Manuscript NO.: 62969, Observational Study
Title: Gut Microbiota Dysbiosis in Chinese Children with Type 1 Diabetes Mellitus: A Case-control Study

Authors: Xia Liu; Yi-wen Cheng; Li Shao; Shu-hong Sun; Jian Wu; Qing-hai Song; Hong-sheng Zou; Zong-xin Ling

February 17, 2021

Dear editor,

Thank you very much for your help to deal with our manuscript. Most of the comments from the Science editor and four reviewers are helpful and valuable. We have revised our manuscript and would like to re-submit it for your consideration. We have addressed the comments raised by the three reviewers by point to point to the best of our ability. We hope that this revision is acceptable, and we look forward to hearing from you soon.

Best wishes,

Sincerely yours,
Zongxin Ling, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, 310003, China. E-mail: lingzongxin@zju.edu.cn. Tel.: +86-571-8723-6436; Fax: +86-571-8723-6436.

Response to Science editor:

1 Scientific quality: The manuscript describes an observational study of the fecal microbiota dysbiosis in Chinese children with type 1 diabetes mellitus. The topic is within the scope of the WJG. (1) Classification: Grade A, two Grades B, and Grade C;
Thank you so much.

(2) Summary of the Peer-Review Report: The overall structure of the manuscript is complete. The text and figures are well structured and the methods employed are logical and well described. In regard to the ethical considerations, the text appears to be appropriate. The questions raised by the reviewers should be answered; and
Thank you so much.

(3) Format: There is 1 table and 8 figures. A total of 87 references are cited, including 35 references published in the last 3 years. There are no self-citations.
Thank you so much.

2 Language evaluation: Classification: Grade A and three Grades B. A language editing certificate issued by Charlesworth was provided.
Thank you so much.

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form, and the written informed consent. No academic misconduct was found in the Bing search.
Thank you so much.

4 Supplementary comments: This is an invited manuscript. The study was supported by National Natural Science Foundation of China. The topic has not previously been published in the WJG.
Thank you so much.

5 Issues raised:
(1) The key word “gut” is missing in the title. Please add it;
Thank you so much for your comments. we have revised the title into: Gut Microbiota Dysbiosis in Chinese Children with Type 1 Diabetes Mellitus: A Case-control Study

(2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);
Thank you so much. We have provided the Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s)

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Thank you so much. We have provided the Figures using PowerPoint.

(4) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text;
We have added the article highlights section at the end of the main text.

and (5) Authors should always cite references that are relevant to their study. Please check and remove any references that not relevant to this study.
We have checked the references.

6 Recommendation: Conditional acceptance.
Thank you so much.

Response to Reviewer #1

Thank you very much for reviewing our manuscript. Thank you for your help to revise my manuscript. Your valuable comments were helpful for us to revise the manuscript. The questions you raised were answered by point-to-point as follows.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: To the editors/authors, This case control study indicates that, in Chinese, T1DM displayed bacterial diversities differently from healthy control subjects. Further, some bacteria might predict clinical onset and treatment outcome.

Comments 1. Hb1C should be reported and its correlation with some bacteria may be useful more than fasting blood glucose, otherwise supporting each other.

Thank you so much for your comments. The clinical indicator, Hb1Ac, was not detected in all these T1DM children and not detected in the healthy controls, while the FBG were detected in all children. There were so many missing data in these participants, that's why we only selected the FBG for the following correlation analysis. In our future studies, we will collected these clinical indicators into our microbiota analysis.

2. Introduction and methods should be concise and referred to previous reports instead. Discussion should not repeat methods and results description and be concise.

Thank you so much for your suggestion. We have revised the parts of introduction and methods. We have compressed the two parts, especially the methods. The bioinformatic analysis is similar with our previous studies, we have referred these studies in this part. You can find them in the revised manuscript. In addition, we have deleted the repeated parts with methods and results in the discussion. We have discussed this results from bacterial diversity, taxonomic composition, key functional bacteria and its clinical relevance, and study limitations. Of course, most of the key differential functional bacteria were discussed more in this section. We tried our best to illustrate the roles and mechanisms of these taxa in the development of T1DM in children. We hope that these revisions can be acceptable.

