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Relationship of miRNA-146a to systemic lupus erythematosus

A PRISMA-compliant meta-analysis

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Abstract

Background and objective: miRNA-146a is a microRNA that plays an important role in systemic lupus erythematosus (SLE). Several studies have examined the role of miRNA-146a in SLE, but have demonstrated equivocal or even contradictory conclusions. Therefore, this meta-analysis aimed to assess the role of miRNA-146a in SLE by examining data from previous studies.

Methods: A meta-analysis of relevant papers published before August 31, 2019, in the WanFang, Chinese Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure (CNKI), PubMed, EMBASE, and Web of Science databases was performed to verify the relationship of miRNA-146a expression level to SLE. Two investigators independently extracted the data and conducted a quality assessment of the studies. All statistical analyses were performed using Stata 14.0. Trial sequence analysis (TSA) was conducted to assess the quality and strength of the studies using the TSA software.

Results: Six publications, involving 151 SEL patients and 132 healthy individuals as controls were included in this meta-analysis. The results showed that the expression of miRNA-146a was associated with SLE risk [standard mean difference (SMD) = −1.21, 95% confidence interval (95% CI) (−2.18, −0.23), $P = .015$]. The stratified analysis revealed that the expression of miRNA-146a was highly related to higher SLE risk among Asian (SMD = −1.30, 95% CI (−2.52, −0.07), $P = .038$) and Caucasian (SMD = −0.72, 95% CI (−1.20, −0.24), $P = .003$) populations. Besides, the serum levels of miRNA146a were significantly different (SMD = −1.73, 95% CI (−3.11, −0.36), $P = .014$). The TSA revealed that the cumulative Z-curve crossed the typical boundary value, and reached the TSA monitoring boundary, but did not reach the required information size. This indicates that even if the cumulative sample size did not meet required information size, no more trials were needed and a reliable conclusion was reached in advance. Sensitivity analyses indicated the instability of the meta-analysis.

Conclusions: Overall, the expression of miRNA-146a is associated with SLE risk. Therefore, miRNA-146a is a promising candidate for the effective diagnosis of SLE. But, due to the limitations of this study, it is necessary to cautiously explain the results of this study.

Systematic review registration: PROSPERO CRD42019151381.

Abbreviations: 95% CI = 95% confidence interval, PBMC = peripheral blood mononuclear cell, RIS = required information size, SLE = systemic lupus erythematosus, SMD = standard mean difference, TSA = trial sequence analysis.

Keywords: meta-analysis, miRNA-146a, systemic lupus erythematosus

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YF and YJ contributed equally to this study and are co-first authors.

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Data supporting our findings are contained within the manuscript.

The datasets generated during and/or analyzed during the current study are publicly available.

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1. Introduction

The systemic lupus erythematosus (SLE) is a common autoimmune disease condition that can affect many systems and organs including the central nervous system, kidneys, joints, and skin regions.[1] Recent estimates have shown that the highest incidence and prevalence of SLE were 23.2/100 000 person-years and 241/100 000 people, respectively in North America. Early diagnosis and effective treatment are extremely important for improved outcomes of SLE and improve on the patient's quality of life.[3–5] However, due to the heterogeneous nature of SEL, and the lack of standard criteria, diagnosis poses a great challenge. Besides, there is a lack of sensitive and specific diagnostic methods in clinical practice for SEL.[2]

The exact cause of SLE remains elusive, however, evidence suggests that miRNA is involved in its pathogenesis.[6,7] miRNA is an important molecule involved in the regulation of gene expression. They are evolutionally conserved single-stranded noncoding RNAs that function in post-transcriptional regulation of gene expression.[8] miRNAs also function in RNA silencing where they negatively regulate gene expression at the mRNA and protein level by regulating molecules that bind to a specific mRNA of interest, inhibiting mRNA translation or degrading target mRNA. The control of miRNA mediated gene expression is critical for normal cellular functions. They also play important roles in biological processes such as cell cycle, differentiation, apoptosis, metabolism among others.[9] Recent evidence suggests changes in miRNA expression have been implicated in various human diseases such as cancer, heart diseases, and autoimmune diseases.[10–14] miRNA-146a is a member of the miRNA-146 family and participates in the reverse modulation of immune and inflammatory responses.[15,16] Recent research suggests that miR-146a plays an essential function in the initiation of various autoimmune diseases, and maybe a potential biomarker for diagnosis.[17,18] miR-146a is a novel marker to evaluate autoimmune disease progression.

