

Aberrant expression of miR-21 in patients with inflammatory bowel disease

A protocol for systematic review and meta analysis

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Abstract

Background: microRNAs have drawn more attention due to their function on the inflammatory process. The association between microRNA-21 (miR-21) expression and risk of inflammatory bowel diseases (IBD) remain inconclusive. This study was aimed to acquire a more exact estimation of this relationship.

Methods: Relevant studies were identified through searching PubMed, Embase, Wanfang, and China National Knowledge Infrastructure database. Pooled standardized mean difference and 95% confidence intervals were calculated using a random-effect model. Publication bias test, sensitivity analysis and subgroup analysis were carried out.

Results: A total of 20 relevant articles comprising 540 patients with ulcerative colitis (UC), 459 patients with Crohn disease (CD) and 511 non-IBD controls were included in this analysis. The expression of miR-21 was significantly increased in colon tissue of both UC and CD patients compared with non-IBD controls. However, there were no significant differences between patients with UC and CD. Moreover, increased miR-21 expression was associated with disease activity status in UC patients, but not in CD patients.

Conclusions: This meta-analysis demonstrates that the higher miR-21 expression in colon tissue is positively associated with the development of UC and CD, and miR-21 might serve as a disease marker of IBD.

Abbreviations: CD = Crohn disease, CI = confidence interval, HC = healthy control, IBD = inflammatory bowel diseases, miR-21 = microRNA-21, miRNAs = microRNAs, NOS = Newcastle-Ottawa scale, SMD = standardized mean difference, UC = ulcerative colitis.

Keywords: Crohn disease, inflammatory bowel disease, meta-analysis, microRNA-21, ulcerative colitis

1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn disease (CD), is characterized by chronic and recurring intestinal inflammation. In recent years, the incidence of IBD is gradually increasing, and it has a major

impact on the patients quality of life.^[1] Although the pathogenesis mechanism of IBD has not been fully clarified, numerous studies have demonstrated that genetic factors play an important role in disease susceptibility, progression, and outcome.^[2]

MicroRNAs (miRNAs) are a class of small endogenous non-coding RNAs that negatively regulate target gene expression at the post-transcriptional level.^[3,4] They are involved in multiple biological processes, such as cellular growth, differentiation, proliferation, apoptosis, and metabolism.^[5–7] Changes in miRNAs expression have been described to be associated with the increased risk of cancer, inflammatory, and autoimmune diseases.^[8–10]

Among the miRNAs involved in the inflammatory responses, miRNA-21 (miR-21) is considered as a representative mediator. Some studies have indicated that miR-21 contributes to the process of inflammation in sepsis and nonalcoholic steatohepatitis.^[11,12] In contrast, miR-21 has been found to prevent excessive inflammation in cardiovascular and renal diseases.^[13,14] These results suggest that the role of miR-21 in inflammatory diseases seems to be complex. Recently, several studies have investigated the relationship between miR-21 expression and IBD,^[15–18] but the results are inconclusive and controversial. Thus, we performed a systematic literature review and meta-analysis to precisely evaluate the implication of miR-21 expression in patients with IBD.

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2. Materials and methods

Ethical approval was not necessary because the data were obtained from previous studies.

2.1. Search strategy

This systematic review and meta-analysis was conducted according to the PRISMA statement. A systematic literature search in Pubmed, Embase, and 2 Chinese databases: China National Knowledge Internet (CNKI) and Wanfang databases, was carried out to identify studies that analyzed the association between miR-21 expression and UC and/or CD. The search terms were as follows: (“inflammatory bowel disease” OR “IBD” OR “ulcerative colitis” OR “UC” OR “Crohn disease” OR “CD”) AND (“microRNA-21” OR “miRNA-21” OR “miR-21”). Two independent authors conducted the search. Manual search was also performed to identify additional studies through the reference lists of published articles. The languages were limited to English and Chinese. The latest search was updated on May 1, 2019.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as following:

1. studies investigating the association between miR-21 expression and risk of IBD;
2. the study must be designed as a case-control study that enrolled CD or UC patients and non-IBD controls;
3. patients should be confirmed by pathological examination;
4. miR-21 expression was detected by qRT-PCR.

The exclusion criteria were as following:

1. studies assessing the miR-21 expression for IBD diagnosis;
2. reviews, animal experiments, or conference abstracts;
3. duplication of previous publications and unqualified data;
4. neither English nor Chinese articles.

2.3. Data extraction and quality assessment

The studies were reviewed and the data were extracted independently by 2 authors according to the above inclusion and exclusion criteria. The following information was collected: the first author, year of publication, country, number of participants, sample type, miR-21 detection method, miR-21 expression levels of both patients, and controls. Any discrepancies were resolved via discussion and consensus before the final analysis.

The quality of the included studies was assessed according to Newcastle-Ottawa Quality Assessment Scale for case-control studies.^[19] Quality scores ranged from 0–9, and the studies with 6 or more scores were considered high-quality studies.

2.4. Statistical analysis

The standardized mean difference (SMD) and 95% confidence intervals (95% CI) were calculated to estimate the relationship between miR-21 expression and IBD risk. Analysis of the heterogeneity among studies was carried out using Cochran Q test and Higgins I^2 statistic. Heterogeneity was considered statistically significant when

$P < .10$ and/or $I^2 > 50\%$. A fixed effect model was conducted when heterogeneity was negligible, whereas the random effect model was used if heterogeneity existed. Sensitivity analyses were carried out to identify the potential sources of heterogeneity. Additionally, potential publication bias was investigated through Egger test. $P < .05$ was considered as statistically significant. All statistical analyses were conducted using STATA 12.0 software.

3. Results

3.1. Study selection

A total of 325 potentially relevant papers were initially identified by searching the electronic databases. After searching for duplicates, 68 publications were removed. By detailed screening of the title and abstract, 235 irrelevant articles were excluded. We further excluded 2 studies for lack of necessary data. Finally, 20 potentially eligible studies were selected for the final analysis.^[15–18,20–35] The flow diagram for the study selection process is presented in Figure 1.

3.2. Characteristics of eligible studies

Of the 20 included studies, 6 studies evaluated the association of miR-21 expression with UC only, 4 with CD only, 10 with both UC and CD. For disease state, 5 studies compared the level of miR-21 expression of UC patients with active phase, in remission and controls, while 3 in CD patients. Samples of 4 studies were from peripheral blood (serum/plasma), 14 from colon tissue, 2 from immune cells, and 4 from 2 of 3 types. Seventeen of these studies were published in English, and the others were in Chinese. All cases were reliably diagnosed based on clinical, endoscopic, and histological criteria. The non-IBD controls were patients who underwent colonoscopy and did not suffer from IBD. Each of these studies obtained a score ≥ 6 in methodological assessment, which indicates high quality. The basic characteristics of each study are listed in Table 1.

