Association between polymorphisms in microRNAs and ischemic stroke in an Asian population: evidence based on 6,083 cases and 7,248 controls

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Introduction
Stroke is a significant worldwide problem. An estimated 80% of the patients survive for at least 1 year after stroke, yet >70% have enduring disabilities.1,2 Ischemic stroke (IS) and intracerebral hemorrhage account for ~80–85% and 15~20% of all stroke cases, respectively.3 IS is a complex syndrome whose pathological development involves multiple components, which include environmental and genetic factors.4 Established environmental risk factors include age, sex, body mass index, hypertension, diabetes mellitus, smoking, and hyperlipidemia. However, recent studies suggested that genetics may contribute more than environment to IS, considering that a number of single-gene disorders are related to IS.5–8 Nevertheless, the factors defining genetic susceptibility to IS remain unclear.

MicroRNAs (miRNAs) represent a group of short non-coding RNA molecules, 18–25 nucleotides in length. Bioinformatics data indicate that a single miRNA can bind to as many as 200 gene targets, and miRNAs may regulate the expression of approximately one-third of protein-coding mRNAs. A single-nucleotide polymorphism (SNP) in the miRNA gene can result in a single-nucleotide change in the miRNA sequence, which may lead to a change in the target binding specificity of the miRNA. This change can affect the regulation of target gene expression and, consequently, the risk of disease.

Background: Polymorphisms in miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832) and miR-499 (rs3746444) have been associated with ischemic stroke (IS), but studies have given inconsistent results.

Methods: This meta-analysis investigated the possible association between IS risk and the four polymorphisms. A total of 14 case-control studies from Asian populations involving 6,083 cases and 7,248 controls for the four polymorphisms were included.

Results: Results showed that the GG genotype of miR-146a (rs2910164) may be associated with increased IS risk according to the recessive model (OR=1.20, 95% CI=1.02–1.42, P=0.03). Similarly, the CC genotype of miR-149 (rs2292832) may be associated with increased IS risk according to the recessive model (OR=1.28, 95% CI=1.08–1.52, P=0.005) and the homozygous model (OR=1.31, 95% CI=1.09–1.58, P=0.004). In contrast, miR-196a2 (rs11614913) and miR-499 (rs3746444) polymorphisms did not show significant association with IS risk in any of the five genetic models.

Conclusion: These results indicate that the GG genotype of miR-146a (rs2910164) and CC genotype of miR-149 (rs2292832) may confer increased susceptibility to IS, while miR-196a2 (rs11614913) and miR-499 (rs3746444) polymorphisms may not be associated with IS risk in Asian populations. These conclusions should be verified in large and well-designed studies.

Keywords: miRNAs, polymorphism, ischemic stroke, meta-analysis
(SNP) in miRNA may create a mismatch, leading to gene expression disorder and diseases. Evidence has indicated that miRNAs regulate various IS-related biological processes, such as atherosclerosis, hypertension, and plaque rupture.

In fact, altered miRNA expression has been observed in IS in preclinical animal models and patients, suggesting a potential role in predicting the diagnosis and prognosis of IS.

More specifically, the literature suggests an association between IS and polymorphisms in miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444). However, these associations are controversial because individual studies relied on relatively small samples. Therefore, to obtain a more comprehensive understanding of the available evidence, we conducted this meta-analysis of 14 case–control studies to evaluate the possible association between IS risk and miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444) in Asian populations.

Materials and methods

Search strategy
All clinical and experimental case–control studies of miRNA polymorphisms and IS risk published through February 1, 2018 were identified through systematic searches in PubMed, EMBASE, Google Scholar, and the Chinese National Knowledge Infrastructure (CNKI) databases using English and Chinese. The search terms used were as follows: microRNA; miRNA; these two terms in combination with polymorphism, polymorphisms, SNP, variant, variants, variation, genotype, genetic, or mutation; and all the above-mentioned terms in combination with stroke or ischemic stroke. Reference lists in identified articles and reviews were also searched manually to identify additional eligible studies.

Inclusion criteria
To be included in our review and meta-analysis, studies had to 1) have a case–control design for assessing the association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444); 2) be accessible as a full-text article and report sufficient data for estimating ORs with 95% CIs; 3) report genotype frequencies; and 4) involve humans rather than animal models.

