Association Between MicroRNAs Polymorphisms and Risk of Ischemic Stroke: A Meta-Analysis in Chinese Individuals

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Objective: Previous studies have demonstrated that some single-nucleotide polymorphisms (SNPs) in miRNAs are related to the risk of ischemic stroke (IS), but the conclusions are still controversial and inconclusive. We performed this meta-analysis to further assess the association between miR-146a C>G (rs2910164), miR-149 T>C (rs2292832), miR-196a2 T>C (rs11614913), miR-499 A>G (rs3746444) and risk of IS in Chinese individuals.

Methods: Relevant studies were identified in the databases of PubMed, Embase. The strength of correlation between microRNAs polymorphisms and IS risk was assessed by odds ratios (ORs) and 95% confidence intervals (95% CIs) under five genetic models.

Results: 5 studies, containing 2,632 cases and 3,191 controls, were included in this meta-analysis. The overall results of meta-analysis indicated that there were no significant association between miR-146a C>G (rs2910164), miR-149 T>C (rs2292832), miR-196a2 T>C (rs11614913), and the IS risk in the overall analyses. MiR-499 A>G (rs3746444) was associated with an increased IS risk under allele model (OR = 1.30, 95% CI = 1.02–1.66), heterozygous model (OR = 1.35, 95% CI = 1.01–1.79) and dominant model (OR = 1.36, 95% CI = 1.02–1.80) in Chinese. The sensitivity analysis results of these four polymorphisms were similar to the overall results.

Conclusion: MiR-499 A>G (rs3746444) G allele and AG, AG + AA genotype might be risk factors of IS in Chinese. No significant association was observed between miR-146a C>G (rs2910164), miR-149 T>C (rs2292832), miR-196a2 T>C (rs11614913), and IS risk. The associations may be different due to geographical factors of China. More explorations in more diverse geographically regions with large sample size are expected to further verify the findings in the future.

Keywords: miR-146a, miR-149, miR-196a2, miR-499, polymorphism, ischemic stroke, risk, meta-analysis

INTRODUCTION

Neurological disorders are the second leading cause of death and a major cause of functional disability worldwide, among which stroke accounted for the highest proportion, imposing a huge burden for the whole society as well as the healthcare system (GBD Neurological Disorders Collaborator Group, 2017). Ischemic stroke (IS) occupies approximately 85%
of all types of strokes (Kotlega et al., 2017). The risk factors of IS include hypertension, smoking, waist-to-hip ratio, diet, diabetes mellitus, etc. (O’Donnell et al., 2010). Moreover, a large range of genetic factors mingled with environmental factors stimulated the complex disease, and it is indicated that genetic ones play a significant role in the cause of IS (Boehme et al., 2017; Pan et al., 2017; Xiang et al., 2017).

MicroRNAs (miRNAs) are non-coding, endogenous ~22 nt RNAs, which can induce either translational repression or miRNA degradation, and regulate post-transcriptional gene expression by binding to the 3′-untranslated region (3′-UTR) of target genes (Bartel, 2009). The most abundant form of DNA variation in the human genome are single nucleotide polymorphisms (SNPs), which can potentially change various biological processes by affecting the maturation process or target selection of miRNAs (Duan et al., 2007). Previous studies have demonstrated that miR-146a G, miR-149 T, miR-196a2 C, miR-499 G alleles are possible genetic predisposing factors (Jeon et al., 2012; Min et al., 2012; Park et al., 2012; Wang et al., 2012). These four SNPs in miRNAs (miR-SNPs) can influence vascular damage responses, and regulate miRNA targets related to thrombosis and inflammation pathways in the circulation system (Luthra et al., 2008; El Gazzar et al., 2011; Yang et al., 2012; Wu et al., 2013). Among them, miR-146a is closely related to regulation of tumor necrosis factor-α (TNF-α) (El Gazzar et al., 2011), meanwhile miR-149 can regulate the expression of MTHFR (Wu et al., 2013), miR-196a2 can target annexin A1 (Luthra et al., 2008), and miR-499 can affect the inflammatory reaction through modulating C-reactive protein (CRP) (Yang et al., 2012) respectively. Several studies aimed to explore the influence of these four SNPs on IS risk, but the conclusions are still controversial and inconclusive. We performed this meta-analysis to further assess the association between miR-146a C>G (rs2910164), miR-149 T>C (rs2292832), miR-196a2 T>C (rs11614913), miR-499 A>G (rs3746444) and risk of IS in Chinese individuals, which may be likely to be utilized as clinical indexes to evaluate the risk of occurrence, development and responses to the treatments of IS.