Some studies[19–22] have reported that the expression of miRNA-146a is associated with SLE risk, while other studies[23] show no association. Therefore, this meta-analysis aims to assess the role of miRNA-146a in SLE by examining data from previous studies.

2. Study protocols

The protocol and registration information are available at http://www.crd.york.ac.uk/PROSPERO/ (registration number: CRD42019151381).

2.1. Literature search strategy

The following databases were interrogated; WanFang, Chinese Biomedical Literature Database (CBM), Chinese National Knowledge Infrastructure (CNKI), PubMed, EMBASE, and Web of Science databases to identify articles that addressed the relationship of miRNA-146a to SEL and were published before August 31, 2019. The search was performed using the following keywords "miRNA-146a," "systemic lupus erythematosus," by 2 independent investigators (Yihua Fan and Yue Ji). The search strategy illustrated in Supplemental Digital Content (see Table S1, http://links.lww.com/MD/E939, Supplemental Content, Search strategy). A manual search was also performed to identify additional studies from the reference list of the originally identified articles. Suitable studies for full-text review were identified based on the title and abstract. The selected articles were read in full to determine whether they were appropriate for inclusion in the meta-analysis. Data extraction and analysis were performed by trained researchers and meta-analysis was performed only on studies published as primary articles.

2.2. Inclusion criteria and exclusion criteria

2.2.1. Inclusion criteria. Studies were included if they met the criteria below:

(1) case-control studies assessing the association between the miRNA-146a expression level and SLE risk,
(2) patients diagnosed with SLE in the case group and healthy people in the control group,
(3) detailed miRNA expression level data provided
(4) if serial studies from the same group of people were reported, included the latest study.
(5) the search was not limited to the language or date publication.

2.2.2. Exclusion criteria. The articles were excluded if they met the exclusion criteria below:

(1) Duplicate data;
(2) lack of adequate information to calculate the statistical index standard mean difference (SMD).

2.3. Quality assessment and data extraction

Two authors (Yihua Fan and Yue Ji) independently extracted the data from the eligible studies. Data entry was performed using the EpiData software (version 3.0; The EpiData Association, Odense, Denmark). Disagreements were resolved by discussion or consensus with a third author (Xuyan Wang). If the miRNA expression level data was not directly reported, data was extracted from the statistical graph using Engauge Digitizer version 4.1 (Http://digitizer.sourceforge.net/). The following information was extracted: the first author, publishing year, country, ethnicity, diagnostic criteria, specimen, detection method, and sample size, etc.

Studies quality was judged by 2 independent reviewers according to the Newcastle-Ottawa Quality Assessment Scale (NOS) for case-control studies. Rating criteria were as follows: Low quality = 0 to 5; medium quality = 6 to 7; and high quality = 8 to 9.

2.4. Data synthesis and statistical analysis

SMD corresponding to 95% confidence interval (95% CI) was used to assess the strength of the association between miRNA-46a expression level and SLE risk. The heterogeneity between studies was tested using the Q test and I^2 statistics.

If $P > .10$, $I^2 < 50\%$, it indicated that there was no statistical heterogeneity among the studies, and the fixed effect model was adopted for the combined analysis. If not, the random effect model was used. Meanwhile, in order to deal with the heterogeneity between studies, the subgroup analysis was carried out to explore the causes. Subgroup analyses were performed based on ethnicity and specimen characteristics. A sensitivity analysis was performed to evaluate the stability and reliability of the meta-analysis. Egger and Begg test was used to determine

potential publication bias. All statistical analyses were performed by STATA 14.0 software (STATA Corporation, College Station, TX). All the statistical tests were two-sided, and $P < .05$ was considered to be statistically significant.

