

Peer Review File

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Reviewer A

Comment 1: For study population, the GWAS datasets used for exposure and CRC appeared to derive from the same population (UK Biobank). Based on the manuscript, it is not appropriate to use a two-sample MR method when the exposure and outcome populations are the same, as this could introduce one-sample bias (which could make the results invalid). I would suggest either using the one-sample MR method or selecting a different GWAS dataset.

Reply 1: Using CRC GWAS data from FinnGen R11 for a new MR study, we found that the causal relationship between BT and an increased risk of CRC still holds. However, reverse MR showed that CRC has no effect on BT levels.

Changes in the text: we have modified our text (Result, Conclusion, Data sources, Discussion)

Comment 2: The manuscript should specify how much variance the selected SNPs explained, as it is crucial for interpreting the results. I am not sure if authors touched on this matter or not, because I was unable to download the supplementary Table S1.

Reply 2: Table S1 contains the proportion of variance explained by each SNP.

Changes in the text: None

Comment 3: The authors did not differentiate between cis- and trans-SNPs, which is important for understanding whether the SNPs directly regulate testosterone levels or are merely statistically associated with. And this distinction is critical for elucidating the biological mechanisms of CRC development.

Reply 3: Testosterone, as a steroid hormone, has a complex synthesis process and does not correspond to cis- or trans-SNPs in the same way that proteins do. In the original GWAS data, no distinction was made either. If relevant data becomes available in the future, further analysis can be conducted.

Changes in the text: None

Comment 4: The authors reported no causal effect of TT on CRC, but MR Egger suggested otherwise. This inconsistency in the results has not been sufficiently addressed or explained in the manuscript.

Reply 4: This problem no longer exists after replacing the CRC GWAS data.

Changes in the text: None

Reviewer B

1. Please provide the full name of OR, CI in the abstract.

Reply: The modifications have been made.

2. Main text

1) Please revise the subheading “Material and Methods” to “Methods”.

Reply: The modifications have been made.

- 2) A subheading “**Statistical analysis**” description in the Methods section must be provided for an Original Article. Please add.

Reply: Statistical analysis has been added.

- 3) The following information is repeated. Please check and revise.

125 confounding factors (ulcerative colitis, Crohn's disease, inflammatory bowel disease, **BMI**,
 26 cholesterol, LDL, HDL, VLDL, **body mass index**, cancer, smoking, alcohol, height, education).

Reply: The modifications have been made.

- 4) Please check all abbreviations in the main text, such as NHGRI-EBI, CI, etc. All abbreviated terms should be full when they first appear.

Reply: The modifications have been made.

3. Figures

- 1) Please confirm your Figures/Tables are original and created/made by the authors. Please note that for reproduced/adapted material, authors are responsible for obtaining permission and related permission files should be provided to the Editorial Office for a record. Please give us a response on this point.

Reply: We confirm our Figures/Tables are original and created/made by us.

- 2) Please provide the figure legends of Figure 1 and 2.

Reply: The modifications have been made.

- 3) Please list all Figure legends on a separate manuscript page after the references list.

Reply: The modifications have been made.

- 4) Please provide the figures in the format of JPG, TIFF or PPT.

Reply: Provided in the attachment.

- 5) Please define all abbreviations in all figure legends.

Reply: The modifications have been made.

- 6) Figure 1: please revise “Outcome” to “Outcome Group”.

Reply: The modifications have been made.

- 7) Figure 2: please revise “Exposure” to “Exposure Group”.

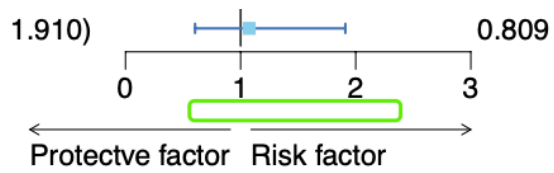
Reply: The modifications have been made.

- 8) Figure 1-2: There is a spelling mistake.

←—————→
Protective factor Risk factor

Reply: The modifications have been made.

- 9) Figure 1-2: Please add the description of x-axis. “OR”?



Reply: The modifications have been made.

10) Figure S2-S5: Please add the description of x-axis.

Reply: The modifications have been made.

4. Tables

1) Please define all abbreviations in the table footnote.

Reply: The modifications have been made.

2) Table 1: please provide a table title.

Reply: The modifications have been made.