MATERIALS AND METHODS

Search Strategy

We conducted a comprehensive literature search of PubMed, Embase up to November 30, 2017. The searched terms are as follows: [“ischemic stroke” AND (“microRNA” OR “miRNA” OR “miR”) AND (“polymorphism” OR “SNP” OR “mutation” OR “variant”)]. In order to identify other studies which may be relevant with this from references, all studies were retrieved in the manual way. We performed the meta-analysis according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement in reporting meta-analysis.

Eligibility Criteria

Eligible studies should conform with the following criteria: (1) the exposure was microRNAs polymorphisms; (2) populations were divided into case and control groups, consisting of IS and without IS respectively; (3) the outcome was incident of IS; (4) the study design was accordance with case-control study; (5) study provided sufficient published data to calculate the odds ratios (ORs) with corresponding 95% confidence intervals (CIs). The exclusion conditions include: (1) repeated publications (studies recently published or with more participants were included); (2) abstract, reviews, meta-analysis, case reports and comments. (3) animal model research. (4) not about Chinese individuals; (5) not published in English.

Data Extraction

Information was extracted from all included studies for analysis, including first author's name, published year, country, ethnicity, source of controls, genotype frequency of case and control, age, genotyping method, and HWE for controls by two independent researchers (Li and Weng). Discrepancy was resolved by referring to original studies in discussion with a third reviewer (Zheng).

Statistical Analysis

Chi-square test was used to determine Hardy-Weinberg equilibrium (HWE) in the control groups, which could be considered disequilibrium when P value is less than 0.05. The strength of correlation between microRNAs polymorphisms and IS risk was assessed by ORs and 95% confidence intervals (95% CIs) under five genetic models. Heterogeneity between the studies was evaluated by both the Cochran’s Q statistic and the I² statistic (Higgins, 2008). P < 0.10 and/or I² > 50% indicated significant heterogeneity, and random-effects model (the DerSimonian and Laird method) was applied for ORs calculation; otherwise, fixed-effects model using the Mantel-Haenszel was utilized. The sensitivity analysis was performed according to HWE status of controls (Thakkinstian et al., 2005). Furthermore, Begg’s funnel plot and Egger’s test were used to inspect potential publication bias among included studies. The statistical significance of the ORs was determined by Z-test, with P < 0.05 suggesting a significant difference. All statistical analyses were conducted with the software Stata 14.0.

RESULTS

Characteristics of Included Studies

We yielded 85 papers initially and 5 studies, containing 2,632 cases and 3,191 controls, were included in this meta-analysis finally. Figure 1 shows the progress of study selection. Among these studies, five studies focused on miR-146a G>C (rs2910164) (Liu et al., 2014; Zhu et al., 2014; Huang et al., 2015; Qu et al., 2016; Luo et al., 2017), one study focused on miR-149 T>C (rs2292832) (Luo et al., 2017), four studies focused on miR-196a2 T>C (rs11614913) (Liu et al., 2014; Zhu et al., 2014; Huang et al., 2015; Luo et al., 2017) and three studies focused on miR-499 A>G (rs3746444) (Liu et al., 2014; Huang et al., 2015; Luo et al., 2017). The characteristics of the included studies are presented in Table 1. Among them, despite one study (Qu et al., 2016) in miR-146a G>C (rs2910164), one study (Huang et al., 2015) in miR-499 A>G (rs3746444), all conformed with HWE.

Heterogeneity Test

The result of between-study heterogeneity of genetic models of miR-146a G>C (rs2910164) polymorphism was significant
(Table 2) under three models (G vs. C, $I^2 = 60.1, P = 0.04$; GG vs. CC, $I^2 = 61.4, P = 0.04$; GG vs. CG + CC, $I^2 = 50.2, P = 0.09$). The similar trend can be observed in miR-499 A$\rightarrow$G (rs3746444) polymorphism under allele model (G vs. A, $I^2 = 52.8, P = 0.12$), heterozygous model (AG vs. AA, $I^2 = 54.3, P = 0.11$) and dominant model (AG + GG vs. AA, $I^2 = 56.1, P = 0.10$), thus random-effects model was used for meta-analysis.