Trial sequence analysis using trial sequence analysis (TSA) 0.9.5.5 Beta was used to calculate the appropriate sample size for the meta-analysis, and to assess the statistical boundaries for futility and efficacy.[24] TSA can reduce the error probability of type I statistics through cumulative analysis, and can judge whether the sample size is sufficient by TSA cutoff value and required information size (RIS), which reflects the reliability of the conclusion. If the cumulative Z value does not cross the TSA boundary value or RIS, the research sample size is insufficient and the conclusion is unstable. If the cumulative Z value crosses the TSA boundary value and does not reach the RIS, it means that even if the sample size is insufficient and there is no need to increase the sample size, a reliable conclusion can be drawn in advance. If the cumulative Z value crosses both the TSA boundary value and the RIS, value, the sample size is sufficient and the conclusion is reliable.[24]

3. Results

3.1. Characteristics of included studies

Two hundred eighty articles were identified using the Web of Science, EMBASE, PubMed, Chinese National Knowledge Infrastructure (CNKI), Chinese Biomedical Literature Database (CBM) and WanFang databases. After reviewing the title and abstract and eliminating the duplicates, nine studies were selected for full-text review. Six studies[19,25] were finally included in the meta-analysis with a total of 132 healthy controls and 151 patient cases (Fig. 1). One of these studies (Yin 2012) conducted both peripheral blood mononuclear cell (PBMC) and Serum tests. The

