## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Gut Microbial Dysbiosis in Rheumatoid Arthritis: A Systematic Review Protocol of Case-Control Studies |
| **AUTHORS** | Wang, Dan-Wen; Pang, Xiang-tian; Zhang, Heng; Gao, Hai-xia; Leng, Yu-fei; Chen, Feng-qin; Zhang, Rui; Feng, Yun; Sun, Zhi-ling |

## VERSION 1 – REVIEW

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| **REVIEWER** | Gabriel Horta-Baas<br>Instituto Mexicano del Seguro Social |
| **REVIEW RETURNED** | 22-Sep-2021 |

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| **GENERAL COMMENTS** | 1. In the abstract and in the text, the authors consider the possibility of a meta-analysis of the data. Still, there is no information on how they will perform the statistical analysis of the data.<br>2. The meaning of the abbreviation EULAR should be "European League Against Rheumatism" or "European League of Associations for Rheumatology."<br>3. "It is very important to reduce the occurrence of RA, delay joint injury and avoid disability, through the improvement of intestinal flora imbalance." This paragraph overestimates the possible effects of gut microbiota manipulation in the treatment of rheumatoid arthritis. (ref. DOI 10.1007/s11926-021-01031-9)<br>4. "PICOS will be scientifically modified by substituting the item "Intervention" for "Investigation." My suggestion is to change by the acronym PECO. (ref. DOI 10.1016/j.envint.2018.07.015)<br>5. In the intervention, the authors consider including studies in which the microbiome was estimated using shotgun sequencing, 16s rRNA sequencing techniques, and/or real-time polymerase chain reaction (rt-PCR). In the case of meta-analysis, how will the statistical analysis be performed?<br>6. Authors should consider the variability due to microbiota estimation methods and the lack of assessment of confounding factors (e.g., diet, smoking, medications, etc.) in existing studies on the subject.<br>7. Outcome. The authors consider assessing alpha and beta diversity, relative abundance, and even metabolites derived from the microbiota. The authors should specify which is the primary outcome of the study and which are the secondary outcomes. The relative abundance of bacteria at the family? or phylum? or genus? level. Alpha diversity indexes: OTUs? Shannon Index? Chao 1 Index? To the best of my knowledge, there is no study in RA patients that analyzes the amount of SCFAs. |

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| **REVIEWER** | Jaeyun Sung<br>Mayo Clinic, Division of Rheumatology |
| **REVIEW RETURNED** | 07-Oct-2021 |

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| **GENERAL COMMENTS** | This manuscript has the following major flaws and should not be considered for publication in its current state:<br><br>- It is not clear what the eventual goal is. The authors state that they plan on doing a meta-analysis, but this is a very broad term. Rather, the authors should clearly state what the specific aims or hypotheses are for the meta-analysis. What will the meta-analysis achieve and why is this significant?<br>- The manuscript suffers from grammatical errors and serious flaws in paragraph coherency, especially in the Introduction of the main text. The reviewer suggests that the manuscript goes through a professional proofreading service prior to publication.<br>- The description in the 'Data synthesis and analysis' section is not clearly described; not thorough; and not persuasively justified. This gives little confidence to this reviewer that the study will be rigorously performed and reproducible.<br><br>Requested major revisions:<br>- p. 1, lines 49–53: The reviewer is confused whether this manuscript is a protocol for a comprehensive literature review or a meta-analysis (as mentioned later). Please state clearly what the purpose of this protocol is.<br>- p. 2, lines 40–51: The authors state two strengths of this study. However, these can hardly be seen as strengths (hence, explaining why this study is important). First, the authors say that this review will "elucidate the characteristics of gut dysbiosis in patients with rheumatoid arthritis". How will a literature review/meta-analysis do so? Simply re-packaging others' works is not a scientific advancement unless a groundbreaking discovery is obtained. Second, the authors state that "the findings of this study will provide a scientific basis for exploring the biomolecular link between the gut microbiota and the pathogenesis of rheumatoid arthritis". There are enough review papers that suggest links between gut microbiota and rheumatoid arthritis. This paper is not needed to bolster an already established scientific basis for studying gut microbiota in rheumatoid arthritis.<br>This reviewer cannot agree with the limitations either:<br>- p. 2, lines 54–56: "Data pooled may be heterogeneous between studies"...this is simply stating what is already known in the field and cannot be viewed as a specific limitation of this particular study.<br>- p. 2, line 59: "Some studies published in non-English languages may be missed.". Such studies should not be considered in the first place, as their peer review process is not credible. Is there any respectable study *not* written in English?<br>- The second and third paragraph of the Introduction has major issues in writing coherency, making this totally unreadable to this reviewer.<br>- p. 5, lines 23-30: Is the purpose of this study to do a literature review? What is the goal for the "meta-analysis"? Note that a "meta-analysis" is not a tool, as the authors seemingly imply here. Lastly, "biomarkers of dysbiosis" seems a very odd thing to say; "dysbiosis" *is* the biomarker for the disease (please think carefully about what a "biomarker" is supposed to do).<br>- p. 5, lines 35-41:<br>-- This Objective needs major proofreading.<br>-- What is a "case-control trains"?<br>- 'Data synthesis and analysis' section:<br>-- p. 9, line 59: How does the author expect to calculate a 'pooled effect estimate' with highly sparse, negative binomial distributed data? Please elaborate and justify. |

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| | -- p. 10, line 17: Please explain why a random-effects analysis is needed here. In particular, the reviewer would like to know what the fixed and random effects are deemed to be in the meta-analysis.<br>-- p. 10, line 20: Among many suitable approaches, why forest plots in particular?<br>-- p. 10, line 30–35: In what circumstance does the author think a meta-analysis would not be feasible? And what precisely is a "narrative synthesis"?<br>-- p. 10, 'Assessment of publication bias'. Please justify the methods. Is the author familiar with the purpose and implementation of these tests?<br><br>Requested minor revisions:<br>- A thorough proofreading is desired for the manuscript. The reviewer shall provide a few examples:<br>-- please change all instances of "the rheumatoid arthritis" to simply "rheumatoid arthritis" (including in the title).<br>-- please be consistent in how references are cited. Most of the time, spaces are provided before the reference number, but there are a few cases where this is not so.<br>- p. 1, line 33: "social" should be "societal"<br>- p. 1, line 43: I am not aware of any clinical practice where "restoring intestinal homeostasis by altering microbiota" is used as an approach to prevent and treat rheumatoid arthritis. The sentence is therefore incorrect. Did the authors mean "could be" rather than "is"?<br>- p. 1, line 54: Please remove "of".<br>- p. 2, line 7: Please define "grey literature" or reword.<br>- p. 2, line 14: Meta-analysis is a very broad term with no single goal or expected outcome. Thus "meta-analyses will be performed..." seems very vague, and it would be helpful to mention what the authors are specifically looking for.<br>-p. 2, line 28: Please change "patients' individuals" to "patients".<br>-p. 2, line 53: Please change "due to gender..." to "due to differences in gender..."<br>- p. 3, line 38: Please correctly explain the acronym for "EULAR". This is something the authors *cannot* be allowed to get wrong.<br>- by convention in any microbiology paper, please italicize names of genera or species. |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1

In the abstract and in the text, the authors consider the possibility of a meta-analysis of the data. Still, there is no information on how they will perform the statistical analysis of the data.

Response：

Thank you very much for your comments, which are very important for the revision of the manuscript. In order to elaborate the methods of data statistics, we have added corresponding contents in the 'Data extraction', and 'Data synthesis and analysis' section.

Data extraction

To conduct the meta-analysis, we involve trials that have available and sufficient data to calculate the standardized mean difference (SMD) with 95% confidence interval (CI) in RA patients and healthy controls in the analysis of the pooled data set. If additional data or data transformations will be required for analysis, we will download the publicly available raw data from online repositories or links provided in the original publications. If there is no relevant data in the original literature, we will acquire it after personal communication with the authors of the manuscripts. If the authors do not

reply, we will use Web Plot Digitizer (v.4.42 ) to digitize and extract sufficient data from graphs and plots in the articles 1 2.
Data synthesis and analysis
When the number of studies for a single bacterium was five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with health controls. We will adopt SMD with 95% CI of microbiota abundance as summary statistics when gut microbiota was detected by different techniques in the included studies 3-5. The included studies will be analyzed at the phylum or genus levels for consitency. The forest plots will be used to visualize the results. We will assess heterogeneity between studies using the Higgin I2 statistic. In relative terms, I2 values are proportional to heterogeneity: I2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity 6. Data analysis will be performed by a random-effect model when there is substantial heterogeneity (I2 > 50%); otherwise, a fixed-effects model will be used 7. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies.
If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline checklist 8. In order to define the characteristics of the gut microbiota in RA, we will perform compositional analysis based on the abundance, diversity, and specific bacterial detection of gut microbiota in RA patients and healthy controls.

