Associations between miR-146a rs2910164 polymorphisms and risk of ischemic cardio-cerebrovascular diseases

Dongdong Zhao, MD\textsuperscript{a}, Yuerong Li, MD\textsuperscript{a}, Xiuyan Yu, MD\textsuperscript{a}, Yuezhi Zhu, MD\textsuperscript{b}, Baoxin Ma, PhD\textsuperscript{a,*}

Abstract

Background: Many studies investigated the association between miR-146a rs2910164 polymorphisms and risk of ischemic cardio-cerebrovascular diseases. However, the results were inconsistent.

Methods: We searched the PubMed, EMBASE, Cochrane library, Web of Science, Chinese National Knowledge Infrastructure, VIP, and Wanfang databases for appropriate studies. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the associations. Heterogeneity, sensitivity, and publication bias were conducted to measure the robustness of our findings.

Results: We conducted a meta-analysis to evaluate the relationship between miR-146a rs2910164 polymorphisms and risk of ischemic cardio-cerebrovascular diseases. A total of 26 related studies involving 11,602 cases and 14,016 controls were identified and included in our meta-analysis. After considering the heterogeneity of the global analysis, we inferred that rs2910164 polymorphisms were associated with a lower risk of coronary heart disease (CHD) significantly in all genetic models. In addition, it was also found that the miR-146a rs2910164 polymorphisms were associated with the low risk of ischemic cardio-cerebrovascular diseases in large sample size subgroup analysis.

Conclusion: These results indicate that miR-146a rs2910164 polymorphisms were significantly associated with a lower risk of ischemic cardio-cerebrovascular. The miR-146a rs2910164 might be recommended as a predictor for susceptibility of ischemic cardio-cerebrovascular diseases.

Abbreviations: ACS = acute coronary syndrome, CAD = coronary artery disease, CHD = coronary heart disease, CI = confidence interval, HWE = Hardy–Weinberg equilibrium, IS = ischemic stroke, OR = odds ratio, SNP = single-nucleotide polymorphism.

Keywords: ischemic cardio-cerebrovascular diseases, meta-analysis, miR-146a, single nucleotide polymorphism

1. Introduction

Ischemic cardio-cerebrovascular disease is a chronic disease that develops imperceptibly throughout life and usually progressing to an advanced stage when symptoms occur.\textsuperscript{[1]} It is a concept of a series of diseases with the same pathological basis and different clinical manifestations, including coronary heart disease (CHD), ischemic cerebrovascular disease, and other diseases.\textsuperscript{[2,3]} Although scientific progress has been made in disease prevention, diagnosis, and treatment, ischemic cardiovascular and cerebrovascular diseases are still the main causes of morbidity and mortality in developed and developing countries.\textsuperscript{[4–6]} It has been estimated that more than 80% of ischemic cardio-cerebrovascular diseases now occur in developing countries.\textsuperscript{[7]} Epistemologically, ischemic cardio-cerebrovascular disease was supposed to be a highly complex disease caused by multiple environmental and genetic risk factors.\textsuperscript{[8]} To date, more and more attention has been paid to the study of susceptibility genes, increasing molecular epidemiological studies also have revealed the important role of genetic factors in ischemic cardio-cerebrovascular diseases.

MicroRNAs (miRNAs and miRs) are endogenous, conservative, small (about 22-nucleotide), single-strand, non-coding RNA molecules, attaching to the 3’ untranslated region (3’-UTR) of target miRNAs, which regulate gene expressions through translation repression or degradation of target genes.\textsuperscript{[9]} Epide-

miological studies demonstrated that miRNA-associated genetic sequence polymorphism played an important role in the development and progression of diseases including neurological disorders, cardiovascular diseases, autoimmune diseases, and cancers.\textsuperscript{[10]} Accumulating evidences have demonstrated that miR-146a participates in inflammatory processes to interfere with the pathology of cardiovascular diseases. In addition, miR-146a also exerts a neuroprotective effect through astrocytes to
downregulated miR-146a in patients with ischemic stroke (IS) in the acute phase.\cite{11,12}

Some studies have examined the associations between miR-146a rs2910164 polymorphisms and ischemic cardio-cerebrovascular diseases. However, the results of these studies were inconsistent due to the limited sample size in a single study. Therefore, we conducted a meta-analysis of all eligible case–control studies to investigate the relationship between single-nucleotide polymorphisms (SNPs) in the miR-146a rs2910164 polymorphisms and ischemic cardio-cerebrovascular diseases.

