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Associations of miR-146a, miR-149, miR-196a2, and miR-499 Polymorphisms with Ischemic Stroke in the Northern Chinese Han Population

Authors' Contribution:
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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Recently, *miR-146a* C>G, *miR- 149* T>C, *miR-196a2* T>C and *miR-499* A>G polymorphisms have been associated with susceptibility to many diseases, including ischemic stroke (IS). However, results have been reported inconsistency in IS, especially in the Chinese population. This study aimed to investigate the polymorphisms of the 4 *miRNAs* and IS risk in the Chinese population.

Material/Methods: We used a case-control study to explore these associations in 396 patients with IS and 378 healthy controls. According to TOAST standards, the selected patients were divided into subgroups: the large artery atherosclerosis (LAA) subgroup and the small artery occlusion (SAO) subgroup. The method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the genotypes.

Results: The *miR-146a* C>G polymorphism was remarkably different (CC *vs.* CG+GG: P=0.027; CC+CG *vs.* GG: P=0.020; C *vs.* G: P=0.006). The *miR-149* T>C polymorphism was also remarkably different (TT *vs.* TC+CC: P=0.017; TT+TC *vs.* CC: P=0.020; T *vs.* C: P=0.004). The *miR-146a* and *miR-149* polymorphisms were also remarkably different in the LAA subgroup (P<0.05). However, we did not find an association of *miR-196a2* T>C or *miR-499* A>G polymorphisms with IS (P>0.05); we did not find any association in the LAA subgroup or the SAO subgroup (P>0.05).

Conclusions: Our study suggested that *miR-146a* C>G and *miR-149* T>C polymorphisms might remarkably increase the risk of IS, which might be mainly associated with an increased risk in LAA stroke; however, the *miR-196a2* T>C and *miR-499* A>G polymorphisms might not be associated with IS risk in the northern Chinese Han population.

MeSH Keywords: Adams-Stokes Syndrome • Genes, vif • MicroRNAs

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Background

Worldwide, stroke is the second leading cause of disability and death [1]. In China, the annual mortality rate of stroke is about 1.6 million, approximately 157 per 100 000, and it has become the main cause of death and adult disability [2]. The incidence rate was reported to be the highest (486 per 100 000) in northern China, whereas in southern China, the incidence was significantly lower (136 per 100 000) [2]. Ischemic stroke (IS) is the most important type of stroke. IS is a multifactorial disease, which is influenced by many environmental and genetic factors. Environmental risk factors of IS include age, sex, body mass index (BMI), smoking, hyperlipidemia, diabetes, and hypertension [2]. Single-nucleotide polymorphisms (SNPs) have played crucial roles in the development of IS. Recent studies have found that many gene polymorphisms have been shown to be obviously associated with the IS risk, such as CC11, paraoxonase 1 (PON1), angiotensin converting enzyme (ACE), and methylenetetrahydrofolate reductase (MTHFR), and that these gene polymorphisms could affect inflammatory reaction and promote the occurrence of atherosclerosis (AS) [3–5].

Many studies have shown that the microRNAs (miRNAs) are related to human diseases such as metabolism, differentiation, apoptosis, and AS [6,7]. MiRNAs are small noncoding RNA molecules of 21 to 24 nucleotides which have been proven to be negative regulators controlling diverse biological processes; inhibiting their translation and/or stability can regulate about one-third of the human genes [8]. Recent studies have indicated that miRNAs have participated in the pathogenesis of AS, including endothelial integrity, lipid metabolism, inflammatory response, and extracellular matrix remodeling [9,10]. Several miRNAs have been found to be useful as biomarkers for the prognosis with IS in humans, including miR-21 and miR-24 [11]. MiRNAs play important roles in the pathophysiology of AS both *in vivo* and *in vitro*. For example, the low expression of miR-181 in the aortic intima of ApoE$^{-/-}$ mice with a high fat diet might promote the formation of AS [12]. The downregulation of miR-149 in osteo-arthritism chondrocytes can be correlated to increased expression of proinflammatory cytokines [13]. In our previous study, miR-126 expression was found to be significantly downregulated in atherosclerotic ApoE$^{-/-}$ mice, and we found that atorvastatin might exert its anti-inflammatory effects by upregulating the expressions of miR-126 *in vivo* [14].