4. Results of the meta-analysis

4.1. Changes in miR-21 expression in UC patients

Pooled data showed that UC patients had significantly higher miR-21 expression than non-IBD controls (SMD=3.60, 95% CI=1.95–5.26) (Fig. 2). Egger test suggested that significant publication bias existed ($P < .001$). After adjusting for publication bias using the trim and fill method, the association remained significant.

In view of the significant heterogeneity, subgroup analysis was conducted according to sample type. The result showed that miR-21 expression in colon tissues of UC patients was notably increased compared with non-IBD controls (SMD=5.13, 95% CI=3.16–7.10). However, there was no significant difference between the 2 groups in peripheral blood (SMD=1.09, 95% CI=–2.79–4.98), and immune cells (SMD=2.63, 95% CI=–0.66–5.92) (Fig. 3A).

Subgroup analysis was also conducted according to the type of control group (Fig. 3B). The result showed that miR-21 expression was significantly higher in UC patients compared to healthy controls (SMD=0.61, 95% CI=–1.19–2.42). However, there was no significant difference between UC

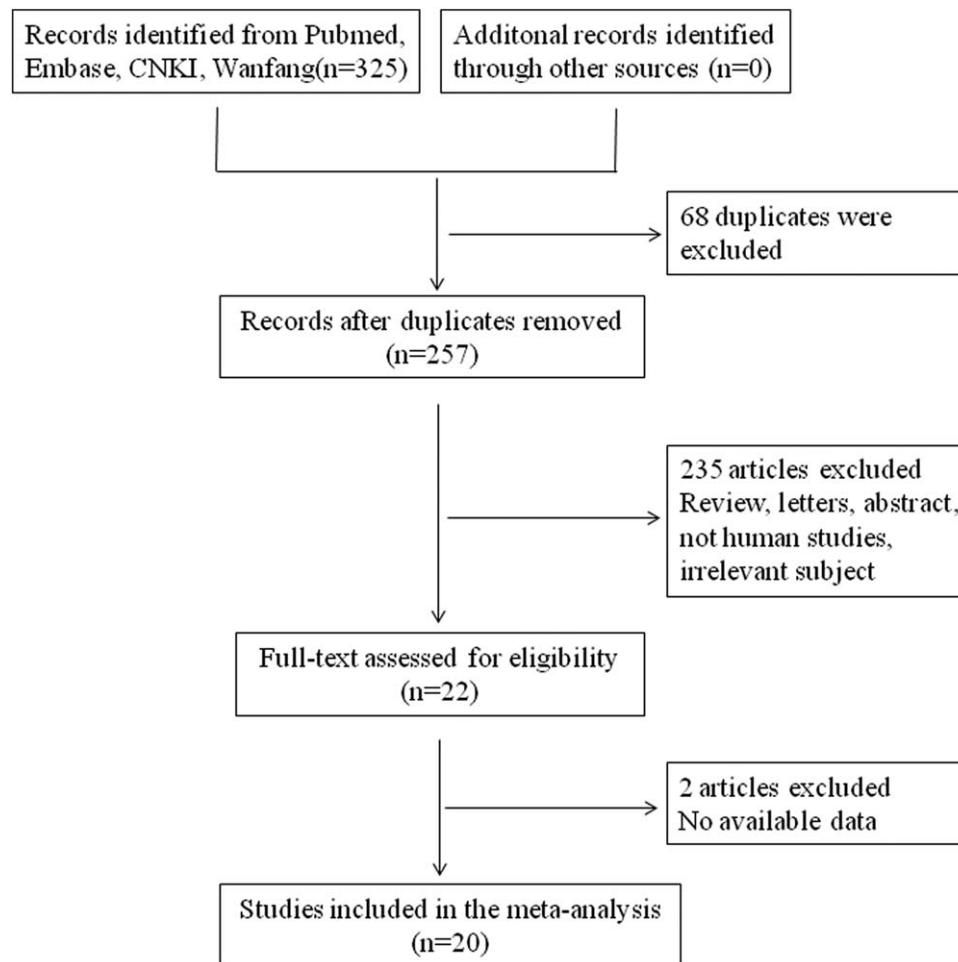


Figure 1. Flowchart summarizing literature search strategy and selection of studies.

patients and non-IBD patients (SMD=6.67, 95% CI=3.75–9.58).

4.2. Change in miR-21 expression in CD patients

Pooled data showed that CD patients had significantly higher miR-21 expression than non-IBD controls (SMD=3.54, 95% CI=2.06–5.03) (Fig. 4). Egger test suggested that significant publication bias existed ($P=.044$). After adjusting for publication bias using the trim and fill method, the association remained significant.

In view of the significant heterogeneity, subgroup analysis was conducted according to sample type. The result showed that miR-21 expression in colon tissues of CD patients was notably increased compared with non-IBD controls (SMD=5.08, 95% CI=3.04–7.11). However, there was no significant difference between the 2 groups in peripheral blood (SMD=0.47, 95% CI=–1.76–2.71), and immune cells (SMD=4.38, 95% CI=–3.32–12.08) (Fig. 5A).

Subgroup analysis was also conducted according to the type of control group (Fig. 5B). The result showed that CD patients had significantly higher miR-21 expression than non-IBD patients and healthy controls (SMD=2.56,

95% CI=0.47–4.64, SMD=4.94, 95% CI=2.28–7.59, respectively).

4.3. miR-21 expression and disease activity

Five studies evaluated the association of miR-21 expression with disease activity in UC patients. The samples were all from colon tissues. Pooled data demonstrated that patients with active phase had significantly increased miR-21 expression when compared with patients in remission (SMD=2.97, 95% CI=0.40–5.53) (Fig. 6).

Three studies evaluated the association between miR-21 expression and disease activity of CD. Pooled data showed that there was no significant difference in miR-21 expression between CD patients with active phase and patients in remission (SMD=0.38, 95% CI=–0.54–1.29) (Fig. 6).

4.4. Comparison of miR-21 expression between UC and CD

Five studies were available to compare miR-21 expression between UC and CD patients. Pooled data showed that there was

Table 1
The baseline characteristics of included studies.