3. This study excluded differences in dietary habits and race, the factors that can affect gut microbial diversities. How to apply the prediction result to the general Chinese? A-month dietary control may not be practical. Discussion should be added this concern.

Thank you so much for your comments. The present descriptive study give the readers a novel understanding about the changing patterns of the T1DM-assocaited fecal microbiota. With these key functional bacteria, we could used these taxa as novel targets for non-invasive diagnostic biomarkers and personalized treatment of T1DM in the future. Of course, the clinical application of these diagnostic biomarkers might be go through a long process.

We agreed with your viewpoint that a-month dietary control may not be practical. We did not change their dietary habits but only ask them not to change their dietary habits deliberately. However, those possible microbiota changing factors such as antibiotics, probiotics, prebiotics, or synbiotics, and active infections, and other gut disorders will be excluded. We think that these strict inclusion criteria are helpful for microbiota research.

4. English need carefully rechecked and edited.

Thank you so much for your comments. We have revised the language of the manuscript thoroughly, please check it in the manuscript.

Response to Reviewer #2

Thank you very much for reviewing our manuscript. Thank you for your help to revise my manuscript. Your valuable comments were helpful for us to revise the manuscript. The questions you raised were answered by point-to-point as follows.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This is an observational study by Liu and colleagues, examining the potential role of the gut microbiota in the development of type 1 diabetes mellitus (T1DM) in a cohort of Chinese children. The authors found that the fecal bacterial diversity increased significantly in T1DM children, and that several key functional bacteria correlated with fasting blood glucose (FBG). The investigators claim that the microbiota profile found could be used as non-invasive diagnostic biomarker to discriminate T1DM from healthy controls, and that the findings may provide novel insights into the pathogenesis of T1DM. The gut microbiome is suggested to play a role in the pathogenesis of autoimmune disorders including T1DM. Evidence of anti-islet cell autoimmunity in T1DM appears in the first years of life, but little is known regarding the establishment of the gut microbiome in early infancy. In addition, a clear relationship between T1DM and intestinal microbiota is yet to be determined. Although the subject is not entirely new, the investigators performed an extensive and detailed study on the composition of the gut microbiota at various levels, and attempted to correlate the findings with fasting blood glucose. The overall structure of the manuscript is complete, as requested by the Editorial instructions of the journal. The text and figures are well structured and the methods employed are logical and well described. In regard to the ethical considerations, the text appears to be appropriate. Regarding the selection of patients, the authors indicate a multi-center origin, but they still end up with a relatively small number of cases, one of the limitations of this study, acknowledged in the Discussion section.

Major points: 1) The investigators should explain in more detail the origin of the patients and samples. Where are the centers (hospitals) located? Are they in the same city? Or they are from different Districts? How far they are form each other? In the Discussion section, the authors comment on the importance of geography. Therefore, it would be interesting to add more details concerning this issue in the manuscript.

Thank you so much for your comments. Indeed, the geography will affect the composition and structure of the gut microbiota. in our present study, our samples were collected from Linyi, Shandong Province and Hangzhou, Zhejiang Province. (another hospital in Shandong has been removed as no participants enrolled in present study) The two hospital enrolled T1DM children and healthy controls. After sequencing, we have divided these participants into two parts according to the origin of the samples. We could not find the differences between the two parts after data analysis. That's why we did not mention it in our discussion. In our discussion section, I mentioned the importance of geography mainly to illustrate the disparity between Chinese children and western population. We hope this response can be acceptable.

2) Regarding limitations of the study, it seems important to acknowledge that the findings of

fecal microbiota and FBG do not indicate an obvious clear primary or secondary relationship. Moreover, although the investigators show statistical significance, the isolated findings show relatively weak associations.

Thank you so much for your comments. We agreed with your viewpoints. We have added this limitation into our discussion. Thank you so much.

Thirdly, the relatively weak correlations between key differential functional bacteria and FBG could not indicate an obvious clear primary or secondary relationship. More clinical indicators should add into these correlation analyses in future studies.