In recent years, it has been shown that *miRNAs* polymorphisms could influence the processing and maturity of miRNAs, which might influence the occurrence and/or prognosis of a disease. Recently, the polymorphisms *miR-146a* C>G, *miR-196a2* T>C, *miR-149* T>C, and *miR-499* A>G have been associated with susceptibility to a variety of diseases, including IS [15,16]. The polymorphisms m*iR-146a* C>G, *miR-149* T>C, *miR-196a2* T>C, and *miR-499* A>G have been remarkably associated to regulation of tumor necrosis factor-α (TNF-α) [17], methylenetetrahydrofolatereductase (MTHFR) [18], annexin A1 (ANXA1) [19], and C-reactive protein (CRP) [20]. The 4 miRNAs targets have been related to inflammation pathways and/or thrombosis in the circulation system. TNF-α, MTHFR, ANXA1, and CRP have been related to AS and thrombosis [20–23]. The associations of these 4 known gene polymorphisms with IS risk has been reported, however, the reported results have been inconsistency, especially in the Chinese population [24,25]. So further research is needed on the associations among these 4 *miRNAs* polymorphisms with IS risk, especially the associations with the subtypes of large artery atherosclerosis (LAA) and small artery occlusion (SAO) in the Chinese population.

In our present study, we sought to research the associations of the aforementioned 4 *miRNAs* polymorphisms with IS risk, as well as analyze the 2 subtypes LAA and SAO in the northern Chinese Han study population.

Material and Methods

Participants

A case-control study design was used; the study included 396 patients with IS and 378 controls. The patients were recruited from the Department of Neurology, the affiliated Hiser hospital, and the affiliated hospital of Qingdao University from September 2013 and March 2016, if they were from the northern Chinese Han population and met the study criteria. The diagnosis of IS met the criteria approved at the Fourth National Cerebrovascular Disease Conference in 1995. At least 2 clinically experienced physicians made the final diagnosis through the characteristics of clinical syndrome, brain computed tomography (CT), and magnetic resonance imaging (MRI). The patients with IS were excluded if IS was caused by transient ischemic attack, hemorrhagic cerebral infarction, cardiogenic cerebral embolism, tumors, cardiovascular malformations, peripheral arterial occlusive disease, trauma, drugs, blood or infectious diseases, or they had been taking lipid-lowering drugs within the last half of the year. According to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) system, the selected patients were divided into 2 subgroups: the LAA subgroup and the SAO subgroup. The participants in the control group were healthy individuals recruited during a physical health examination in the Hiser hospital; they were matched with the patients by age, sex, race, and region and they were without medical history of IS and/or coronary heart disease. The study was performed with the approval of the ethics committee of the 2 hospitals. Clinical information from the study population included BMI, hypertension, diabetes, drinking, and smoking.

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Table 1. Sequences of PCR primers and product length of PCR.

SNPS	Sequences of PCR primers	Product length of PCR
miR-146a (rs2910164)	F: 5'-CATGGGTTGTGTCAGTGTCAGAGCT-3' R: 5'-TGCCTTCTGTCTCCAGTCTTCCAA-3'	147bp
miR-196a2 (rs11614913)	F: 5'-CCCCTTCCCTTCTCCTCCAGATA-3' R: 5'-CGAAAACCGACTGATGTAACTCCG-3'	149bp
miR-499 (rs3746444)	F: 5'-CAAAGTCTTCACTTCCCTGCCA-3' R: 5'-GATGTTTAACTCCTCTCCACGTGATC-3'	146bp
miR-149 (rs2292832)	F: 5'-TGTCTTCACTCCCGTGCTTGTC-3' R: 5'-GCCCGAAACACCCGTAAGATAT-3'	250bp

Plasma lipid measurements

Approximately 3 mL samples of fasting blood were collected from each study participant. Serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were analyzed.