2. Materials and methods

2.1. Search strategy and eligibility of relevant studies

We conducted a systematic search using the PubMed, EMBASE, Cochrane library, Web of Science, Chinese National Knowledge Infrastructure, VIP, and Wanfang databases. The last search was performed on July 2018, with keywords including (“miR146a” OR “miR-146a” OR “miRNA146a” OR “microRNA146a” OR “rs2910164”) AND (“ischemic cardio-cerebrovascular diseases” OR “ischemic heart and cerebrovascular diseases” OR “ischemic stroke” OR “IS” OR “cerebral ischemic stroke” OR “CIS” OR “cerebral infarction” OR “CI” OR “lacunar infarction” OR “LI” OR “coronary heart disease” OR “CHD” OR “ischemic heart disease” OR “IHD” OR “coronary atherosclerotic heart disease” OR “coronary artery disease” OR “CAD” OR “acute coronary syndrome” OR “ACS” OR “acute myocardial infarction” OR “AMI” OR “myocardial infarction” OR “MI”) AND (“gene” OR “genetic” OR “single-nucleotide polymorphism” OR “SNP” OR “allele” OR “variation” OR “variant” OR “mutation”). We also manually searched the reference lists of relevant reports to identify additional studies. All identified studies that met the inclusion criteria have been assessed by 2 independent reviewers using standardized forms.

2.2. Inclusion and exclusion criteria

Eligible studies should conform with the following criteria: the design was accordance with case–control study; the exposure was miR-146a rs2910164; the outcome was incident of ischemic cardio-cerebrovascular diseases; and study provided sufficient published data to calculate odds ratios (ORs) with corresponding 95% confidence intervals (CIs). Exclusion criteria were as follows: reviews, case reports, meta-analysis, and animal model research; repeated publications; not relevant to ischemic cardio-cerebrovascular diseases; not a case–control study; and lacking sufficient data for quantitative analyses. Moreover, non-English and non-Chinese articles were also excluded.

2.3. Data extraction and quality assessment

Data were extracted independently by 2 reviewers (Zhao and Li). All discrepancies were resolved by discussion with a 3rd reviewer (Ma). The following information was extracted: first author’s name, published year, country of origin and ethnicity of study participants, type of ischemic cardio-cerebrovascular diseases, genotyping methods, number of cases and controls, and genotype frequency of case and control, Hardy–Weinberg equilibrium (HWE) for controls by 2 independent researches (Zhao and Li). Detailed characteristics of included studies were shown in Table 1. We assessed the quality of all included studies according to the Newcastle–Ottawa quality assessment scale.\cite{13} Quality score ranged from 0 to 9, a final score ≥6 was regarded as high quality.

2.4. Statistical analysis

HWE was assessed via Chi-square test in the control populations of each study, which could be considered disequilibrium when \( P \) value is less than .05. Pooled ORs and corresponding 95% CIs were calculated to estimate associations between miR-146a rs2910164 polymorphism and ischemic cardio-cerebrovascular diseases. The strength of the association was determined using the following models: allelic model (G vs C), homozygote model (GG vs CC), heterozygote model (GG vs CG), dominant model (GG + CG vs CC), and recessive model (GG vs CG + CC). Subgroup analyses were conducted by ethnicity (Asian and Caucasian), disease types (IS and CHD subgroups), and sample size (small sample: the total number of controls and cases not less than 1000; large sample: the total number of controls and cases not less than 1000). We used the Cochran Q-statistic and the \( I^2 \) statistic to evaluate statistical heterogeneity among studies.\cite{13} If \( P \) ≥.1 suggested a lack of heterogeneity among studies. A random-effect model (the DerSimonian and Laird method) was used in the presence of heterogeneity (\( P < .1 \)); otherwise, a fixed-effect model using the Mantel–Haenszel method was utilized. We conducted a sensitivity analysis to assess the stability of the results. Furthermore, Begg funnel plot and Egger test were used to evaluate publication bias among included studies. Z-test was used to assess the overall effect and \( P < .05 \) was considered statistically significant difference. Analyses were carried out with Review Manager 5.3 and Stata 12.0 software (Stata Corporation, College Station, TX).

