Peer Review File

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Reviewer A

Comment 1: Can authors give more detail about the power analysis described in lines 109-111? What is the quoted beta value referring to? What is the usefulness of performing a power calculation in this case, where we are not prospectively collecting data?

Reply 1: Thank you for your comments. Because genetic variations sometimes do not fully account for the observed phenotypic traits, statistical power calculations are necessary. These calculations help adjust the selection of instrumental variables and determine whether the sample size is adequate. Many prior studies have also conducted power calculations before embarking on two-sample Mendelian randomization (MR) studies(Larsson et al., 2020; Liu et al., 2023; Nounu et al., 2022). Therefore, we believe that power calculations are essential in MR research. In our study, we referred to this article for power calculations. We computed the expected beta value when achieving a power of 80%, without referencing beta values from observational studies. There may have been some confusion due to our unclear wording. We have revised our description of power calculations for clarification.

Changes in the text: We modified our description(see Page 7, line 115-116).

Reference:

Larsson, S. C., Carter, P., Kar, S., Vithayathil, M., Mason, A. M., Michaëlsson, K., & Burgess, S. (2020). Smoking, alcohol consumption, and cancer: A mendelian randomisation study in UK Biobank and international genetic consortia participants. *PLoS Medicine*, *17*(7), e1003178. https://doi.org/10.1371/journal.pmed.1003178

Liu, X., Lv, Z., Wang, Q., Yu, J., Wang, J., Zhou, Y., Sui, M., Hao, C., Xue, D., & Zhang, Y. (2023). IL1RA mediated the effects of aspirin on COVID-19 severity: A Mendelian randomization study. *The Journal of Infection*, *86*(4), 410–411. https://doi.org/10.1016/j.jinf.2023.01.025

Nounu, A., Kar, S. P., Relton, C. L., & Richmond, R. C. (2022). Sex steroid hormones and risk of breast cancer: A two-sample Mendelian randomization study. *Breast Cancer Research : BCR*, *24*(1), 66. https://doi.org/10.1186/s13058-022-01553-9

Comment 2: Lines 51-52 describe a lung cancer GWAS with 31,700 cases and 192,324 controls, while lines 115-116 describe a lung cancer GWAS with 11,348 cases and 15,861 controls. What accounts for the discrepancy? As currently written, this discrepancy is confusing to the reader.

Reply 2: We thank for the comment and deeply apologize for our mistake. We have rectified the data in lines 51-52 of our revised manuscript. We sincerely apologize for the data error once again.

Changes in the text: We modified our data and highlight it(see Page 4, line 54).

Comment 3: The last two paragraphs of the methods section are confusing to this reader.

Specifically the sentences: “therefore, we considered those mentioned factors as confounding factors between BBs and lung cancer. It allowed us to test the (iii) assumption more fully”. Is this describing some analysis that tested for the causal relationship between beta blockers and lung cancer via Mendelian randomization while also modeling those confounders? If so, that model should be described in more detail. Secondarily, I am not clear on the sentence “we have further selected patients who were required taking BBs for the res of their life… then we evaluated them with similar MR methods”. Is this describing a subset analysis, perhaps where the GWAS sample was subsetted to only those with confirmed lifelong prescriptions of beta blockers? If so, this should be clarified.

Reply 3: Thank you for the suggestions and we apologize for the ambiguity in our wording. In fact, we did employ Mendelian randomization analysis to explore the association between BBs and each confounding factor. The results indicated no significant confounding interference with the causal relationship between BBs and lung cancer. Furthermore, we did not conduct subset analysis. What we intended to convey is that the GWAS study identified individuals who had a lifelong intake of BBs, and based on the GWAS summary data, we conducted a Mendelian randomization study. We have revised the wording in the Methods section accordingly

Changes in the text: We improved our description in the Methods section(see Page 8-9, line 154-156 and 157-158).

Comment 4: In the analysis described in lines 190 and 191, it appears that authors conducted additional MR analyses for the confounders, in which those same 22 SNPs that were chosen as instruments for beta blockers are used in a Mendelian randomization analysis for the confounders. I am not necessarily convinced that this is evidence that the confounders did not influence the BB-lung cancer association. Recall that inverse variance weighting has a 0% breakdown level; i.e., if even one selected SNP is non-causal, the IVW estimator is biased. It is highly unlikely that, even if some of the selected SNPs for BB do modulate lung cancer risk via a pathway including one of the confounders, all of them do so. Thus the IVW MR estimate for the confounders effect on lung cancer is likely not meaningful. Can authors provide a citation for this approach, i.e., conducting MR for the confounders with the same set of instruments, and using a non-significant association as evidence against confounding? This same critique applies to the analysis of the pharmacological factors, i.e., section 3.5.