The fixed-effects model was used to calculate ORs for miR-196a2 T$\rightarrow$C (rs11614913) polymorphism due to the low heterogeneity under all genetic models. No evidence of significant heterogeneity was detected in miR-146a C$\rightarrow$G (rs2910164) polymorphism under allele model (G vs. C, OR = 0.99, 95% CI = 0.87–1.12; GG vs. CC: OR = 0.99, 95% CI = 0.74–1.31; CG vs. CC: OR = 0.99; 95% CI = 0.88–1.11; GG vs. CC + CG: OR = 0.99, 95% CI = 0.79–1.24, Table 2).

The results of sensitivity analysis were similar to the overall analyses (Figure 2).

**MiR-149 T$\rightarrow$C (rs2292832) and the Risk of Is**

No significant correlation was found in the overall analyses or sensitive analyses in any one of these models (C vs. T: OR = 0.88, 95% CI = 0.70–1.12; CC vs. TT: OR = 0.80, 95% CI = 0.49–1.31;
TABLE 1 | Major characteristics of included studies.

| First author | Year | Country | Ethnicity | Source of control | Age (Case/Control) | Sample size (Case/Control) | Genotyping method | P for HWE |
|---------------|------|---------|-----------|-------------------|-------------------|---------------------------|------------------|-----------|
| miR-146a C>G (rs2910164) | | | | | | | | |
| Liu | 2014 | China | Asian | Healthy | 67.52 ± 10.29/66.34 ± 11.07 | 296/391 | PCR-RFLP | 0.650 |
| Zhu | 2014 | China | Asian | Healthy | 61.62 ± 0.98/62.05 ± 0.982 | 368/381 | PCR-LDR | 0.952 |
| Huang | 2015 | China | Asian | Healthy | 63 (54, 70)/61 (54, 68) | 531/531 | Taqman | 0.107 |
| Qu | 2016 | China | Asian | Healthy | 61.30 ± 9.40/59.50 ± 8.50 | 1,139/1,585 | PCR-LDR | <0.01 |
| Luo | 2017 | China | Asian | Healthy | 60.70 ± 12.33/60.17 ± 10.32 | 298/303 | PCR | 0.672 |
| miR-149 T>C (rs2292832) | | | | | | | | |
| Luo | 2017 | China | Asian | Healthy | 60.70 ± 12.33/60.17 ± 10.32 | 298/303 | PCR | 0.447 |
| miR-196a2 T>C (rs11614913) | | | | | | | | |
| Luo | 2017 | China | Asian | Healthy | 60.70 ± 12.33/60.17 ± 10.32 | 298/303 | PCR | 0.385 |
| miR-499 A>G (rs3746444) | | | | | | | | |
| Liu | 2014 | China | Asian | Healthy | 67.52 ± 10.29/66.34 ± 11.07 | 296/391 | PCR-RFLP | 0.060 |
| Zhu | 2014 | China | Asian | Healthy | 61.62 ± 0.98/62.05 ± 0.982 | 368/381 | PCR-LDR | 0.384 |
| Huang | 2015 | China | Asian | Healthy | 63 (54, 70)/61 (54, 68) | 531/531 | Taqman | 0.856 |
| Luo | 2017 | China | Asian | Healthy | 60.70 ± 12.33/60.17 ± 10.32 | 298/303 | PCR | 0.132 |

HWE, Hardy-Weinberg equilibrium; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; PCR-LDR, polymerase chain reaction–ligase detection reaction; PCR, polymerase chain reaction.

TABLE 2 | Overall and sensitivity analyses of miR-146a C>G (rs2910164) and risk of IS.