3. Results

3.1. Characteristics of included studies

Figure 1 shows our study selection process. In total, 2540 studies were retrieved from the PubMed, EMBASE, Cochrane library, Web of Science, Chinese National Knowledge Infrastructure, VIP, and Wanfang databases. Of these, 405 duplicates were excluded from this study. Another 1932 studies were excluded after reviewing the titles and abstracts. A total of 203 eligible studies were evaluated for full-text review. A total of 179 of them were excluded due to nonreporting of available data, duplicate data, meta-analyses, and case reports. Twenty-four original articles containing 26 studies were retained after reviewing the full text (Table 1).\cite{14-37}

HWE was assessed via Chi-square test in the control subjects of each study. Genotype distributions among controls were consistent with HWE in only 23 studies.\cite{15-30,32-37} These 23 studies investigated 2 diseases (IS and CHD) and were included in the final meta-analysis. However, there were 3 types of CHDs have been hit after literature review as coronary artery disease (CAD), acute coronary syndrome (ACS), and myocardial infarction. There were 3 types of ISs, which were silent brain infarction, lacunar infarction, and atherosclerotic cerebral infarction (ACI). The 23 studies included 9780 cases and 10411 controls, 12 studies focused on IS and 11 focused on CHD. Subgroup analyses were also conducted by ethnicity (Asian and Caucasian) and sample size (the total number of controls and cases less than 1000 was used as small, otherwise as large).
According to the Newcastle–Ottawa quality assessment scale, the summarized quality scores ranged from 6 to 9, which suggested that the evidence had a good quality.

3.2. Results from meta-analysis

The results of meta-analysis for the association between miR-146a rs2910164 polymorphism and ischemic cardio-cerebrovascular diseases risk are shown in Figures 2 and 3 and Table 2. Twenty-three eligible studies including 9780 cases and 10411 controls were included in this analysis. Heterogeneities were observed in 5 types of genetic models’ analysis. We used the random-effect model to evaluate the association between rs2910164 polymorphisms and the risk of ischemic cardio-cerebrovascular diseases in all genetic models, there was no significant associations based on the global analysis of these studies (allelic model: OR = 0.95, 95% CI = 0.87–1.03, P = .19; homozygote model: OR = 0.90, 95% CI = 0.76–1.07, P = .24; heterozygous model: OR = 0.93, 95% CI = 0.85–1.01, P = .09; dominant model: OR = 0.92, 95% CI = 0.83–1.02, P = .12; and recessive model: OR = 0.95, 95% CI = 0.84–1.08, P = .45) (Fig. 2, Table 2).

However, because of the heterogeneity of the global meta-analysis, we cannot infer that rs2910164 polymorphism was completely unrelated to ischemic cardio-cerebrovascular diseases only from the results of global analysis. Therefore, to reduce the influence of heterogeneity and further clarify the relationship between rs2910164 polymorphisms and the risk of ischemic cardio-cerebrovascular diseases, we performed subgroup analysis based on the type of disease, ethnic origin, and sample size. For the analysis of IS, heterogeneities were observed in allelic (I^2 = 78%, P < .00001), homozygote (I^2 = 79%, P < .00001), dominant (I^2 = 66%, P = .0007), and recessive (I^2 = 72%, P < .0001) models, while there was no significant heterogeneity in heterozygous (I^2 = 34%, P = .12) model. And there was also no significant association among all kinds of genetic models as allelic, homozygote, heterozygous, dominant, and recessive models (allelic model: OR = 1.04, 95% CI = 0.92–1.18, P = .55; homozygote model: OR = 1.08, 95% CI = 0.82–1.42, P = .59; heterozygous model: OR = 1.04, 95% CI = 0.95–1.14, P = .37;
Table 1
Main characteristic of studies included in the meta-analysis.