First author, year	Country	Disease	Control	Sample source	Detection method	Sample size	NOS
Ando 2016 ^[27]	Sweden	UC, CD	non-IBD	Colonic CD3+ T cells	qRT-PCR	UC/CD/non-IBD: 19/17/5	9
Beres 2017 ^[33]	Hungary	UC, CD	non-IBD	Colon tissue	qRT-PCR	UC/CD/non-IBD: 25/10/11	7
Feng 2012 ^[21]	China	UC	HC	Colon tissue	qRT-PCR	UC/HC: 22/15	8
Gunaltay 2014 ^[17]	Sweden	UC	non-inflamed	Colon tissue	qRT-PCR	UC/non-IBD: 16/11	9
He 2016 ^[28]	China	UC	HC	Serum	qRT-PCR	UC/HC: 25/20	6
Jensen 2015 ^[25]	USA	CD	non-CD	Plasma	qRT-PCR	CD/non-CD: 69/33	9
Mohammadnia-Afrouzi 2016 ^[30]	Iran	UC	HC	Peripheral Foxp3+ Treg cells	qRT-PCR	UC/HC: 30/30	8
Paraskevi 2012 ^[22]	Greece	UC, CD	HC	Whole blood	qRT-PCR	UC/CD/HC: 88/128/162	6
Schaefer 2015 ^[29]	USA	UC, CD	non-IBD	Colon tissue, Whole blood	qRT-PCR	UC/CD/non-IBD Tissue: 23/33/34 Blood: 30/30/30	9
Schonauen 2018 ^[35]	Germany	UC, CD	non-IBD	Serum	qRT-PCR	UC/CD/non-IBD: 15/36/20	9
Shi 2015 ^[26]	China	UC, CD	HC	Colon tissue, Peripheral CD4+ T cells	qRT-PCR	UC/CD/HC: 23/26/19	7
Takagi 2010 ^[16]	Japan	UC	HC	Colon tissue	qRT-PCR	UC/HC: 12/12	9
Thorlacius-Ussing 2017 ^[18]	Denmark	UC, CD	non-IBD	Colon tissue	qRT-PCR	UC/CD/non-IBD: 10/8/9	8
Wu 2008 ^[15]	USA	UC, CD	HC	Colon tissue	qRT-PCR	UC/CD/HC: 30/5/15	8
Wu 2010 ^[20]	USA	CD	HC	Colon tissue	qRT-PCR	CD/HC: 6/6	6
Wu 2017 ^[34]	China	CD	HC	Colon tissue	qRT-PCR	CD/HC: 9/9	7
Yang 2013 ^[23]	China	UC	HC	Colon tissue, serum	qRT-PCR	UC/HC: 15/15	7
Yin 2016 ^[31]	China	UC, CD	HC	Colon tissue	qRT-PCR	UC/CD/HC: 13/3/8	6
Zahm 2014 ^[24]	USA	UC, CD	non-IBD	Colon tissue, serum	qRT-PCR	UC/CD/non-IBD Tissue: 12/7/14 serum: 18/11/18	9
Zhao 2016 ^[32]	China	CD	HC	Colon tissue	qRT-PCR	CD/HC: 20/20	8

CD = Crohn disease, HC = healthy control, IBD = inflammatory bowel disease, NOS = Newcastle-Ottawa scale, UC = ulcerative colitis.

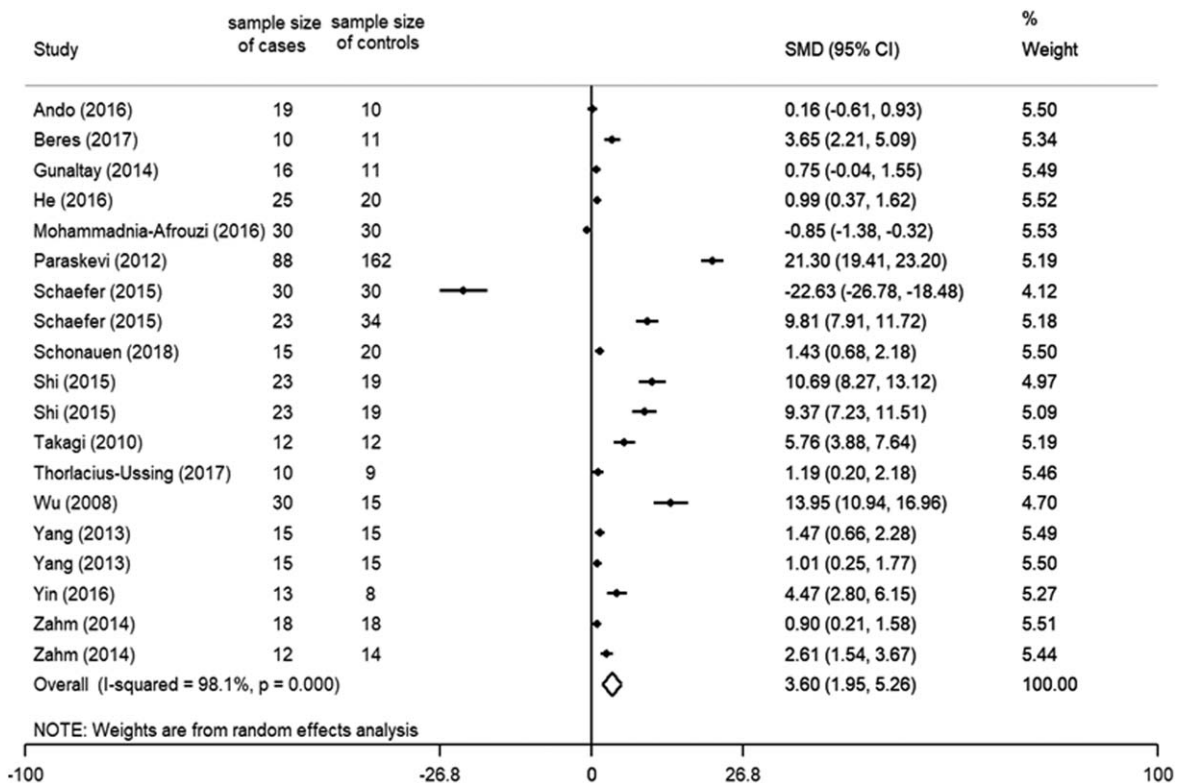
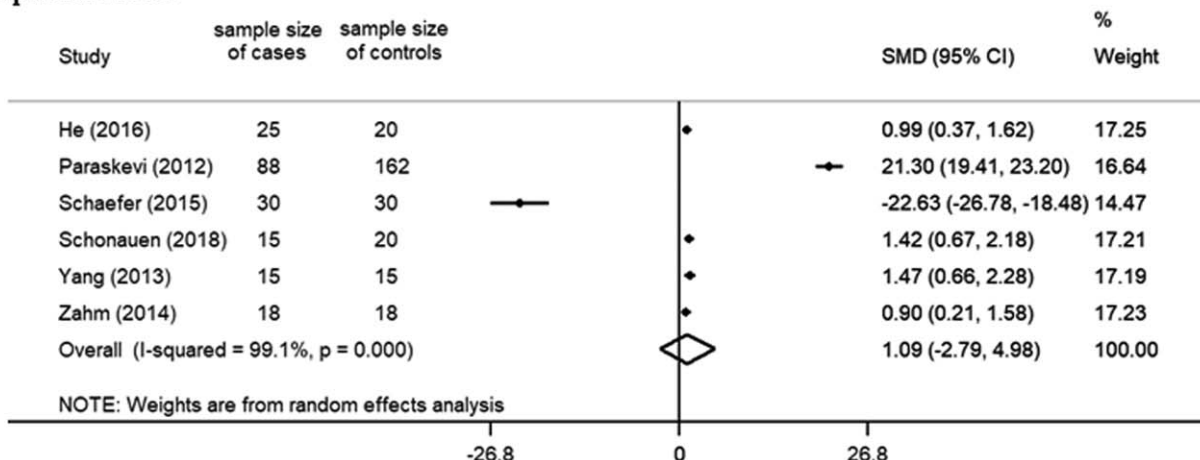
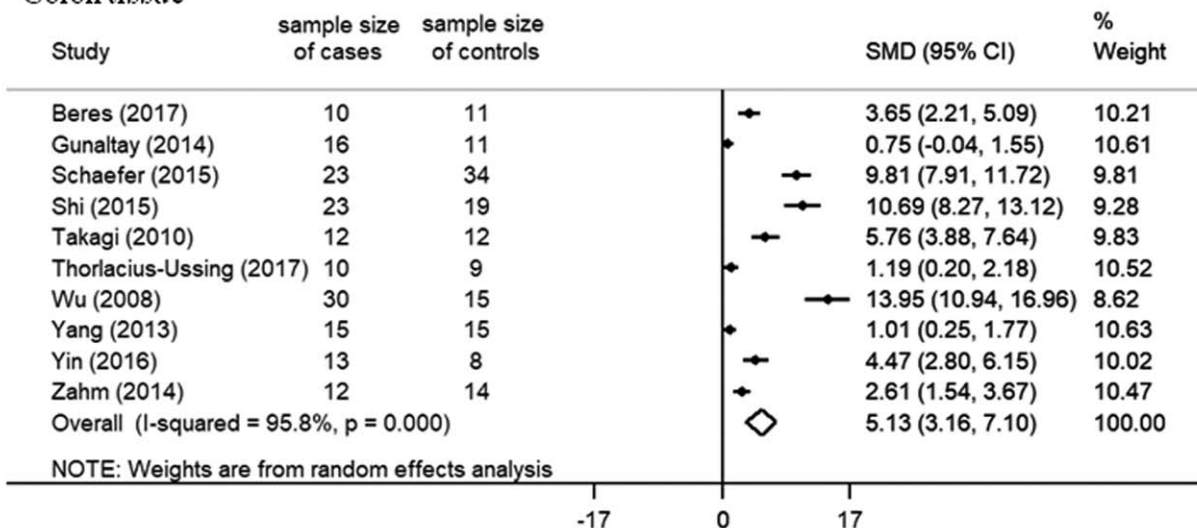


