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Original Article

Matrix metalloproteinase 10 gene polymorphism and atherothrombotic cerebral infarction risk in a Han Chinese population

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Abstract: Background: Matrix metalloproteinase 10 (MMP10) plays an important role in ischemic stroke and has a close relationship with some stroke risk factors. The aim of this study was to investigate the relationship between two single nucleotide polymorphisms (SNP) in the exon regions of the *MMP10* gene and atherothrombotic cerebral infarction risk. Methods: Five hundred and thirty-seven hospital-based patients who had suffered first atherothrombotic cerebral infarction and 580 unrelated healthy controls were enrolled. Demographic and clinical features of the subjects were recorded, and two polymorphisms, rs17435959 (G>C), rs17293607 (C>T) were chosen to be genotyped by real-time polymerase chain reaction-restriction TaqMan probes using the ABI 7300 TaqMan platform. Results: There were several clinical parameters, such as blood pressure, fasting blood glucose, total cholesterol, homocysteine, as well as carotid plaque and smoking, but not average age and sex ratios that showed significant differences between patients and control subjects. For rs17435959, there was no significant difference between the ischemic stroke group and the healthy control group in genotype frequency (OR=1.295, *P*=0.187, 95% CI (0.882-1.899)) or allele frequency (OR=1.267, *P*=0.202, 95% CI (0.881-1.823)). Moreover, in smoking, none smoking, having carotid plaque, no carotid plaque, male or female subtypes, there was significant difference between patients and control subjects in genotype frequencies or allele frequencies. The minor allele frequency of rs17293607 was 0.92%, prohibiting further study of this allele. Conclusions: These findings suggest that the rs17435959 SNP may not associated with atherothrombotic cerebral infarction risk. We also found that rs17293607 is not polymorphic in our study population.

Keywords: Matrix metalloproteinase 10, polymorphism, genetic, genetic predisposition to disease, atherothrombotic cerebral infarction

Introduction

Ischemic stroke has become one of the leading causes of morbidity and mortality in China and has an increasing incidence year by year, in which atherothrombotic cerebral infarction is one of the most common. If timely and effective interventions cannot be carried out, the Chinese people and China's economy will suffer heavy healthcare burdens [1]. There are many well-documented and modifiable risk factors for stroke, such as hypertension, diabetes, dyslipidemia, atrial fibrillation, asymptomatic carotid stenosis, smoking, and so on [2]. Among these factors, carotid atherosclerosis has a close association with the incidence and recurrence of ischemic stroke [3]. Carotid atherosclerosis can lead to a direct infarction at the narrowest part of the constricted vessel, or an unstable plaque can have an unpredictable sudden breakage, rupture, fissure or ulceration that leads to platelet activation, thrombosis, shedding and the obstruction of the distal vasculature [4].

Matrix metalloproteinases (MMPs), a family of more than twenty endopeptidases, play a central pathologic role in stroke by degrading ECM substrates that are essential for normal signaling and homeostasis within the neurovascular

unit [5]. Moreover, the degradation of ECM substrates causes the loss of ECM components, leading to a thinning of a plaque's fibrous cap, exposing the lipid-filled core, making the plaque more unstable, and leading to the obstruction of the distal vasculature [6, 7].

Matrix metalloproteinase 10 (MMP10) plays a role in a wide range of substrate cleavage, include collagen, gelatin, nestin, laminin, proteoglycans and elastin. MMP-10 can also activate pro-MMP-1, pro-MMP-7, pro-MMP-8 and pro-MMP-9, which then degrade extracellular collagen in different pathological conditions together with other MMPs [8]. MMP10 is notably increased in neurons of the ischemic brain but not in healthy areas [9]. However, unlike most MMPs, it can effectively reduce infarct size in experimental stroke by enhancing fibrinolysis via a thrombin-activatable fibrinolysis inhibitor mediated mechanism [10], which indicates that MMP10 may have a novel profibrinolytic role in ischemic stroke. Besides, serum MMP10 levels also has a close relationship with some risk factors for ischemic stroke, such as carotid intima-media thickness, the presence of carotid plaques, inflammatory markers and smoking [11-13].

