP-1, 2, 9, and 12 using the gene library GenBank (http://www.ncbi.nlm.nih.gov/omim/) and Mass Array assay design 2.0 software by Sangon Biotech Co, Ltd, Shanghai, China (Table 1).

#### 2.5 SNP detection

Blood samples (5 ml, arm vein) from both patients and controls were drawn into sterile tubes containing sodium citrate and were stored at −80°C. DNA extraction and MMP-1, 2, 9, 12 SNP detection was completed by Sangon Biotech Co, Ltd, Shanghai, China. An Axyprep-96 whole blood genomic DNA Kit (AXYGEN company) was used to extract DNA, and was separated by 0.8% agarose gel electrophoresis. The DNA was concentrated at 5 mg/L and stored at −80°C. SNPs of the eight variants of MMP-1, 2, 9, and 12 were detected using the matrix-assisted laser desorption/ionization-time of flight mass spectrometry method. Genotyping was performed in real time with Mass ARRAY RT software version 3.0.0.4. Analysis was performed using mass array typer software version 3.4 (sequenom Inc., San Diego, CA, USA).

### 2.6 Statistical analysis

All statistical tests were analyzed using SPSS software for Windows version 23.0 (SPSS Inc., Chicago, IL). Each variant, and genotype distributions of the eight variants between IS and control were analyzed with a Chi-squared test. A chi-squared analysis was used to compare all categorical data. Normally distributed, continuous data were compared with a student’s t-test and expressed as mean ± standard deviation. The BH (Benjamini–Hochberg) method of FDR (False discovery Rate) was used to correct type I errors. The generalized multifactor dimensionality reduction (GMDR) beta v0.7 software package was used to analyze gene–gene interactions (http://www.healthsystem.virginia.edu/internet/addiction-genomics/Software), as previously described (Lou et al., 2007; Yi et al., 2019). GMDR software obtains the best model combination from multiple genes and behavioral indicators through the factor dimensionality reduction principle. The optimal model is obtained from the following results: (1) The model is meaningful only when the p-value is less than 0.05; (2) The larger the testing balance accuracy is, the better the model effect is; (3) The closer the cross validation (CV) consistency is to 10, the better. The influence of high-risk interactive genotypes on functional outcomes was investigated with multivariable logistic regression analysis, after adjusting for the main baseline variables related to each main variable in the univariate analysis (enter approach and probability of entry p < .2). A p-value of less than .05 was considered a statistically significant difference (bilateral test).

### 3 RESULTS

#### 3.1 Hardy–Weinberg equilibrium

The frequency distribution of the eight variants did not deviate from HWE (p > .05), indicating that gene frequency of the selected study population is representative of the gene distribution of the general population (Table 2).

#### 3.2 Clinical characteristics of IS and controls

Demographic characteristics are summarized in Table 3. The proportion of hypertension and diabetes were higher in the IS group than in the control group. The TC, HDL, LDL, and HCY were higher in the IS group than in the control group. There was no significant difference between the two groups in terms of gender, age, smoking habits, alcohol consumption, or triglyceride levels (p > .05) (Table 3).

#### 3.2.1 Single-locus analysis

The genotype distributions of MMP-1 (rs1799750), MMP-2 (rs243865, rs2285053, and rs2241145), MMP-9 (rs17576), and MMP-12
### TABLE 1  
Amplification and extension primers used in this study

| SNPs    | Forward primer and reverse primer (5′→3′)                  | Extension primer (5′→3′)                  |
|---------|-------------------------------------------------------------|------------------------------------------|
| rs1799750 | F:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | GATTTGAGATAAGTCATATC                     |
| rs243865  | F:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | CCCCACCCACACCTCTGTCAAGACTG               |
| rs225053  | F:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | ACCGAGAATGGCGAC                         |
| rs2241145 | R:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | TATTGACACCTCCACTCAGACTG                 |
| rs17576   | F:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | GCCCCAGGACTCTACACCC                     |
| rs660599  | R:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | AATGTAAAGCTCTGTCTCTTT                    |
| rs2276109 | R:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | TGCTCAAGGATGATATCAA                      |
| rs652438  | F:ACGTTGGATGTTCTTCTCGTCAAGACTG GATTGATTTGAGATAAGTCATATC | TTTGGCTCTTGCTCTTAAAA                    |

Abbreviations: F, forward primer; R, reverse primer; SNPs, SNPs.