Figure 2. Forest plot for the overall evaluation of the association between miR-21 expression and UC.

Peripheral blood



Colon tissue



Immune cells

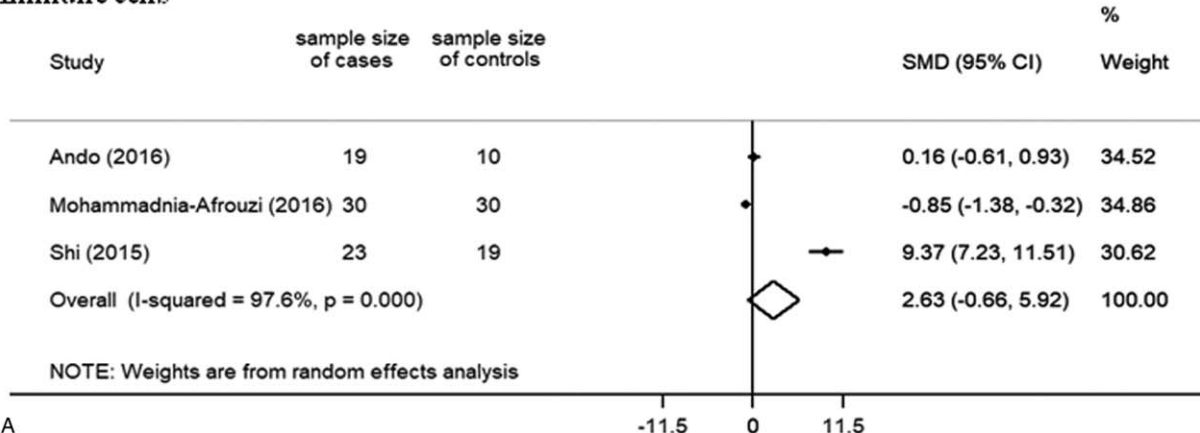
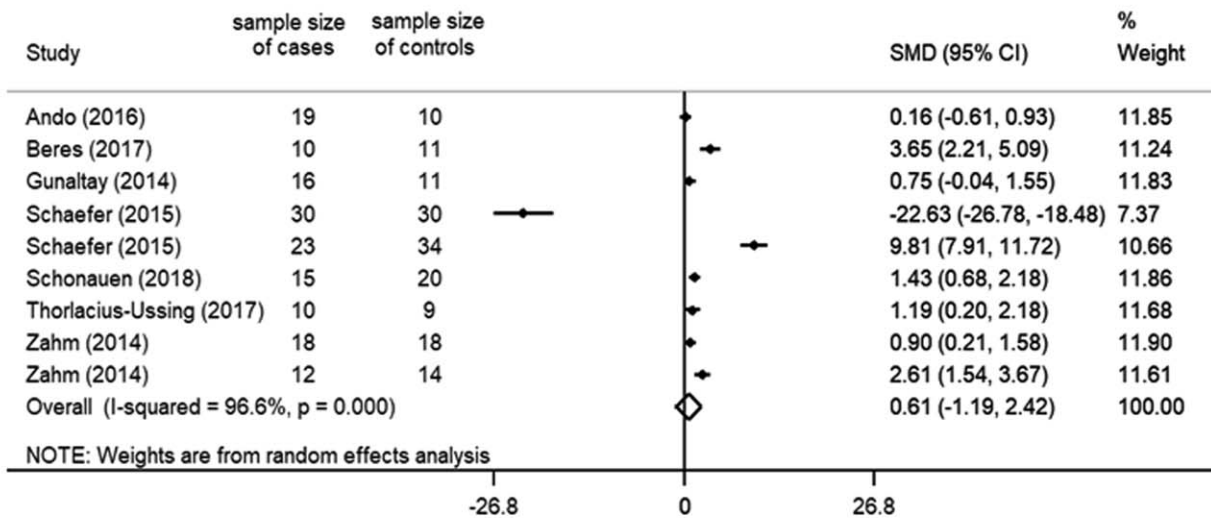