| Genetic model | Subgroup | No. of studies | Meta-analysis | P for Egger's test | Heterogeneity |
|---------------|----------|----------------|---------------|-------------------|--------------|
|               |          |                | OR (95%CI)    | P-value           | I² (%)       | P-value       |
| G vs. C       | Overall  | 5              | 0.99 (0.87–1.12) | 0.87              | 0.77         | 60.1          | 0.04         |
|               | HWE-Yes  | 4              | 0.99 (0.82–1.19) | 0.88              |              | 69.8          | 0.02         |
| GG vs. CC     | Overall  | 5              | 0.99 (0.74–1.31) | 0.92              | 0.85         | 61.4          | 0.04         |
|               | HWE-Yes  | 4              | 0.98 (0.65–1.48) | 0.94              |              | 70.8          | 0.02         |
| CG vs. CC     | Overall  | 5              | 0.99 (0.88–1.11) | 0.85              | 0.50         | 0             | 0.46         |
|               | HWE-Yes  | 4              | 1.01 (0.86–1.18) | 0.93              |              | 13.7          | 0.32         |
| CG + GG vs. CC| Overall  | 5              | 0.99 (0.89–1.11) | 0.87              | 0.31         | 39.3          | 0.16         |
|               | HWE-Yes  | 4              | 0.99 (0.80–1.23) | 0.94              |              | 53.5          | 0.09         |
| GG vs. CG + CC| Overall  | 5              | 0.99 (0.79–1.24) | 0.94              | 0.85         | 50.2          | 0.09         |
|               | HWE-Yes  | 4              | 0.99 (0.71–1.37) | 0.94              |              | 62.6          | 0.05         |

OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

TC vs. TT: OR = 0.86; 95% CI = 0.61–1.22; TC + CC vs. TT: OR = 0.85, 95% CI = 0.61–1.17; CC vs. TC + TT: OR = 0.87, 95% CI = 0.55–1.37, Table 3, Figure 3).

MiR-196a2 T>C (rs11614913) and the Risk of IS

There was no significant association between this SNP and the IS susceptibility under all genetic models (C vs. T: OR = 0.97, 95% CI = 0.88–1.07; CC vs. TT: OR = 0.93, 95% CI = 0.76–1.15; TC vs. TT: OR = 0.97; 95% CI = 0.82–1.15; TC + CC vs. TT: OR = 0.96, 95% CI = 0.82–1.13; CC vs. TC + TT: OR = 0.96, 95% CI = 0.80–1.14, Table 4, Figure 4).

MiR-499 A>G (rs3746444) and the Risk of IS

The overall analysis showed that the miR-499 A>G (rs3746444) polymorphism increased the risk of IS under allele model (G vs. A, OR = 1.30, 95% CI = 1.02–1.66), heterozygous model (AG vs. AA, OR = 1.35; 95% CI = 1.01–1.79) and dominant model (AG + GG vs. AA: OR = 1.36, 95% CI = 1.02–1.80). No significant association was observed between miR-499 A>G (rs3746444) polymorphism and risk of IS under homozygous model (GG vs.
AA: OR = 1.02, 95% CI = [0.71–1.46]) and recessive model (GG vs. AG + AA, OR = 1.51, 95% CI = [0.82–2.75]) (Table 5, Figure 5).

Sensitivity analysis was conducted. However, no change of the result was detected.

**Publication Bias**
It is shown from Tables 2–5 that no significant publication bias was observed because the P-values for Egger’s linear regression tests are no less than 0.05. Moreover, Begg’s funnel plots did not show any significant asymmetry (Figure 6).

**DISCUSSION**
MiRNAs play key roles in many physiological and pathological processes, including tumorigenesis, proliferation, hematopoiesis, metabolism, immune function, epigenetics, and neurodegenerative diseases (Miska, 2005; O’Connell et al., 2010). SNP in miRNAs correlated closely with traditional risk factors of IS, such as atherosclerosis, hypertension, diabetes and hyperlipidemia (high cholesterol) (Boucherat et al., 2015; Li et al., 2015; Gómez-Banoy and Mockus, 2016; Schober and Weber, 2016). The four miRNAs we focused on—miR-146a, miR-149, miR-196a2, and miR-499—are intimately related to regulation of TNF-α (El Gazzar et al., 2011), MTHFR (Wu et al., 2013), annexin A1 (ANXA1) (Luthra et al., 2008), and CRP (Yang et al., 2012) respectively, which were general causes of cerebral ischemia. Above all, these miRNAs might be associated with risk of IS.

In the present meta-analysis, we found that the miR-499 A > G (rs3746444) G allele presented 1.24-fold higher risk of IS than A allele, as well as the AG and GG carriers presented 1.35- and 1.36-fold higher risk than AA carriers, respectively. Previous researches have revealed that miR-499 involved in cell death under the anoxia and ischemia condition through its suppression of calcineurin-mediated dephosphorylation of dynamin-related protein-1 (Drp1), as a result the Drp1 accumulation in mitochondria and the Drp1-mediated activation of the mitochondrial fission machinery reduced (Wang et al.,...
FIGURE 3 | Forest plot for the association between miR-149 T>C (rs2292832) polymorphism and ischemic stroke risk in allele genetic model.