Some single nucleotide polymorphisms (SNPs) of other *MMP* genes are thought to have some relationship with the risk of ischemic stroke and carotid atherosclerosis [14, 15]. However, to our knowledge, studies that definite relationships between the SNPs in *MMP10* gene and ischemic stroke, or regard the structure of MMP10 protein since the SNPs produce amino acid changes which may have different roles on MMP10 secretion and activation have not been established. The rs17435959 SNP causes a leucine (Leu) to valine (Val) transition at codon 4 in exon 1 of the *MMP10* gene, and rs1729-3607 leads to a transition of glycine (Gly) to arginine (Arg) at codon 65 in exon2 of the gene. These two polymorphisms lead to the substitution of specific amino acids and may have functional effects. After studying the relationship between a number of MMPs and carotid plaques or atherothrombotic cerebral infarction [16-18], we now focus on whether these two SNPs have a relationship with the risk of atherothrombotic cerebral infarction in a hospital-based patient cohort.

Materials and methods

Study subjects

Between April 2010 and March 2012, 537 hospital-based first-time atherothrombotic cerebral infarction subjects were recruited from the Department of Neurology, while 580 unrelated healthy controls were identified at our hospital at the same time. The symptomatic stroke patients were confirmed to have atherothrombotic cerebral infarctions with brain imaging using a computed tomography scan (CT) and/or magnetic resonance imaging (MRI).

The following exclusion criteria were used in our study: 1) to ensure the subjects suffered stroke because of vascular disease instead of blood or heart problems, patients with atrial fibrillation, cardiac valve abnormalities, recent myocardial infarction (3 months), heart failure, recent cerebral hemorrhage (3 months), recent surgery (3 months), severe liver disease, renal failure, hematologic or autoimmune diseases, or pregnancy were excluded; 2) patients with a history of tumors, chronic inflammatory diseases, autoimmune diseases or aneurysm, were also excluded because we could not be sure whether the two SNPs have a relationship with the susceptibility to these uncommon diseases.

The Medical Ethics Committee of Taizhou Hospital approved the study and all participants provided written informed consent.

Data collection

Among the obtained clinical data, age, sex and race were determined from the patient's ID card, while smoking and drinking status were self-reported. Height and weight were measured to calculate the body mass index (BMI), reported as weight (kg)/height^2 (m^2). Blood pressure was measured three times on the screening day, and the average systolic blood pressure (SBP) and the average diastolic blood pressure (DBP) were used in the analyses. Blood samples were obtained early in the morning without fasting after last supper. In these samples, fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid, hypersensitivity C-reactive protein (Hs-CRP), fibrinogen

Table 1. Demographic and clinical features of the study subjects

Characteristics	Ischemic stroke group (n=537)	Healthy control group (n=580)	*t* or *u* or χ^2	*P*
Age (year)	67.4±10.5	67.0±10.6	0.58	0.565
SBP (mmHg)	152.5±23.1	140.2±22.7	8.96	0.000
DBP (mmHg)	87.0±12.1	82.0±11.6	7.10	0.000
FBG (mmol/L)	6.26±2.57	5.56±1.58	5.82	0.000
BMI (kg/m^2)	22.51±2.38	22.39±2.25	0.86	0.392
TG (mmol/L)	1.40 (1.02, 2.04)	1.34 (0.92, 1.95)	-2.09[a]	0.036
TC (mmol/L)	4.75±1.00	4.63±0.89	2.23	0.026
LDL-C (mmol/L)	2.58±0.81	2.56±0.77	0.38	0.702
HDL-C (mmol/L)	1.28±0.29	1.27±0.31	0.34	0.737
Uric Acid (μmol/L)	302.8±99.3	314.2±96.9	-1.91	0.056
Hs-CRP (g/L)	3.2 (3.1, 7.0)	3.1 (3.1, 4.0)	-3.82[a]	0.000
Fibrinogen (g/L)	3.61±1.42	3.52±1.62	0.98	0.327
Homocysteine (μmol/L)	12.4 (10.1, 15.7)	11.0 (9.3, 13.8)	-5.01[a]	0.000
Gender (male/female)	319/218	319/261	2.21[b]	0.137
CP (1/0)[c]	427/110	412/168	10.7[b]	0.001
Smoker (%)	200 (37.2)	160 (27.6)	11.9[b]	0.001
Alcohol drinker (%)	135 (25.1)	109 (18.8)	6.58[b]	0.010

[a]*u* value, [b]χ^2 value, [c]CP Carotid plaque, 1 having plaque(s), 0 no plaque. SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Hs-CRP, hypersensitivity C-reactive protein.

and homocysteine concentrations were measured at the clinical laboratory of our hospital. Carotid ultrasound examinations were performed in pre-defined areas, including the right distal common carotid artery, left distal common carotid artery, right bulb, left bulb, right proximal internal carotid artery and left proximal internal carotid artery using a 7–12 MHz scanning frequency ultrasound machine in B-mode (Sonos 5500; Agilent, Santa Clara, CA, USA), and subjects were classified as "having plaque(s)" or "no plaque", according to the scanned images and previous ultrasonic criteria [19].