### TABLE 2  
Hardy–Weinberg equilibrium of SNPs genotype in IS group and control group

| SNPs    | Genotype | IS group [n(%)] | p value | control group [n(%)] | p value |
|---------|----------|----------------|---------|----------------------|---------|
| rs1799750 | CC       | 249 (45.6)   | .907    | 144 (41.1)           | .555    |
|          | CT       | 241 (44.0)   |         | 157 (44.9)           |         |
|          | TT       | 57 (10.4)    |         | 49 (14.0)            |         |
| rs243865  | CC       | 420 (76.7)   | .896    | 274 (78.3)           | .762    |
|          | CT       | 119 (21.8)   |         | 72 (20.6)            |         |
|          | TT       | 8 (1.5)      |         | 4 (1.1)              |         |
| rs225053  | CC       | 310 (56.7)   | .942    | 194 (55.4)           | .546    |
|          | CT       | 204 (37.3)   |         | 136 (38.9)           |         |
|          | TT       | 33 (0.60)    |         | 20 (5.7)             |         |
| rs2241145 | CC       | 133 (24.3)   | .339    | 75 (21.4)            | .423    |
|          | CG       | 262 (47.9)   |         | 182 (52.0)           |         |
|          | GG       | 152 (27.8)   |         | 93 (26.6)            |         |
| rs17576   | AA       | 23 (4.2)     | .177    | 29 (8.3)             | .061    |
|          | AG       | 204 (37.3)   |         | 119 (34.0)           |         |
|          | GG       | 320 (58.5)   |         | 202 (57.7)           |         |
| rs660599  | AA       | 17 (3.1)     | .922    | 4 (1.2)              | .061    |
|          | AG       | 157 (28.7)   |         | 102 (29.1)           |         |
|          | GG       | 373 (68.2)   |         | 244 (69.7)           |         |
| rs2276109 | CC       | 0 (0)        | .453    | 1 (0.3)              | .208    |
|          | CT       | 29 (5.3)     |         | 19 (5.4)             |         |
|          | TT       | 518 (94.7)   |         | 330 (94.3)           |         |
| rs652438  | CC       | 11 (2)       | .926    | 2 (0.6)              | .103    |
|          | CT       | 135 (24.7)   |         | 82 (23.4)            |         |
|          | TT       | 401 (73.3)   |         | 266 (76.0)           |         |
TABLE 3  Clinical characteristics in IS group and control group

| Characteristics                  | IS group (n = 547) | Control group (n = 350) | p value |
|----------------------------------|--------------------|-------------------------|---------|
| Gender (male) (n,%)              | 278/269            | 185/165                 | .552    |
| Age (years, mean ± standard deviation) | 62.21 ± 8.92   | 61.75 ± 8.67            | .451    |
| Cigarette smoking (n,%)         | 193 (35.3)         | 114 (22.5)              | .404    |
| Alcohol drinking (n,%)           | 117 (21.3)         | 79 (22.5)               | .676    |
| Hypertension (n,%)               | 405 (74.5)         | 168 (48.0)              | <.005   |
| Diabetes (n,%)                   | 193 (35.3)         | 53 (15.1)               | <.005   |
| TG (mmol/L, mean ± standard deviation) | 1.81 ± 1.38     | 1.79 ± 1.75             | .858    |
| TC (mmol/L, mean ± standard deviation) | 5.39 ± 0.90      | 5.24 ± 1.18             | .029    |
| HDL (mmol/L, mean ± standard deviation) | 1.30 ± 0.43     | 1.38 ± 0.49             | .005    |
| LDL (mmol/L, mean ± standard deviation) | 3.79 ± 0.78     | 3.44 ± 0.93             | <.005   |
| HCY (mmol/L, mean ± standard deviation) | 13.18 ± 7.58    | 10.49 ± 3.48            | <.005   |

Abbreviations: HCY, Homocysteine; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TG, triglycerides.