Data extraction
Two authors (DHZ and CBL) independently extracted the following data from the included studies: first author’s family name, year of publication, ethnicity, testing methods, control source, age, sex, P-value for Hardy–Weinberg equilibrium (HWE) in controls, numbers and genotypes of cases and controls, and frequencies of genotypes in cases and controls. Discrepancies were resolved by consensus. Only those studies that met the predetermined inclusion criteria were included.

Assessment of methodological quality
To assess the quality of the studies included in this analysis, the Newcastle–Ottawa scale was used by two independent assessors (JRW and LC). For the Newcastle–Ottawa scale, a full score is nine stars; a score range of 5–9 stars is considered to indicate generally high methodological quality, whereas a range of 0–4 stars is considered to indicate poor quality.

The quality of all the included studies is summarized in Table 1. Any disagreements about Newcastle–Ottawa scores were resolved by other authors following a comprehensive reassessment. Only high-quality studies were included in our meta-analysis.

Statistical analyses
The unadjusted OR with 95% CI was used to assess the strength of the association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444) based on genotype frequencies in cases and controls. The significance of pooled ORs was determined using the Z-test, with P<0.05 defined as the significance threshold. Meta-analysis was conducted using a fixed-effect model when P>0.10 for the Q-test, indicating the lack of heterogeneity among studies; otherwise, a random-effect model was used. All these statistical tests were performed using Review Manager 5.2 (Cochrane Collaboration, Oxford, England).

Publication bias was assessed using Begg’s funnel plot and Egger’s weighted regression, with P<0.05 considered statistically significant. Begg’s funnel plots and Egger’s weighted regression were calculated using Stata 12.0 (StataCorp LP, College Station, TX, USA).

Results

Description of studies
Figure 1 is a flow diagram illustrating the process of searching for and selecting studies. A total of 184 potentially relevant publications up to February 1, 2018 were systematically identified through searches of the PubMed, EMBASE, Google Scholar, and CNKI databases in English.
| Study                | Selection (score) | Comparability (score) | Exposure (score) | Total score<sup>b</sup> |
|---------------------|-------------------|-----------------------|------------------|------------------------|
| Adequate definition of patient cases | Representativeness of controls | Selection of controls | Definition of controls | Control for important factor or additional factor | Ascertainment of exposure (blinding) | Same method of ascertainment for participants | Non-response rate<sup>a</sup> |
| Sun<sup>13</sup>    | 1                 | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Li<sup>4</sup>      | 1                 | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 5          |
| He and Han<sup>15</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Jeon et al<sup>16</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Hu et al<sup>17</sup> | 1               | 1                     | 0                | 1                       | 1                      | 0                  | 1                  | 1 | 6          |
| Liu et al<sup>18</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Zhu et al<sup>19</sup> | 1                | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Huang et al<sup>20</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Zhong et al<sup>21</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Qu et al<sup>22</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 5          |
| Lyu et al<sup>23</sup> | 1                | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Zhu<sup>24</sup>    | 1                 | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Luo et al<sup>25</sup> | 1               | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 7          |
| Zhu et al<sup>26</sup> | 1                | 1                     | 0                | 1                       | 2                      | 0                  | 1                  | 1 | 6          |

Notes: <sup>a</sup>When there was no significant difference in the response rate between both groups based on a chi-squared test ($P>0.05$), one point was awarded. <sup>b</sup>Total score was calculated by adding up the points awarded in each item.
184 potentially relevant studies identified through PubMed, EMBASE, Google Scholar, and the Chinese National Knowledge Infrastructure databases using English and Chinese up to February 1, 2018

161 excluded during first screening by titles and abstracts

23 potentially relevant studies included for full-text analysis

Nine excluded with reasons
Not in English or Chinese (n = 2)
Not case–control study (n = 2)
Not the concerned miRNA polymorphisms (n = 3)
Lack of precise genotypes (n = 2)

14 studies included in the meta-analysis

Number of studies included for each polymorphism
For miR-146a (rs2910164) (n = 13)
For miR-196a2 (rs11614913) (n = 7)
For miR-149 (rs2292832) (n = 6)
For miR-499 (rs3746444) (n = 7)

Figure 1 Flowchart of study selection.

and Chinese. Of these, we excluded 161 studies during initial screening based on review of the titles and abstracts. During analysis of the full text of the remaining articles, two studies were excluded for not being case–control studies, three studies were excluded because they did not report precise genotypes, and two articles were excluded because they investigated polymorphisms of miRNAs other than miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), or miR-499 (rs3746444). A further two studies were excluded because they were not written in English or Chinese.