| Author          | Year | Country | Ethnicity | Disease | Genotyping methods | Sample size (case/control) | GG (case/control) | GG (case/control) | CC (case/control) | CC (case/control) | G (case/control) | C (case/control) | HWE of control | NOS |
|-----------------|------|---------|-----------|---------|-------------------|----------------------------|-------------------|-------------------|-------------------|-------------------|----------------|----------------|----------------|------|
| Li (1)          | 2010 | China   | Asian     | CAD     | PCR-RFLP          | 268/1010                   | 110/465           | 79/210           | 79/345           | 268/875          | 268/1145        | 0.009          | 8               |
| Li (2)          | 2010 | China   | Asian     | CAD     | PCR-RFLP          | 415/1010                   | 184/455           | 149/345          | 349/875          | 482/1145         | 0.009          | 8               |
| Sun             | 2011 | China   | Asian     | CAD     | PCR-RFLP          | 381/650                    | 65/118            | 170/304          | 146/228          | 300/540          | 462/760         | 0.345          | 6               |
| Yang            | 2012 | China   | Asian     | CAD     | Taqman            | 829/917                    | 165/189           | 392/457          | 272/271          | 722/835          | 936/999         | 0.885          | 8               |
| Chen            | 2013 | China   | Asian     | CAD     | Taqman            | 658/658                    | 181/194           | 305/330          | 172/124          | 667/718          | 649/598         | 0.769          | 8               |
| Jeon (1)        | 2013 | Korea   | Asian     | CAD     | Taqman            | 678/552                    | 128/767           | 227/311          | 215/323          | 583/418          | 773/688         | 0.597          | 7               |
| Jeon (2)        | 2013 | Korea   | Asian     | CAD     | SBI               | 373/553                    | 57/77             | 179/266          | 137/211          | 293/418          | 453/688         | 0.597          | 8               |
| Chen            | 2014 | China   | Asian     | MI      | PCR-LDR           | 919/689                    | 269/301           | 463/435          | 187/152          | 101/037          | 837/741         | 0.946          | 8               |
| Harmann         | 2014 | Germany | Caucasian | CAD     | HRM               | 206/200                    | 120/117           | 74/73            | 12/10            | 314/207          | 98/93           | 0.748          | 7               |
| Hu              | 2014 | China   | Asian     | CAD     | PCR-RFLP          | 196/205                    | 34/26             | 87/82            | 75/97            | 155/134          | 237/276         | 0.193          | 6               |
| Li              | 2014 | China   | Asian     | CAD     | SNApshot          | 173/298                    | 15/51             | 85/136           | 73/111           | 115/238          | 231/358         | 0.401          | 6               |
| Liu             | 2014 | China   | Asian     | CAD     | PCR-RFLP          | 296/391                    | 52/77             | 159/198          | 85/116           | 263/352          | 329/430         | 0.650          | 7               |
| Ramkaran        | 2014 | South   | Asian     | CAD     | PCR-RFLP          | 106/100                    | 50/45             | 43/46            | 13/19            | 69/64            | 0.569          | 8               |

Table 2
Meta-analysis of miR-146a rs2910164 polymorphism with ischemic cardio-cerebrovascular diseases.

| N               | OR (95%CI) | P     | GG vs CC | OR (95%CI) | P     | CC vs CC | OR (95%CI) | P     | CC vs CC | OR (95%CI) | P     |
|-----------------|------------|-------|----------|------------|-------|----------|------------|-------|----------|------------|-------|
| Overall         | 26.09(0.89,1.03) | <0.0001 | 0.74 | 0.39(0.83,1.08) | <0.0001 | 0.72 | 0.35(0.93,1.07) | 0.05 | 0.34 | 0.79(0.49,1.31) | 0.0001 |
| HWE             | 23.09(0.87,1.03) | <0.0001 | 0.74 | 0.39(0.83,1.07) | <0.0001 | 0.72 | 0.35(0.93,1.07) | 0.05 | 0.34 | 0.79(0.49,1.31) | 0.0001 |
| Disease         | 12.04(0.92,1.18) | <0.0001 | 0.78 | 0.51(0.92,1.42) | <0.0001 | 0.79 | 0.51(0.92,1.42) | 0.12 | 0.34 | 0.79(0.49,1.31) | 0.0001 |
| CHD Ethnicity   | 11.07(0.82,1.02) | 0.27 | 0.0001 | 0.77(0.69,0.87) | 0.19 | 0.27 | 0.0001 | 0.77(0.69,0.87) | 0.19 | 0.27 | 0.0001 | 0.77(0.69,0.87) | 0.19 |
| Asian           | 21.09(0.86,1.04) | <0.0001 | 0.74 | 0.39(0.93,1.07) | <0.0001 | 0.72 | 0.35(0.93,1.07) | 0.05 | 0.34 | 0.79(0.49,1.31) | 0.0001 |
| Caucasian       | 14.09(0.68,1.03) | 0.00 | 0.0001 | 0.73(0.67,1.14) | 0.00 | 0.0001 | 0.73(0.67,1.14) | 0.00 | 0.0001 | 0.73(0.67,1.14) | 0.00 |

CHD = coronary heart disease, 95%CI = 95% confidence interval, HWE = Hardy-Weinberg equilibrium, IS = ischemic stroke, N = number of comparisons, OR = odds ratio, P = P-value, P0 = P-value of heterogeneity test random-effects model was used when P value for heterogeneity test P<0.1; otherwise, fixed-effect model was used.