Figure 3. Forest plot for subgroup analysis based on sample type (A) and the type of control group (B) of the association between miR-21 expression and UC.

non-IBD controls



Healthy controls

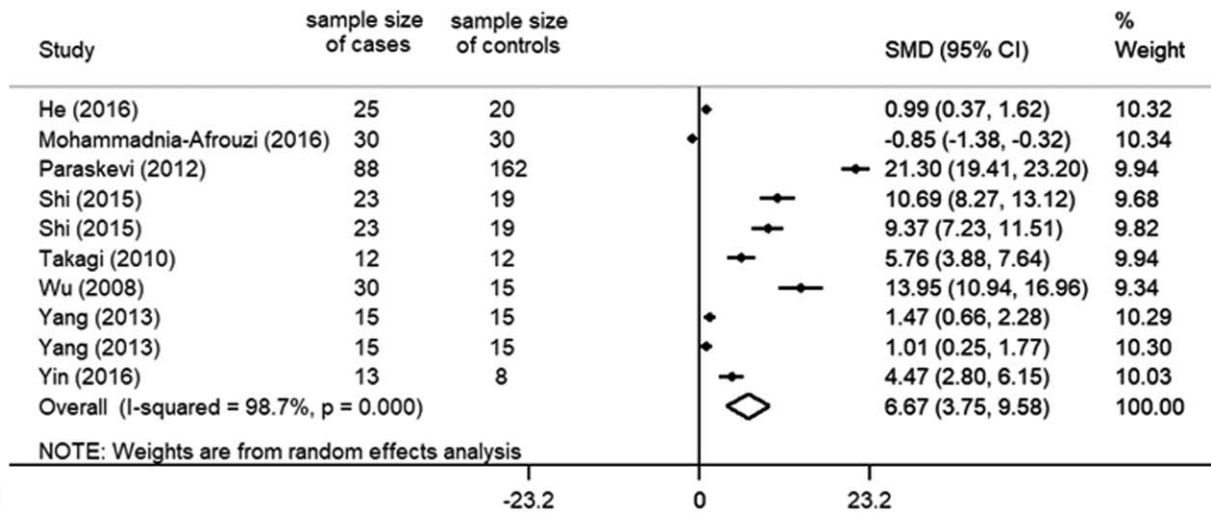


Figure 3. (Continued).

no difference between the 2 groups (SMD=1.76, 95% CI=-0.12-3.64) (Fig. 7).

5. Discussion

miR-21 is a unique miRNA and abundantly expresses in multiple tissues and cells.^[14,36] The dysregulation of miR-21 expression is associated with a wide variety of diseases, such as cancer, infection and inflammatory disease.^[37-39] The present meta-analysis is the first to extensively review the literature and estimate the relationship between miR-21 expression and risk of IBD. The results revealed that both UC and CD patients had

higher miR-21 level than non-IBD controls. Moreover, active UC patients had elevated miR-21 expression compared with patients in remission. These findings could be explained by several reasons. Accumulating evidence has suggested that miR-21 could lead to an increase in intestinal epithelial permeability, *which promoting tissue inflammation*.^[40,41] Studies in animal models have shown that the deletion of miR-21 in mice improves the survival rate through protecting against tissue injury.^[40,42] Moreover, miR-21 has been discovered to express on immune cells and promote the production of inflammatory cytokines, such as TNF- α , IFN- γ and IL-1 β , which are closely related to the pathogenesis of IBD.^[43-45] These findings provided

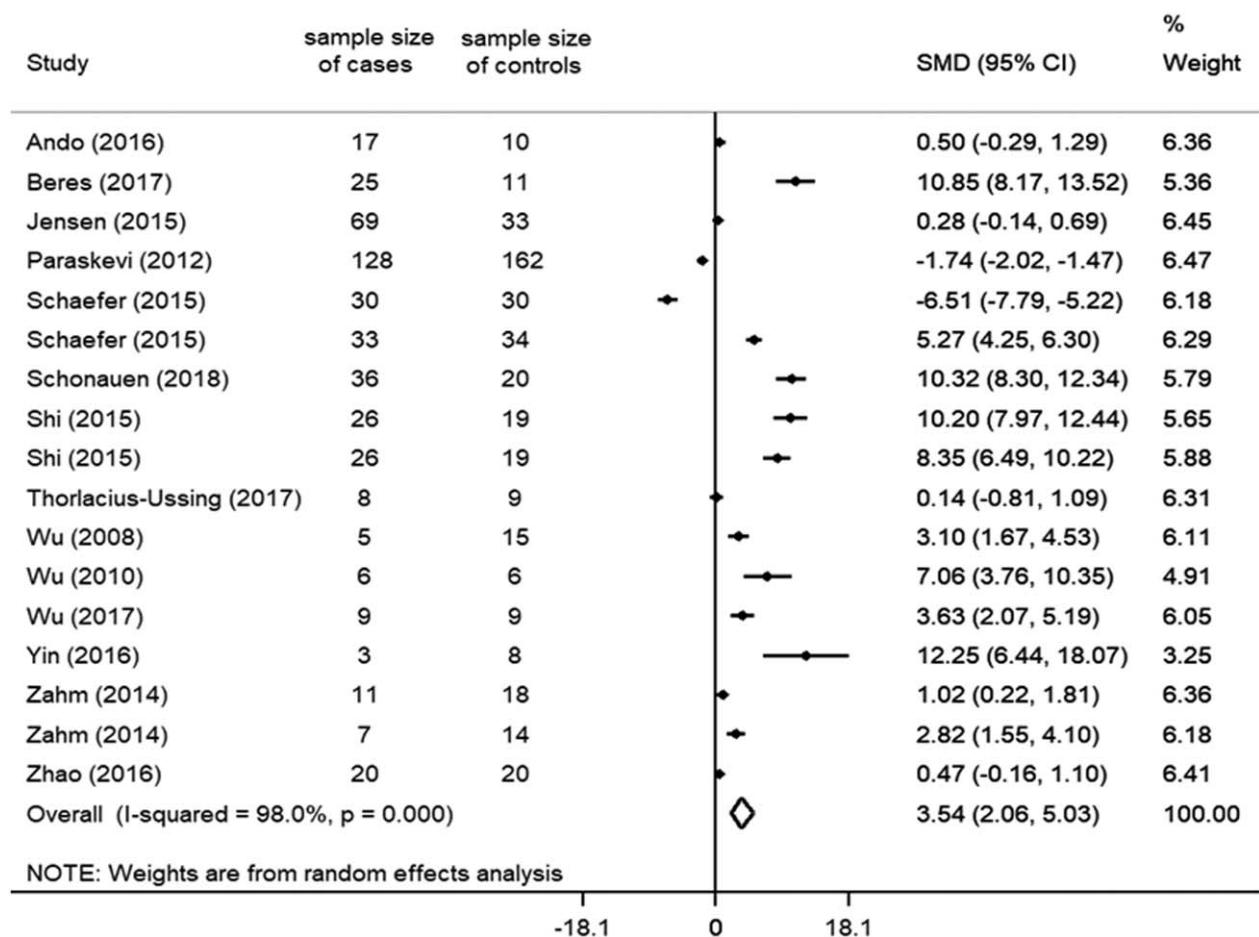


Figure 4. Forest plot of the association between miR-21 expression and CD.

evidence that miR-21 could play important roles in the pathogenesis of IBD.