3) The investigators should explain in more detail why the findings of this study could be used as "non-invasive diagnostic biomarkers to distinguish between patients with T1DM and healthy controls"? What exactly do authors mean? Since the diagnosis of T1DM does depend on microbial studies, what could we expect regarding the findings? Early predisposition to the development of T1DM? If this were the idea, how practical would be investigating the fecal microbiota of the population? We understand that the findings of this study are much more important in terms disease pathogenesis, and the potential development of microbiota-targeted treatments, as adjuvant therapies or perhaps influencing preventive measures.

Minor comments:

1) A general language revision will be necessary. There are many minor mistakes in several parts of the manuscript.

Thank you so much for your comments. We have revised the language of the manuscript thoroughly, please check it in the manuscript.

Response to Reviewer #3

Thank you very much for reviewing our manuscript. Thank you for your help to revise my manuscript. Your valuable comments were helpful for us to revise the manuscript. The questions you raised were answered by point-to-point as follows.

Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The manuscript reported an original paper in title "Fecal Microbiota Dysbiosis in Chinese Children with Type 1 Diabetes Mellitus: A Case-control Study". The authors aimed to investigate the alterations of gut microbiota in Chinese children with T1DM and its associations with the fasting blood glucose (FBG). They concluded that the fecal microbiota of T1DM patients altered with positively and negatively correlated to the FBG. In addition, they proposed that T1DM-associated fecal microbiota can provide novel insights into the diagnosis and treatment of T1DM. This article is a well-designed, and clear writing paper.

However, some criticisms are listed below. The major comments

1. This paper compare the microbiota difference of T1DM and controls at bacterial composition and functional level. It is a comprehensive and detail study but the entire manuscript is too complicated to read. It need to be concise.

Thank you so much for your comments. Your suggestion is valuable and helpful. As mentioned by other experts, we have compressed the manuscript, especially the part of methods and discussion. The methods have been referred by our previous study. We have deleted the repeat results in the part of the discussion. Indeed, the part of discussion is still long, which discuss the bacterial diversity, the taxonomic alterations of fecal microbiota, the possible roles and mechanisms of the key differential functional bacteria, and the possible clinical application of these key differential functional bacterial for non-invasive diagnosis of T1DM. The outline of the discussion is relatively clear. We hope our revision can be acceptable.

2. It is interesting to analyze the T1DM-associated microbial functional alteration between the microbiota of T1DM and controls. The results find that several metabolic pathways differ between two groups, especial in glycan metabolism are associated with the pathogenesis and development of T1DM. Do you find the significantly different bacteria (Bacteroides vulgatus ATCC8482, Bacteroides ovatus, the Eubacterium hallii group, and Anaerostipes hadrus) contribute to the functional metabolism?

Thank you so much for your comments. You have mentioned the microbial functional alterations in T1DM-associated fecal microbiota. In fact, the microbial function was inferred by PiCRUSt analysis. PiCRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) is based on 16S rRNA sequencing data, which can analyze the microbial functions in fermentation system. So far, we could not predict the microbial function for specific taxa, even with the metagenomics. Our present study found that several metabolic pathways differ between two groups, especial in glycan metabolism are associated with the pathogenesis and development of T1DM. And we also found that several taxa differed

significantly between the two groups. These altered bacteria contributed to the changing metabolic pathways. However, It was still impossible to link specific bacteria with metabolic functions directly. As the function of specific bacteria was very complicate. Thank you so much for your helpful suggestion.

3. Because this study is a cross-sectional study, the main limitation of this study is that does the alteration of gut microbiota in T1DM patients cause or trigger the T1DM develop? Therefore, I think this association is a preliminary result, further causal-development study desires further investigation.

Thank you so much for your comments. We acknowledged that this descriptive study is a cross-sectional study. We agreed with your viewpoint. Indeed, We did not explore the mechanism of these altered bacteria in the development of T1DM. Most of the results were correlation analysis. The research on cause and effect for these bacteria were not conducted in present study. We will perform causal-development study in the future study.

The minor comments

1. In Page 4, AIM, line 59. "Our present study aims to investigated the alterations of…." The investigated change to investigate.