TaqMan® SNP-Genotyping Assays

Genomic DNA was extracted from whole-blood samples using the Blood Genomic DNA Isolation Mini Kit (Shanghai General Biotech Co., Ltd, Shanghai, China) according to the manufacturer's protocol. The DNA concentration was determined by absorption at 260 nm in a BioPhotometer Plus (Eppendorf, Hamburg, Germany). All DNA samples were frozen at –80°C until use.

Genotyping was performed with real-time polymerase chain reaction-restriction TaqMan probes using the ABI 7300 TaqMan platform (Applied Biosystems, Carlsbad, California, America) according to the manufacturer's protocol. TaqMan Universal PCR Master Mix and the 40 X SNP Genotyping Assay was also from Applied Biosystems (ABI). ABI's assay ID C_22274980_10 is for the SNP ID rs17435959 while C_25628162_20 is for rs17293607. Allelic discrimination was automatically completed using the Sequence Detection System 1.4.0 software with 100% concordant. Ten percent of the DNA samples were randomly selected to repeat the assay to confirm consistency, and genotyping operators and allelic recorders were blind to the clinic data of each sample.

Statistical analysis

Statistical analysis was performed using the SPSS 18.0 software package. Data are presented as the mean±standard deviation (for normal variables) or median (25% quartile, 75% quartile) (for skewed variables) or percent frequency. Student's t test (for normal variables) or Mann-Whitney test (for skewed variables) was used to compare measurement data between two groups, while a chi-square test was used for count data. An exact test of Hardy-Weinberg equilibrium provided by Wigginton

Table 2. Logistic regression analysis estimating the risk factors for vulnerable plaque

Independent variable (X)	β	SE	Wald	*P*	OR (95% CI)
Age (year)	-0.278	0.167	2.786	0.095	0.757 (0.546-1.050)
SBP (mmHg)	0.831	0.145	32.797	0.000	2.297 (1.728-3.053)
DBP (mmHg)	0.236	0.146	2.597	0.107	1.266 (0.950-1.686)
FBG (mmol/L)	0.816	0.187	19.077	0.000	2.262 (1.568-3.262)
BMI (kg/m^2)	0.137	0.214	0.409	0.523	1.146 (0.754-1.743)
TG (mmol/L)	-0.096	0.149	0.416	0.519	0.909 (0.679-1.216)
TC (mmol/L)	0.256	0.172	2.211	0.137	1.291 (0.922-1.809)
LDL-C (mmol/L)	0.064	0.143	0.201	0.654	1.066 (0.805-1.412)
HDL-C (mmol/L)	-0.254	0.187	1.843	0.175	0.776 (0.538-1.119)
Uric Acid (μmol/L)	-0.250	0.146	2.927	0.087	0.779 (0.585-1.037)
Hs-CRP (g/L)	0.501	0.190	6.943	0.008	1.650 (1.137-2.394)
Fibrinogen (g/L)	0.120	0.179	0.445	0.505	1.127 (0.793-1.601)
Homocysteine (μmol/L)	0.633	0.189	11.527	0.001	1.883 (1.301-2.726)
Gender (male/female)	0.044	0.161	0.075	0.784	1.045 (0.762-1.434)
Carotid plaque	0.391	0.164	5.562	0.017	1.478 (1.071-2.039)
Smoker (%)	0.409	0.172	5.649	0.017	1.506 (1.074-2.111)
Alcohol drinker (%)	0.185	0.181	1.039	0.308	1.203 (0.843-1.717)

Divide age by 60, divide SBP by 140 mmHg, divide DBP by 90 mmHg, divide FBG by 7.1 mmol/L, divide BMI by 23 kg/m^2, divide TG by 1.80 mmol/L, divide TC by 5.5 mmol/L, divide LDL-C by 3.10 mmol/L, divide HDL-C by 1.15 mmol/L, divide Uric Acid by 350 μmol/L, divide Hs-CRP by 8.0 g/L, divide Fibrinogen by 4.0 g/L, divide Homocysteine by 15.0 μmol/L, all of the cut values indicating whether NO or YES are the reference values for our hospital. The logistic regression model: $\ln(p/(1-p))=\alpha+\beta X+e$, β is the estimated logit coefficient, SE is the standard error of the coefficient, Wald=$(\beta/SE)^2$, Exp(b) is the ''odds ratio (OR)'' of the individual coefficient. More detail introduction to logistic regression is available at http://en.wikipedia.org/wiki/Logistic_regression.