TABLE 4 | Overall analyses of miR-196a2 T>C (rs11614913) and risk of IS.

| Genetic model | Subgroup | No. of studies | Meta-analysis | P for Egger’s test | Heterogeneity |
|---------------|----------|----------------|---------------|-----------------|--------------|
|               |          |                | OR (95%CI) | P-value | I² (%) | P-value |
| C vs. T       | Overall  | 4              | 0.97 (0.88–1.07) | 0.56   | 0.21   | 0     | 0.45   |
| CC vs. TT     | Overall  | 4              | 0.93 (0.76–1.15) | 0.52   | 0.44   | 0     | 0.45   |
| TC vs. TT     | Overall  | 4              | 0.97 (0.82–1.15) | 0.76   | 0.61   | 0     | 0.56   |
| TC + CC vs. TT| Overall  | 4              | 0.96 (0.82–1.13) | 0.64   | 0.59   | 0     | 0.73   |
| CC vs. TC + TT| Overall  | 4              | 0.96 (0.80–1.14) | 0.61   | 0.83   | 49.1  | 0.12   |

OR, odds ratio; CI, confidence interval.

2011). MiR-499 activates the production of CRP and regulates the expression of many inflammatory cytokines, including IL-17RB, IL-23a, IL-2R, IL-6, IL-2 and IL-18R, so that it is able to influence the inflammatory reaction (Yang et al., 2012; Hashemi et al., 2013). Its increase of the concentration payed on serum or plasma CPR is likely to cause the increase of the incidence of stroke through regulating the level of risk factors of IS, such as inflammation, hypertension, and hyperlipidemia (Luo et al., 2017). Liu et al. (2014) found the association between increased IS risk and rs3746444 A/G variant genotypes (GG + AG) was more notable in younger subjects, never smokers, non-obesity (BMI ≤ 24), non-hypertension non-diabetes, non-coronary heart disease and non-hyperlipidemia. The results are inconsistent and its other potential mechanisms are still uncertain.

There was no significant association between miR-146a C>G (rs2910164) and IS risk in the overall analyses. According to the previous studies, miR-146a inhibits interleukin-1 receptor-associated kinase 1 (IRAK-1) and tumor necrosis factor receptor associated factor 6 (TRAF-6) expression through negative feedback regulation (Ramkaran et al., 2014), causing decreased levels of pro-inflammatory cytokines and critical transcription factor in atherosclerosis such as IL-1, IL-6, IL-8, TNF-α, and resulting inhibition of nuclear factor (NF)-κB via the Toll-like receptor pathway (Guo et al., 2010). Therefore, the down-regulation of miR-146a tends to reduce inflammation-related atherosclerosis and have an effect on vascular damage response. The polymorphisms of miRNA will affect the further processing of pre-miRNA into a mature miRNA, therefore influence occurrence and development of disease (Saunders et al., 2007). Furthermore, miR-146a G allele may decrease the level of mature miRNA expression (Shen et al., 2008). Zhu et al. (2014) found that miR-146a CC genotype and C allele are associated with an increased incidence of large-artery atherosclerotic stroke in the northern Chinese Han population. However, Qu et al. (2016) conducted a study with 1,311 patients in six provinces and found rs2910164 has no association with IS incidence under several genetic models, stroke subtype and sex. It appears to be a strong predictor of stroke prognosis but not stroke incidence. Further reasons for the different results may include: the difference of geographical factors, sample size, study design, selection criteria of the patients or the diversity of genotyping methods, as well as not all studies obtained their results after
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FIGURE 4 | Forest plot for the association between miR-196a2 T>C (rs11614913) polymorphism and ischemic stroke risk in allele genetic model.