In the end, 14 studies13–26 were included in this meta-analysis based on our search strategy and inclusion criteria. Their characteristics are summarized in Table 2. Of these, 13 studies13,14,16–26 (Table 3) involving 5,726 cases and 7,175 controls evaluated the association between miR-146a (rs2910164) polymorphism and IS risk. Seven studies16,18–20,24–26 (Table 3) involving 3,090 cases and 3,047 controls evaluated the association between miR-196a2 (rs11614913) polymorphism and IS risk. Six studies15–17,24–26 (Table 3) involving 2,448 cases and 2,322 controls evaluated miR-149 (rs2292832) polymorphism and IS risk. The remaining seven studies16,18,20,23–26 (Table 3) involving 3,082 cases and 3,044 controls evaluated miR-499 (rs3746444) polymorphism and IS risk. The distribution of genotypes in controls was consistent with HWE (P > 0.05) in all but three studies.14,20,22 The overall quality of the included studies was adequate, and the mean Newcastle–Ottawa score for the included studies was 6.57 (Table 1).

Quantitative data synthesis
IS risk and miR-146a (rs2910164) polymorphism
The overall results for miR-146a (rs2910164) are summarized in Table 4 and Figure 2. On the basis of 5,726 cases and 7,175 controls from 13 studies,13,14,16–26 the overall results indicated that the GG genotype of miR-146a (rs2910164) may be associated with increased IS risk according to the recessive model (OR = 1.20, 95% CI = 1.02–1.42, P = 0.03; Figure 2B).

Table 2 Characteristics of the studies included in the meta-analysis

| Study            | Year | Ethnicity | Country | Testing method | Control source                    | Age (years, mean±SD) | Male, n (%) | SNP                  |
|------------------|------|-----------|---------|----------------|-----------------------------------|----------------------|-------------|----------------------|
| Sun13            | 2011 | Asian     | China   | PCR-RFLP       | Hospital-based healthy volunteers | 63±12                | 236 (61.9)  | miR-146a (rs2910164) |
| Li14             | 2010 | Asian     | China   | PCR-RFLP       | Hospital-based healthy volunteers | 64±11                | 188 (67.2)  | miR-146a (rs2910164) |
| He and Han15     | 2013 | Asian     | China   | PCR-RFLP       | Hospital-based healthy volunteers | 65.7±11.5            | 205 (55.0)  | miR-149 (rs2292832)  |
| Jeon et al16     | 2013 | Asian     | South Korea | TaqMan         | Hospital-based healthy volunteers | 64.16±11.90         | 336 (49.6)  | miR-146a (rs2910164); miR-196a2 (rs11614913); |
| (Continued)      |      |           |         |                |                                   |                      |             | (Continued)          |

(Continued)
Table 2 (Continued)

| Study   | Year | Ethnicity | Country | Testing method | Control source | Age (years, mean±SD) | Male, n (%) | SNP |
|---------|------|-----------|---------|----------------|----------------|----------------------|-------------|-----|
| Hu et al17 | 2014 | Asian     | China   | PCR-RFLP       | Hospital-based healthy volunteers | 64±11.7 | 63±10.5 | 94 (48.0) | 95 (46.3) |
| Liu et al18 | 2014 | Asian     | China   | PCR-RFLP       | Hospital-based healthy volunteers | 67.52±10.29 | 66.34±11.07 | 227 (58.06) | 180 (60.81) |
| Zhu et al19 | 2014 | Asian     | China   | PCR-LDR        | Hospital-based healthy volunteers | 61.62±0.986 | 62.05±0.982 | 253 (68.75) | 261 (68.50) |
| Huang et al20 | 2015 | Asian     | China   | TaqMan         | Hospital-based healthy volunteers | 63 (54–70)* | 61 (54–68)* | 327 (61.6) | 327 (61.6) |
| Zhong et al21 | 2016 | Asian     | China   | PCR            | Hospital-based healthy volunteers | 62.6±8.63 | 61.1±9.58 | 177 (59.6) | 170 (56.7) |
| Qu et al22  | 2016 | Asian     | China   | PCR-LDR        | Hospital-based healthy volunteers | 61.30±9.40 | 59.50±8.50 | 718 (63.0) | 903 (57.0) |
| Lyu et al23 | 2016 | Asian     | China   | TaqMan         | Hospital-based healthy volunteers | 58±11.9 | 58±11.9 | 210 (55.6) | 210 (55.6) |
| Zhu24       | 2016 | Asian     | China   | PCR-RFLP       | Hospital-based healthy volunteers | 63.74±4.49 | 63.31±4.84 | 215 (54.3) | 202 (53.4) |
| Luo et al25 | 2017 | Asian     | China   | PCR            | Hospital-based healthy volunteers | 67.70±12.33 | 60.17±10.32 | 196 (65.8) | 181 (59.8) |
| Zhu et al26 | 2017 | Asian     | China   | TaqMan         | Hospital-based healthy volunteers | 61.0±10.2 | 59.7±9.9 | 321 (62.9) | 311 (59.4) |

Note: *These data are expressed as median (25th, 75th quartiles).