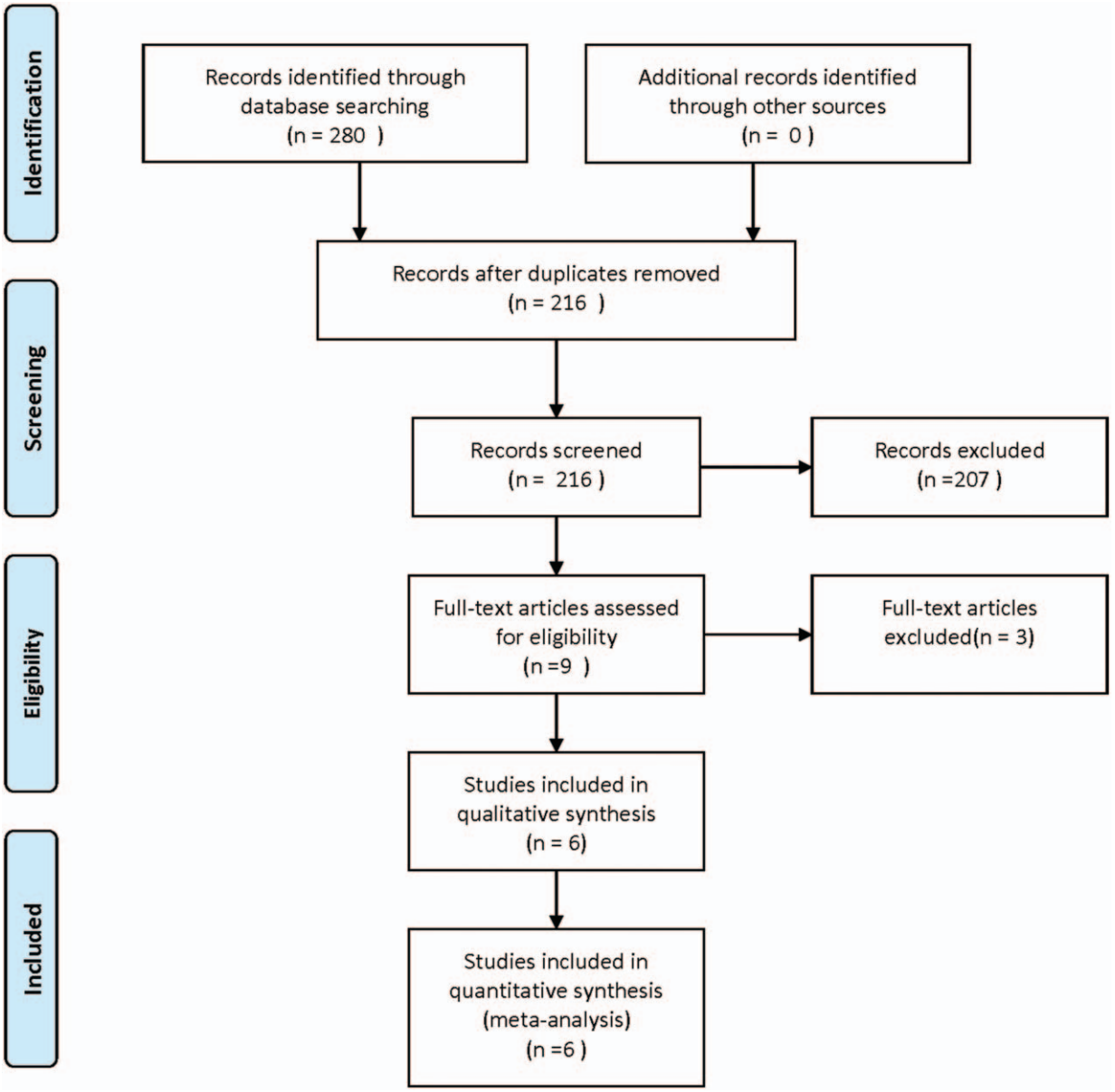

Figure 1. The flow diagram.

Table 1

Characteristics of eligible studies in meta-analysis.

Author (reference)	Country	Ethnicity	Diagnostic criteria	Specimen	Detection method	sample size (Case /Control)	Age (Case /Control)	NOS score
Wang 2010	China	Asian	ARA	Serum	RT-PCR	40/40	48.4±12.6/34.3±6.7	6
Du 2013	China	Asian	ARA	PBMC	RT-PCR	22/17	32.2±13.8/29.5±8.5	6
Yin 2012	China	Asian	ARA	PBMC	RT-PCR	20/15	35.75±11.59/43.13±9.76	7
Yin 2012	China	Asian	ARA	Serum	RT-PCR	19/15	NA/43.13±9.76	7
Shumnalieva 2018	Bulgaria	Caucasian	ARA	Serum	RT-PCR	40/32	43.6±12.01/39.15±12.07	8
Zhang 2019	China	Asian	ARA	Serum	RT-PCR	22/20	22–25/22–25	7
Wang 2011	China	Asian	ARA	PBMC	RT-PCR	8/8	15–42/25–30	6

ARA = American College of Rheumatolog criteria, NA = Data not available, NOS = Newcastle-Ottawa Quality Assessment Scale, PBMC = Peripheral Blood Mononuclear Cell, RT-PCR = reverse transcriptionpolymerase chain reaction.

6 studies were conducted from 2010 to 2019, 5 studies were conducted in China while one study was conducted in Bulgaria. All SLE patients met the diagnostic criteria of the American College of Rheumatology and Newcastle-Ottawa Quality Assessment Scale (NOS) Scores were 6 to 8. The characteristics of the included studies are summarized in Table 1.

3.2. Quantitative synthesis

The random-effects model showed that high risk of SLE was dependent on miRNA-146a expression [SMD = −1.21, 95%CI (−2.18, −0.23), $P = .015$] (Fig. 2). The TSA revealed that the cumulative Z-curve crossed the conventional boundary value, and reached the TSA monitoring boundary, but did not meet the RIS (2582 cases) (Fig. 3). This means that although the cumulative sample size did not meet expectations, no more trials were needed and a positive conclusion was reached in advance.