DNA extraction and genotyping

We extracted genomic DNAs by using a DNA extraction kit (TianGen Biotech Beijing Co., Ltd., China) following the manufacturer's instructions. We used PCR- RFLP assays to analyze the polymorphisms. The sequences of primers for PCR are shown from Sangon Biotech Co., Ltd. (Shanghai) in Table 1. The reactivation condition of PCR was as follows (for example, *miR-146a*): 94°C predenaturation for 3 minutes; 35-cycle reaction (94°C for 1 minute, 65°C for 45 seconds, 72°C for 1 minute); 72°C for 2 minutes; 4°C preservation. The annealing temperatures of *miR-196a2*, *miR-499I,* and *miR-149* were 56°C, 65°C, and 58°C respectively. According to the online software analysis of endonuclease, the restriction enzymes were selected, which were Sac I (*miR-146a* C>G), Msp I (*miR-149* T>C), Bcl I (*miR-196a2* T>C), and Pvu II (*miR-499* A>G) respectively.

Statistical analysis

We used SPSS statistical software version 12.0 for the statistical analysis. Continuous variables were displayed as mean ±SD. The χ^2 goodness of fit test was adopted to test the deviation of genotypes distribution in the study population from Hardy-Weinberg equilibrium (HWE). We used odds ratio (OR) and 95% confidence intervals (95%CI) to assess the relativity between each allele and genotype distribution frequencies. A multivariate logistic regression was used to assess the relevance by the adjustment of the full risk factors. $P<0.05$ was considered to be obviously statistical significance.

Results

The characteristics of the participants are shown in Table 2. The patients with IS and the control participants were not remarkably different in age, gender, BMI, or the levels of TC and TG ($P>0.05$). However, the patients with IS were significantly different in smoking, drinking diabetes, hypertension, and the levels of HDL-c and LDL-c ($P<0.001$).

Subgroup analyses were carried out according to LAA and SAO subtypes. Compared with the control group, we did not find a remarkable difference in age, gender, BMI, or TG in the LAA and SAO subgroups ($P>0.05$); however, there were remarkable differences in drinking, smoking, hypertension, and diabetes ($P<0.05$). The levels of HDL-c in the LAA and SAO subgroups were remarkably lower ($P<0.05$). The level of LDL-c in the LAA subgroup was remarkably higher ($P<0.001$); however, there was no remarkable difference in the SAO subgroup compared with the control group ($P>0.05$). The level of TC in the LAA subgroup was remarkably higher ($P<0.05$); however, there was no remarkable difference in the SAO subgroup compared with the control group ($P>0.05$). There were no remarkable differences in TG between the 2 subgroups and the control group ($P>0.05$).

We investigated the *miR-146a* C>G, *miR-196a2* T>C, *miR-499* A>G, and *miR-149* T>C polymorphisms in IS patients and control participants (Table 3). The 4 *miRNAs* genotype frequencies of the control group were consistent with the HWE (*miR-146a* C>G, $P=0.512$; *miR-196a2* T>C, $P=0.354$; *miR-499* A>G, $P=0.910$; *miR-149* T>C, $P=0.720$). We evaluated the *miRNAs* genotype frequencies after adjusting for age, gender, hypertension, diabetes, BMI, smoking, drinking, TC, TG, HDL-c, and LDL-c by multivariate logistic regression analyses. Compared with the control group, the *miR-146a* C>G polymorphism was remarkably different (CC *vs.* GG: OR was 1.86, 95% CI was 1.19–2.88, $P=0.006$; CC *vs.* CG+GG: OR was 1.39, 95% CI was 1.04–1.86, $P=0.027$; CC+CG *vs.* GG: OR was 1.62, 95% CI was 1.08–2.42, $P=0.020$; CC *vs.* CG *vs.* GG: OR was 1.34, 95% CI was 1.09–1.65, $P=0.006$; C *vs.* G: OR was 1.33, 95% CI was 1.09–1.64, $P=0.006$). The *miR-149* T>C polymorphism was remarkably different between the IS

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Table 2. Baseline characteristics of the enrolled population.