Abbreviations: LDR, ligase detection reaction; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single-nucleotide polymorphism.
| Study      | Year | P-value for HWE          | Sample size (cases/controls) | No of cases | Allele frequencies of cases, n (%) | No of controls | Allele frequencies of controls, n (%) |
|------------|------|--------------------------|-----------------------------|-------------|-----------------------------------|----------------|---------------------------------------|
| miR-146a   |      |                          |                             |             |                                   |                |                                       |
| Sun et al  | 2011 | 0.345                    | 358/650                     | 136 161     | 433 (60.5)                         | 283 (39.5)     |                                        |
| Li et al   | 2010 | 0.009                    | 268/1,010                   | 79 110      | 268 (50.0)                         | 268 (50.0)     |                                        |
| Jeon et al | 2013 | 0.589                    | 678/553                     | 223 327     | 773 (57.0)                         | 583 (43.0)     |                                        |
| Hu et al   | 2014 | 0.193                    | 196/205                     | 75 87       | 237 (60.5)                         | 155 (39.5)     |                                        |
| Liu et al  | 2014 | 0.650                    | 296/391                     | 85 159      | 329 (55.6)                         | 263 (44.4)     |                                        |
| Zhu et al  | 2014 | 0.952                    | 368/381                     | 145 173     | 463 (63.0)                         | 273 (37.0)     |                                        |
| Huang et al| 2015 | 0.106                    | 531/531                     | 189 261     | 639 (60.2)                         | 423 (39.8)     |                                        |
| Zhong et al| 2016 | 0.133                    | 297/300                     | 141 128     | 410 (69.0)                         | 184 (31.0)     |                                        |
| Qu et al   | 2016 | <0.001                   | 1,139/1,585                 | 355 618     | 1,328 (58.3)                       | 950 (41.7)     |                                        |
| Lyu et al  | 2016 | 0.079                    | 378/378                     | 119 198     | 436 (57.7)                         | 320 (42.3)     |                                        |
| Zhu et al  | 2016 | 0.521                    | 396/378                     | 131 194     | 456 (57.6)                         | 336 (42.4)     |                                        |
| Luo et al  | 2017 | 0.672                    | 298/303                     | 129 130     | 388 (65.1)                         | 208 (34.9)     |                                        |
| Zhu et al  | 2017 | 0.085                    | 523/510                     | 170 267     | 607 (58.0)                         | 439 (42.0)     |                                        |
| miR-196a2  |      |                          |                             |             |                                   |                |                                       |
| Jeon et al | 2013 | 0.126                    | 678/553                     | 139 352     | 630 (46.5)                         | 726 (53.5)     |                                        |
| Liu et al  | 2014 | 0.060                    | 296/391                     | 51 181      | 283 (47.8)                         | 309 (52.2)     |                                        |
| Zhu et al  | 2014 | 0.384                    | 368/381                     | 71 189      | 331 (45.0)                         | 405 (55.0)     |                                        |
| Huang et al| 2015 | 0.856                    | 531/531                     | 100 265     | 465 (43.8)                         | 597 (56.2)     |                                        |
| Zhu et al  | 2016 | 0.354                    | 396/378                     | 112 205     | 429 (54.2)                         | 363 (45.8)     |                                        |
| Luo et al  | 2017 | 0.385                    | 298/303                     | 73 138      | 284 (47.7)                         | 312 (52.3)     |                                        |
| Zhu et al  | 2017 | 0.548                    | 523/510                     | 150 273     | 573 (54.8)                         | 473 (45.2)     |                                        |
| miR-149    |      |                          |                             |             |                                   |                |                                       |
| He and Han | 2013 | 0.303                    | 357/373                     | 138 162     | 438 (66.6)                         | 276 (41.4)     |                                        |
| Jeon et al | 2013 | 0.921                    | 678/553                     | 299 303     | 961 (45.5)                         | 545 (54.5)     |                                        |
| Hu et al   | 2014 | 0.199                    | 196/205                     | 79 76       | 234 (59.7)                         | 158 (40.3)     |                                        |
| Zhu et al  | 2016 | 0.720                    | 396/378                     | 165 179     | 509 (63.5)                         | 283 (36.5)     |                                        |
| Luo et al  | 2017 | 0.447                    | 298/303                     | 131 127     | 389 (65.3)                         | 207 (34.7)     |                                        |
| Zhu et al  | 2017 | 0.351                    | 523/510                     | 232 221     | 685 (65.5)                         | 361 (34.5)     |                                        |
| miR-499    |      |                          |                             |             |                                   |                |                                       |
| Jeon et al | 2013 | 0.740                    | 678/553                     | 460 195     | 1,115 (82.2)                       | 241 (17.8)     |                                        |
| Liu et al  | 2014 | 0.170                    | 296/391                     | 181 96      | 458 (77.4)                         | 134 (22.6)     |                                        |
| Huang et al| 2015 | 0.002                    | 531/531                     | 398 133     | 929 (87.5)                         | 133 (12.5)     |                                        |
| Lyu et al  | 2016 | 0.621                    | 378/378                     | 257 110     | 624 (82.5)                         | 132 (17.5)     |                                        |
| Zhu et al  | 2016 | 0.910                    | 396/378                     | 255 123     | 633 (79.9)                         | 159 (20.1)     |                                        |
| Luo et al  | 2017 | 0.131                    | 298/303                     | 215 78      | 508 (85.2)                         | 88 (14.8)      |                                        |
| Zhu et al  | 2017 | 0.380                    | 505/510                     | 349 124     | 840 (80.3)                         | 206 (19.7)     |                                        |