3.3. Subgroup analysis

Subgroup analyses were based on ethnic origin and specimen of included studies. The Asian and Caucasian populations were found to exhibit statistical significant differences (SMD = −1.30,

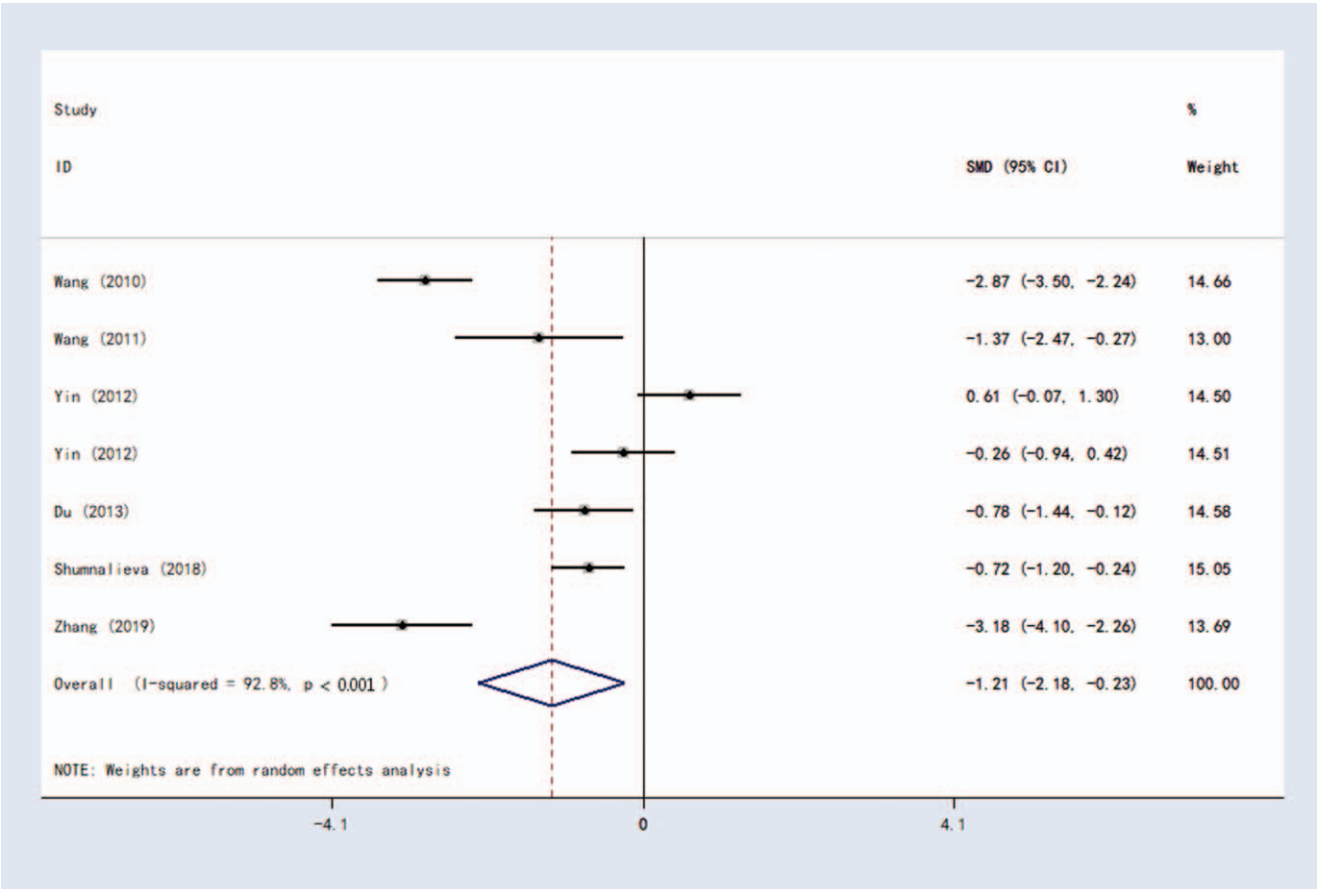

Figure 2. Forest plot for miRNA-146a and the risk for systemic lupus erythematosus.