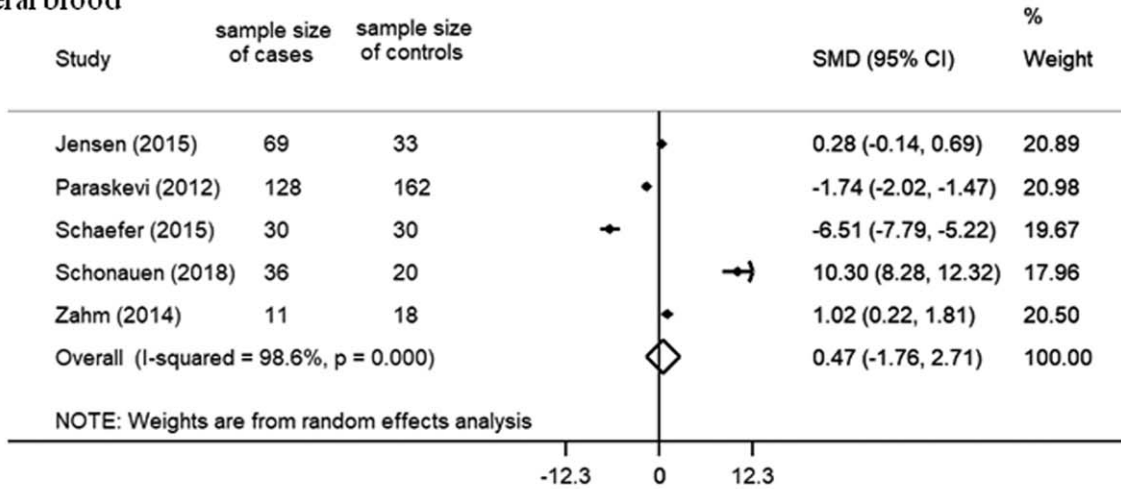
The association of miR-21 expression with disease status was analyzed. In UC patients, a higher miR-21 expression was found in the active phase, while there was no difference in remission phase compared with non-IBD controls, indicating that the upregulation of miR-21 expression could be a potential indicator of UC disease activity. However, we did not observe a similar result in CD patients. The reason for this was most likely due to low statistical power because of the relatively small number of included studies. More researches are needed to verify the exact value of miR-21 as a marker of CD activity.

To address the heterogeneity among studies, we carried out a subgroup analysis according to the type of controls. Pooled results showed that both UC and CD patients had a higher miR-21 expression than healthy controls. However, only CD patients showed an increased miR-21 expression than non-IBD patients. These results suggested that upregulation of miR-21 might have a role in multiple chronic intestinal inflammatory diseases. Subgroup analysis based on sample types was also performed. A number of studies have shown that miRNA

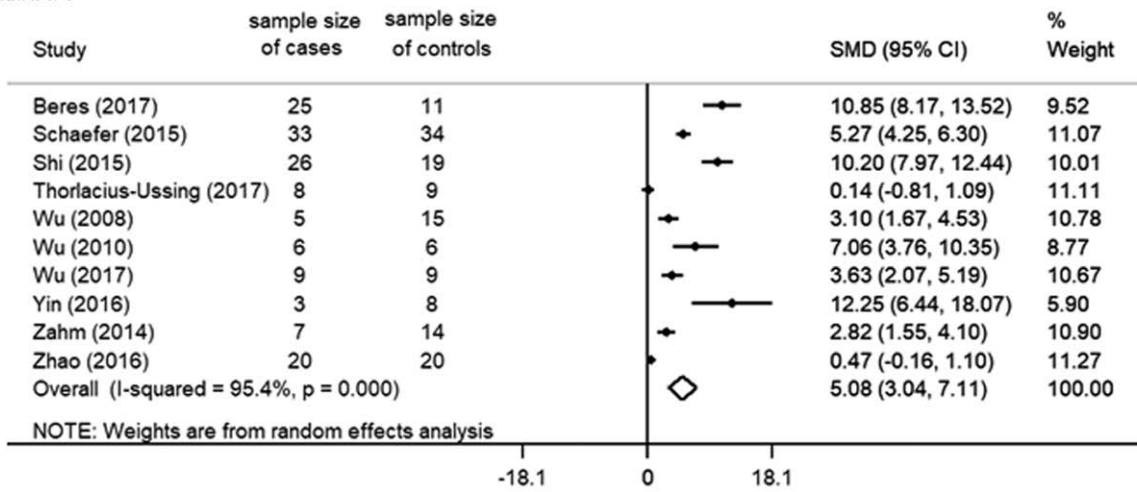
expression profile is significantly changed according to different sample types, including peripheral blood, tissue, and immune cells.^[46,47] Many studies have reported that miR-21 expression was upregulated in colon tissue of IBD patients,^[18,20,26,33] and this tendency was confirmed by our meta-analysis. However, the previous findings on miR-21 expression in peripheral blood are contradictory,^[23–25] and our pooled results showed that there was no difference between IBD patients and non-IBD patients. It suggests that circulating miR-21 does not reflect the alteration of expression in remote tissues. Despite there were many advantages of using peripheral blood to detect miRNAs, the use of circulating miR-21 as a predictive biomarker for IBD should be further discussed.

miR-21 has been found to play a crucial role in the differentiation, apoptosis, and activation of T cells^[27,48,49] that contribute to the pathogenesis of IBD. The aberrant expression of miR-21 in T cells from UC and CD patients has been reported in 3 recent studies.^[26,27,30] However, pooled results failed to demonstrate any difference between both patient groups and controls. One possible explanation may be the difference of immune cell population used in different