Abbreviation: HWE, Hardy–Weinberg equilibrium.

Table 4 Overall meta-analysis of the association between ischemic stroke and polymorphisms in miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444)

| Genetic model         | OR [95% CI] | Z (P-value) | Heterogeneity of study design | Analysis model |
|-----------------------|-------------|-------------|--------------------------------|----------------|
| Allelic model         | 1.10 [0.99–1.22] | 1.74 (0.08) | 47.91 | Random |
| Recessive model       | 1.20 [1.02–1.42] | 2.16 (0.03) | 31.55 | Random |
| Dominant model        | 0.91 [0.80–1.04] | 1.41 (0.16) | 34.76 | Random |
| Homozygous model      | 1.24 [1.00–1.53] | 1.95 (0.05) | 43.43 | Random |
| Heterozygous model    | 1.06 [0.95–1.17] | 1.00 (0.32) | 20.79 | Random |

(Continued)
Table 4 (Continued)

| Genetic model | OR [95% CI] | Z (P-value) | Heterogeneity of study design | Analysis model |
|---------------|-------------|-------------|-------------------------------|----------------|
|               |             |             |                               | 1(df) df (P-value) I² (%) |
| miR-196a2 (rs11614913) from 7 case–control studies (3,090 cases and 3,047 controls) | | | | |
| Allelic model (C-allele vs T-allele) | 1.04 [0.97–1.12] | 1.10 (0.27) | 3.20 6 (0.78) | 0 Fixed |
| Recessive model (CC vs TC+TT) | 1.04 [0.93–1.17] | 0.73 (0.46) | 4.60 6 (0.60) | 0 Fixed |
| Dominant model (TT vs TC+CC) | 0.95 [0.85–1.08] | 0.77 (0.44) | 2.86 6 (0.83) | 0 Fixed |
| Homozygous model (CC vs TT) | 1.07 [0.92–1.24] | 0.91 (0.36) | 2.85 6 (0.83) | 0 Fixed |
| Heterozygous model (TC vs TT) | 1.07 [0.93–1.23] | 0.90 (0.37) | 2.72 5 (0.74) | 0 Fixed |
| miR-149 (rs2292832) from 6 case–control studies (2,448 cases and 2,322 controls) | | | | |
| Allelic model (C-allele vs T-allele) | 1.09 [1.00–1.18] | 1.91 (0.06) | 4.84 5 (0.44) | 0 Fixed |
| Recessive model (CC vs TC+TT) | 1.28 [1.08–1.52] | 2.80 (0.005) | 6.14 5 (0.29) | 19 Fixed |
| Dominant model (TT vs TC+CC) | 0.89 [0.79–1.00] | 1.99 (0.05) | 6.31 5 (0.28) | 21 Fixed |
| Homozygous model (CC vs TT) | 1.31 [1.09–1.58] | 2.92 (0.004) | 8.27 5 (0.14) | 40 Fixed |
| Heterozygous model (TC vs TT) | 1.07 [0.95–1.21] | 1.12 (0.26) | 4.22 5 (0.52) | 0 Fixed |
| miR-149 (rs3746444) from 7 case–control studies (3,082 cases and 3,044 controls) | | | | |
| Allelic model (G-allele vs A-allele) | 1.09 [0.95–1.25] | 1.28 (0.20) | 12.36 6 (0.05) | 51 Random |
| Recessive model (GG vs AG+AA) | 1.21 [0.91–1.61] | 1.31 (0.19) | 3.81 5 (0.58) | 0 Fixed |
| Dominant model (TT vs TC+CC) | 0.93 [0.78–1.12] | 0.77 (0.44) | 16.43 6 (0.01) | 63 Random |
| Homozygous model (GG vs AA) | 1.20 [0.90–1.60] | 1.25 (0.21) | 4.47 5 (0.48) | 0 Fixed |
| Heterozygous model (AG vs AA) | 1.06 [0.87–1.28] | 0.56 (0.57) | 17.10 6 (0.009) | 65 Random |

