

## Peer Review File

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### Reviewer Comments

**Comment 1:** The authors state repeatedly throughout the manuscript that the analysis is identifying a 'causal' relationship. However, causality is not determined through a statistical analysis. The word 'causal' should be removed throughout the manuscript.

**Response:** We appreciate the valuable feedback from the reviewers on our research. Upon further review, we realized that we indeed used the term "causal relationship" to describe the relationship between branched-chain amino acids and the three types of tumors. However, after reading other relevant studies, we believe that the term "causal relationship" is not entirely accurate. Therefore, we have made corresponding modifications to the article based on your suggestions. We hope our explanation can be understood.

**Changes in the text:** We have made corresponding modifications to the abstract, methodology, results, and discussion sections of the article, and the reviewers may re-examine the full text.

**Comment 2:** line 59 - awkward sentence

**Response:** We appreciate the valuable suggestions from the reviewers. After re-examination, we found that this sentence indeed has issues. Our original intention was to indicate that amino acids can be involved in the occurrence and development of tumors. However, due to our oversight, this sentence has caused some misunderstanding. Therefore, we have made corresponding modifications based on your suggestions. We hope our revisions can gain your approval.

**Changes in the text:** We have modified our text as advised (please see Page 6, line 117).

**Comment 3:** It would be helpful to state how much variability in blood BCAAs was explained by SNPs identified.

**Response:** Thank you to the reviewers for their valuable comments on our research. We have not fully understood your intended meaning. However, we would like to provide the following explanation regarding the use of SNPs as tools for branched-chain amino acids in the blood: The Mendelian randomization method we employed is an effective approach for studying the causal relationship between the two, utilizing Genome-Wide Association Studies (GWAS) to replace exposure with disease using single-nucleotide polymorphisms (SNPs), thereby exploring the relationship between them. Therefore, in our study, SNPs are used to replace the

associations of branched-chain amino acids with prostate cancer, kidney cancer, and bladder cancer. We hope the reviewers can understand and acknowledge our explanation regarding your feedback. If you have any comments, please feel free to contact us.

**Comment 4:** Analysis does not consider the impact of BCCAs on aggressive vs. low-grade prostate cancer, which is typical in prostate cancer research.

**Response:** We appreciate you taking the time to provide valuable feedback on our research. Our study indeed focused solely on prostate cancer and did not further classify it. This is because the Mendelian randomization method primarily directly uses GWAS data from other researchers, and the GWAS data we included did not provide a more detailed classification of prostate cancer. This is a limitation of our study, and based on your comments, we have made corresponding revisions to the section discussing the limitations of the research. We hope our responses and explanations can gain your understanding and approval. Thank you!

**Changes in the text:** We have modified our text as advised (please see Page 22, line 464-466).

**Comment 5:** Authors need to help the reader to interpret an OR=1.0003 or similar very small ORs. Perhaps adding a few sentences in the results section to provide needed context.

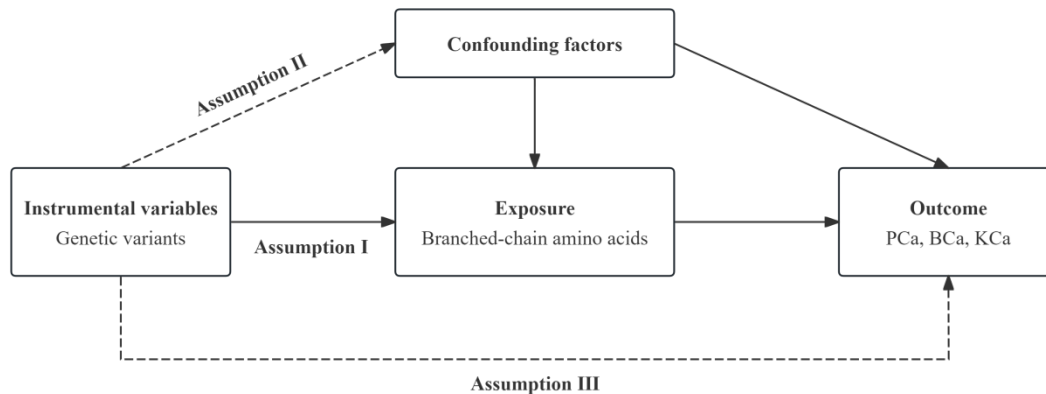
**Response:** We appreciate the valuable feedback from the reviewers on our research. After re-examining our study, we realized that we did not provide an explanation for the smaller ORs, which may lead to some misunderstanding among readers. Therefore, we have made supplementary additions to the results section. We hope our revisions can gain your understanding.