Characteristics	Control (n=378) (%)	Ischemic stroke			P_1	P_2	P_3
		LAA (n=268) (%)	SAO (n=128) (%)	Total (n=396) (%)			
Age, y (mean ±SD)	63.31±4.84	63.88±4.8	63.68±4.35	63.74±4.49	0.542	0.255	0.200
Gender (n, %)					0.122	0.603	0.812
Male	202 (53.4)	150 (56)	65 (50.8)	215 (54.3)			
Female	176 (46.6)	118 (44)	63 (49.2)	181 (45.7)			
Smoking (n, %)					<0.001	<0.001	<0.001
No	327 (86.5)	192 (71.6)	91 (71.1)	283 (71.5)			
Yes	51 (13.5)	76 (28.4)	37 (28.9)	113 (28.5)			
Drinking (n, %)					0.005	<0.001	<0.001
No	323 (85.4)	206 (76.9)	89 (69.5)	295 (74.5)			
Yes	55 (14.6)	62 (23.1)	39 (30.5)	101 (25.5)			
Hypertension (n, %)					<0.001	<0.001	<0.001
No	328 (86.8)	179 (66.8)	83 (64.8)	262 (66.2)			
Yes	50 (13.2)	89 (33.2)	45 (35.2)	134 (33.8)			
Diabetes (n, %)					<0.001	<0.001	<0.001
No	349 (92.3)	219 (81.7)	104 (81.2)	323 (81.6)			
Yes	29 (7.7)	49 (18.3)	24 (18.8)	73 (18.4)			
BMI	24.25±1.07	24.1±1.12	24.26±1.07	24.21±1.09	0.946	0.160	0.550
Lipedema (mean ±SD, mmol/L)							
TG	1.50±0.41	1.54±0.6	1.56±0.76	1.56±0.71	0.143	0.326	0.140
TC	4.49±0.88	4.69±0.99	4.41±0.89	4.60±0.97	0.008	0.389	0.104
HDL-c	1.24±0.18	1.03±0.23	1.1±0.25	1.08±0.24	<0.001	<0.001	<0.001
LDL-c	2.88±0.34	3.01±0.46	2.93±0.4	2.98±0.44	<0.001	0.199	<0.001

P_1 – LAA *vs.* Control; P_2 – SAO *vs.* Control; P_3 – total *vs.* control.

patients and the control groups (TT *vs.* CC: OR was 2.00; 95% CI was 1.22–3.28, *P*=0.006; TT *vs.* TC+CC: OR was 1.42, 95% CI was 1.07–1.88, *P*=0.017; TT+TC *vs.* CC: OR was 1.75, 95% CI was 1.09–2.82, *P*=0.020; TT *vs.* TC *vs.* CC: OR was 1.37, 95% CI was 1.11–1.7, *P*=0.004; T *vs.* C: OR was 1.37, 95% CI was 1.11–1.7, *P*=0.004). There were no statistical differences in the polymorphisms of *miR-196a2* T>C and *miR-499* A>G between the IS patients and control groups (*P*>0.05). According to TOAST classification, we further assessed the effects of each *miRNA* polymorphism to the IS risk in the LAA and SAO subtypes. Our study found that the *miR-146a* C>G polymorphism in the LAA subtype was remarkably different compared with the control group (CC *vs.* GG: OR was 2.04, 95% CI was1.26–3.3, *P*=0.004; CC *vs.* CG+GG: OR was 1.51, 95% CI was 1.08–2.09, *P*=0.015; CC+CG *vs.* GG: OR was 1.7, 95% CI was 1.1–2.63, *P*=0.018; CC *vs.* CG *vs.* GG: OR was 1.41, 95% CI was 1.09–1.82, *P*=0.008; C *vs.* G: OR was 1.4, 95% CI was 1.12–1.76, *P*=0.003); however, there was no remarkably difference between the SAO subtype and the control group (*P*>0.05). The *miR-149* T>C polymorphism was remarkably different between the LAA subtype and the control group (TT *vs.* CC: OR was 2.47; 95% CI was 1.45–4.21, *P*=0.001; TT *vs.* TC+CC: OR was 1.7, 95% C was 1.23–2.34, *P*=0.001; TT+TC *vs.* CC: OR was 1.98, 95% CI was 1.19–3.27, *P*=0.008; TT *vs.* TC *vs.* CC: OR was 1.57, 95% CI was 1.23–1.99, *P*<0.001; T *vs.* C: OR was 1.55, 95% CI was 1.23–1.96, *P*<0.001); however, there was not a remarkable difference between the