F means quality assurance measure of the degree of difference among multiple research effects.
errors. Therefore, we conducted stratified subgroup analysis based on sample sizes to further analyze the relationship between miR-146a rs2910164 polymorphisms and ischemic cardio-cerebrovascular diseases, which showed that miR-146a rs2910164 polymorphisms were associated with decreased risk of ischemic cardio-cerebrovascular diseases in a large sample size (N ≥ 1000) under the heterozygous model (OR = 0.89, 95% CI = 0.82–0.97, P = .009). We found there was no association in small sample size (N < 1000) under all genetic models (allelic model: OR = 0.94, 95% CI = 0.82–1.07, P = .33; homozygote model: OR = 0.87, 95% CI = 0.67–1.14, P = .31; heterozygous model: OR = 0.96, 95% CI = 0.84–1.10, P = .56; dominant model: OR = 0.93, 95% CI = 0.79–1.10, P = .40; and recessive model: OR = 0.91, 95% CI = 0.75–1.11, P = .35) (Table 2).

3.3. Sensitivity and publication bias analyses
Sensitivity analysis was performed by omitting each study to examine the impact on the pooled ORs. The corresponding pooled OR was not significantly altered in all genetic models, suggesting that our meta-analysis results were accurate (Fig. 4). Potential publication bias in the current study was evaluated with Begg funnel plot and Egger test. No obvious asymmetry of funnel plots was observed in any comparisons, which indicated that no publication bias was observed in the current meta-analysis (Fig. 5 and Table 3).

4. Discussion
In the present meta-analysis, we found that the miR-146a polymorphisms were not significantly associated with the risk of
Figure 3. Forest plots of odds ratios for the association between miR-146a rs2910164 and risk of coronary heart disease (CHD). (A) G versus C; (B) GG versus CC; (C) CG versus CC; (D) CG + GG versus CC; and (E) GG versus CG + CC.

Figure 4. Sensitivity analysis of each study performed by omitting each data from the analysis (G vs C).
ischemic cardio-cerebrovascular diseases in all genetic models. Our subgroup study stratified by disease type showed that rs2910164 polymorphisms were associated with CHD. We found an association between these polymorphisms and CAD based on 2 studies evaluated CAD in Caucasian populations, demonstrating that lower risks for GG carriers versus CC carriers, while rs2910164 was not associated with ischemic cardio-cerebrovascular diseases in Asian populations. Subgroup analysis based on sample size also revealed that the CG of rs2910164 was associated with the lower risks of ischemic cardio-cerebrovascular diseases in the large sample group.

Several studies have assessed the effects of miR-146a SNPs on ischemic cardio-cerebrovascular diseases, but the results were inconsistent. Although a recent meta-analysis was performed to reveal the relationship of miR-146a polymorphisms and CAD and IS, little study has been included on ischemic cardiovascular and cerebrovascular diseases. To obtain conclusive results on the relationship between miR-146a polymorphisms and ischemic cardio-cerebrovascular diseases, we included 18 more studies with 8464 cases and 10919 controls for ischemic cardio-cerebrovascular diseases analysis. We found a similar lower CHD risk for the G allele and GG, CG, CG+GG of rs2910164, our meta-analysis also provided further evidence for the lower risk of ischemic cardio-cerebrovascular diseases for CG of rs2910164 in large sample size. In the sensitivity analysis, no significant effects were altered after omitting each study at a time, suggesting that our meta-analysis results were reliable.