[19] was performed to compare the observed and expected genotype frequencies. The genotypes and alleles frequencies between the patients and controls were compared using a chi-square test. Logistic regression analysis was performed to extract the most prognostic factors for atherothrombotic cerebral infarction, in which we divide age by 60, SBP by 140 mmHg, DBP by 90 mmHg, FBG by 7.1 mmol/L, BMI by 23 kg/m^2, TG by 1.80 mmol/L, TC by 5.5 mmol/L, LDL-C by 3.10 mmol/L, HDL-C by 1.15 mmol/L, Uric Acid by 350 μmol/L, Hs-CRP by 8.0 g/L, Fibrinogen by 4.0 g/L, Homocysteine by 15.0 μmol/L, and all of the cut values indicating whether NO or YES are the reference values for our hospital. The logistic regression model: $\ln(p/(1-p))=\alpha+\beta X+e$, β is the estimated logit coefficient, SE is the standard error of the coefficient, Wald=$(\beta/SE)^2$, Exp(b) is the ''odds ratio (OR)'' of the individual coefficient. More detail introduction to logistic regression is available at http://en.wikipedia.org/wiki/Logistic_regression. A *P* value <0.05 was considered as statistically significant.

Results

Demographic and clinical features of the study subjects are summarized in **Table 1**. There were no statistically significant differences between the two groups in average age and sex ratios. However, there were several clinical parameters that showed significant differences between the two groups. Subjects in the ischemic stroke group had higher SBP, DBP, FBG, TG, TC, Hs-CRP and homocysteine, as well as more carotid plaques. The stroke group also included more smokers and alcohol drinkers.

The logistic regression analysis showed that higher level of SBP, FBG, Hs-CRP, homocysteine, more carotid plaque(s), and smoking were risk factors for the ischemic stroke group compared to the healthy control group, as listed in **Table 2**.

The results of the polymorphism validation show that rs17293607 is not polymorphic in our study population, as it's minor allele frequency was 0.85%, leading us to cease the

Table 3. Genotype and allele frequencies of rs17435959

	Total (n=1117)						
	Cases	Controls	Single analysis[a]			Logistic regression analysis[b]	
	n (%)	n (%)	χ^2	*P*	OR (95% CI)	*P*	OR (95% CI)
Genotype							
GC+CC	60+3 (11.7)	51+3 (9.3)	1.74	0.187	1.295 (0.882-1.899)	0.172	1.333 (0.882-2.016)
GG	474 (88.3)	526 (90.7)			Reference		Reference
Allele							
C	66 (6.1)	57 (4.9)	1.62	0.202	1.267 (0.881-1.823)		
G	1008 (93.9)	1103 (95.1)			Reference		

[a]Chi-square test comparing genotypes and alleles frequencies between the cases and controls. [b]adjusted for age, SBP, DBP, FBG, BMI, TG, TC, LDL-C, HDL-C, Uric Acid, Hs-CRP, fibrinogen, homocysteine, gender (male/female), carotid plaque, smoking and alcohol drinking, and the cut values for continuous variable are same as **Table 2**.

Table 4. Genotype and allele frequencies of rs17435959 in smoking and none smoking subtypes

		Cases	Controls	Single analysis[a]			Logistic regression analysis[b]	
		n (%)	n (%)	χ^2	*P*	OR (95% CI)	*P*	OR (95% CI)
Smoking subtypes								
Genotype	GC+CC	17+0 (8.5)	12+0 (7.5)	0.12	0.729	1.145 (0.531-2.474)	0.500	1.351 (0.564-3.237)
	GG	183 (88.3)	148 (90.7)			Reference		Reference
Allele	C	17 (4.3)	12 (3.8)	0.11	0.735	1.139 (0.536-2.420)		
	G	383 (95.8)	308 (96.3)			Reference		
None smoking subtypes								
Genotype	GC+CC	43+3 (13.6)	39+3 (10.0)	2.42	0.119	1.423 (0.913-2.217)	0.214	1.355 (0.839-2.188)
	GG	291 (86.4)	378 (90.0)			Reference		Reference
Allele	C	49 (90.7)	45 (90.7)	2.35	0.125	1.351 (0.913-2.101)		
	G	625 (90.7)	795 (90.7)			Reference		

[a]Chi-square test comparing genotypes and alleles frequencies between the cases and controls. [b]adjusted for age, SBP, DBP, FBG, BMI, TG, TC, LDL-C, HDL-C, Uric Acid, Hs-CRP, fibrinogen, homocysteine, gender (male/female), carotid plaque and alcohol drinking, and the cut values for continuous variable are same as **Table 2**.

analysis of rs17293607. However, rs17435959 had a minor allele frequency of 5.5% and was further analyzed.