(rs660599, rs2276109, and rs652438) were compared between the IS and control group. The genotype distribution of rs17576 was statistically different between the two groups (p < .05). However, the genotype distribution of MMP-1 (rs1799750), MMP-2 (rs243865, rs2285053, and rs2241145), and MMP-12 (rs660599, rs2276109, and rs652438) were not statistically different between the two groups (p > .05) (Table 4).

3.3 | Logistic analysis of risk factors of IS

From a univariate analysis, four SNPs candidates were selected for comparison with a logistic regression analysis between the IS and control groups for hypertension, diabetes, TC, HDL, LDL and HCY levels. Hypertension, diabetes, TC, HDL, LDL, and HCY were all independently associated with an increased risk of IS (Table 5). The AG genotype of rs17576 and the GG genotype of rs17576 were associated with a higher risk for IS with an OR and CI of 2.490 (1.251–4.959) and 2.494 (1.274–4.886), respectively (Table 5).

3.4 | GMDR model for gene interactions

The GMDR model of a gene–gene interaction between rs17576 and rs660599 was deemed the best. The cross-validation consistency was 8/10 and the sign test was nine (p = 0.0107) (Table 6). The optimal model of interaction between rs17576 and rs660599 by GMDR is shown in Figure 1.

3.5 | Interaction analysis for rs17576 and rs660599 using logistic regression

According to nine different combinations of rs17576 and rs660599 genotypes in Figure 1, they are divided into high risk and low risk, high risk combinations are indicated by darker coloring (1), low risk combination are indicated by lighter coloring (0) (Figure 2) (assignment: high risk = 1; low risk = 0). After adjusting the factors of hypertension, diabetes, TC, HDL, LDL, and HCY by multivariate logistic regression analysis, it was found that having the combination of rs17576 and rs660599 SNPs was correlated with higher risk for IS with an OR of 1.568 and a CI of 1.152–2.135 (Table 7).

4 | DISCUSSION

This case-control study is one conducted on the Han Hakka population that aimed to investigate the association between MMP-1 (rs1799750), MMP-2 (rs243865, rs2285053, and rs2241145), MMP-9 (rs17576), and MMP-12 (rs660599, rs2276109, and rs652438) genotypes. It is the first one conducted on SNP–SNP interactions among MMP genes and the risk of IS in the Han Hakka population in Western Fujian, China. Haplotype variation in MMP-1 (rs1799750), MMP-2 (rs243865,
rs2285053, and MMP-12 (rs660599, rs2276109, and rs652438) were not associated with a higher risk for IS, however, haplotype variation in MMP-9 rs17576 correlated with an increased risk of IS. GMDR analysis revealed that having both rs17576 and rs660599 SNPs gives rise to an increased risk of IS as well.

Several studies have reported no association between MMP-1, 2, or 12 polymorphisms and ischemic stroke risk, which is consistent with this study. Chehaibi et al. showed that there was no significant correlation between the MMP-1-1607G/2G gene polymorphism and atherosclerosis in Tunisian patients (Chehaibi et al., 2014). Yunhua Hao et al. revealed that MMP-2 rs243865 was not related to cerebral infarction in the Chinese Han population (Hao et al., 2015). Simultaneously, Yeon Jung Kim et al. demonstrated that MMP-2 rs243865 was not related to cerebral infarction (Kim et al., 2020). Furthermore, Marc Fatar et al. showed that there is an association of the MMP-2 gene (rs1030868, rs2241145, rs2287074, rs2287076, and rs7201) with the development of lacunar stroke, but no association of MMP-2 with other stroke subtypes (Fatar et al., 2008). Weiling Li et al. showed that MMP-12 rs2276109 was not associated with cerebral infarction in the Chinese Han population (Hao et al., 2015). Simultaneously, Yeon Jung Kim et al. demonstrated that MMP-2 rs243865 was not related to cerebral infarction (Kim et al., 2020). Furthermore, Marc Fatar et al. showed that there is an association of the MMP-2 gene (rs1030868, rs2241145, rs2287074, rs2287076, and rs7201) with the development of lacunar stroke, but no association of MMP-2 with other stroke subtypes (Fatar et al., 2008).
atherosclerosis (Li et al., 2012). This study found that MMP-9 rs17576 was related to IS. Mehrdad Sheikhvatan et al. also showed that MMP-9-C1562T and MMP-9 rs17576 gene polymorphisms were related to coronary atherosclerosis (Sheikhvatan et al., 2018). Additionally, Xianjing Feng et al. revealed MMP-9 rs17576 may be associated with the risk of intracranial atherosclerotic stenosis (Feng et al., 2021). Nevertheless, Alexey Polonikov et al. found that MMP-9 rs17576 did not increase the risk of cerebral infarction alone but increased the risk of cerebral infarction as a part of a gene network (Polonikov et al., 2019). However, there are many studies that are inconsistent with these findings (Djuric et al., 2012; Manso et al., 2010; Traylor et al., 2014). The diversity of these outcomes may be due to ethnic differences, study design, and sample size as well as fortuity. As a matter of fact, it is likely that there are multiple variations in the pathogenesis of cerebral infarction, each with a slight or potentially undetectable effect (Schork et al., 2009). Due to gene–gene and gene–environment interactions, linkage analyses are commonly used for single-gene disease studies and may not be suitable for genetic studies of stroke.