Because of the common characteristics diseases such as similar pathophysiology of ischemic events, we combine the cardiovascular and cerebrovascular diseases to analyses and draw corresponding conclusions. Considering that the heterogeneity of the overall meta-analysis, and the risk factors and treatment effects of cardiovascular and cerebrovascular are quite different, we performed subgroup analysis of cardiovascular and cerebrovascular diseases, respectively. We observed that rs2910164 was associated with the risk of ischemic cardio-cerebrovascular diseases analysis in the large sample group, so the rs2910164 was associated with the risk of CHD. In addition, we also found a lower risk of ischemic cardio-cerebrovascular diseases in Caucasian. However, only 2 studies were included in the Caucasian subgroup and 2 studies focused on CAD. Although the subgroup analysis for Caucasian population in this study

Table 3

| Group        | G vs C | GG vs CC | CG vs CC | CG+GG vs CC | GG vs CG+CC |
|--------------|--------|----------|----------|-------------|-------------|
| Overall      | .673   | .943     | .751     | .850        | .833        | .966        | .717        | .958        | .834        |
| Disease      |        |          |          |             |             |             |             |             |             |
| IS           | .732   | .704     | .945     | .593        | .631        | .735        | .837        | .963        | .732        | .474        |
| CHD          | .436   | .405     | .436     | .263        | .640        | .491        | .161        | .235        | .876        | .683        |
| Sample size  |        |          |          |             |             |             |             |             |             |
| ≥1000        | .602   | .459     | .602     | .436        | .754        | .992        | .466        | .954        | .466        | .322        |
| <1000        | .361   | .659     | .743     | .538        | .511        | .461        | .661        | .425        | .511        | .690        |
| Ethnicity    |        |          |          |             |             |             |             |             |             |
| Asian        | .928   | .847     | .976     | .804        | .566        | .816        | .976        | .992        | .880        | .844        |

P_B = P-value of Begg test, P_E = P-value of Egger test.
found that rs2910164 was associated with the risk of ischemic cardio-cerebrovascular diseases, the conclusions drawn in this subgroup analysis are still not broadly representative, further studies are still needed to confirm the associations.

Ischemic cardio-cerebrovascular diseases are a series of diseases caused by atherosclerosis. Atherosclerosis is a chronic inflammatory disease of the arteries, it is principally a lipid-driven process initiated by the accumulation of lipoprotein particles and an inflammatory process in focal areas of arteries as well.\[18\] Recently, miR-146a was recognized as a potent regulator in many physiological and pathological processes, including immune function, inflammatory reaction, metabolism, oxidative stress, neurodegenerative, and cardiovascular diseases.\[19\] Recent studies have shown that miR-146a can regulate the synthetic phenotype and proliferation of vascular smooth muscle cells by targeting Krüppel-like factor 4.\[40,41\] In addition, Eyleten et al suggested the combination of plasma high-sensitivity C-reactive protein and serum miR-146b gained a better sensitivity/significance for the prediction of IS.\[42\] MiR-146a expression has been reported to mediate inflammatory response and promote astrocyte proliferation.\[43\] In several studies, the expression level of miR-146a in peripheral blood mononuclear cells was significantly increased in patients with CHD.\[44\] The high level of miR-146a concentration in peripheral blood mononuclear cells may directly affect the differentiation and activities of Th1 cells, which has been implicated in the progression and the onset of the ACS.\[23\] Pordzik et al demonstrated that hyperglycemia-associated downregulation of miR-146a mediates platelet activation in diabetics, favoring IS.\[45\] In addition, other studies also revealed that miR-146a could reduce the production of pro-inflammatory cytokines via downregulating interleukin-1 receptor-associated kinase 1 and tumor necrosis factor (TNF) receptor associated factor 6 in macrophages.\[46,47\] Therefore, the down-regulation of miR-146a may increase vascular damage response and inflammation-related atherosclerosis by increasing the levels of interleukin-1 receptor-associated kinase 1, TNF receptor associated factor 6, and TNF-α. SNP rs2910164 involves a C-to-G nucleotide substitution, which can cause a mismatch in the stem structure of the miR-146a precursor, then decrease the expression of miR-146a.

The results of our meta-analysis should be interpreted attentively because of the following limitations. First of all, the relatively small number of patients may affect the outcomes. After a comprehensive literature search, only 26 eligible studies were included. Genotype distributions among controls were consistent with HWE in only 23 studies. Secondly, there was limited information about clinicopathological features or disease subtypes in the original studies, which made it difficult to conduct more subgroup meta-analysis. Thirdly, ischemic cardio-cerebrovascular diseases were multifactorial disease influenced by both genetic and environmental factors, the gene–gene and gene–environment interactions may significantly affect the function of miR-146a rs2910164 SNP. Fourthly, most of the patients in this study were Asian, which would limit the comprehensiveness and veracity of the results. Finally, an SNP might be in linkage disequilibrium with other genetic variations of susceptibility genes to ischemic cardio-cerebrovascular diseases, which may present stronger effect when considered together with other variations.