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Table 3. Associations of *miR-146a* C>G, *miR-196a2* T>C, *miR-499* A>G, and *miR-149* T>C polymorphisms between IS risk and control groups.

Genotypes	Control (n,%)	IS (n,%)	OR (95% CI)	*P*
miR-146a (C>G)				
CC	154 (40.7)	131 (33.1)	1	
CG	179 (47.4)	194 (49)	1.27 (0.94, 1.74)	0.125
GG	45 (11.9)	71 (17.9)	1.86 (1.19, 2.88)	0.006
CC *vs.* (CGG)			1.39 (1.04, 1.86)	0.027
(CC+CG) *vs.* GG			1.62 (1.08, 2.42)	0.020
CC *vs.* CG *vs.* GG			1.34 (1.09, 1.65)	0.006
C	487 (64.4)	456 (57.6)	1	
G	269 (35.6)	336 (42.4)	1.33 (1.09, 1.64)	0.006
miR-196a2 (T>C)				
TT	110 (29.1)	112 (28.3)	1	
TC	196 (51.9)	205 (51.8)	1.03 (0.74, 1.43)	0.872
CC	72 (19)	79 (19.9)	1.08 (0.71, 1.63)	0.723
TT *vs.* (TC+CC)			1.04 (0.76, 1.42)	0.801
(TT+TC) *vs.* CC			1.06 (0.74, 1.51)	0.752
TT *vs.* TC *vs.* CC			1.04 (0.85, 1.27)	0.728
T	416 (55)	429 (54.2)	1	
C	340 (45)	363 (45.8)	1.04 (0.85, 1.27)	0.734
miR-499 (A>G)				
AA	249 (65.9)	255 (64.4)	1	
AG	116 (30.7)	123 (31.1)	1.04 (0.76, 1.41)	0.825
GG	13 (3.4)	18 (4.5)	1.35 (0.65, 2.82)	0.421
AA *vs.* (AG+GG)			1.07 (0.79, 1.44)	0.666
(AA+AG) *vs.* GG			1.34 (0.65, 2.77)	0.434
AA *vs.* AG *vs.* GG			1.09 (0.85, 1.39)	0.524
A	614 (81.2)	633 (79.9)	1	
G	142 (18.8)	159 (20.1)	1.09 (0.84, 1.4)	0.521
miR-149 (T>C)				
TT	190 (50.3)	165 (41.7)	1	
TC	158 (41.8)	179 (45.2)	1.31 (0.97, 1.76)	0.081
CC	30 (7.9)	52 (13.1)	2.00 (1.22, 3.28)	0.006
TT *vs.* (TC+CC)			1.42 (1.07, 1.88)	0.017
(TT+TC) *vs.* CC			1.75 (1.09, 2.82)	0.020
TT *vs.* TC *vs.* CC			1.37 (1.11, 1.7)	0.004
T	538 (71.2)	509 (64.3)	1	
C	218 (28.8)	283 (35.7)	1.37 (1.11, 1.7)	0.004

OR – adjusted odds ratio; 95% CI – 95% confidence interval. OR based on the risk factors, including age, sex, hypertension, diabetes, BMI, smoking, drinking, TC, TG, LDL-c, and HDL-c.

