Peer Review File

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Reviewer A

This review focuses on initial studies on causal relationship between gut microbiota and pancreatic cancer using MR analysis. While this study is important in establishing the relationship between gut microbiota and pancreatic cancer, this study should include more complete details and key information. The MR analysis alone does not provide compelling evidence on establishing this causal relationship and more in-depth analysis is extremely important. It is important to note that this would only provide initial workup information for further in-depth studies. The following suggestions are provided to help strengthen this work.

1.The study should include more details on the patients like age, gender, type of pancreatic cancer, stage of cancer, treatment, duration of cancer, co-existing conditions, etc.

Response: Thank you so much for your comment and recommendation. Genome-wide association study (GWAS) refers to finding out the sequence variation within the whole human genome, that is, single nucleotide polymorphism (SNP), and screening out SNPs related to diseases. Statistical comparisons were made to identify SNP or genes associated with specific traits (that is, a statistical association between SNP and phenotypic data of the population). Mendelian randomization is a method of using these genetic data to assess the causal relationship between various risk factors. we did not find more detailed information.

2.Please include more details on how the 2559 SNPs were narrowed down further.

Response: Thank you for your important advice. We have added more details to the METHODS section.

Changes in the text: Page 8, line 150-155.

3.There is no subgroup or sub classifications in this study which might certainly affect the results and analysis.

Response: We appreciate your constructive advice. We used summary statistics in our analysis rather than the original data, so subgroup analysis could not be performed.

4.There is no compelling evidence on pin-pointing the exact causal relationship between the mentioned gut microbiota and pancreatic cancer.

Response: Thank you for your valuable advice. At present, randomised controlled trials are the gold standard for testing causality, but such trials are difficult to perform because of ethical and moral problems and the generally high costs. Mendelian randomisation provides a new opportunity for observational research to test causality. This method is a causal inference method developed under the framework of instrumental variable theory, which is used to test or estimate the causal relationship between exposure and related outcomes. However, we now refer to “potential causality” in the manuscript to account for the reviewer’s concern.

5.As mentioned in the study, demographic bias is a major limitation as only the European population is included.
Response: Thank you for your important comment. Most of the data from online databases and major cancer alliances come from Europe, and the bias introduced by population stratification has to be avoided when using two-sample MR analysis. Therefore, all MR results in our study are based on the European population. Mendelian randomisation studies on East Asian and other populations need to be developed further to prove whether previous MR findings are equally applicable to non-European populations.

6.The methods used for analysis should be explained more clearly and concisely for better understanding and clarity.
Response: Thank you so much for your suggestion. We have made the METHODS section more concise.

Reviewer B
Overall, the paper titled "Causal Relationship between Gut Microbiota and Pancreatic Cancer: A Two-Sample Mendelian Randomisation Study" demonstrates a commendable effort to investigate the potential causal links between gut microbiota composition and pancreatic cancer using Mendelian randomization (MR) methodology. The study addresses an important and contemporary research question with potential implications for cancer prevention and treatment. While the paper has several notable strengths, there are also areas where improvements are needed to enhance its rigor and impact.

1. The introduction of the paper effectively establishes the significance of the research, highlights the research gap, and outlines the study's objectives and key findings. To enhance clarity, a more in-depth exploration of these mechanisms, with supporting references, would enhance the paper’s depth.
Response: Thank you for your important comment. We have enhanced the INTRODUCTION section accordingly.
Changes in the text: see Page 4, line 60-62.

2. The Methods section provides a comprehensive and detailed description of the research methodology, particularly the use of Mendelian randomization (MR) to investigate the causal relationship between gut microbiota and pancreatic cancer. The section is well-structured and offers clarity in explaining the steps involved in the study. However, there are some areas where additional clarification and attention to detail would improve the comprehensibility and rigor of the methods. The section appropriately mentions the three key assumptions of Mendelian randomization, but it would be helpful to provide a brief explanation of each assumption to ensure that readers understand their importance in the analysis. While the section mentions the use of PhenoScanner to screen for phenotypes related to the SNPs, it would be beneficial to provide more details on the specific phenotypes screened and the criteria used to

exclude them. Discuss how this step helped mitigate the influence of confounding factors on the MR analysis. Moreover, The authors provides an overview of various MR analysis methods used, such as IVW, MR-Egger, weighted median, and weighted modes. However, consider briefly explaining the strengths and limitations of each method to help readers understand why multiple approaches were employed.

Response: We appreciate your constructive advice. We have added the respective content to the METHODS section.

Changes in the text: see Page 5, line 82-86. Page 6, line 105-111. Page 7, line 120-130.

3. Discussion: The paper acknowledges discrepancies with previous studies but does not delve into potential reasons for these differences. Providing possible explanations for discrepancies would improve the discussion. Ensuring consistent and accurate citation of previous studies, especially when discussing related research, would enhance the paper's scholarly rigor.

Response: Thank you for your important advice. We have described the limitations of our study in the DISCUSSION section.

Changes in the text: see Page 11, line 208-219.