1. Safadi J, Quinton A, Lennox B, et al. Gut dysbiosis in severe mental illness and chronic fatigue: a novel trans-diagnostic construct? A systematic review and meta-analysis. 2021 doi: 10.1038/s41380-021-01032-1
2. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573
3. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. Lipids Health Dis 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]
4. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. Nutrients 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]
5. Creedon AC, Hung ES, Berry SE, et al. Nuts and their Effect on Gut Microbiota, Gut Function and Symptoms in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Nutrients 2020;12(8) doi: 10.3390/nu12082347 [published Online First: 2020/08/13]
6. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Bmj 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557 [published Online First: 2003/09/06]
7. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]
8. Campbell M, McKenzie J, Sowden A, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. 2020;368:l6890. doi: 10.1136/bmj.l6890

2. The meaning of the abbreviation EULAR should be "European League Against Rheumatism" or "European League of Associations for Rheumatology."

Response：
Thank you very much for pointing out this mistake and giving me the opportunity to realize the wrong expression. The sentence has been amended to read:

European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to achieve the goal of continuous remission or low disease activity 1.

1. Smolen JS, Landewe RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis 2020 doi: 10.1136/annrheumdis-2019-216655 [published Online First: 2020/01/24]

3. "It is very important to reduce the occurrence of RA, delay joint injury and avoid disability, through the improvement of intestinal flora imbalance." This paragraph overestimates the possible effects of gut microbiota manipulation in the treatment of rheumatoid arthritis. (ref. DOI 10.1007/s11926-021-01031-9)

Response：
Thank you very much for your suggestions on the correction of this misleading expression. By reading the references, we have modified this sentence to read:
Regulating the gut microbiota to slow the progression of the disease, especially in the preclinical phase of RA, may be a promising approach for the treatment of RA in the future 1 2.

1. Horta-Baas G, Sandoval-Cabrera A, Romero-Figueroa MJCrr. Modification of Gut Microbiota in Inflammatory Arthritis: Highlights and Future Challenges. 2021;23(8):67. doi: 10.1007/s11926-021-01031-9
2. Gupta VK, Cunningham KY, Hur B, et al. Gut microbial determinants of clinically important improvement in patients with rheumatoid arthritis. Genome Med 2021;13(1):149. doi: 10.1186/s13073-021-00957-0 [published Online First: 2021/09/15]

4. "PICOS will be scientifically modified by substituting the item "Intervention" for "Investigation." My suggestion is to change by the acronym PECO. (ref. DOI 10.1016/j.envint.2018.07.015 Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes.)。

Response：
Thank you very much for your suggestion. We have replaced PICOS with PECOS, and modified the “Eligibility criteria” in the manuscript. The amendments are as follows:
Eligibility criteria
The studies, written in English as eligible, will be selected and screened based on PECOS steps (Population, Exposure, Comparator, Outcomes, and Study design) 1 2. The data items will be extracted as following:
Types of participants (P)
The population of interest of the eligible studies should be adults (≥18 years old) with met the diagnostic criteria(the ACR/EULAR 2010) for RA 3 or established RA (1987 classification criteria) 4 in the experimental group，the control group is a healthy population.
Type of exposure (E)
Trials were applied to assess the gut microbiota. Quantitative synthesis of microbiota in fecal samples was performed by using metagenomic shotgun sequencing, 16s rRNA sequencing techniques and/or real-time polymerase chain reaction (rt-PCR).
Comparison (C)
Only healthy adults will be considered eligible for the control group.
Type of outcomes (O)

The primary outcome of the study will be the composition of the gut microbiome and the relative abundance of bacteria in RA. The secondary outcomes will be considered: changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the effects of different gender and region on the relative abundance of gut microbiota.
Type of studies (S)
We will only include studies with the case-control design, written in English and published in the original peer-reviewed journals. The animal studies, reviews, case reports, and the full text unachieved will be excluded from the qualitative and quantitative synthesis.

1. Morgan RL, Thayer KA, Bero L, et al. GRADE: Assessing the quality of evidence in environmental and occupational health. Environment international 2016;92-93:611-6. doi: 10.1016/j.envint.2016.01.004 [published Online First: 2016/02/02]
2. Morgan RL, Whaley P, Thayer KA, et al. Identifying the PECO: A framework for formulating good questions to explore the association of environmental and other exposures with health outcomes. Environment international 2018;121(Pt 1):1027-31. doi: 10.1016/j.envint.2018.07.015 [published Online First: 2018/09/01]
3. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62(9):2569-81. doi: 10.1002/art.27584 [published Online First: 2010/09/28]
4. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31(3):315-24. doi: 10.1002/art.1780310302 [published Online First: 1988/03/01]

5. In the intervention, the authors consider including studies in which the microbiome was estimated using shotgun sequencing, 16s rRNA sequencing techniques, and/or real-time polymerase chain reaction (rt-PCR). In the case of meta-analysis, how will the statistical analysis be performed?

Response：
Thank you very much for your comments. We added the following to the manuscript:
Data synthesis and analysis
When the number of studies for a single bacterium was five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with health controls. We will adopt SMD with 95% CI of microbiota abundance as summary statistics when gut microbiota was detected by different techniques in the included studies 1-3. The included studies will be analyzed at the phylum or genus levels for consitency. The forest plots will be used to visualize the results. We will assess heterogeneity between studies using the Higgin I2 statistic. In relative terms, I2 values are proportional to heterogeneity: I2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity 4. Data analysis will be performed by a random-effect model when there is substantial heterogeneity (I2 > 50%); otherwise, a fixed-effects model will be used 5. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies.
If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline checklist 6. In order to define the characteristics of the gut microbiota in RA, we will perform compositional analysis based on the abundance, diversity, and specific bacterial detection of gut microbiota in RA patients and healthy controls.

1. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. Lipids Health Dis 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]

2. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. Nutrients 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]
3. Creedon AC, Hung ES, Berry SE, et al. Nuts and their Effect on Gut Microbiota, Gut Function and Symptoms in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Nutrients 2020;12(8) doi: 10.3390/nu12082347 [published Online First: 2020/08/13]
4. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Bmj 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557 [published Online First: 2003/09/06]
5. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]
6. Campbell M, McKenzie J, Sowden A, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. 2020;368:l6890. doi: 10.1136/bmj.l6890

6. Authors should consider the variability due to microbiota estimation methods and the lack of assessment of confounding factors (e.g., diet, smoking, medications, etc.) in existing studies on the subject.

Response：
Thank you very much for your comments.
The variability of results may be a dilemma faced by many researchers engaged in gut microbiota studies and related systematic reviews and meta-analyses. The results of gut microbiota examination can be affected by differences in gender, age, diet, drugs and specimen measurement methods, even in the same disease. Therefore, we consider that there may be heterogeneity in data collection between studies. In order to reduce the extent of heterogeneity, we have performed relatively strict inclusion criteria. The percentage and relative abundance of phyla or genus levels in gut microbiota will be used in this analysis to avoid potential variation due to different detection methods of the microbiome in the included studies. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies. We hope to minimize the variability of research results through the above measures.

7. Outcome. The authors consider assessing alpha and beta diversity, relative abundance, and even metabolites derived from the microbiota. The authors should specify which is the primary outcome of the study and which are the secondary outcomes. The relative abundance of bacteria at the family? or phylum? or genus? level. Alpha diversity indexes: OTUs? Shannon Index? Chao 1 Index? To the best of my knowledge, there is no study in RA patients that analyzes the amount of SCFAs.