In conclusion, our meta-analysis suggests the CG genotype of rs2910164 may be a decreased risk of ischemic cardio-cerebrovascular diseases. Thus, we suggest rs2910164 could be recommended as a protective factor for the susceptibility of ischemic cardio-cerebrovascular diseases.

Author contributions
Data curation: Dongdong Zhao.
Formal analysis: Dongdong Zhao, Yuerong Li.
Methodology: Yuerong Li.
Software: Dongdong Zhao, Yuerong Li, Xiuyan Yu, Yuezhi Zhu, Baoxin Ma.
Supervision: Yuerong Li, Yuezhi Zhu.
Writing – original draft: Dongdong Zhao.
Writing – review & editing: Baoxin Ma.

References
[1] Gan Y, Liu Q, Wu W, et al. Ischemic neurons recruit natural killer cells that accelerate brain infarction. Proc Natl Acad Sci U S A 2014;111:2704–9.
[2] Isapoulos C, Kucianski T, Mayr HL, et al. The AUStalian MEDiterranean Diet Heart Trial (AUSMED Heart Trial): a randomized clinical trial in secondary prevention of coronary heart disease in a multiethnic Australian population: study protocol. Am Heart J 2018;203:4–11.
[3] Karikari TK, Ghayour-Mobarad A, Höglund K, et al. Commentary: global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Front Neurol 2018;9:201.
[4] O’Donnell MJ, Xavier D, Liu L, et al. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet 2010;376:112–23.
[5] Witzum JL, Lichtman AH. The influence of innate and adaptive immune responses on atherosclerosis. Annu Rev Pathol 2014;9:73–102.
[6] Wu Y, Benjamin EJ, MacMahon S. Prevention and control of cardiovascular disease in the rapidly changing economy of China. Circulation 2016;133:2545–60.
[7] Han C, Liu F, Yang X, et al. Ideal cardiovascular health and incidence of atherosclerotic cardiovascular disease among Chinese adults: the China-PAR project. Sci China Life Sci 2018;61:504–14.
[8] Fiorito G, Vlaanderen J, Poldoro S, et al. Oxidative stress and inflammation mediate the effect of air pollution on cardio- and cerebrovascular disease: a prospective study in nonsmokers. Environ Mol Mutagen 2018;59:234–46.
[9] Chen KS, Stroup EF, Budhipramono A, et al. Mutations in microRNA processing genes in Wilms tumors derepress the IGF2 regulator PLAG1. Genes Dev 2018;32:996–1007.
[10] Sullivan TB, Robert LC, Tseebay PA, et al. Spatiotemporal microRNA profile in peripheral nerve regeneration: miR-138 targets vimentin and inhibits Schwann cell migration and proliferation. Neural Regen Res 2018;13:1253–62.
[11] Bao MH, Xiao Y, Zhang QS, et al. Meta-analysis of miR-146a polymorphisms association with coronary artery diseases and ischemic stroke. Int J Mol Sci 2015;16:14305–17.
[12] Li SH, Su SY, Liu JL. Differential regulation of microRNAs in patients with ischemic stroke. Curr Neurovasc Res 2015;12:214–21.
[13] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.
[14] Li L Association of miRNA-146a polymorphism with risk of cardiovascular disease and ischemia stroke and the mechanisms. Master’s Thesis. Hunan, China: Central South University; 2010.
[15] Sun J. Association of miR-146a and EPHX2 polymorphisms with risk of ischemic stroke in Changsha Han population and the mechanisms. Master’s Thesis. China: Central South University, Hunan; 2011.
[16] Yang Y. Common genetic variations in pre-miRNAs and the risk of coronary heart disease in a Chinese Han population. PhD Thesis. Peking, China: Peking Union Medical College; 2012.
[17] Chen L, Wu YT. Association of genetic polymorphisms in microRNAs precursor with the risk and prognosis of coronary heart diseases. J Xian Jiaotong Univ 2013;34:495–9.
[18] Jeon YJ, Kim OJ, Kim SY, et al. Association of the miR-146a, miR-149, miR-196a2, and miR-499 polymorphisms with ischemic stroke and
silent brain infarction risk genetic polymorphisms in pre-microRNAs and risk of ischemic stroke in a Chinese population. Arterioscler Thromb Vasc Biol 2013;33:420–30.