GMDR is a tool used to study gene–gene interactions and has become a hot tool in gene interaction research (Lou et al., 2007). Nevertheless, significant observations were made in this study using the GMDR method. Through GMDR study, it was found that the interaction between MMP-9 rs17576 and MMP-12 rs660599 increases the risk of IS by 1.568-fold. This result suggests that the interaction of these two gene polymorphisms may play a key role in genetic susceptibility to IS. Recent genome-wide association studies have shown the presence of common genetic variants increases the risk of ischemic stroke, but most of the research has focused only on single genes. These findings add to the evidence that genes–gene interactions can increase the risk of complex diseases, such as ischemic stroke. The combinatorial analysis used in this study may be helpful in the elucidation of complex genetic risk factors for common diseases like IS.

MMP-9 is a 92-kDa protein that belongs to a family of zinc- and calcium-dependent endopeptidases (Fenhalls et al., 1999; Pourmotabbed et al., 1994) and plays a key role in all stages of atherosclerosis through monocyte recruitment influence, ECM degradation, endothelial cell migration, and activation of vascular smooth muscle cells (Blankenberg et al., 2003; Hirose et al., 2008; Ye, 2006). MMP-9 gene polymorphisms encode and regulate the transcription of the MMP-9 protein, and correlate with the concentration of MMP-9 in plasma (Luizon et al., 2016). Genetic polymorphisms located in promoter regions of MMP genes can lead to increased gene expression and may be associated with susceptibility to various diseases (Blankenberg et al., 2003). MMP-9 R279Q polymorphism is a glutamine-arginine substitution in the catalytic domain of MMP-9, which may affect substrate binding. Although the interaction between MMP-9 rs17576 and MMP-12 rs660599 can increase the risk of IS, the interactions between the two gene variants are unclear. Previous studies have shown that both MMP-9 and MMP-12 participate in the process of monocyte recruitment and ECM degradation (Pérez-Ria et al., 2013). Furthermore, MMP-9 and MMP-12 cause n-cadherin shedding and thereby beta-catenin signaling, with subsequent vascular smooth muscle cell proliferation (Dwivedi et al., 2009). Moreover, joint effects or haplotypes of MMP polymorphisms are stronger than the individual effect of each polymorphism. MMPs serve as target genes for gene regulatory networks driving molecular and cellular pathways related to a multistep pathogenesis of cerebrovascular disease (Polonikov et al., 2019).

The results of this study once again indicate that the MMP-9 rs17576 polymorphism is significantly associated with atherosclerotic ischemic stroke. This study provides a theoretical basis for future research on MMP gene polymorphisms and their role in ischemic stroke. Previous studies mainly focused on a single gene polymorphism, with only a few studies on gene–gene interactions. The main discovery of this study is that interaction between MMP-9 rs17576 and MMP-12 rs660599 is associated with increased risk of ischemic stroke. These results provide a theoretical basis for the role of gene–gene interaction in ischemic stroke.

Despite these interesting findings, there are of course limitations to this study. First, these results need to be verified with larger, multicenter studies because of limited sample size and the general nature of single center studies. It is planned to cooperate with other local hospitals to expand the sample size for relevant content research. Second, only a few MMP polymorphisms were studied, many important polymorphic genes of MMPs and their interactions were excluded in the study, begging the need for further research to be performed. Third, only patients with atherothrombosis were enrolled in this study, the other subtypes of stroke and transient ischemic attacks were excluded. The association
of an MMP9 SNP or MMP SNP-SNP interaction with other subtypes of stroke or transient ischemic attacks is unknown. Therefore, further studies are necessary.