Response：
Thank you very much for your comments.
The primary outcome of the study will be the composition of gut microbiome and the relative abundance of bacteria in RA. The secondary outcomes will be considered: changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the effects of different gender and region on the relative abundance of gut microbiota. The analysis will be performed at phylum or genus levels of the relative abundance of bacteria. Results of this study may include at least one alpha diversity index (OTUs, Shannon and Chao 1) if sufficient analyzable data are available.
Thank you very much for pointing out my mistake about “SCFAs”, which is very important for the revision of the manuscript. The analysis of SCFA is currently carried out in animal experiments of RA, and there is no study in clinical trials of RA patients. I apologize for my carelessness and have withdrawn the content.

Reviewer: 2
- It is not clear what the eventual goal is. The authors state that they plan on doing a meta-analysis, but this is a very broad term. Rather, the authors should clearly state what the specific aims or hypotheses are for the meta-analysis. What will the meta-analysis achieve and why is this significant?

Response：
Thank you very much for your comments, which made us review and revise the manuscript in depth, and elaborated in the relevant comments. The purpose of this protocol is to outline a systematic review and meta-analysis, which evaluates the changes in the diversity of gut microbiota and the relative abundance of bacterial phyla and genera in patients with RA. When revising the manuscript, we elaborated on the necessity and purpose of meta-analysis on gut microbiota of RA patients.
In the “INTRODUCTION” section:
The identification of specific microbial profiles and patterns that may contribute to the pathogenesis of RA remains a major challenge due to the inconsistent results of studies on the gut microbiota. The conflicting results may stem from inter-study batch effects, such as various biological factors influencing gut microbiome composition, different data processing and analysis methods 1 2. Through a quantitative review of the existing literature, the changes of RA gut microbiota can be understood more clearly and comprehensively. Recently, several meta-analyses of gut microbiota have identified specific microbial biomarkers associated with disease 3-8. However, there has been no systematic review and meta-analysis focusing on the characteristic dysbiosis of gut microbiota in RA to date. Therefore, we will perform a systematic review and meta-analysis to identify characteristic alterations in the gut microbiota of RA patients.
In the “Type of outcomes (O)” section:
The primary outcome of the study will be the composition of the gut microbiome and the relative abundance of bacteria in RA. The secondary outcomes will be considered: changes in the gut microbiota diversity (alpha-diversity, beta-diversity), the effects of different gender and region on the relative abundance of gut microbiota.

1. Wu Y, Jiao N, Zhu R, et al. Identification of microbial markers across populations in early detection of colorectal cancer. Nat Commun 2021;12(1):3063. doi: 10.1038/s41467-021-23265-y [published Online First: 2021/05/26]
2. Najafi S, Abedini F, Azimzadeh Jamalkandi S, et al. The composition of lung microbiome in lung cancer: a systematic review and meta-analysis. BMC microbiology 2021;21(1):315. doi: 10.1186/s12866-021-02375-z [published Online First: 2021/11/13]
3. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun 2018;9(1):4169. doi: 10.1038/s41467-018-06473-x [published Online First: 2018/10/12]
4. Shen T, Yue Y, He T, et al. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. Front Aging Neurosci 2021;13:636545. doi: 10.3389/fnagi.2021.636545 [published Online First: 2021/03/02]
5. Wirbel J, Pyl P, Kartal E, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. 2019;25(4):679-89. doi: 10.1038/s41591-019-0406-6
6. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573
7. Iglesias-Vazquez L, Van Ginkel Riba G, Arija V, et al. Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Nutrients 2020;12(3) doi: 10.3390/nu12030792 [published Online First: 2020/03/21]
8. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]

- The manuscript suffers from grammatical errors and serious flaws in paragraph coherency, especially in the Introduction of the main text. The reviewer suggests that the manuscript goes through a professional proofreading service prior to publication.

Response：
Thank you very much for your advice. I will try my best to improve my writing in English. I have rewritten the “Introduction" and invited the professor of medical English to review the manuscript. In order to ensure the intelligibility and preciseness of language expression, this manuscript will be proofread professionally before publication.
INTRODUCTION
RA is a chronic disease characterized by persistent synovitis, inflammatory and autoantibody changes 1. The prevalence of RA is about 1% globally, and 1.02% in China 2. The prevalence of RA in women is 2-3 times higher than that in men 3. Delays in diagnosis and treatment are associated with worse outcomes, including irreversible joint destruction, disability and disease-related non-articular outcomes such as reduced life span 4 5. In China, 77.6% of RA patients had disabilities, among which moderate and severe disabilities accounted for about 39%, seriously affecting the quality of life of patients 6. The gradual deterioration of RA leads to a sharp increase in the cost of the disease, which imposes a heavy societal and economic burden on individuals and the country 7-9.
RA is an ancient disease with a complex pathogenesis and is currently an incurable disease 10. European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to achieve the goal of continuous remission or low disease activity 11. The prognosis of RA has improved in recent decades with advances in diagnosis and treatment. However, as the etiology and pathogenesis of RA are not fully understood, the therapeutic effect is greatly reduced, which seriously hinders the effective remission of RA patients 1 11-15. Therefore, it is particularly important to explore the etiology and pathogenesis of RA.
Environmental factors are considered to play an important role in RA 16. The gut microbiota is considered an important environmental factor in the development of RA 17. Almost all studies on autoimmune rheumatic diseases show abnormal microbial community structure (i.e. dysbiosis) 18. Dysbiosis not only affects the pro-inflammatory and anti-inflammatory process of the intestinal mucosa, but also affects the distal joint through the intestinal-joint axis 19-21. The studies have found dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance of intestinal flora has occurred before the onset of RA 17 22. Dysbiosis has been involved in the pathogenesis of RA in the decade before its diagnosis 23. The intestinal flora imbalance also appeared in the initial peak and relapse stage of RA 24. Dysbiosis is related to the inflammatory response and disease activity of RA, which can be partially recovered by effective treatment 25-27. As a first-line treatment for RA, methotrexate (MTX) may act in part by modulating the human gut microbiota 27. The results of animal experiments suggest that interventions targeting intestinal microbiota may have the potential to prevent RA in the preclinical stage 28. Probiotics supplementation as adjunctive therapy improves the inflammatory state of RA in human and animal studies 29-32. Therefore, gut microbiota plays an important role in the development of RA, and may be a new therapeutic target 33 34. Gut microbiome studies of RA are essential to elucidate etiology and pathophysiological mechanisms and to develop potential therapeutic strategies. Regulating the gut microbiota to slow the progression of the disease, especially in the preclinical phase of RA, may be a promising approach for the treatment of RA in the future 35 36.
Although numerous studies have shown that dysbiosis of the gut microbiome is a key hallmark of RA, the distinct composition of the gut microbiome in RA patients remains controversial. The abundance of Prevotella increased in patients with early RA, which hurt the development and prognosis of RA 17 37-40. However, it has been reported that the abundance of Prevotella did not significantly change in RA patients 41. Moreover, P. copri and P. histicola of Prevotella have different effects on RA 17. Bacteroidetes were enriched in female patients with RA, while Actinomycetes and Collinsella were

enriched in healthy subjects 41. However, the abundance of Bacteroides and Bifidobacterium was found to be reduced in RA patients and animal experiments 42 43. It follows that the results of studies on the gut microbiota of RA patients are contradictory. The identification of specific microbial profiles and patterns that may contribute to the pathogenesis of RA remains a major challenge due to the inconsistent results of studies on the gut microbiota. The conflicting results may stem from inter-study batch effects, such as various biological factors influencing gut microbiome composition, different data processing and analysis methods 44 45. Through a quantitative review of the existing literature, the changes of RA gut microbiota can be understood more clearly and comprehensively. Recently, several meta-analyses of gut microbiota have identified specific microbial biomarkers associated with disease 46-51. However, there has been no systematic review and meta-analysis focusing on the characteristic dysbiosis of gut microbiota in RA to date. Therefore, we will perform a systematic review and meta-analysis to identify characteristic alterations in the gut microbiota of RA patients.

1. Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis: A Review. JAMA 2018;320(13):1360-72. doi: 10.1001/jama.2018.13103 [published Online First: 2018/10/05]
2. Alamanos YD, A. A. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005;4(3):130-6. doi: 10.1016/j.autrev.2004.09.002 [published Online First: 2005/04/13]
3. Gerlag DM, Norris JM, Tak PP. Towards prevention of autoantibody-positive rheumatoid arthritis: from lifestyle modification to preventive treatment. Rheumatology (Oxford) 2016;55(4):607-14. doi: 10.1093/rheumatology/kev347 [published Online First: 2015/09/17]
4. Taskforce ELAR. Rheuma Map A Research Roadmap to transform the lives of people with Rheumatic and Musculoskeletal Diseases. 2019
5. Veale DJ, Orr C, Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. Semin Immunopathol 2017;39(4):343-54. doi: 10.1007/s00281-017-0633-1 [published Online First: 2017/05/17]
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41. Jeong Y, Kim JW, You HJ, et al. Gut Microbial Composition and Function Are Altered in Patients with Early Rheumatoid Arthritis. J Clin Med 2019;8(5) doi: 10.3390/jcm8050693 [published Online First: 2019/05/19]
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- The description in the 'Data synthesis and analysis' section is not clearly described; not thorough; and not persuasively justified. This gives little confidence to this reviewer that the study will be rigorously performed and reproducible.

Response：
Thank you very much for your comments, which are very important for the revision of the manuscript. In order to elaborate the methods of data statistics, we have added corresponding contents in the “Data extraction”, and “Data synthesis and analysis” section.
Data extraction
To conduct the meta-analysis, we involve trials that have available and sufficient data to calculate the standardized mean difference (SMD) with 95% confidence interval (CI) in RA patients and healthy controls in the analysis of the pooled data set. If additional data or data transformations will be required for analysis, we will download the publicly available raw data from online repositories or links provided in the original publications. If there is no relevant data in the original literature, we will acquire it after personal communication with the authors of the manuscripts. If the authors do not reply, we will use Web Plot Digitizer (v.4.42 ) to digitize and extract sufficient data from graphs and plots in the articles 1 2.
Data synthesis and analysis
When the number of studies for a single bacterium was five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with health controls. We will adopt SMD with 95% CI of microbiota abundance as summary statistics when gut microbiota was detected by different techniques in the included studies 3-5. The included studies will be analyzed at the phylum or genus levels for consitency. The forest plots will be used to visualize the results. We will assess heterogeneity between studies using the Higgin I2 statistic. In relative terms, I2 values are proportional to heterogeneity: I2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity 6. Data analysis will be performed by a random-effect model when there is substantial heterogeneity (I2 > 50%); otherwise, a fixed-effects model will be used 7. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies.
If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline checklist 8. In order to define the characteristics of the gut microbiota in RA, we will perform compositional analysis based on the abundance, diversity, and specific bacterial detection of gut microbiota in RA patients and healthy controls.

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2. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573
3. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. Lipids Health Dis 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]

4. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. Nutrients 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]
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7. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]
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- p. 1, lines 49–53: The reviewer is confused whether this manuscript is a protocol for a comprehensive literature review or a meta-analysis (as mentioned later). Please state clearly what the purpose of this protocol is.

Response：
Thank you very much for your comments and reminders. This part was amended to:
The purpose of this protocol is to outline a systematic review and meta-analysis, which evaluates the changes in the diversity of gut microbiota and the relative abundance of bacterial phyla or genera in patients with RA.

- p. 2, lines 40–51: The authors state two strengths of this study. However, these can hardly be seen as strengths (hence, explaining why this study is important). First, the authors say that this review will "elucidate the characteristics of gut dysbiosis in patients with rheumatoid arthritis". How will a literature review/meta-analysis do so? Simply re-packaging others' works is not a scientific advancement unless a groundbreaking discovery is obtained. Second, the authors state that "the findings of this study will provide a scientific basis for exploring the biomolecular link between the gut microbiota and the pathogenesis of rheumatoid arthritis". There are enough review papers that suggest links between gut microbiota and rheumatoid arthritis. This paper is not needed to bolster an already established scientific basis for studying gut microbiota in rheumatoid arthritis.

Response：
Thank you very much for your comments. With the rapid growth of microbiome analysis technology, it has greatly promoted the development of human health-related disciplines. Despite the increasing amount of research on the microbiome, researchers currently have limited understanding of the precise relationship between the human gut microbiome and disease 1. Although gut microbiota is considered to play an important role in the pathogenesis of RA, the characteristic composition of gut microbiota in RA patients remains controversial. For example, some studies have shown a link between Prevotella and RA, but others have found no link between RA and Prevotella 2. The inconsistent results of dysbiosis may be partly due to different study designs and methods, which hinder the consistency of microbial results for the same disease 3. In addition, due to the limitations of research funds and other conditions, such as the small sample size, the repeatability and reliability of the results of microbiome detection are weakened, which limits the clinical utility of microbiota biomarkers 4 5. A meta-analysis is a statistical process that illustrates consistency between studies by summarizing comparable data from a number of scientific papers 1 6. Therefore, we hope to clarify the characteristics of gut dysbiosis in RA patients through a systematic review and meta-analysis.

And based on this, further scientific relationship between gut microbial markers and RA pathogenesis will be explored. Therefore, we think these are two strengths of this article.
After carefully elaborating your comments, we think that your comments are persuasive. We have withdrawn it from" Strengths and limitations of this study". This part was amended to:
This systematic review will identify the characteristic changes in the composition and diversity of gut microbiota in patients with RA, a significant but controversial clinical issue.
The percentage and relative abundance of phyla or genus levels in the gut microbiota will be used in this analysis to avoid potential variation due to different detection methods of the microbiome in the included studies.

1. Duvallet C, Gibbons SM, Gurry T, et al. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nature Communications 2017;8(1) doi: 10.1038/s41467-017-01973-8
2. Jeong Y, Kim JW, You HJ, et al. Gut Microbial Composition and Function Are Altered in Patients with Early Rheumatoid Arthritis. J Clin Med 2019;8(5) doi: 10.3390/jcm8050693 [published Online First: 2019/05/19]
3. Ho NT, Li F, Wang S, et al. metamicrobiomeR: an R package for analysis of microbiome relative abundance data using zero-inflated beta GAMLSS and meta-analysis across studies using random effects models. BMC bioinformatics 2019;20(1):188. doi: 10.1186/s12859-019-2744-2 [published Online First: 2019/04/18]
4. Ruan R, Deng X, Dong X, et al. Microbiota Emergencies in the Diagnosis of Lung Diseases: A Meta-Analysis. Front Cell Infect Microbiol 2021;11:709634. doi: 10.3389/fcimb.2021.709634 [published Online First: 2021/10/09]
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6. Kushkevych I, Martinkova K, Vitezova M, et al. Intestinal Microbiota and Perspectives of the Use of Meta-Analysis for Comparison of Ulcerative Colitis Studies. J Clin Med 2021;10(3) doi: 10.3390/jcm10030462 [published Online First: 2021/02/04]

- p. 2, lines 54–56: "Data pooled may be heterogeneous between studies"...this is simply stating what is already known in the field and cannot be viewed as a specific limitation of this particular study.

Response：
Thank you very much for your comments. The results of gut microbiota examination can be affected by differences in gender, age, diet, drugs and specimen measurement methods, even in the same disease. Therefore, we consider that there may be heterogeneity in data collection between studies. In order to reduce the extent of heterogeneity, we have performed relatively strict inclusion criteria and plan to perform subgroup analyses. However, heterogeneity of data synthesis may reduce the value of gut microbiome markers in RA pathogenesis. So we put it in the Limitations. After receiving your comments, we consider that your comments are convincing and, after careful adjudication, we have withdrawn them from" Strengths and limitations of this study".

- p. 2, line 59: "Some studies published in non-English languages may be missed.". Such studies should not be considered in the first place, as their peer review process is not credible. Is there any respectable study *not* written in English?

Response：
Thank you very much for your comments. Cochrane Handbook for Systematic Reviews of Interventions suggestion that searches should capture as many studies as possible that meet the eligibility criteria with no restricted by language 1. English is regarded as the universal language of

science. The inclusion criteria for studies written in English may make many studies published in non-English missed. Although excluding non-English learning has been proved not to change the conclusions of most systematic reviews, it is still considered as a limiting condition of the study 2 3.