Reply 4: Thank you for your suggestion. We thoroughly searched and read previous MR studies before embarking on our research. Some prior studies employed a similar approach to control for confounders, namely, conducting repeated two-sample MR analyses using the same set of IVs(Zhou et al., 2019). Similarly, there have been analogous methods used to explore intermediate factors(Yang et al., 2021). Our team has also employed this approach in previous MR research(Huo et al., 2021). Therefore, we believe that utilizing the same set of IVs does not compromise the reliability of our results

Refference:

Zhou, H., Zhang, Y., Liu, J., Yang, Y., Fang, W., Hong, S., Chen, G., Zhao, S., Zhang, Z., Shen, J., Xian, W., Huang, Y., Zhao, H., & Zhang, L. (2019). Education and lung cancer: A Mendelian randomization study. *International Journal of Epidemiology*, *48*(3), 743–750. https://doi.org/10.1093/ije/dyz121

Yang, F., Chen, S., Qu, Z., Wang, K., Xie, X., & Cui, H. (2021). Genetic Liability to Sedentary Behavior in Relation to Stroke, Its Subtypes and Neurodegenerative Diseases: A Mendelian Randomization Study. *Frontiers in Aging Neuroscience*, *13*, 757388. https://doi.org/10.3389/fnagi.2021.757388

Huo, Z., Ge, F., Li, C., Cheng, H., Lu, Y., Wang, R., Wen, Y., Yue, K., Pan, Z., Peng, H., Wu, X., Liang, H., He, J., & Liang, W. (2021). Genetically predicted insomnia and lung cancer risk: A Mendelian randomization study. *Sleep Medicine*, *87*, 183–190. https://doi.org/10.1016/j.sleep.2021.06.044

Comment 5: In general, I am somewhat unclear on the concept of using a medication as the exposure in a Mendelian randomization framework. It seems apparent that there is no 'unmediated' association between genetic variation and the use of a medication, and instead all association must be due to genetic risk of some conditions which necessitate the use of that medication. This makes the analysis very fraught with respect to the three assumptions of MR, as the authors have noted. This makes it all the more essential for the authors to provide evidence that the observed causal association between BB and lung cancer is unconfounded.

Reply 5: Thank you for your comments. We believe that the causal relationship between medication and the disease may not be solely mediated by the condition where medication is used. Our data is derived from a large-scale GWAS study. Our MR results indicate that the relationship between Beta-Blockers (BBs) and lung cancer is not associated with the common pharmacological actions of BBs. There is no causal link between BBs and common factors in lung cancer. To ensure the reliability of the causal relationship, we employed various sensitivity analysis methods and found no evidence of confounding. Currently, there are numerous articles employing drug-related SNPs as instrumental variables for MR analysis, many of which are well-designed and scientifically rigorous(Rosoff et al., 2021; Zheng et al., 2023). Furthermore, there are studies that use SNP variants related to substance intake(Yuan & Larsson, 2022) and individual lifestyle habits(Dixon-Suen et al., 2022) as instrumental variables (IVs) in Mendelian randomization (MR) analysis. SNP IVs do not necessarily need to be directly associated with the exposure trait; they serve as tools representing the exposure in the analysis. Therefore, we consider our findings to be credible. For potential mediators or undiscovered mechanisms, we will make efforts to conduct further analysis and exploration in future research.

Refference:

Rosoff, D. B., Smith, G. D., & Lohoff, F. W. (2021). Prescription Opioid Use and Risk for Major Depressive Disorder and Anxiety and Stress-Related Disorders: A Multivariable Mendelian Randomization Analysis. *JAMA Psychiatry*, *78*(2), 151–160. https://doi.org/10.1001/jamapsychiatry.2020.3554

Zheng, J., Ni, C., Zhang, Y., Huang, J., Hukportie, D. N., Liang, B., & Tang, S. (2023). Association of regular glucosamine use with incident dementia: Evidence from a longitudinal cohort and Mendelian randomization study. *BMC Medicine*, *21*(1), 114. https://doi.org/10.1186/s12916-023-02816-8

Yuan, S., & Larsson, S. C. (2022). Coffee and Caffeine Consumption and Risk of Kidney Stones: A Mendelian Randomization Study. *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation*, *79*(1), 9-14.e1.

https://doi.org/10.1053/j.ajkd.2021.04.018
Dixon-Suen, S. C., Lewis, S. J., Martin, R. M., English, D. R., Boyle, T., Giles, G. G., Michailidou, K., Bolla, M. K., Wang, Q., Dennis, J., Lush, M., Investigators, A., Ahearn, T. U., Ambrosone, C. B., Andrulis, I. L., Anton-Culver, H., Arndt, V., Aronson, K. J., Augustinsson, A., … Lynch, B. M. (2022). Physical activity, sedentary time and breast cancer risk: A Mendelian randomisation study. *British Journal of Sports Medicine*, *56*(20), 1157–1170. https://doi.org/10.1136/bjsports-2021-105132

Reviewer B

1. Please ensure all abbreviated terms are defined the first time they appear in the Abstract. Like "OR, CI…"
Reply: Thank you for your comment. We have reviewed the abbreviations. We made corrections and highlighted them in the revised manuscript.
Changes in the text: We made the corrections (see Page 4, line 72).

2. Please provide the **figure legends** of **Supplementary Figure B2-B3 and Supplementary Figure C2-C3** in your manuscript.
Reply: Thank you for your suggestion. We have figure legends of provided Supplementary Figure B2-B3 and Supplementary Figure C2-C3 in revised manuscript.
Changes in the text: We have added the information (see Page 19-20, line 388-391, 394-397)

3. And please cite **Supplementary Figure B2-B3 and Supplementary Figure C2-C3** consecutively in your manuscript.
Reply: Thank you for your suggestion. We have cited Supplementary Figure B2-B3 and Supplementary Figure C2-C3 consecutively in revised manuscript.
Changes in the text: We have modified the citation (see Page 12, line 215, 232-234)