5 | CONCLUSION

This study shows the MMP-9 rs17576 gene polymorphism is associated with increased IS risk while the other seven gene polymorphisms studied were not significantly associated with increased risk for IS in the Han Hakka population. Simultaneously, it was revealed, through GMDR analysis, that interaction between MMP-9 rs17576 and MMP-12 rs660599 is associated with increased IS risk in the Han Hakka population.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All data generated during the project will be made available upon the request from the corresponding author. There are no security, licensing, or ethical issues related to these data.

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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. https://doi.org/10.1136/jmg.2006.040808

Adams, H. P., Bendixen, B. H., Kappelle, L. J., Biller, J., Love, B. B., Gordon, D. L., & Marsh, E. E. (1993). Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in acute stroke treatment. Stroke: A Journal of Cerebral Circulation, 24(1), 35–41. https://doi.org/10.1161/01.STR.24.1.35

Blankenberg, S., Rupprecht, H. J., Poirier, O., Bickel, C., Smieja, M., Hafner, G., Meyer, J., Cambien, F., & Tretel, L. (2003). Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation, 107(12), 1579–1585. https://doi.org/10.1161/01.STR.0100058700.41738.12

Chang, J., Stanfill, A., & Pourmotabbed, T. (2016). The role of matrix metalloproteinase polymorphisms in ischemic stroke. International Journal of Molecular Sciences, 17(8), 1323. https://doi.org/10.3390/ijms17081323

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., Wu, J.,... Wang, L.-D. (2021). Stroke prevention and control system in China: CSPPC-Stroke program. International Journal of Stroke, 16(3), 265–272. https://doi.org/10.1177/1747493020913557

Chehaibi, K., Hrira, M. Y., Nouira, S., Maatouk, F., Hamda, K. B., & Slimane, M. N. (2014). Matrix metalloproteinase-1 and matrix metalloproteinase-12 gene polymorphisms and the risk of ischemic stroke in a Tunisian population. Journal of the Neurological Sciences, 342(1-2), 107–113. https://doi.org/10.1016/j.jns.2014.04.036

Djuric, T., Stojkovic, L., Zivkovic, M., Koncar, I., Stankovic, A., Djordjevic, A., & Alavantic, D. (2012). Matrix metalloproteinase-1 promoter genotypes and haplotypes are associated with carotid plaque presence. Clinical Biochemistry, 45(16-17), 1353–1356. https://doi.org/10.1016/j.clinbiochem.2012.05.032

Dwivedi, A., Slater, S. C., & George, S. J. (2009). MMP-9 and -12 cause N-cadherin shedding and thereby beta-catenin signalling and vascular smooth muscle cell proliferation. Cardiovascular Research, 81(1), 178–186. https://doi.org/10.1093/cvr/cvn278

Fatar, M., Strockic, M., Steffens, M., Senn, E., Reuter, B., Bukow, S., Griebe, M., Alonso, A. Lichtner, P., Bugert, P., Meitingter, T., Wienker, T. F., & Hennerici, M. G. (2008). Single-nucleotide polymorphisms of MMP-2 gene in stroke subtypes. Cerebrovascular Diseases, 26(2), 113–119. doi.org/10.1159/000139657

Feigin, V. L., Nguyen, G., Cercy, K., Johnson, C. O., Alam, T., Parmar, P. G., Abajobir, A. A., Abate, K. H., Abd-Allah, F., Abeje, A. N., Abyu, G. Y., Ademi, Z., Agarwal, G., Ahmed, M. B., Akinyemi, R. O., Al-Raddadi, R., Aminde, L. N., Amlie-Lefond, C., Ansari, H., ... Roth, G. A. (2018). Global, regional, and country-specific lifetime risks of stroke, 1990 and 2016. New England Journal of Medicine, 379(25), 2429–2437.