1. Lefebvre C GJ, Briscoe S, Littlewood A, Marshall C, Metzendorf M-I, Noel-Storr A, Rader T, Shokraneh F, Thomas J, Wieland LS. Chapter 4: Searching for and selecting studies. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.2 (updated February 2021). Cochrane 2021;Available from www.training.cochrane.org/handbook.
2. Morrison A, Polisena J, Husereau D, et al. The effect of English-language restriction on systematic review-based meta-analyses: a systematic review of empirical studies. 2012;28(2):138-44. doi: 10.1017/s0266462312000086
3. Henrich J, Heine SJ, Norenzayan A. The weirdest people in the world? Behav Brain Sci 2010;33(2-3):61-83; discussion 83-135. doi: 10.1017/S0140525X0999152X [published Online First: 2010/06/17]

- The second and third paragraph of the Introduction has major issues in writing coherency, making this totally unreadable to this reviewer.

Response：
Thank you very much for your comments. I have rewritten the “Introduction” section in a wide range and will continue to work hard to improve my writing. The rewritten content is in the reply to the second comment. It is not described here to avoid unnecessary duplication. I apologize again for my poor English writing.

- p. 5, lines 23-30: Is the purpose of this study to do a literature review? What is the goal for the "meta-analysis"? Note that a "meta-analysis" is not a tool, as the authors seemingly imply here. Lastly, "biomarkers of dysbiosis" seems a very odd thing to say; "dysbiosis" *is* the biomarker for the disease (please think carefully about what a "biomarker" is supposed to do).

Response：
Thank you very much for your question that gave me a deeper insight into “dysbiosis” and the meta-analysis of gut microbiota in RA.
An increasing number of studies have shown that gut microbial “dysbiosis” is involved in the progression of RA. However, the term “dysbiosis” is a vague definition with varying interpretations. The so-called dysbiosis of the gut microbiota refers to changes in the composition and representation of individual species of microorganisms in comparison with healthy individuals 1. Dysbiosis also defined as an alteration in the diversity and abundance of intestinal microbes 2. In microbial ecology, dysbiosis can be defined as the disruption of potential ecological links between microorganisms, such as competition and inhibition 3. Dysbiosis in this study refers to the abnormal alterations in the diversity and abundance of gut microbes in RA patients compared with healthy individuals. Although the gut microbiota is profoundly important in the pathogenesis of RA, the studies on dysbiosis in RA patients have reported inconsistent or even opposing results. The distinct composition of gut microbiota in RA patients remains controversial 4. The objective of the meta-analysis is to determine the consistency of various microbiota studies and provide reliable results 5. Characteristic changes in gut microbiota associated with a disease are considered its microbial biomarkers. Recently, several meta-analyses of gut microbiota have identified specific microbial biomarkers associated with disease 6-11. Therefore we will perform a systematic review and meta-analysis to identify characteristic biomarkers of the gut microbiota in RA patients.

1. Kushkevych I, Martinkova K, Vitezova M, et al. Intestinal Microbiota and Perspectives of the Use of Meta-Analysis for Comparison of Ulcerative Colitis Studies. J Clin Med 2021;10(3) doi: 10.3390/jcm10030462 [published Online First: 2021/02/04]
2. Kameli N, Borman R, Lpez-Iglesias C, et al. Characterization of Feces-Derived Bacterial Membrane Vesicles and the Impact of Their Origin on the Inflammatory Response. Front Cell Infect Microbiol 2021;11:667987. doi: 10.3389/fcimb.2021.667987 [published Online First: 2021/05/25]
3. Olesen SW, Alm EJ. Dysbiosis is not an answer. Nat Microbiol 2016;1:16228. doi: 10.1038/nmicrobiol.2016.228 [published Online First: 2016/11/26]
4. Duvallet C, Gibbons SM, Gurry T, et al. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nature Communications 2017;8(1) doi: 10.1038/s41467-017-01973-8
5. Ho NT, Li F, Wang S, et al. metamicrobiomeR: an R package for analysis of microbiome relative abundance data using zero-inflated beta GAMLSS and meta-analysis across studies using random effects models. BMC bioinformatics 2019;20(1):188. doi: 10.1186/s12859-019-2744-2 [published Online First: 2019/04/18]
6. Ho NT, Li F, Lee-Sarwar KA, et al. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. Nat Commun 2018;9(1):4169. doi: 10.1038/s41467-018-06473-x [published Online First: 2018/10/12]
7. Shen T, Yue Y, He T, et al. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. Front Aging Neurosci 2021;13:636545. doi: 10.3389/fnagi.2021.636545 [published Online First: 2021/03/02]
8. Wirbel J, Pyl P, Kartal E, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. 2019;25(4):679-89. doi: 10.1038/s41591-019-0406-6
9. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573
10. Iglesias-Vazquez L, Van Ginkel Riba G, Arija V, et al. Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Nutrients 2020;12(3) doi: 10.3390/nu12030792 [published Online First: 2020/03/21]
11. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]

--This Objective needs major proofreading.

Response：
Thank you very much for your comments. This objective has been modified to: The purpose of this protocol is to outline a systematic review and meta-analysis, which evaluates the changes in the diversity of gut microbiota and the relative abundance of bacterial phyla or genera in patients with RA.

-- What is a "case-control trains"??

Response：
Thank you very much for your reminding. It has been revised in the corresponding part of the manuscript.

- 'Data synthesis and analysis' section:
-- p. 9, line 59: How does the author expect to calculate a 'pooled effect estimate' with highly sparse, negative binomial distributed data? Please elaborate and justify.

Response：

Thank you very much for your comments, which are very important for the revision of the manuscript. In order to elaborate the methods of data statistics, we have added corresponding contents in the 'Data extraction', and 'Data synthesis and analysis' section.

Data extraction

To conduct the meta-analysis, we involve trials that have available and sufficient data to calculate the standardized mean difference (SMD) with 95% confidence interval (CI) in RA patients and healthy controls in the analysis of the pooled data set. If additional data or data transformations will be required for analysis, we will download the publicly available raw data from online repositories or links provided in the original publications. If there is no relevant data in the original literature, we will acquire it after personal communication with the authors of the manuscripts. If the authors do not reply, we will use Web Plot Digitizer (v.4.42 ) to digitize and extract sufficient data from graphs and plots in the articles 1 2.

Data synthesis and analysis

When the number of studies for a single bacterium was five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with health controls. We will adopt SMD with 95% CI of microbiota abundance as summary statistics when gut microbiota was detected by different techniques in the included studies 3-5. The included studies will be analyzed at the phylum or genus levels for consitency. The forest plots will be used to visualize the results. We will assess heterogeneity between studies using the Higgin I2 statistic. In relative terms, I2 values are proportional to heterogeneity: I2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity 6. Data analysis will be performed by a random-effect model when there is substantial heterogeneity (I2 > 50%); otherwise, a fixed-effects model will be used 7. Additionally, we will conduct subgroup analysis of different genders (male/female) and regions (east/west) included in the studies.

If meta-analysis is not feasible, we will conduct narrative synthesis to summarize the relevant evidence between RA and gut dysbiosis. The quantitative narrative synthesis will be conducted according to the Synthesis Without Meta-analysis (SWiM) guideline checklist 8. In order to define the characteristics of the gut microbiota in RA, we will perform compositional analysis based on the abundance, diversity, and specific bacterial detection of gut microbiota in RA patients and healthy controls.

1. Safadi J, Quinton A, Lennox B, et al. Gut dysbiosis in severe mental illness and chronic fatigue: a novel trans-diagnostic construct? A systematic review and meta-analysis. 2021 doi: 10.1038/s41380-021-01032-1

2. Nikolova V, Smith M, Hall L, et al. Perturbations in Gut Microbiota Composition in Psychiatric Disorders: A Review and Meta-analysis. 2021 doi: 10.1001/jamapsychiatry.2021.2573

3. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. Lipids Health Dis 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]

4. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. Nutrients 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]

5. Creedon AC, Hung ES, Berry SE, et al. Nuts and their Effect on Gut Microbiota, Gut Function and Symptoms in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Nutrients 2020;12(8) doi: 10.3390/nu12082347 [published Online First: 2020/08/13]

6. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Bmj 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557 [published Online First: 2003/09/06]

7. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]

8. Campbell M, McKenzie J, Sowden A, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. 2020;368:l6890. doi: 10.1136/bmj.l6890

-- p. 10, line 17: Please explain why a random-effects analysis is needed here. In particular, the reviewer would like to know what the fixed and random effects are deemed to be in the meta-analysis.