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Table 4. Associations of *miR-146a* C>G, *miR-196a2* T>C, *miR-499* A>G, and *miR-149* T>C polymorphisms between the subgroups and the control group.

Genotypes	Control n (%)	LAA			SAO		
		n (%)	OR (95% CI)	P	n (%)	OR (95% CI)	P
miR-146a (C>G)							
CC	154 (40.7)	84 (31.3)	1		47 (36.7)	1	
CG	179 (47.4)	134 (50)	1.37 (0.97, 1.94)	0.074	60 (46.9)	1.1 (0.71, 1.7)	0.675
GG	45 (11.9)	50 (18.7)	2.04 (1.26, 3.3)	0.004	21 (16.4)	1.53 (0.83, 2.82)	0.174
CC *vs.* (CG+GG)			1.51 (1.08, 2.09)	0.015		1.19 (0.78, 1.79)	0.422
(CC+CG) *vs.* GG			1.7 (1.1, 2.63)	0.018		1.45 (0.83, 2.55)	0.193
CC *vs.* CG *vs.* GG			1.41 (1.09, 1.82)	0.008		1.2 (0.9, 1.62)	0.218
C	487 (64.4)	302 (56.3)	1		154 (60.2)	1	
G	269 (35.6)	234 (43.7)	1.4 (1.12, 1.76)	0.003	102 (39.8)	1.2 (0.9, 1.6)	0.222
miR-196a2 (T>C)							
TT	110 (29.1)	75 (28)	1		37 (28.9)	1	
TC	196 (51.9)	139 (51.9)	1.04 (0.72, 1.5)	0.833	66 (51.6)	1 (0.63, 1.59)	0.996
CC	72 (19)	54 (20.1)	1.1 (0.7, 1.74)	0.684	25 (19.5)	1.03 (0.57, 1.86)	0.916
TT *vs.* (TC+CC)			1.06 (0.75, 1.49)			1.01 (0.65, 1.57)	0.967
(TT+TC) *vs.* CC			1.07 (0.72, 1.59)	0.728		1.03 (0.62, 1.71)	0.904
TT *vs.* TC *vs.* CC			1.05 (0.84, 1.32)	0.686		1.01 (0.76, 1.36)	0.923
T	416 (55)	289 (53.9)	1		140 (54.7)	1	
C	340 (45)	247 (46.1)	1.05 (0.84, 1.31)	0.693	116 (45.3)	1.01 (0.76, 1.35)	0.925
miR-499 (A>G)							
AA	249 (65.9)	172 (64.2)	1		83 (64.8)	1	
AG	116 (30.7)	83 (30.9)	1.04 (0.74, 1.46)	0.84	40 (31.2)	1.03 (0.67, 1.6)	0.879
GG	13 (3.4)	13 (4.9)	1.45 (0.66, 3.2)	0.36	5 (3.9)	1.15 (0.4, 3.33)	0.791
AA *vs.* (AG+GG)			1.08 (0.78, 1.5)	0.656		1.05 (0.69, 1.59)	0.832
(AA+AG) *vs.* GG			1.43 (0.65, 3.14)	0.371		1.14 (0.4, 3.27)	0.805
AA *vs.* AG *vs.* GG			1.1 (0.84, 1.45)	0.491		1.05 (0.73, 1.5)	0.792
A	614 (81.2)	427 (79.7)	1		206 (80.5)	1	
G	142 (18.8)	109 (20.3)	1.1 (0.84, 1.46)	0.487	50 (19.5)	1.05 (0.73, 1.5)	0.792
miR-149 (T>C)							
TT	190 (50.3)	100 (37.3)	1		65 (50.8)	1	
TC	158 (41.8)	129 (48.1)	1.55 (1.11, 2.17)	0.010	50 (39.1)	0.93 (0.61, 1.42)	0.719
CC	30 (7.9)	39 (14.6)	2.47 (1.45, 4.21)	0.001	13 (10.2)	1.27 (0.62, 2.57)	0.514
TT *vs.* (TC+CC)			1.7 (1.23, 2.34)	0.001		0.98 (0.66, 1.46)	0.92
(TT+TC) *vs.* CC			1.98 (1.19, 3.27)	0.008		1.31 (0.66, 2.6)	0.437
TT *vs.* TC *vs.* CC			1.57 (1.23, 1.99)	<0.001		1.04 (0.76, 1.42)	0.796
T	538 (71.2)	329 (61.4)	1		180 (70.3)	1	
C	218 (28.8)	207 (38.6)	1.55 (1.23, 1.96)	<0.001	76 (29.7)	1.04 (0.76, 1.42)	0.795