Thank you so much for your comments. We have revised it according your suggestion.

2. In Page 7, lines 132-137. "Leiva-Gea et al. demonstrated…. the production of mucin [15]." This information in background seems not related to the study design and results, do you mean that lactate- and butyrate-producers bacteria may associated with T1DM? However, in the results (functional assay), there are no data presenting this correlation.

Thank you so much for your comments. We agreed with your viewpoint and we have decided to delete this sentence.

3. In Page 8, lines 141-142. "Furthermore, maintaining the eubiosis of early-life gut microbiota in children can reduce the risk of developing T1DM." I do not see any evidence to support this conclusion.

Thank you so much for your comments. We agreed with your viewpoint and we have decided to delete this sentence.

4. In Page 9, Participants selection, line 154. "A total of 51 confirmed T1DM children…." Are the enrolled children newly diagnosed or in treatment? It needs to clearly present.

Thank you so much for your comments. Most of the T1DM children were newly diagnosed and treated with only insulin. Several T1DM children were diagnosed T1DM for nearly 6 month, but also not treated with other options.

5. In Page 9, Participants selection, lines 162-63. "The levels of fasting blood glucose (FBG) of these participants were detected in the morning." Were they asked to fasting?

Thank you so much for your comments. Yes, all participants provide their fasting blood for following analysis.

6. In Page 9, Participants selection, lines 166-168. “The protocols for the present study were approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University.” Because this study is multicenters trial, the approval of the Ethics Committee needs to obtain from all participant units.

Thank you so much for your comments. We agreed with your viewpoints that the clinical trial in multicenters should be approve by their Ethics Committee. In fact, we have check the samples for microbiota analysis carefully, and we found that the samples from Roncheng People’s Hospital were not included in our present study, as they have been excluded by the strict inclusion criteria. The protocols were also approved by the Ethics Committee of Linyi People’s Hospital (reference no. YX10075)

7. The Discussion section is too redundant, please concise this section.

Thank you so much for your comments. We have deleted the repeat results in the part of the discussion. Indeed, the part of discussion is still long, which discuss the bacterial diversity, the taxonomic alterations of fecal microbiota, the possible roles and mechanisms of the key differential functional bacteria, and the possible clinical application of these key differential functional bacterial for non-invasive diagnosis of T1DM. The outline of the discussion is relatively clear. We hope our revision can be acceptable.

8. A total of 87 references seems too many, please to delete some if possible.

Thank you so much for your comments. The references have been reduced into 69. We hope that this revision can be acceptable.

Response to Reviewer #4

Thank you very much for reviewing our manuscript. Thank you for your help to revise my manuscript. Your valuable comments were helpful for us to revise the manuscript. The questions you raised were answered by point-to-point as follows.

Reviewer #4:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (High priority)

Specific Comments to Authors: Evaluation of the ORIGINAL ARTICLE paper Manuscript NO: 62969 entitled "Fecal Microbiota Dysbiosis in Chinese Children with Type 1 Diabetes Mellitus: A Case-control Study". The aim of this study was to investigate if alterations in the gut microbiota correlate with fasting blood glucose (FBG) in Chinese children with T1DM. The authors suggest that the study showed that the investigation of T1DM-and association of fecal microbiota provide novel insights into the pathogenesis of the disease, which would shed light on the diagnosis and treatment of T1DM.

Comments for the authors

1. This is well written paper on an interestingness issue with findings: Alterations of gut microbiota plays vital role in the development of autoimmune diseases such as type 1 diabetes mellitus (T1DM).

Thank you so much for your positive comments.

2. The text, the table and the large number of figures are appropriate and highly informative.

Thank you so much for your positive comments.

3. The number of references is huge and they are up to date.

Thank you so much for your positive comments.

4. There are substantial practical implications of the results of the paper in T1DM patients.

Thank you so much for your positive comments.

5. Adding the following paper will strengthen the results of the paper: Vatanen T, Franzosa EA, Schwager R, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature. 2018 Oct;562(7728):589-594

We have added this reference into our manuscript.