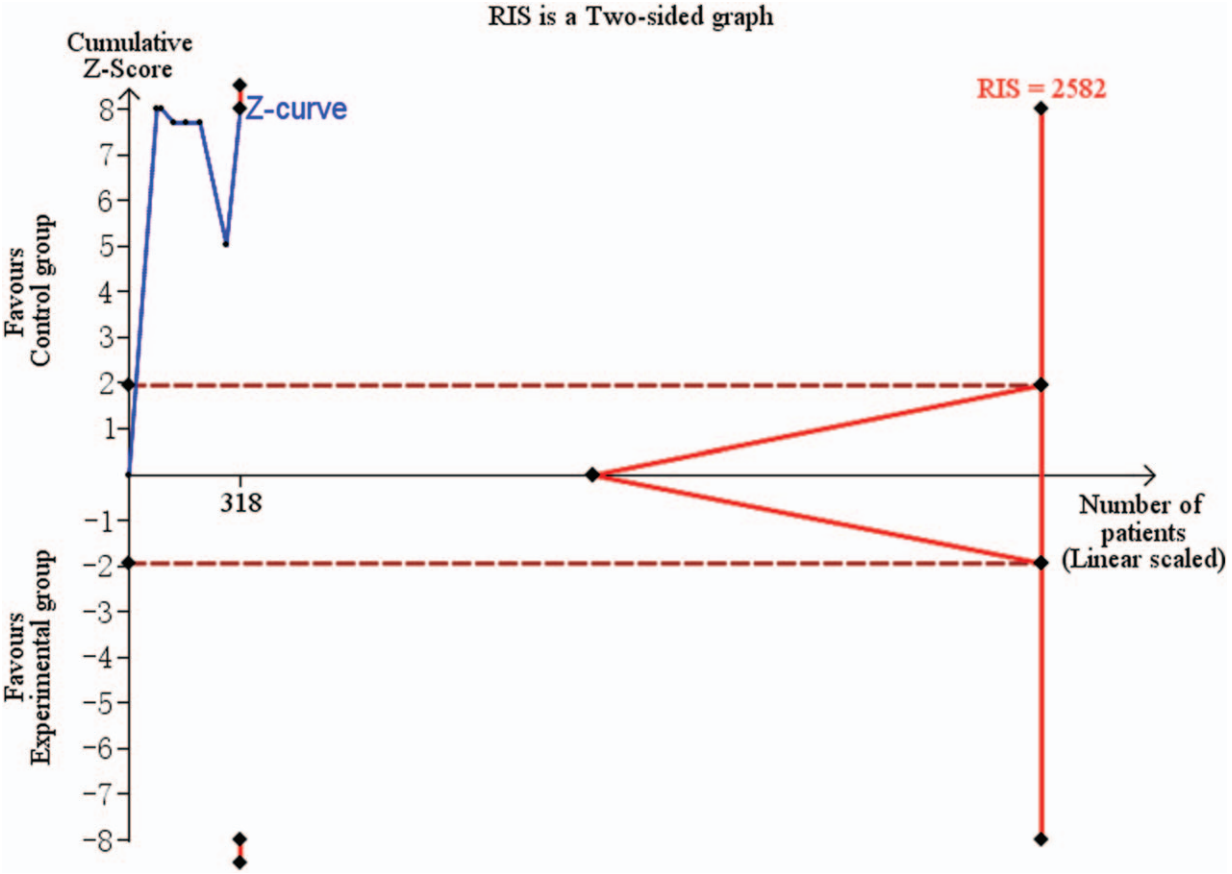

Figure 3. Trial sequence analysis results.

95% CI (−2.52, −0.07), $P=.038$. vs SMD = −0.72, 95% CI (−1.20, −0.24), $P=.003$) (see Fig. S1, http://links.lww.com/MD/E935, Supplemental Content, Subgroup analysis of Ethnicity). However, the Caucasian population had been included in only one study. If classified by the specimen, we found that the serum met the statistical difference (SMD = −1.73, 95%CI (−3.11, −0.36), $P=.014$), but the PBMC did not exhibit statistical difference (SMD = −0.46, 95% CI (−1.60, −0.67), $P=.425$) (see Fig. S2, http://links.lww.com/MD/E936, Supplemental Content, Subgroup analysis of Specimen).