IS risk and miR-196a2 (rs11614913) polymorphism

The overall results are summarized in Table 4 and Figure 3. On the basis of 3,090 cases and 3,047 controls from seven studies,16,18–20,24–26 miR-196a2 (rs11614913) polymorphism did not show significant association with IS risk in any of the following five genetic models: allelic model, OR=1.04, 95% CI=0.97–1.12, P=0.27 (Figure 3A); recessive model, OR=1.04, 95% CI=0.93–1.08, P=0.44 (Figure 3B); dominant model, OR=0.95, 95% CI=0.85–1.12, P=0.44 (Figure 3C); homozygous model, OR=0.95, 95% CI=0.85–1.08, P=0.44 (Figure 3D); and heterozygous model, OR=1.07, 95% CI=0.93–1.23, P=0.37 (Figure 3E).

IS risk and miR-149 (rs2292832) polymorphism

The overall results for miR-149 (rs2292832) are summarized in Table 4 and Figure 4. On the basis of 2,448 cases and 2,322 controls, the overall results showed significant association with IS risk in the following five genetic models: allelic model, OR=1.09, 95% CI=1.00–1.18, P=0.06 (Figure 4A); recessive model, OR=1.28, 95% CI=1.08–1.52, P=0.005 (Figure 4B); dominant model, OR=0.89, 95% CI=0.79–1.00, P=0.19 (Figure 4C); homozygous model, OR=1.31, 95% CI=0.92–1.84, P=0.04 (Figure 4D); and heterozygous model, OR=1.07, 95% CI=0.79–1.21, P=0.12 (Figure 4E).
Figure 2 (Continued)
Polymorphisms in miRNAs and IS risk

Figure 2 Forest plot describing the association between the miR-146a (rs2910164) polymorphism and ischemic stroke risk according to different genetic models: (A) allelic (G-allele vs C-allele), (B) recessive (GG vs GC+CC), (C) dominant (CC vs GC+GG), (D) homozygous (GG vs CC), and (E) heterozygous (GC vs CC).

Figure 3 (Continued)
**Figure 3** Forest plot describing the association between the miR-196a2 \((rs11614913)\) polymorphism and ischemic stroke risk according to different genetic models: (A) allelic, (B) recessive, (C) dominant, (D) homozygous, and (E) heterozygous.
Polymorphisms in miRNAs and IS risk

2,322 controls from six studies,16,18,20,23–26 the overall results indicated that the CC genotype of miR-149 (rs2292832) may be associated with increased IS risk according to the recessive model (OR=1.28, 95% CI=1.08–1.52, P=0.005; Figure 4B) and homozygous model (OR=1.31, 95% CI=1.09–1.58, P=0.004; Figure 4D).