Response：
Thank you very much for your comments. Statistical models commonly used to combine data in the meta-analysis are the fixed-effect model and the random-effects model. In a meta-analysis, a fixed-effect model assumes that there is no statistical heterogeneity in the population value of all outcomes being assessed 1. A random-effects model assumes that the results of all studies are different due to random factors as well as the heterogeneity of the studies 1. I2 describes the percentage of total variation in the study due to heterogeneity, I2 values of 25%, 50%, and 75% means low, moderate, and high heterogeneity 2. Fixed and random effects models give the same result when there is no heterogeneity among the studies , I2 = 0 3. A random-effects model was applied when there was substantial heterogeneity (I 2 ≥ 50%); otherwise, a fixed-effects model was used (I 2< 50%) 4-8. Considering the inherent heterogeneity among the included studies, we will use a random-effects model for data analysis.

1. Andrade C. Understanding the Basics of Meta-Analysis and How to Read a Forest Plot: As Simple as It Gets. The Journal of clinical psychiatry 2020;81(5) doi: 10.4088/JCP.20f13698 [published Online First: 2020/10/08]
2. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Bmj 2003;327(7414):557-60. doi: 10.1136/bmj.327.7414.557 [published Online First: 2003/09/06]
3. Deeks JJ HJ, Altman DG (editors). Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.1 (updated September 2020). Cochrane 2020;Available from www.training.cochrane.org/handbook
4. Miao L, Du J, Chen Z, et al. Effects of Microbiota-Driven Therapy on Circulating Trimethylamine-N-Oxide Metabolism: A Systematic Review and Meta-Analysis. Front Cardiovasc Med 2021;8:710567. doi: 10.3389/fcvm.2021.710567 [published Online First: 2021/09/24]
5. Xu M, Xu X, Li J, et al. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry 2019;10:473. doi: 10.3389/fpsyt.2019.00473 [published Online First: 2019/08/14]
6. Li F, Ye J, Shao C, et al. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. Lipids Health Dis 2021;20(1):22. doi: 10.1186/s12944-021-01440-w [published Online First: 2021/02/28]
7. Kim KN, Yao Y, Ju SY. Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans with Obesity: A Systematic Review and Meta-Analysis. Nutrients 2019;11(10) doi: 10.3390/nu11102512 [published Online First: 2019/10/23]
8. Creedon AC, Hung ES, Berry SE, et al. Nuts and their Effect on Gut Microbiota, Gut Function and Symptoms in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Nutrients 2020;12(8) doi: 10.3390/nu12082347 [published Online First: 2020/08/13]

--- p. 10, line 30–35: In what circumstance does the author think a meta-analysis would not be feasible? And what precisely is a "narrative synthesis"?

Response：
Thank you very much for your comments. Meta-analysis is a statistical method that combines data from independent studies. It is essential to ensure that sufficient data sets are combined so that heterogeneous or confounding results do not drive the results of the meta-analysis 1. A meta-analysis

is infeasible if relatively few data are available. Therefore, when the number of studies on a single bacterium is more than five, we will perform a meta-analysis 2 3.
Narrative synthesis refers to an approach to the systematic review and synthesis of findings from multiple studies that relies primarily on the use of words and text to summarize and explain the findings of the synthesis 4. Narrative synthesis is an excellent alternative to quantitative data synthesis when meta-analysis is not feasible 5. Narrative synthesis is often used to describe the results of included studies in systematic evaluations of public health 6-8. Therefore, we will carry out narrative synthesis according to the Synthesis Without Meta-analysis guideline, if meta-analysis is not feasible 9.
1. Glass GV. Primary, secondary, and meta-analysis of research. Educat Res 1976;5: 3–8
2. Ji R, Zhao X, Cao X, et al. Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol. BMJ Open 2021;11(3):e045810. doi: 10.1136/bmjopen-2020-045810 [published Online First: 2021/03/04]
3. Shen T, Yue Y, He T, et al. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. Front Aging Neurosci 2021;13:636545. doi: 10.3389/fnagi.2021.636545 [published Online First: 2021/03/02]
4. Rodgers M, Arai L, Britten N, et al. Guidance on the conduct of narrative synthesis in systematic reviews: a comparison of guidance-led narrative synthesis versus meta-analysis. 2001
5. Campbell M, Katikireddi SV, Sowden A, et al. Lack of transparency in reporting narrative synthesis of quantitative data: a methodological assessment of systematic reviews. Journal of clinical epidemiology 2019;105:1-9. doi: 10.1016/j.jclinepi.2018.08.019 [published Online First: 2018/09/10]
6. Dharmaratne P, Rahman N, Leung A, et al. Is there a role of faecal microbiota transplantation in reducing antibiotic resistance burden in gut? A systematic review and Meta-analysis. Annals of medicine 2021;53(1):662-81. doi: 10.1080/07853890.2021.1927170 [published Online First: 2021/06/26]
7. Rao R, Dsouza JM, Mathew JL. Comparison of microbiota in the upper versus lower respiratory tract in children during health and respiratory disease: protocol for a systematic review. Syst Rev 2021;10(1):253. doi: 10.1186/s13643-021-01806-2 [published Online First: 2021/09/23]
8. Chan M, Baxter H, Larsen N, et al. Impact of botanical fermented foods on metabolic biomarkers and gut microbiota in adults with metabolic syndrome and type 2 diabetes: a systematic review protocol. BMJ Open 2019;9(7):e029242. doi: 10.1136/bmjopen-2019-029242 [published Online First: 2019/08/02]
9. Campbell M, McKenzie JE, Sowden A, et al. Synthesis without meta-analysis (SWiM) in systematic reviews: reporting guideline. Bmj 2020;368:l6890. doi: 10.1136/bmj.l6890 [published Online First: 2020/01/18]

-- p. 10, 'Assessment of publication bias'. Please justify the methods. Is the author familiar with the purpose and implementation of these tests?

Response：
Thank you very much for pointing out this mistake and giving me the opportunity to realize the wrong expression of "Begg's tunnel plot". Some published documents described "tunnel plot" as "Begg's tunnel plot", which makes me misunderstand. I deeply apologize for my lack of knowledge, negligence and carelessness. We refer to similar literature and intend to change this part to the following: We will apply funnel plots and Egger's test to assess publication bias. If funnel plots present asymmetry, we will use Egger's test to statistically examination 5 6.
Funnel plots are often used to detect publication bias because of their intuitiveness and simplicity 7 8. Funnel plots presenting asymmetry are considered publication bias 8. However, different observers may have different interpretations of graphs from the same funnel plots due to the subjectivity of the graphs. Therefore, it is recommended that multiple testing methods should be applied to assess publication bias 9. The asymmetry of the funnel plots was statistically evaluated by three test

methods: Begg’s, Egger’s, and Macaskill’s 10. Among the methods to detect publication bias, Egger’s test is used more frequently than other tests 11. Therefore, we plan to assess publication bias visually by drawing funnel plots and statistically by performing Egger's test.

1. Wang L, Alammar N, Singh R, et al. Gut Microbial Dysbiosis in the Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis of Case-Control Studies. J Acad Nutr Diet 2020;120(4):565-86. doi: 10.1016/j.jand.2019.05.015 [published Online First: 2019/09/02]
2. Ji R, Zhao X, Cao X, et al. Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol. 2021;11(3):e045810. doi: 10.1136/bmjopen-2020-045810
3. Andrade C. Understanding the Basics of Meta-Analysis and How to Read a Forest Plot: As Simple as It Gets. The Journal of clinical psychiatry 2020;81(5) doi: 10.4088/JCP.20f13698 [published Online First: 2020/10/08]
4. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. Bmj 1997;315(7109):629-34. doi: 10.1136/bmj.315.7109.629 [published Online First: 1997/10/06]
5. Lin L, Chu H, Murad MH, et al. Empirical Comparison of Publication Bias Tests in Meta-Analysis. J Gen Intern Med 2018;33(8):1260-67. doi: 10.1007/s11606-018-4425-7 [published Online First: 2018/04/18]
6. Herrmann D, Sinnett P, Holmes J, et al. Statistical controversies in clinical research: publication bias evaluations are not routinely conducted in clinical oncology systematic reviews. Annals of oncology : official journal of the European Society for Medical Oncology 2017;28(5):931-37. doi: 10.1093/annonc/mdw691 [published Online First: 2017/01/01]
7. van Enst WA, Ochodo E, Scholten RJ, et al. Investigation of publication bias in meta-analyses of diagnostic test accuracy: a meta-epidemiological study. BMC Med Res Methodol 2014;14:70. doi: 10.1186/1471-2288-14-70 [published Online First: 2014/06/03]

-- p. 10, line 20: Among many suitable approaches, why forest plots in particular?