The genotype and allele frequencies of rs17435959 in the ischemic stroke group and healthy control group are shown in **Table 3**. The observed genotype frequencies were at Hardy-Weinberg equilibrium (*P*=0.441 for the ischemic stroke group, *P*=0.153 for the healthy control group). There was no significant difference between the ischemic stroke group and the healthy control group in genotype frequency (11.7% vs. 9.3%, *P*=0.187, OR=1.295, 95% CI (0.882-1.899)) or allele frequency (6.1% vs. 4.9%, *P*=0.202, OR=1.267, 95% CI (0.881-1.823)). Moreover, in smoking, none smoking, having carotid plaque(s), no carotid plaque, male, or female subtypes, there was no significant difference between patients and control subjects in genotype frequencies or allele frequencies (**Tables 4**-**6**).

Discussion

In the present case-control study, we investigated the relationship between a *MMP10* gene polymorphism, rs17435959, and atherothrombotic cerebral infarction risk. We found that the genotype and allele frequencies were not associated with atherothrombotic cerebral infarction risk in the total cohort of patients or in subtype analysis.

Stroke is creating a heavier and heavier human and economic burden in China and the world. In China, approximately 2 million people have a stroke each year, 1.5 million people die of stroke annually, and 7 million people are stroke survivors, among which three quarters lose the

Table 5. Genotype and allele frequencies of rs17435959 in having carotid plaque(s) and no carotid plaque subtypes

		Cases	Controls	Single analysis[a]			Logistic regression analysis[b]	
		n (%)	n (%)	χ^2	*P*	OR (95% CI)	*P*	OR (95% CI)
Having carotid plaque(s) subtypes								
Genotype	GC+CC	50+3 (12.4)	39+3 (10.2)	1.03	0.311	1.248 (0.813-1.917)	0.148	1.406 (0.886-2.231)
	GG	374 (87.6)	370 (89.8)			Reference		Reference
Allele	C	56 (6.6)	45 (5.4)	0.89	0.345	1.215 (0.811-1.820)		
	G	798 (93.4)	779 (94.5)			Reference		
No carotid plaque subtypes								
Genotype	GC+CC	10+0 (9.1)	12+0 (7.1)	0.35	0.556	1.300 (0.542-3.115)	0.993	1.005 (0.356-2.841)
	GG	100 (90.9)	156 (92.9)			Reference		Reference
Allele	C	10 (4.5)	12 (3.6)	0.33	0.565	1.286 (0.547-3.023)		
	G	210 (95.5)	324 (96.4)			Reference		

[a]Chi-square test comparing genotypes and alleles frequencies between the cases and controls. [b]adjusted for age, SBP, DBP, FBG, BMI, TG, TC, LDL-C, HDL-C, Uric Acid, Hs-CRP, fibrinogen, homocysteine, gender (male/female), smoking and alcohol drinking, and the cut values for continuous variable are same as **Table 2**.

Table 6. Genotype and allele frequencies of rs17435959 in male and female subtypes

		Cases	Controls	Single analysis[a]			Logistic regression analysis[b]	
		n (%)	n (%)	χ^2	*P*	OR (95% CI)	*P*	OR (95% CI)
Male subtypes								
Genotype	GC+CC	38+2 (12.5)	32+1 (10.3)	0.76	0.384	1.243 (0.762-2.026)	0.208	1.410 (0.826-2.408)
	GG	279 (87.5)	286 (89.7)			Reference		Reference
Allele	C	42 (6.6)	33 (5.3)	0.90	0.344	1.252 (0.786-1.994)		
	G	596 (93.4)	779 (94.7)			Reference		
Female subtypes								
Genotype	GC+CC	22+1 (10.6)	19+2 (8.0)	0.89	0.344	1.348 (0.726-2.504)	0.977	0.989 (0.479-2.042)
	GG	195 (89.4)	240 (92.0)			Reference		Reference
Allele	C	24 (4.5)	23 (3.6)	0.61	0.433	1.264 (0.704-2.270)		
	G	412 (95.5)	499 (96.4)			Reference		