OR – adjusted odds ratio; 95% CI – 95% confidence interval. OR based on the risk factors, including age, sex, hypertension, diabetes, BMI, smoking, drinking, TC, TG, LDL-c, and HDL-c.

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SAO subtype and the control group ($P>0.05$). Compared with the control group, there were no statistical differences in the polymorphisms of *miR-196a2* T>C and *miR-499* A>G in the LAA and SAO subgroups ($P>0.05$) (Table 4).

Discussion

In the present study, our purpose was to evaluate the associations of *miR-146a*, *miR-149*, *miR-196a2,* and *miR-499* polymorphisms with the risk of IS in the northern Chinese Han population. Our findings showed that the polymorphisms of *miR-146a* C>G and *miR-149* T>C might remarkably increase the risk of IS in the northern Chinese Han population, which might be mainly associated with the LAA stroke risk. However, the polymorphisms of *miR-196a2* T>C and *miR-499* A>G might not be associated with the risk of IS in the northern Chinese Han population.

A previous study found that miRNAs were involved in various crucial biological pathological processes and could regulate about 30% of human genes expression [26]. The SNPs or genetic mutations of miRNAs existing in pre-miRNAs can affect the expression of mature miRNAs, and can then cause mutative miRNAs expression and lead to the occurrence of diseases [27]. The SNPs of miRNAs are closely related to many diseases including AS, hyperlipidemia, and coronary heart disease (CHD) [28,29].