Response：
Thank you very much for your comments.
Visual display and presentation of data is especially important for transparent reporting in systemic review and meta-analysis. Cochrane Handbook for Systematic Reviews of Interventions introduces the following modes of visual display and representation of data: structured tabulation of results across studies, forest plots, box-and-whisker plots and bubble plots, albatross plot, harvest and effect direction plots 1. The results of the meta-analysis are usually presented graphically in a forest plot 2 3. Forest plots used tabular and graphical information to show results from individual studies and pooled analyses 4. The visual representation about estimates of comparisons or associations, corresponding precision and statistical significance makes it easier to see variations between individual study results 5. Therefore, as the most prominent and widely used graphic display in systemic review and meta-analysis, forest plots are considered as the gold standard for result visualization, and has a high utilization rate in medical journals 6 7. Based on the above, we will choose forest plots to visualize the results.

1. McKenzie JE BS. Chapter 12: Synthesizing and presenting findings using other methods. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane 2019; Available from www.training.cochrane.org/handbook
2. Andrade C. Understanding the Basics of Meta-Analysis and How to Read a Forest Plot: As Simple as It Gets. The Journal of clinical psychiatry 2020;81(5) doi: 10.4088/JCP.20f13698 [published Online First: 2020/10/08]

3. Dettori JR, Norvell DC, Chapman JR. Seeing the Forest by Looking at the Trees: How to Interpret a Meta-Analysis Forest Plot. Global spine journal 2021;11(4):614-16. doi: 10.1177/21925682211003889 [published Online First: 2021/05/04]
4. Alavi M, Hunt G, Visentin D, et al. Seeing the forest for the trees: How to interpret a meta-analysis forest plot. 2021;77(3):1097-101. doi: 10.1111/jan.14721
5. Li G, Zeng J, Tian J, et al. Multiple uses of forest plots in presenting analysis results in health research: A Tutorial. 2020;117:89-98. doi: 10.1016/j.jclinepi.2019.09.021
6. Schild AH, Voracek M. Finding your way out of the forest without a trail of bread crumbs: development and evaluation of two novel displays of forest plots. Research synthesis methods 2015;6(1):74-86. doi: 10.1002/jrsm.1125 [published Online First: 2015/06/04]
7. Schild A, Voracek MJRsm. Less is less: a systematic review of graph use in meta-analyses. 2013;4(3):209-19. doi: 10.1002/jrsm.1076

- please change all instances of "the rheumatoid arthritis" to simply "rheumatoid arthritis" (including in the title).

Response：
Thank you very much for your advice. The error has been corrected in the manuscript.

- please be consistent in how references are cited. Most of the time, spaces are provided before the reference number, but there are a few cases where this is not so.

Response：
Thank you very much for your advice. I have modified the format of the references.

- p. 1, line 33: "social" should be "societal"

Response：
Thank you very much for your advice. The sentence has been amended to read:
Rheumatoid arthritis (RA) has a huge societal impact due to the high prevalence, irreversible joint damage and systemic complications.

- p. 1, line 43: I am not aware of any clinical practice where "restoring intestinal homeostasis by altering microbiota" is used as an approach to prevent and treat rheumatoid arthritis. The sentence is therefore incorrect. Did the authors mean "could be" rather than "is"?

Response：
Thank you very much for your suggestions on the correction of this misleading expression. The sentence has been amended to read:
Restoring intestinal homeostasis by altering the microbiota could be an attractive strategy for the prevention and treatment of RA.

- p. 1, line 54: Please remove "of".

Response：
Thank you very much for your advice. The sentence has been amended to read:

Therefore, we aim to elucidate the characteristic change in the diversity and composition of gut microbiota in RA.

- p. 2, line 7: Please define "grey literature" or reword.

Response：
Thank you very much for your advice. The sentence has been amended to read:
We will systematically search through PubMed, EMBASE, Web of Science and Cochrane Library, as well as dissertations and conference proceedings. The reference lists of all included studies will be also reviewed to retrieve additional relevant studies.

- p. 2, line 14: Meta-analysis is a very broad term with no single goal or expected outcome. Thus "meta-analyses will be performed..." seems very vague, and it would be helpful to mention what the authors are specifically looking for.

Response：
Thank you very much for your advice. The sentence has been amended to read:
The expected outcomes will be the specific changes in composition and diversity of the gut microbiota in patients with RA.

-p. 2, line 28: Please change "patients' individuals" to "patients".

Response：
Thank you very much for your advice. The sentence has been amended to read:
Ethical approval is unnecessary as this review does not address the data and privacy of patients.

-p. 2, line 53: Please change "due to gender..." to "due to differences in gender..."

Response：
Thank you very much for your advice. The sentence has been withdrawn.

- p. 3, line 38: Please correctly explain the acronym for "EULAR". This is something the authors *cannot* be allowed to get wrong.

Response：
Thank you very much for pointing out this mistake. The sentence has been amended to read:
European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to achieve the goal of continuous remission or low disease activity

1. Smolen JS, Landewe RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis 2020 doi: 10.1136/annrheumdis-2019-216655 [published Online First: 2020/01/24]

- by convention in any microbiology paper, please italicize names of genera or species.

Response：
Thank you very much for your advice. The sentences have been amended to read:
The abundance of Prevotella increased in patients with early RA, which hurt the development and prognosis of RA 1-5. However, it has been reported that the abundance of Prevotella did not significantly change in RA patients 6. Moreover, P. copri and P. histicola of Prevotella have different effects on RA 5. Bacteroidetes were enriched in female patients with RA, while Actinomycetes and Collinsella were enriched in healthy subjects 6. However, the abundance of Bacteroides and Bifidobacterium was found to be reduced in RA patients and animal experiments 7 8.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Jaeyun Sung<br>Mayo Clinic, Division of Rheumatology |
| **REVIEW RETURNED** | 27-Dec-2021 |

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|---|---|
| **GENERAL COMMENTS** | Overall, greatly improved manuscript. This reviewer commends the authors for their efforts. I do have the following minor editorial suggestions to further improve the manuscript:<br><br>- In the Introduction, the author proposes a few reasons underlying the inter-study variability in RA gut microbiome observations. One important factor that has not yet been included (but should not be overlooked) is the differences in demographics of the study cohorts, e.g., sex, age, ethnicity, geography, and diet. It would be appropriate to mention this.<br><br>- Please change "The primary outcome of the study will be the composition of the gut microbiome..." to "The primary outcome of the study will be the identification of the composition of the gut microbiome...". The outcome is what results from the study, so saying "the identification of" would make more sense. Same for "The expected outcomes will be the specific changes in composition and diversity of<br>the gut microbiota in patients with RA.".<br><br>- "RA is an ancient disease...". This sounds odd. How long ago is ancient? If difficult to clarify, please truncate.<br><br>- "However, as the etiology and pathogenesis of RA are not fully understood, the therapeutic effect is greatly reduced, ..." This doesn't make logical sense. Just because one doesn't know the etiology and pathogenesis of RA, that doesn't mean the a therapy will have drastic reductions in efficacy. Please reword this segment while thinking more carefully about cause and effect.<br><br>- "The studies have found dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance of intestinal flora has occurred before the onset of RA 17 22." Please change "has occured before" to "could have occured before".<br><br>- "When the number of studies for a single bacterium was five or more". Please change "was" to "is" if you're indicating future tense.<br><br>- Please change "health controls" to "healthy controls".<br><br>- "The percentage and relative abundance of phyla or genus levels in the gut microbiota will be used in this analysis to avoid potential variation due to different detection methods of the microbiome in the |

| | |
|---|---|
| | included studies." Using proportional data, or conducting this analysis for phyla or genus taxonomic ranks, will not eliminate the risk of "potential variation due to different detection methods of the microbiome in the included studies." (any good microbiome bioinformatician will understand why.) I suggest changing this sentence to simply "The relative abundances of phyla and/or genus levels in the gut microbiota will be used in this meta-analysis."<br><br>- Although this reviewer agrees that having a meta-analysis on RA gut microbiome will contribute to the advancement of RA research, it is certainly not guaranteed that the authors will find robust characteristic biomarker signals. Please elaborate a bit further on what the authors will do in case no robust characteristics are observed. I see that a 'narrative synthesis' will be done when the meta-analysis is not feasible, but what conclusions will the authors reach when the meta-analysis is indeed feasible but no noticeably consistent trend is observed? Providing thoughts on potential back-up plans would be insightful to the audience. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 2

Dr. Jaeyun Sung, Mayo Clinic

Comments to the Author:

Overall, greatly improved manuscript. This reviewer commends the authors for their efforts. I do have the following minor editorial suggestions to further improve the manuscript:

- In the Introduction, the author proposes a few reasons underlying the inter-study variability in RA gut microbiome observations. One important factor that has not yet been included (but should not be overlooked) is the differences in demographics of the study cohorts, e.g., sex, age, ethnicity, geography, and diet. It would be appropriate to mention this.