3.4. Sensitivity analysis

Sensitivity analyses were performed to confirm the robustness of the results by the elimination method. The corresponding SMD showed no significant changes (see Fig. S3, http://links.lww.com/MD/E937, Supplemental Content, Sensitivity analyses). However, when Zhang et al studies were excluded, the results of meta-analysis were reversed ($P>.05$). Therefore, the result of meta-analysis needs to be interpreted cautiously.

3.5. Publication bias

Begg funnel plot and Egger test were used to determine the publication bias of the included studies. There was no statistical evidence of publication bias in our meta-analysis (Egger test: $P=.919$; Begg test: $P=.881$) (see Fig. S4, http://links.lww.com/MD/E938, Supplemental Content, The funnel plot for publication bias).

4. Discussion

In recent years, increasing evidence has indicated that miRNAs play essential roles in the pathogenesis of autoimmune diseases. Previous studies have shown increased expression of miR-146a in PBMC and CD4+ T cells in patients with rheumatoid arthritis.[26] Kriegsmann et al,[27] found that the expression of miR-146a in the synovial tissues of rheumatoid arthritis patients was higher compared to that of osteoarthritis patients. In another meta-analysis, miRNA-146a was reported to serve as a novel biomarker for the diagnosis of Sjogren syndrome.[28] However, the expression of human-miR-146a is reported to be decreased in patients with SLE[20] but this remains a controversy. Zhang et al,[21] found that the expression level of miR-146a in PBMC in SLE patients was lower compared with that of healthy individuals. The expression level was also correlated with the activity of the disease. miR-146a is a negative regulator of the interferon signaling pathway, thus may participate in the pathogenesis of SLE.[29] Wang et al,[19] found decreased expression of miR-146a in the serum of individuals with SLE which was also associated with C-Reactive Protein. Yin et al,[23] reported a decreased expression level of miR-146a in the serum of individuals with SLE. Therefore, the current study aimed at conducting a meta-

analysis on the relationship between the expression of miRNA-146a and SLE risk.

In this meta-analysis, data from 6 case-control studies were analyzed to assess the association between the expression of miRNA-146a and SLE risk. The results suggested that the expression of miRNA-146a was associated with the risk of SLE. Therefore, miRNA-146a can be used as a novel biomarker for SLE diagnosis in high-risk populations. A previous meta-analysis showed no significant differences between the expression of miRNA-146a and SLE risk, possibly due to the use of small sample sizes.[30] Therefore, this meta-analysis included new studies and conducted a sequential analysis of the trials to ensure the stability of the meta-analysis results. Subgroup analysis showed that miRNA expression was different in serum. A previous meta-analysis[30] showed no difference between the expression of miRNA-146a and SLE risk in serum. This meta-analysis showed significant differences in miRNA expression and SLE susceptibility between European and Asian populations. However, most of the patients with SLE included in this study were Chinese. Therefore, longitudinal studies with larger sample sizes are needed to further establish the ethnic differences between miRNA-146a and SLE risk.

In this meta-analysis, heterogeneity among the studies was reported which might have affected the interpretation of the results. However, sensitivity analyses and subgroup analysis did not reveal the source of heterogeneity. A possible reason for heterogeneity could be concerning disease duration, severity, and treatment in SEL patients. Due to the limited information provided by the included studies, further analysis was not possible.

5. Limitations

This study has limitations.

(1) Only Chinese and English literature were included, which may lead to insufficient data coverage of other ethnicities;
(2) only Asian and Caucasian data were provided in the included literature of this study. Other races, such as African, were not available, which reduced the comprehensiveness of the results.
(3) The majority of SLE subjects were Chinese, which limited general application to other populations.

6. Conclusions

This meta-analysis shows that the expression of miRNA-146a is associated with the risk of SLE. miRNA-146a is a promising candidate for effective diagnosis of SLE. In view of the limitations of this study, it is necessary to cautiously explain the results of this study.

Author contributions

Data collection: Yihua Fan and Yue Ji
Funding acquisition: Wei Liu
Resources: QiangZhang and Aihua Wang
Software: Xuyan Wang and Jingyu Xu
Supervision: Jingyi Hu and Wei Liu
Writing – original draft: Yihua Fan
Writing – review & editing: Yihua Fan and Yue Ji

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