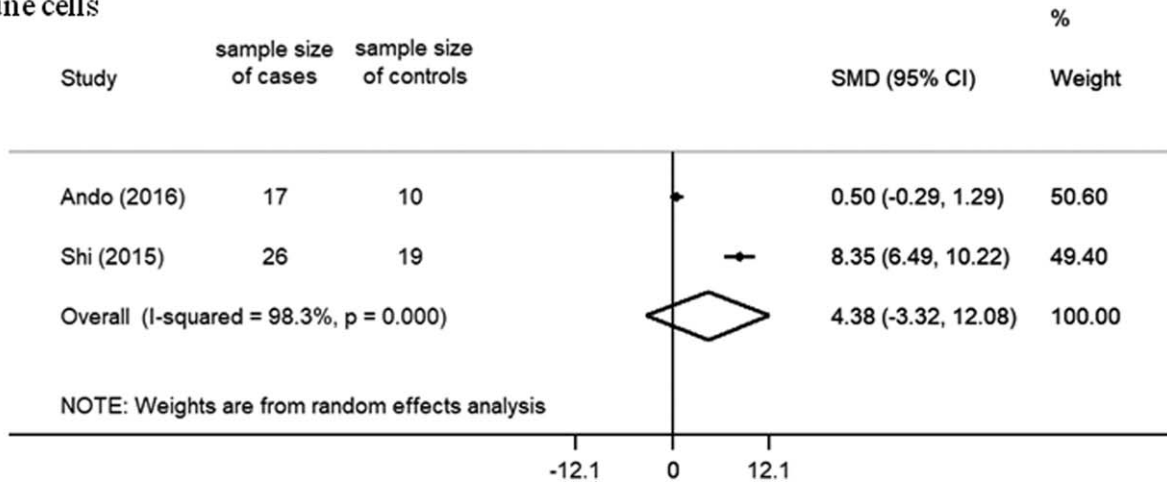
Peripheral blood



Colon tissue



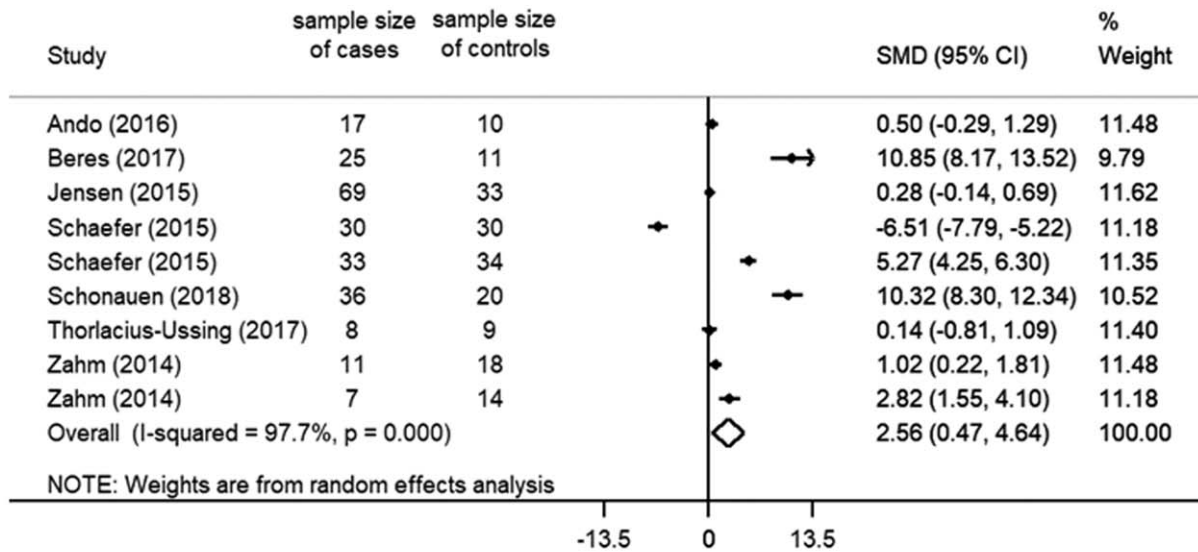
Immune cells



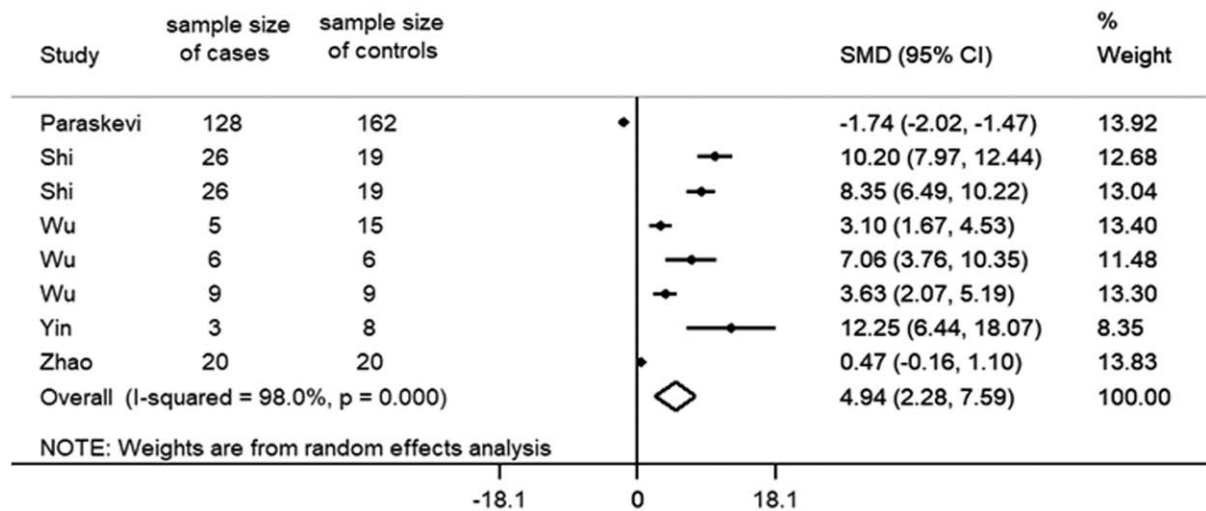
A

Figure 5. Forest plot for subgroup analysis based on sample type (A) and the type of control group (B) of the association between miR-21 expression and CD.

non-IBD controls



Healthy controls



B

Figure 5. (Continued).

studies. The aforementioned 3 studies investigated the miR-21 expression on colonic CD3+, peripheral CD4+, and peripheral regulatory T cells, respectively. Another possible explanation may be due to the relatively limited number of included studies. Therefore, further research should be carried out to determine whether miR-21 affects IBD development via regulating T cells.

There are a few limitations in this study. First, the number of included studies was limited, especially only a few reports were appropriate for analysis of disease activity. This might affect the accuracy of the results. Second, although several literature electronic databases were systematically searched, publication bias was found in this meta-analysis. After adjusting the result using trim and fill method, the conclusion was not altered. Thirdly, significant heterogeneity was

observed among the studies, which may influence the reliability of the pooled results. More studies with large sample sizes are essential to update the findings of this meta-analysis.

In conclusion, our meta-analysis demonstrates that increased miR-21 expression is significantly associated with susceptibility to IBD, including CD and UC. Compared to peripheral blood, this relationship appeared to be more prominent when assessing miR-21 expression in colon tissue. Moreover, colonic miR-21 appeared to be a potential indicator of disease activity in UC patients, but not in CD patients. Further studies are needed to strengthen the conclusions of this study, as well as investigate additional miRNAs involved in IBD pathogenesis.