[a]Chi-square test comparing genotypes and alleles frequencies between the cases and controls. [b]adjusted for age, SBP, DBP, FBG, BMI, TG, TC, LDL-C, HDL-C, Uric Acid, Hs-CRP, fibrinogen, homocysteine, carotid plaque, smoking and alcohol drinking, and the cut values for continuous variable are same as **Table 2**.

ability to work. Approximately ¥ 20 billion per year is spent to the diagnoses and treat stroke [21]. In the United States, approximately 795,000 people have a stroke each year, of which approximately 610,000 are a first attack, resulting in 134,000 deaths annually. Approximately 6.4 million Americans are stroke survivors, and the cost of stroke was $ 34.3 billion (direct and indirect costs) in 2008 [22]. Increasing stroke incidence may be caused by several factors, including the uncontrolled risk factors, the aging population, the unhealthy lifestyles, or the unknown complicated pathologies of stroke. No biomarkers or genetic markers exist for early diagnosis, which makes the search for susceptibility genes a hot topic in the field.

Because MMP10 plays an important role in ischemic stroke and has a close relationship with some stroke risk factors, our study focused on two missense mutations in its exon regions: rs17435959 and rs17293607. Our data shows that for rs17435959, the minor allele frequency was 4.9%~6.1%, and for rs17293607, the minor allele frequency was 0.92%. The minor allele frequency of rs17435959 is similar to Hlatky' study [23] (4.9%~6.1% vs. 4.0%~5.1%). However, the T allelic frequency of rs17293607 is quite different (0.85% vs. 10.4~12.5%), which may be caused by European and Han Chinese ethnic populations having a different mutation frequency.

Our data shows that there was no significant difference between the ischemic stroke group

and the healthy control group in genotype frequency (11.7% vs. 9.3%, P=0.187, OR=1.295, 95% CI (0.882-1.899)) or allele frequency (6.1% vs. 4.9%, P=0.202, OR=1.267, 95% CI (0.881-1.823)) in the total cohort of patients. Moreover, there was no significant difference between patients and control subjects in genotype frequencies or allele frequencies in the four subtypes divided by the risk factors (smoking and carotid plaque) which are both association to MMP-10 and ischemic stroke. These findings may be cause by several reasons. Firstly, ischemic stroke itself should be thought of as a syndrome rather than a specific disease and can be caused by a number of different pathologies [24], so one or even several variants in one or several genes can not show significant relationship with ischemic stroke. Secondly, according to the study of Orbe J et al [10], MMP10 can reduce infarct size in experimental stroke, which it plays a protective role in the pathology of stroke. However, according to the studies of Montero I et al [11], Orbe J et al [12] and Paramo JA et al [13], higher serum MMP10 levels are associated with inflammatory markers, increased carotid intima-media thickness, the presence of carotid plaques, and smoking in asymptomatic subjects, in which smoking, increased carotid intima-media thickness and the presence of carotid plaques are universally acknowledged to be the risk factors of ischemic stroke. So the role of MMP10 in the pathogenesis of stroke is still uncertain, which may leads to the lack of association between *MMP10* polymorphisms and ischemic stroke risk. Thirdly, given the rarity of the minor (C) allele, the limited sample size of this study may not completely exclude that this polymorphism may be a risk factor for ischemic stroke.

Our data also shows that hypertension, diabetes, dyslipidemia, carotid atherosclerosis, smoking, and higher level of Hs-CRP and homocysteine were risk factors for stroke, which is consistent to traditional view and the extensive evidence. More attention should be pay to these well-documented and modifiable risk factors for the primary prevention of stroke [2].

In summary, to our knowledge, this is the first study about the association of SNPs of *MMP10* with atherothrombotic cerebral infarction in a Han Chinese population. Our findings suggest that the rs17435959 SNP may not be associated with atherothrombotic cerebral infarction risk, that rs17293607 is not polymorphic in our study population. However, our study has some limitations, including the few SNPs studied, the limited sample size, and the rarity of minor alleles of our SNPs. More studies are needed to focus on the association of variants in *MMP10* gene with the risk of atherothrombotic cerebral infarction in diverse ethnic populations.

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Disclosure of conflict of interest

None.

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