Dear editor and reviewers

Re: Manuscript ID**: 89200** and Title: **Causal Roles of Gut Microbiota in Cholangiocarcinoma Etiology Suggested by Genetic Study.**

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled “Causal Roles of Gut Microbiota in Cholangiocarcinoma Etiology Suggested by Genetic Study.” (ID: 89200). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised parts are highlighted in red in the manuscript. The main corrections in the paper and the responds to the reviewers' comments are as flowing:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: It's a pleasure to review such great research. I generally agree with the author's opinion. However, I believe that this information needs to be verified through follow-up research.

Author's response: We would like to express our sincere gratitude for your thoughtful review and your support for our paper. Your feedback is greatly appreciated. We fully agree with your suggestion that the information presented in our research needs to be further verified through follow-up research, as well as exploring potential underlying mechanisms. Your insight into the need for validation aligns with our own assessment of the work. In fact, we have already outlined our plans for future research endeavors in this direction. We intend to conduct follow-up studies that will delve deeper into the aspects you've mentioned. These studies will aim to strengthen the validity of our findings and shed more light on the underlying mechanisms.

Here are some plans for potential future research directions:

1. Experimental validation of the candidate microbes identified to be causally associated with

cholangiocarcinoma (CCA) risk. Further in vitro and in vivo studies could be conducted to consolidate the causal effects and explore the underlying molecular mechanisms.

2. Analysis of species-level resolution of gut microbiota through metagenomic shotgun sequencing or other techniques. The current study was limited to genus-level associations due to 16S rRNA gene sequencing. A more detailed characterization at the species level could provide further insights.

3. Exploration of potential interventions targeting the gut microbiota to prevent CCA. Probiotics, prebiotics, antibiotics, fecal microbiota transplantation could be examined as potential approaches to modulate the gut microbiota and reduce CCA risk.

4. Analysis of dietary and other lifestyle factors that may influence the gut microbiota traits implicated in CCA. The interactions between diet, microbiome, and CCA risk could be an avenue for prevention strategies.

5. Examination of the influence of host genetics and epigenetics on the gut microbiota and its causal effects on CCA. integrative multi-omics approaches could help delineate the host-microbiome interactions.

6. Prospective cohort studies tracking individuals over time to analyze changes in the gut microbiota and development of CCA. This could further characterize the temporal relationships and refine risk prediction models.

7. Elucidation of the downstream metabolomic products generated by the gut microbes that may be mechanistically driving tumorigenesis. Metabolomics profiling could identify mediators influencing cancer pathways.

8. Assessment of the utility of the identified microbial markers to improve early detection or prognosis of CCA. Diagnostic and prognostic biomarker discovery efforts leveraging microbiome data may be worthwhile.

9. Investigation of potential synergistic effects between different microbes on CCA outcomes. Analyzing microbial co-occurrence networks could uncover interactive effects.

10. Replication of findings across diverse human populations and ethnicities. Expanding microbiome analyses globally could uncover population-specific associations.

However, we also want to extend our apologies for the current limitations in our experimental conditions, which prevent us from immediately addressing these concerns. Despite these

limitations, we are committed to enhancing the robustness of our research in subsequent studies.

Additionally, we have taken the necessary steps to ensure that our language is clear and concise, and we have carefully reviewed the manuscript for any additional grammatical errors or typos. Thank you for bringing these issues to our attention, and we appreciate your understanding as we strive to produce high-quality work.

Here is the proof of polishing our manuscript (See the attached figure in the appendix "Reply to the reviewer. pdf")

CERTIFICATE OF ENGLISH EDITING

This document certifies that the manuscript entitled

"Causal Roles of Gut Microbiota in Cholangiocarcinoma Etiology Suggested by Genetic Study"

was edited for English language, including grammar, punctuation and spelling by one or more native English-speaking editors of KetengEdit. Neither the research content nor the authors' intentions were altered in any way during the editing process.

Best Regards

Keteng Editing

Team Date Issued

December 13, 2023

Disclaimer: *The changes in the document may be accepted or rejected by the authors in their sole discretion after our editing. However, KetengEdit is not responsible for revisions made to the document after our edit on December 13, 2023.*

To verify the final edited version, or if you have any questions or concerns regarding the edited document, please contact KetengEdit at fudan1392@163.com. For more details regarding our company and current services, please visit: http://www.ketengedu.com

Shanghai Keteng Educational Technology Co.,Ltd.
Room 11505, NO.498, Guoshoujing Road, Pudong New Area, Shanghai, China
Contact us: +86 021-50829828 fudan1392@163.com

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And marked in red in revised manuscript. We deeply appreciate your consideration of our

manuscript, and we look forward to receiving comments from the editor and reviewers. If you have any queries, please don't hesitate to contact me at the address below.

Thank you and best regards.

Yours sincerely.

Corresponding author: Qiyong Li

E-mail: liqiyong@zju.edu.cn