TABLE 5 | Overall and sensitivity analyses of miR-499 A>G (rs3746444) and risk of IS.

| Genetic model | Subgroup      | No. of studies | Meta-analysis | P for Egger’s test | Heterogeneity |
|--------------|--------------|----------------|---------------|--------------------|---------------|
|              |              |                | OR (95%CI)    | P-value            | I² (%)        | P-value       |
| G vs. A      | Overall      | 3              | 1.30 (1.02–1.66) | <0.05              | 0.67          | 52.8          | 0.12          |
|              | HWE-Yes      | 2              | 1.48 (1.20–1.83) | <0.05              |              | 0             | 0.84          |
| GG vs. AA    | Overall      | 3              | 1.69 (0.92–3.10) | 0.09               | –             | 18.6          | 0.27          |
|              | HWE-Yes      | 2              | 1.69 (0.92–3.10) | 0.09               |              | 18.6          | 0.27          |
| AG vs. AA    | Overall      | 3              | 1.35 (1.01–1.79) | <0.05              | 0.17          | 54.3          | 0.11          |
|              | HWE-Yes      | 2              | 1.56 (1.21–2.02) | <0.05              |              | 0             | 0.67          |
| AG + GG vs. AA| Overall     | 3              | 1.36 (1.02–1.80) | <0.05              | 0.36          | 56.1          | 0.10          |
|              | HWE-Yes      | 2              | 1.58 (1.23–2.02) | <0.05              |              | 0             | 0.93          |
| GG vs. AG + AA| Overall    | 3              | 1.51 (0.82–2.75) | 0.19               | –             | 17.7          | 0.27          |
|              | HWE-Yes      | 2              | 1.51 (0.82–2.75) | 0.19               |              | 17.7          | 0.27          |

OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

adjustment of conflicting factors. More explorations in more diverse geographically regions with large sample size are expected to confirm the conclusions.

The present meta-analysis did not find association between miR-149 T>C (rs2292832), miR-196a2 T>C (rs11614913), and IS risk under all genetic models. In fact, no study has elucidated that the genetic polymorphism of miR-196a2 T>C (rs11614913) is associated with stroke. For miR-149 T>C (rs2292832), it might be spurious result because the results were on basis of only one study with limited participants. Whether miR-149 T>C (rs2292832), miR-196a2 T>C (rs11614913), are related to IS risk or not is an interesting question for future investigation. Furthermore, the two SNPs mentioned have been demonstrated to be related to other human diseases, including cancer (Hu et al., 2008), congenital heart disease (Xu et al., 2009) and coronary heart disease (Sung et al., 2016), or correlated with IS when they are combined with other SNPs, suggesting that these polymorphisms tend to influence different human diseases in various ways as well as reflecting different genetic or etiological factors for different diseases.

In addition, some studies explored the gene–gene, gene-environment interaction between miRNA polymorphism and IS risk. Jeon et al. (2013) constructed all possible allele combinations and found miR-146a G/-149 T/-196a2 C/-499 G, miR-146a C/-196a2 T/-499 A, miR-146a C/-196a2T were significantly associated with disease prevalence in Korea. Huang et al. (2015)
focused on the combined effects between the stroke-associated polymorphism (miR-146a) and fasting glucose, HDL-c, and LDL-c levels on the risk of IS and found that the combined effects between miRNA polymorphism and fasting glucose/blood lipid levels may contribute to stroke pathogenesis.

To our knowledge, this is the first meta-analysis that evaluates the association between these four polymorphisms and risk of IS in Chinese. Sensitivity analysis by HWE status of controls made it more precise. Therefore, our findings were more reliable than the conclusions of previous studies. These results might provide further implications for evaluating risk of IS.

However, the limitations in our meta-analysis shouldn’t be ignored when interpreting our findings. First of all, the evidence of between study heterogeneity was apparent, which might distort the conclusion of this meta-analysis. Second, the objects in all included studies were totally enrolled from hospitals,
which might not be sufficient to represent the huge Chinese population and widespread distribution nationwide. Thirdly, IS is a complicated and multifactorial disease influenced by both genetic and environmental factors. But the effects of the combination of genetic and environmental factors are difficult to be detected due to the lack of the accurate individual information in studies included. Lastly, a SNP might be in linkage disequilibrium with other genetic variations of stroke susceptibility genes, which may present stronger effect when considered together with other variations.

In conclusion, miR-499 A → G (rs2910164), miR-149 T → C (rs2292832), miR-196a2 T → C (rs11614913) polymorphism and risk of IS. The associations may be different according to geographical factors of China. More explorations in more diverse geographically regions with large sample size are expected to further verify the findings in the future.

AUTHOR CONTRIBUTIONS
W-JL and C-XL: planned and designed the study; HW, C-XL, and JZ: searched the databases and extracted the data; C-XL, HW, and Z-HF: performed statistical analysis; C-XL: drafted the manuscript; W-JL and J-LO: reviewed the manuscript. All authors approved the final paper for submission and publication.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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