IS risk and miR-499 (rs3746444) polymorphism

The overall results are summarized in Table 4 and Figure 5. On the basis of 3,082 cases and 3,044 controls from seven studies,16,18,20,23–26 miR-499 (rs3746444) polymorphism did not show significant association with IS risk in any of the following five genetic models: allelic model, OR=1.09, 95% CI=0.95–1.25, P=0.20 (Figure 5A); recessive model, OR=1.21, 95% CI=0.91–1.61, P=0.19 (Figure 5B); dominant model, OR=0.93, 95% CI=0.78–1.12, P=0.44 (Figure 5C); homozygous model, OR=1.20, 95% CI=0.90–1.60, P=0.21 (Figure 5D); or heterozygous model, OR=1.06, 95% CI=0.87–1.28, P=0.57 (Figure 5E).

Sensitivity analysis

Sensitivity analysis was conducted for miR-146a (rs2910164) by excluding the studies by Li et al14 and Qu et al;22 the P-value for HWE was less than 0.05 for these two studies. The recessive model gave different results (OR=1.19, 95% CI=0.98–1.45, P=0.07) than those obtained when all studies were meta-analyzed. Sensitivity analysis was conducted for miR-146a (rs2910164) by excluding one study by Jeon et al.16 Again, the recessive model gave different results (OR=1.18, 95% CI=0.99–1.41, P=0.07) than when all studies were included. Therefore, the results for miR-146a (rs2910164) should be interpreted with caution.

Sensitivity analysis was conducted for miR-196a2 (rs11614913) by excluding the study by Jeon et al.16 The results were similar to those obtained with all studies.

Figure 4 (Continued)
regardless of the genetic model. This implies that our meta-analysis results for miR-196a2 (rs11614913) are robust. Similar robustness was observed when we performed sensitivity analysis for miR-149 (rs2292832) and for miR-499 (rs3746444) by excluding the study by Jeon et al.  

Sensitivity analysis was conducted for miR-499 (rs3746444) by excluding a study by Huang et al., 20 in which the P-value of HWE was less than 0.05. The results were not altered in any of the five genetic models.

**Figure 4** Forest plot describing the association between the miR-149 (rs2292832) polymorphism and ischemic stroke risk according to different genetic models: (A) allelic, (B) recessive, (C) dominant, (D) homozygous, and (E) heterozygous.

Publication bias

Begg’s funnel plot and Egger’s test were performed to detect potential publication bias in this meta-analysis. No obvious asymmetry was observed in Begg’s funnel plots in
the recessive model, and Egger’s tests (Figure 6) indicated no publication bias.

**Discussion**

Previous studies have demonstrated that mutations in the pre-miRNA of miR-146a, miR-499, miR-149, and miR-196a2 decrease the levels of the corresponding mature miRNAs.\(^{20,29,30}\) These four miRNAs affect thrombosis or inflammation pathways in the circulatory system by regulating tumor necrosis factor-α (TNF-α),\(^{31}\) methylenetetrahydrofolate reductase,\(^{32}\) annexin A1,\(^{33}\) C-reactive protein,\(^{34}\) the NF-κB pathway, and the MAP kinase pathway.\(^{35}\) Many studies have been conducted to reveal the impact of SNPs on precursor and mature miRNAs and their associations with IS risk.\(^{13–26}\) In fact, several meta-analyses have been conducted to explore the association between miRNA polymorphisms and IS risk. The results have been inconsistent, largely due to limited sample size.\(^{36–39}\) Therefore, we conducted this meta-analysis on all eligible studies to provide a more precise estimate of the association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444). Interestingly, all the case–control studies in our meta-analysis analyzed Asian populations.

A previous meta-analysis by Zhu et al\(^{39}\) found the C allele of miR-146a (rs2910164) to be associated with lower IS risk, but this trend was observed only in Koreans according to the allelic model. Our meta-analysis, in contrast, suggests that this C allele is not significantly associated with IS risk; instead, we found the GG genotype of miR-146a (rs2910164) to be associated with increased risk. Our result may be more reliable than that of the previous meta-analysis by Zhu et al\(^{39}\) because our meta-analysis contained nine more case–control studies\(^{14,15,17,21–26}\) with larger samples.

