Manuscript ID: 89254

Title: Causal associations of anthropometric indicators and risk of Non-alcoholic fatty liver disease: genetic evidence from a two-stage Mendelian randomization

Dear Editors,

Thank you very much for forwarding the reviewers' comments on our manuscript. We are pleased that the reviewer is supportive of the publication and we greatly appreciate for their helpful and insightful comments and critiques. We have modified the text of the manuscript accordingly in the revision. We believe that the quality of the revised manuscript has been significantly enhanced based on reviewers' comments. Here, our detailed point-by-point responses can be found below.

Many thanks for your continued consideration of our work.

Yours sincerely,

Dr. Zheng-Jun Yang

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Comments and Suggestions for Authors

Thank you for the opportunity to review this paper on the causal associations of anthropometric indicators and risk of Non-alcoholic fatty liver disease: genetic evidence from a two-stage Mendelian randomization. In particular, Mendelian randomization, a new epidemiological analysis method, is less susceptible to confounding factors and is the next level of analysis that allows causal inference even in observational studies. However, there are a few questions that need to be answered, and an explanation would make it easier for the reader to understand. Please see some comments below:

Editors:

(1) Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited,

including A, B, arrows, etc. With respect to the reference to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends require a general title and explanation for each figure. Such as A :; B: ; C: .

Response: Thank you for the reminder. We have revised and re-uploaded all supplementary figures in the required format.

(2) Please add the author's contribution section. The format of this section will be as follows: Author contributions: Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; Wang CL, Liang L and Fu JF wrote the paper.

Response: We apologize for the omission of author contributions in our original manuscript. We have included them in the "Declarations" section **(page 17, lines 334-337)**.

(3) Please add the Core tip section. The number of words should be controlled between 50-100 words.

Response: We appreciate the editor's meticulous evaluation of our manuscript and the reminder regarding the absence of a Core tip section. We have now incorporated this section into the revised manuscript **(page 3, lines 50-57)**.

(4) Please provide the PMID numbers to the reference list and list all authors of the references. If there is no PMID or DOI, please provide the website address.

Response: Thank you for your concern, and we appreciate the editor for bringing this to our attention. In the revised

manuscript, we have revised the citation format of references to comply with the journal's requirements **(page 18-22)**.

(5) The article title cannot exceed 18 words.

Response: Thank for your concerns, we are extremely grateful to editor for pointing out this problem. We have revised the title to “Investigating the Causal Associations between Five Anthropometric Indicators and Nonalcoholic Fatty Liver Disease: Mendelian Randomization Study”.

(6) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text (and directly before the References).

Response: Thank you for your careful review, we sincerely apologize for this oversight. In the most recent revised version of the manuscript, we have included the missing section **(page 16, lines 3316-322)**.

(7) Please provide all fund documents.

Response: Thanks for your reminding. This research is primarily based on the science and technology research project of Sichuan Administration of Traditional Chinese Medicine (2023MS419). We have attached the relevant fund documents separately.

(8) Please provide the Biostatistics Review Certificate.

Response: Thanks for your concerns, we have uploaded the biostatistical review certificate as an attachment along with the revised manuscript.

Reviewer #1:

1- Highly relevant references have to be discussed and cited as Doi:10.1038/s43856-022-00196-3, Doi: 10.1186/s12889-023-15467-4, Doi: /10.1016/j.atherosclerosis.2022.06.182.

Response: Thanks for your suggestion, we have added a more detailed discussion of these relevant literature above in our revised version **(page 14-16, lines 278-311)**.

Based on European GWAS pooled data, a previous study reported partially different findings compared with our findings, indicating that WHR may be a potential risk factor for NAFLD[45]. Several factors could explain this difference. First, the WHR (BMI adjusted) data used in the GWAS were corrected for BMI, indicating that changes in WHR independent of BMI might not be fully captured by the selection of WHR (BMI adjusted) SNPs. Second, the causal inferences included fewer SNPs, resulting in limited phenotypic differences and reduced statistical power to detect a true causal association between WHR and NAFLD risk. Furthermore, differences in study populations and sample sizes could have contributed to the inconsistent findings. To address these challenges, cross-repeated validation was performed using two independent GWAS datasets to eliminate bias resulting from data selection and enhance the credibility of our findings.

Although previous studies have extensively investigated the relationship between AO and NAFLD, there are limited studies on the association between other anthropometric measures[45,46]. This study expands and validates these results by incorporating a wider range of anthropometric measures, offering additional supporting evidence for a causal association in early-stage NAFLD. Compared with previous studies, this study has numerous notable advantages. Although the association between WC, BMI, and BMI-adjusted WHR and NAFLD has been partially examined in previous studies, evidence for a causal association between other anthropometric indicators (such as HC and BF) and NAFLD remains limited. To the best of our knowledge, the causal association between the five major anthropometric indicators and NAFLD was comprehensively assessed for the first time in this study using UVMR and MVMR approaches. This study provides valuable causal evidence and directionality for the early prediction and diagnosis of NAFLD. The results of this

study were replicated using GWAS data from a European population to enhance the reliability of the findings and ensure robust conclusions. Data from two distinct sources were examined in this replication, thereby increasing the potential for identifying new opportunities. In our preliminary analysis, several unidirectional causal associations, such as HC, WC, WHR (BMI adjusted), BMI, and BF, were identified. However, nonreproducible causal relationships between four indicators (HC, WHR, BMI, BF) and NAFLD were excluded from our conclusion to enhance the reproducibility of our approach and reinforce the strength of our conclusions.

References

45 Ning L, Sun J. Associations between body circumference and testosterone levels and risk of metabolic dysfunction-associated fatty liver disease: a mendelian randomization study. BMC Public Health 2023; 23: 602. [PMID: 36997893 DOI: 10.1186/s12889-023-15467-4]

46 Gagnon E, Pelletier W, Gobeil É, Bourgault J, Manikpurage HD, Maltais-Payette I, Abner E, Taba N, Esko T, Mitchell PL, Ghodsian N, Després J-P, Vohl M-C, Tchernof A, Thériault S, Arsenault BJ. Mendelian randomization prioritizes abdominal adiposity as an independent causal factor for liver fat accumulation and cardiometabolic diseases. Commun Med (Lond) 2022; 2: 130. [PMID: 36249462 DOI: 10.1038/s43856-022-00196-3]

47 Gagnon E, Pelletier W, Gobeil É, Bourgault J, Manikpurage HD, Arsenault BJ. A multivariable Mendelian randomization analysis disentangling the causal relations between abdominal obesity, non-alcoholic fatty liver disease and cardiometabolic diseases. Atherosclerosis 2022; 355: 33. [DOI: 10.1016/j.atherosclerosis.2022.06.182]

2- Letter “M” must be capitalized in “mendelian” including the title.

Response: Thanks for the constructive suggestion, and we have changed “mendelian” into “Mendelian” throughout in the title.

3- Running Title needs a correction.

Thanks for your suggestion, and we have changed running title “anthropometric indicators and NAFLD” into “causal associations between anthropometric indicators and NAFLD”.