Feng, X., Yu, F., Zhou, X., Liu, Z., Liao, D., Huang, Q., Li, X., Jin, X., & Xia, J. (2021). MMP9 rs17576 is simultaneously correlated with symptomatic intracranial atherosclerotic stenosis and white matter hyperintensities in Chinese population. Cerebrovascular Diseases, 50(1), 4–11. doi.org/10.1159/000511582

Fenhalis, G., Geyp, M., Dent, D. M., & Parker, M. I. (1999). Breast tumour cell-induced down-regulation of type I collagen mRNA in fibroblasts. British Journal of Cancer, 81(7), 1142–1149. https://doi.org/10.1038/sj.bjc.6690821

Fujimoto, S., Hartung, D., Ohshima, S., Edwards, D. S., Zhou, J., Yalamanchili, P., Azure, M., Fujimoto, A., Isobe, S., Matsumoto, Y., Boersma, H., Wong, N., Yamazaki, J., Narula, N., Petrov, A., & Narula, J. (2008). Molecular imaging of matrix metalloproteinase in atherosclerotic lesions: Resolution with dietary modification and statin therapy. Journal of the American College of Cardiology, 52(23), 1847–1857. https://doi.org/10.1016/j.jacc.2008.08.048

Galis, Z. S., Sukhova, G. K., Lark, M. W., & Libby, P. (1994). Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. Journal of Clinical Investigation, 94(6), 2493–2503. https://doi.org/10.1172/JCI117619

Hao, Y., Tian, S., Sun, M., Zhu, Y., Nie, Z., & Yang, S. (2015). Association between matrix metalloproteinase gene polymorphisms and development of ischemic stroke. International Journal of Clinical and Experimental Pathology, 8(9), 11647–11652.

Hirose, Y., Chiba, K., Karasugi, T., Nakajima, M., Kawaguchi, Y., Mikami, Y., Furuchi, T., Mio, F., Miyake, A., Miyamoto, T., Ozaki, K., Takahashi, A., Milzuta, H., Kubo, T., Kimura, T., Tanaka, T., Toyama, Y., & Ikegawa, S. (2008). A functional polymorphism in THBS2 that affects alternative splicing and MMP binding is associated with lumbar-disc herniation. American Journal of Human Genetics, 82(5), 1122–1129. https://doi.org/10.1016/j.ajhg.2008.03.013

Hooper, N. M. (1994). Families of zinc metalloproteases. FEBS Letters, 354(1), 1–6. https://doi.org/10.1016/0014-5793(94)01079-X

Kim, Y.-J., Kim, B. J., Lee, M. H., Lee, H.-B., Lee, J. S., Chang, D.-I., Choi-Kwon, S., Chun, S., Lee, J.-K., Kang, D.-W., Kwon, S. U., & Kim, J. S. (2020). Are genetic variants associated with the location of cerebral arterial lesions in stroke patients. Cerebrovascular Diseases, 49(3), 262–268. https://doi.org/10.1159/000508301
deterioration in patients with atrial fibrillation. Brain & Behavior, 9(6), e01291.
Zhang, G., Li, W., Guo, Y., Li, D., Liu, Y., & Xu, S. (2018). MMP gene polymorphisms, MMP-1 -1607 1G/2G, -519 A/G, and MMP-12 -82 A/G, and ischemic stroke: A meta-analysis. Journal of Stroke and Cerebrovascular Diseases, 27(1), 140–152. https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.08.021
Zhang, M., Zhu, W., Yun, W., Wang, Q., Cheng, M., Zhang, Z., Liu, X., Zhou, X., & Xu, G. (2015). Correlation of matrix metalloproteinase-2 SNPs with the risk of small vessel disease (SVD). Journal of the Neurological Sciences, 356(1-2), 61–64. https://doi.org/10.1016/j.jns.2015.04.056
Zhou, M., Wang, H., Zeng, X., Yin, P., Zhu, J., Chen, W., Li, X., Wang, L., Wang, L., Liu, Y., Liu, J., Zhang, M., Qi, J., Yu, S., Arshin, A., Gakidou, E., Glenn, S., Krish, V. S., Miller-Petrie, M. K., ... Liang, X. (2019). Mortality, morbidity, and risk factors in China and its provinces, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. Lancet, 394(10204), 1145–1158. https://doi.org/10.1016/S0140-6736(19)30427-1
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