## Table A

| Study or subgroup | Events | Total Events | Total | Weight (%) | OR M-H, random, 95% CI | OR M-H, random, 95% CI |
|------------------|--------|--------------|-------|------------|------------------------|------------------------|
| Huang et al (2015)\(^{20}\) | 133    | 1,062 128    | 1,062 | 14.1       | 1.04 (0.81–1.35)        |                        |
| Jeon et al (2013)\(^{18}\) | 241    | 1,356 206    | 1,106 | 17.1       | 0.94 (0.77–1.16)        |                        |
| Liu et al (2014)\(^{18}\) | 134    | 592 127      | 782   | 13.5       | 1.51 (1.15–1.98)        |                        |
| Lyu et al (2016)\(^{21}\) | 132    | 756 143      | 756   | 14.0       | 0.91 (0.70–1.18)        |                        |
| Luo et al (2017)\(^{25}\) | 88     | 596 65       | 606   | 10.4       | 1.44 (1.02–2.03)        |                        |
| Zhu et al (2017)\(^{26}\) | 206    | 1,046 206    | 1,020 | 16.5       | 0.97 (0.78–1.20)        |                        |
| Zhu (2016)\(^{24}\) | 159    | 792 142      | 756   | 14.5       | 1.09 (0.84–1.40)        |                        |

Total (95% CI) 6,200 6,088 100 1.09 (0.94–1.25)

**Figure 5 (Continued)**
**Figure 5** Forest plot describing the association between the miR-149 (rs2292832) polymorphism and ischemic stroke risk according to different genetic models: (A) allelic, (B) recessive, (C) dominant, (D) homozygous, and (E) heterozygous.
between the C allele of miR-146a (rs2910164) and lower IS risk contained only one case–control study, which was by Jeon et al.16 While the meta-analysis by Zhu et al39 reported an association between the A allele of miR-499 (rs3746444) and decreased IS risk in Chinese, our meta-analysis did not detect this association, either across Asian populations or specifically in the Chinese population (data not shown). Our result may be more reliable because our meta-analysis included four more case–control studies23–26 than the one by Zhu et al.39 The results of our meta-analysis are consistent with those reported in the meta-analysis by Xiao et al.37

Figure 6 (Continued)
Our meta-analysis suggests a significant association between the CC genotype of miR-149 (rs2292832) and increased IS risk. In contrast, the meta-analysis of Xiao et al.\(^{37}\) based on two case–control studies indicated that the TT genotype and T allele of miR-149 (rs2292832) are associated with significantly lower IS risk, whereas another meta-analysis\(^{36}\) based on three case–control studies found the CC genotype and C allele of miR-149 (rs2292832) to be significantly associated with IS risk. Our meta-analysis contained three more case–control studies\(^ {24–26}\) than either of these other meta-analyses, which may make it more reliable.

Our meta-analysis did not find a significant association between miR-196a2 (rs11614913) polymorphism and IS risk. This result confirms other meta-analyses\(^ {37–39}\) based on smaller samples.

To the best of our knowledge, the current meta-analysis involves the largest sample (6,083 cases and 7,248 controls) than previous studies\(^ {36–39}\) investigating the possible association of IS risk with miR-146a (rs2910164), miR-196a2 (rs11614913), miR-149 (rs2292832), and miR-499 (rs3746444) in Asian populations. Nevertheless, the meta-analysis is limited by the designs of the included studies. First, the \(P\)-value for HWE was less than 0.05 in two studies\(^ {14,22}\) on miR-146a (rs2910164) and one study\(^ {26}\) on miR-499 (rs3746444). These results suggested that these study populations may not be representative of the broader target population. Second, the results may be affected by both genetic and environmental factors, but most studies did not report environmental exposure, making it impossible to include them in the meta-analysis. Third, our exclusion of unpublished data and of papers published in languages other than English and Chinese may have biased our results. Fourth, the studies may be subject to performance bias, attrition bias, and reporting bias, although Newcastle–Ottawa scores were at least 5 for all 14 studies, indicating high quality. Fifth, stroke is a heterogeneous disease and has different subtypes that may affect the results of genetic association studies, but most case–control studies in our meta-analysis appeared not to use a well-phenotyped population. This may make the results less accurate. Finally, all the patients in this meta-analysis were Asian and this may limit the relevance of the results to other populations. Thus, more large and well-designed studies are warranted in non-Asian populations.

**Conclusion**

This meta-analysis suggests that the GG genotype of miR-146a (rs2910164) and the CC genotype of miR-149 (rs2292832) may confer increased susceptibility to IS in Asian populations, whereas polymorphism in miR-196a2 (rs11614913) and miR-499 (rs3746444) may not be associated with IS risk. These conclusions should be verified in large and well-designed studies.

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**Author contributions**

The study was designed by JRW and LC. The research was performed by DHZ, CBL, and QZ. Statistical analyses were
performed by XFL, GQ, QH, and YSM. The manuscript was written by DHZ. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure
The authors report no conflicts of interest in this work.

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