[19] Chen C, Hong H, Chen L, et al. Association of microRNA polymorphisms with the risk of myocardial infarction in a Chinese population. Tohoku J Exp Med 2014;233:89–94.

[20] Hamann L, Glaeser C, Schulz S, et al. A micro RNA-146a polymorphism is associated with coronary restenosis. Int J Immunogenet 2014;41:393–6.

[21] Hu YM, Li SJ, Jiang XF, et al. Study on the association of miR-146a C>G, miR-149 T>C polymorphism with susceptibility to ischemic stroke. Prog Mod Biomed 2014;14:5648–51.

[22] Li Y, Zhu J, Ma GD, et al. Association between miR-146a gene polymorphism and lacunar infarction. Shandong Med J 2014;34:1–3.

[23] Liu Y, Ma Y, Zhang B, et al. Genetic polymorphisms in pre-microRNAs and risk of ischemic stroke in a Chinese population. J Mol Neurosci 2014;52:473–80.

[24] Ramkaran P, Khan S, Phulukdaree A, et al. Chuturgoon AA. miR-146a polymorphism in coronary artery disease. Mol Med Rep 2016;14:2328.

[25] Lv GT, Wang S, Wang QK. Association of miR-146a rs2910164 and miR-196a2 gene polymorphisms with ischemic stroke in a Chinese population. Oncotarget 2017;8:31295–304.

[26] Wang Y, Wang X, Li Z, et al. Two single nucleotide polymorphisms (rs2431697 and rs2910164) of miR-146a are associated with risk of coronary artery disease. Int J Environ Res Public Health 2017;14.

[27] Zhu HG, Zhang HJ, Bao LG, et al. Analysis of association of genetic polymorphisms of microRNAs with ischemic stroke. Chin J Med Genet 2017;34:261–5.

[28] Johnson EL, Krauss GL, Lee AK, et al. Association between midlife risk factors and late-onset epilepsy: results from the atherosclerosis risk in communities study. JAMA Neurol 2018;75:1375–82.

[29] Xie Y, Chu A, Feng Y, et al. MicroRNA-146a: a comprehensive indicator of inflammation and oxidative stress status induced in the brain of chronic T2DM rats. Front Pharmacol 2018;9:478.

[30] Polimeni A, De Rosa S, Indol C. Vascular miRNAs after balloon angioplasty. Trends Cardiovasc Med 2013;23:9–14.

[31] Indol C, Iaconetti C, Garelli C, et al. Non-coding RNAs in vascular remodeling and restenosis. Vasc Pharmacol 2019;114:49–63.

[32] Eyileten C, Wick Z, De Rosa S, et al. MicroRNAs as diagnostic and prognostic biomarkers in ischemic stroke—a comprehensive review and bioinformatic analysis. Cells 2018;7 pii: E249.

[33] Gomez C, Cunha C, Nascimento F, et al. Cortical neurotoxic astrocytes with early ALS pathology and miR-146a deficit replicate glosis markers of symptomatic SOD1G93A mouse model. Mol Neurobiol 2019;56:2137–58.

[34] Del MA, Arroyo AR, Andrés-Manzano MJ, et al. miR-146a deficiency in hematopoietic cells is not involved in the development of atherosclerosis. PLoS ONE 2018;13:e0198932.

[35] Pordzik J, Pisarz K, De Rosa S, et al. The potential role of platelet-related microRNAs in the development of cardiovascular events in high-risk populations, including diabetic patients: a review. Front Endocrinol (Lausanne) 2018;9:70.

[36] Nguyen LS, Fregene J, Bole-Foyer C, et al. Role of miR-146a in neural stem cell differentiation and neural lineage determination: relevance for neurodevelopmental disorders. Mol Autism 2018;9:38.

[37] Zhou B, Rao L, Peng Y, et al. Common genetic polymorphisms in pre-microRNAs were associated with increased risk of dilated cardiomyopathy. Chin Clin Acta 2010;411:1287–90.