Response：

Thank you very much for pointing out the omissions in the manuscript. The sentence has been amended to read:

The conflicting results may stem from inter-study batch effects, such as various biological factors influencing gut microbiome composition, different data processing and analysis methods. The differences in demographics of the study cohorts (e.g., sex, age, ethnicity, geography, and diet) also have an important influence on the variability of the results of the gut microbiome study.

- Please change "The primary outcome of the study will be the composition of the gut microbiome..." to "The primary outcome of the study will be the identification of the composition of the gut microbiome...". The outcome is what results from the study, so saying "the identification of" would

make more sense. Same for "The expected outcomes will be the specific changes in composition and diversity of the gut microbiota in patients with RA.".

Response：

Thank you very much for your advice. The sentences have been amended to read:

The expected outcomes will be the identification of the specific changes in composition and diversity of the gut microbiota in patients with RA.

The primary outcome of the study will be the identification of the composition of the gut microbiome and the relative abundance of bacteria in RA.

- "RA is an ancient disease...". This sounds odd. How long ago is ancient? If difficult to clarify, please truncate.

Response：

Thank you very much for your suggestions to correct this imprecise expression. We looked at the literature and found that the antiquity of RA remains controversial 1-5. One school of thought is that RA emerged in America since 8000 BC and in Europe since the 7th century 1 6. But another theory postulates that RA is a disease of the modern era and that its pathogenesis is a result of an environmental or genetic stimulus that did not exist in ancient times 4 5. So, we amended the sentence to read:

RA is a lifelong condition and currently no cure for most patients.

1. Domen RE. Peleopathological evidence of rheumatoid arthritis. Jama 1981;246(17):1899. [published Online First: 1981/10/23]

2. Dequeker J, Rico H. Rheumatoid arthritis-like deformities in an early 16th-century painting of the Flemish-Dutch school. Jama 1992;268(2):249-51. [published Online First: 1992/07/08]

3. Domen RJJ. The antiquity and origins of rheumatoid arthritis. 1992;268(19):2649. doi: 10.1001/jama.1992.03490190049018

4. Kwiecinski J, Rothschild BJRi. No rheumatoid arthritis in ancient Egypt: a reappraisal. 2016;36(6):891-5. doi: 10.1007/s00296-015-3405-z

5. Entezami P, Fox DA, Clapham PJ, et al. Historical perspective on the etiology of rheumatoid arthritis. Hand clinics 2011;27(1):1-10. doi: 10.1016/j.hcl.2010.09.006 [published Online First: 2010/12/24]

6. Aceves-Avila FJ, Medina F, Fraga A. The antiquity of rheumatoid arthritis: a reappraisal. The Journal of rheumatology 2001;28(4):751-7. [published Online First: 2001/05/01]

- "However, as the etiology and pathogenesis of RA are not fully understood, the therapeutic effect is greatly reduced, ..." This doesn't make logical sense. Just because one doesn't know the etiology and pathogenesis of RA, that doesn't mean that a therapy will have drastic reductions in efficacy. Please reword this segment while thinking more carefully about cause and effect.

Response：

Thank you very much for helping me to point out the logical error. The sentence has been amended to read:

RA is a lifelong condition and currently no cure for most patients 1 2. European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR) recommend that the purpose of RA treatment should be to enable each patient to achieve the goal of continuous remission or low disease activity 3. Although the prognosis of RA has improved with advances in diagnosis and treatment in recent decades, the exact etiology and pathogenesis of RA are not fully understood. In order to develop more effective treatment strategies for RA, it is essential to explore its underlying etiology and pathogenesis.

1. Smolen J, Landewé R, Bijlsma J, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. 2020;79(6):685-99. doi: 10.1136/annrheumdis-2019-216655

2. Alam J, Jantan I, Bukhari SNA. Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. Biomed Pharmacother 2017;92:615-33. doi: 10.1016/j.biopha.2017.05.055 [published Online First: 2017/06/06]

3. Smolen JS, Landewe RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. Ann Rheum Dis 2020 doi: 10.1136/annrheumdis-2019-216655 [published Online First: 2020/01/24]

- "The studies have found dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance of intestinal flora has occurred before the onset of RA 17 22." Please change "has occured before" to "could have occured before".

Response：

Thank you very much for your advice. The sentence has been amended to read:

The studies have found dysbiosis in both RA patients and high-risk individuals, indicating that the imbalance of intestinal flora could have occured before the onset of RA.

- "When the number of studies for a single bacterium was five or more". Please change "was" to "is" if you're indicating future tense.

Response：

Thank you very much for your advice. The sentence has been amended to read:

When the number of studies for a single bacterium is five or more, we will conduct the meta-analysis by R language Version 3.4.3 to compare the abundance level of gut microbiota in RA patients with healthy controls.

- Please change "health controls" to "healthy controls".

Response：

Thank you very much for your reminding. It has been revised in the corresponding part of the manuscript.

- "The percentage and relative abundance of phyla or genus levels in the gut microbiota will be used in this analysis to avoid potential variation due to different detection methods of the microbiome in the included studies." Using proportional data, or conducting this analysis for phyla or genus taxonomic ranks, will not eliminate the risk of "potential variation due to different detection methods of the microbiome in the included studies." (any good microbiome bioinformatician will understand why.) I suggest changing this sentence to simply "The relative abundances of phyla and/or genus levels in the gut microbiota will be used in this meta-analysis."

Response：

Thank you very much for your advice. The sentence has been amended to read:

The relative abundances of phyla and/or genus levels in the gut microbiota will be used in this meta-analysis.

- Although this reviewer agrees that having a meta-analysis on RA gut microbiome will contribute to the advancement of RA research, it is certainly not guaranteed that the authors will find robust characteristic biomarker signals. Please elaborate a bit further on what the authors will do in case no robust characteristics are observed. I see that a 'narrative synthesis' will be done when the meta-analysis is not feasible, but what conclusions will the authors reach when the meta-analysis is indeed

feasible but no noticeably consistent trend is observed? Providing thoughts on potential back-up plans would be insightful to the audience.

Response:

Thank you very much for your comment, which makes us think more deeply about the possible results of meta-analysis on gut microbiome of RA. The results of a meta-analysis of gut microbiota may be related to sample size, microbiota detection methods, different geographical regions and gender. If we did not observe robust characteristic biomarker signals through meta-analysis, we would do the following: 1. carefully analyze the results, especially the results of subgroup analysis, to explore whether there are significant changes in gut microbiome of RA in different genders or regions; 2. observational prospective, retrospective or cohort studies will be included to increase the sample size 1.

Gut microbiota structure varied within individuals due to a series of factors including dietary habits, geographical region, gender, and so on. According to the results of a large number of clinical and preclinical studies, dysbiosis plays an important role in the development of RA 2-7. Therefore, we will conclude reasonably that the existence of dysbiosis in RA patients cannot be denied even though the meta-analysis did not produce a noticeably consistent trend in the meta-analysis.

**VERSION 3 – REVIEW**

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| **REVIEWER** | Jaeyun Sung<br>Mayo Clinic, Division of Rheumatology |
| **REVIEW RETURNED** | 12-Feb-2022 |

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| **GENERAL COMMENTS** | This reviewer thanks the authors for their hard work in addressing all of my editorial suggestions. Please accept as-is. |