In recent years, many studies found that *miR-146a* C>G polymorphism was closely related to the susceptibility of atherosclerotic diseases. In an Iranian population, *miR-146a* C>G polymorphism was associated with an increased susceptibility to CHD [30]. The gene polymorphism of *miR-146a* C>G was associated with an increased risk of CHD in the Chinese population, which might occur through influencing the expression level of the mature miR-146a [29]. Jeon et al. found that *miR-146a* C>G polymorphism was significantly increased the risk of IS and the G allele could increase the risk, which was significantly associated in the LAA and SAO subgroups in the South Korean population [31]. In the Chinese population, the results of the study were inconsistent. The studies by Huang and Zhu et al found that the polymorphism of *miR-146a* C>G could significantly increase the risk of IS and the G allele could increase the risk of disease in the northern Chinese Han population [24,32]. Our study also found that *miR-146a* C>G polymorphism remarkably increased the risk of IS, and the G allele could increase the risk in the northern Chinese Han population, especially in the LAA subgroup. Another study found that *miR-146a* C>G polymorphism could affect miR-146a, IRAK-1, TRAF6, and TNF-α expression levels and was associated with susceptibility to CHD [33]. The *miR-146a* G allele was associated with decreased mature miR-146a level, which was associated with susceptibility to breast and ovarian cancers [34]. However, other studies by Liu and Luo found no statistical difference between the polymorphism of *miR-146a* C>G and the IS risk in the southern Chinese Han population [25,35]. In addition, studies have found that the polymorphism of *miR-149* T>C could affect the function and level of the mature miR-149 [18,36]. MTHFR is a target gene of miR-149, which plays an important role in the occurrence and development of AS [18]. One study found that the polymorphism of *miR-149* T>C can affect the metabolic pathway of MTHFR by influencing the function and level of mature miR-149, and then further change the level of MTHFR to increase the susceptibility of CHD [18]. One study has shown that the polymorphism of *miR-149* T>C can significantly increase the risk of IS by influencing the plasma level of homocysteine, which could influence the plasma level of homocysteine to increase the risk of IS in the Chinese population [37]. Our study found that the polymorphism of *miR-149* T>C was obviously related to the risk of IS, and the C allele might be a risk factor in the northern Chinese Han population. We further found that the polymorphism of *miR-149* T>C could significantly increase the risk of IS in the LAA subgroup. However, other studies found no correlation between IS risk and the genetic polymorphisms of *miR-149* T>C in the southern Chinese Han population and South Korean population [25]. Our study found that the polymorphisms of *miR-146a* C>G and *miR-149* T>C were associated with the LAA subtype of IS. The possible reason could be that the 2 genetic polymorphisms are mainly related to AS, and AS is the most important factor in the occurrence of LAA stroke [38]. The target gene of *miR-196a2* is ANXA1, and elevated level of miR-196a2 can lead to decreasing the levels of ANXA1 mRNA, and protein [19]. ANXA1 plays an important role in the migration and adhesion of neutrophils and monocytes in the process of atherosclerosis, and also plays an important role in the anti-inflammatory response signaling pathway [23]. The *miR-196a2* T allele has been associated with decreased mature miR-196a2 level [39]. The polymorphism of *miR-196a2* T>C was found to be associated with susceptibility to cancer [40]. However, our study found that the polymorphisms of *miR-196a2* T>C might not be associated with IS in the Chinese Han population. In the LAA and SAO subgroups, an association was also not found. Similarly, no association was found between the polymorphism of *miR-196a2* T>C and the risk of IS, including in the Korean and Chinese populations [31,32]. However, one study found that the polymorphism of *miR-196a2* T>C was associated with susceptibility to CHD [41]. Therefore, the polymorphism of *miR-196a2* T>C might have distinct influence on different organs of atherosclerotic diseases; these findings need to be studied in further research. The polymorphism of *miR-499* A>G has been shown to be related to the decrease of plasma CRP concentrations [20]. It could change the level and function of mature miR-499 expression and affect the combination of target gene and mature miR-499 [42]. The polymorphism of *miR-499* A>G has been

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associated with susceptibility to CHD [43]. There have been fewer reports on the association of the polymorphism of *miR-499* A>G with IS, and study results have been inconsistent. Studies have found that the polymorphism of *miR-499* A>G was correlated with IS, and the G allele could remarkably increase the risk of IS in the Chinese population [25,35]. However, other studies found there were no associations between the polymorphism of *miR-499* A>G with IS, including in the Korean and Chinese populations [24,31]. Similarly, our study did not find remarkable associations between the gene polymorphism of *miR-499* A>G and IS, including in the LAA and SAO subgroups. The results of the aforementioned other studies were different in different ethnic groups, different regions, different countries, and different experimental design; therefore, different study findings might be found.

There were certain limitations in the present study. First, the potential for selective bias was inevitable in this case-controlled study. Second, the study was a single population study with a limited sample size, so the results still need be confirmed in multicenter, different populations, races, and larger samples. Third, the pathological mechanism underlying the gene polymorphisms of *miR-146a* and *miR-149* with IS risk is still unclear, so further research will be needed to study the related functions of *miR-146a* and *miR-149* with IS risk.

Conclusions

In conclusion, this present study showed that the polymorphisms of *miR-146a* C>G and *miR-149* T>C might be related to a remarkable increase of IS risk, which might be mainly associated with LAA stroke; however, the polymorphisms of *miR-196a2* T>C and *miR-499* A>G might not be related to the risk of IS in the northern Chinese Han population.

Conflicts of interest

None.

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