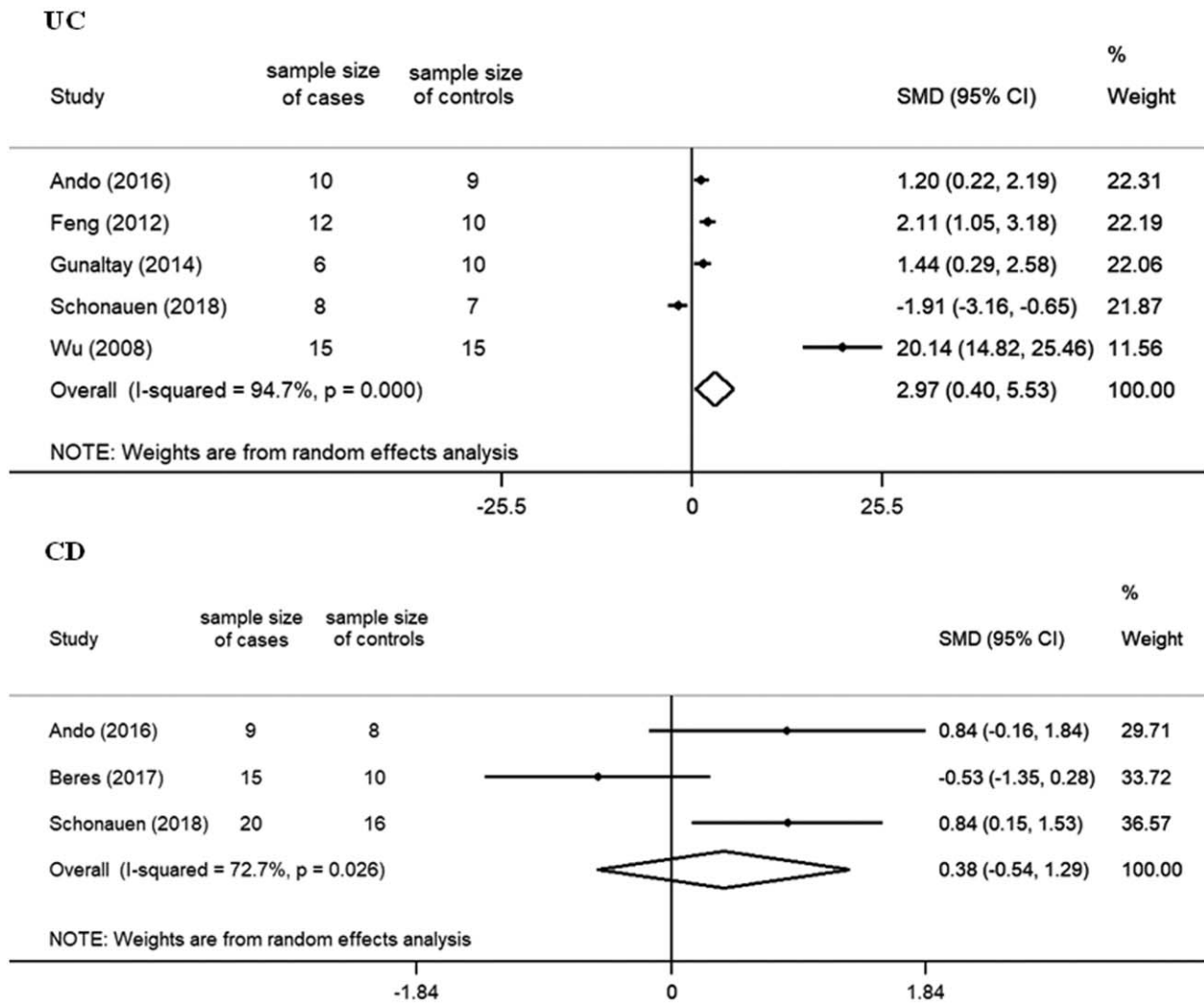


Figure 6. Forest plot for the association between miR-21 expression and disease activity of patients with UC or CD.

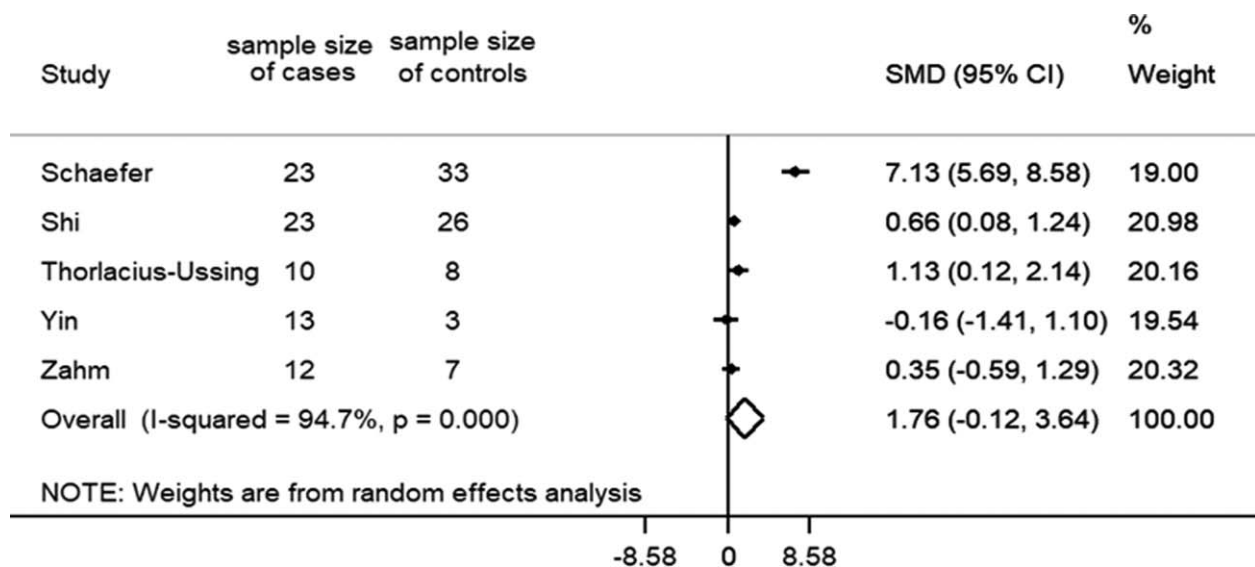


Figure 7. Forest plot for the comparison of miR-21 expression between UC and CD.

Author contributions

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Funding acquisition: Huimin Yan.
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Software: Xinyu Zhang.
Supervision: Huimin Yan.
Validation: Huimin Yan.
Visualization: Huimin Yan.
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