**Changes in the text:** We have modified our text as advised (please see Page 11, line 237-239; Page 13, line 265-267; Page 14, line 291-293; Page 15, line 318-320).

**Comment 6:** Analysis does not control for age, the dominant cancer risk factor. Indeed, no patient demographics or characteristics are provided in the manuscript.

**Response:** We sincerely appreciate the valuable suggestions provided by the reviewers regarding our research. Since we are conducting Mendelian randomization analysis and utilizing GWAS data from other researchers for the relevant analysis, we are unable to obtain specific information about the population. Moreover, due to the peculiarities of the Mendelian randomization method, confounding factors (such as age and sex) did not have a significant impact on the study during the analysis process. The specific analysis workflow can be referenced in the figure below, and the three hypotheses can be found in the line 175-182 of

the Page 9. Thank you for taking the time to provide suggestions for our research, and we hope our responses can gain the reviewers' understanding and approval. Thank you!



**Comment 7:** Regarding p values and CI, the number of significant digits after each decimal point should be reduced to perhaps 3.

**Response:** Thank you for your valuable feedback on our article. We have made corresponding modifications to the tables in the article based on your suggestions to ensure their formatting meets your requirements. We hope to gain your understanding and approval. Thank you!

**Changes in the text:** We have modified our text as advised (please see Page 11, line 231-239; Page 13, line 262-267; Page 14, line 286-292; Page 15, line 312-316; Table 2-5).

**Comment 8:** It is likely that BCAAs represent a dietary pattern rather than specific biological agents. This is briefly mentioned in line 332 for prostate cancer. However, since diet is not a major risk factor for bladder or kidney cancer there is no reason to expect the IVs for BCAAs to be linked with these tumors.

**Response:** We appreciate the valuable suggestions provided by the reviewers regarding our research. Our study aims to explore the relationship between plasma branched-chain amino acids and three types of tumors. Dietary patterns may influence the levels of branched-chain amino acids; however, this does not imply that because diet is not a risk factor for bladder cancer and kidney cancer, the connection between branched-chain amino acids and these two cancers can be dismissed. Therefore, we believe our research indicates that there is no relationship between branched-chain amino acids (BCAA) and bladder cancer and kidney cancer. Moreover, without sufficient research, we should not directly negate the potential relationship between them. We thank the reviewers for taking the time to provide feedback on our study and hope our explanation can gain your approval.

In addition, our study references the research conducted by Wang et al (The role of branched chain amino acids metabolic disorders in tumorigenesis and progression. *Biomedicine & pharmacotherapy* = *Biomedecine & pharmacotherapie* 2022, 153:113390).

**Comment 9:** Much of the discussion of prostate cancer is in regard to tumor tissue expression, which is after tumor initiation. However, the analysis is looking at risk. The authors should be clearer that prior analysis after tumor initiation do not necessary have relevance on their goals in the analysis.

**Response:** We are grateful to the reviewers for their valuable suggestions regarding our research. Upon re-evaluating our study, we found that the discussion section primarily focuses on the effects of branched-chain amino acids on tumor tissues. However, we did not conduct an in-depth discussion on how BCAAs specifically influence tumor development. Therefore, we have decided to include the specific effects of BCAAs on cells in our discussion. We have made corresponding modifications to the discussion section of the paper based on your suggestions, and we hope you will be satisfied with our revisions.

**Changes in the text:** We have modified our text as advised (please see Page 19-20, line 398-419).