Special comments: The manuscript tackles a pressing concern in the medical field: the identification of diagnostic biomarkers for Autism Spectrum Disorder (ASD). By aiming to interpret the causal relationship between ASD and a metabolite profile, the study addresses the complexity of ASD's etiology, acknowledging both genetic and environmental factors. The robust methods employed in this study, along with the transparent presentation of results, make this a significant contribution to the literature. However, a few areas require clarification and elaboration: 1. Offer more context around the choice of populations and the implications of primarily using a European dataset. 2. Elaborate on the technical aspects of the methods, ensuring clarity for readers from diverse backgrounds. 3. Provide deeper discussions on the results, explaining their implications and significance in plain language. 4. Improve visual aids to support and enhance the presentation of findings and your figures 5. Emphasize the broader implications, potential limitations, and future research directions in the discussion section.

Response:

We would like to express our gratitude for your valuable feedback and constructive comments on our manuscript titled "Identifying Diagnostic Biomarkers for Autism Spectrum Disorder: A Two-Sample Mendelian Randomization Analysis." We greatly appreciate your time and effort in reviewing our work. Details of answers on each issue is listed below:

1. **Offer more context around the choice of populations and the implications of primarily using a European dataset.**

   Answer: We understand the need for more context regarding the choice of European datasets in our study. The decision to primarily use a European dataset was driven by the extensive availability of such data, making it the largest and most comprehensive dataset for metabolite GWAS. We recognize that this choice may limit the generalizability of our results to other populations and emphasize this point in the revised manuscript especially in the method, result and discussion section.

2. **Elaborate on the technical aspects of the methods, ensuring clarity for readers from diverse backgrounds.**

   Answer: We appreciate your suggestion to elaborate on the technical aspects of our methods. In our revised manuscript, we provide more detailed explanations of the analytical techniques employed especially about the purpose of LD clumping, the reason why use multi algorithms to estimate the causal association, and the logic for identifying hub genes in the interaction network in the method section.

3. **Provide deeper discussions on the results, explaining their implications and significance in plain language.**

   Answer: We acknowledge the need for a deeper discussion of the results, explaining their implications and significance in plain language. And we make extended explaining in the result section and interpret in the discussion section especially about the insistency of the estimated causal association.

4. **Improve visual aids to support and enhance the presentation of findings and your figures.**

   Answer: Your feedback regarding the improvement of visual aids is well-received. As the regression plots for the causal association and sensitive analysis were presented following standard workflow introduce by the STROBE-MR statement, we try to add estimated effect

size and significance in the leave-one-out analysis plot for each metabolite as shown in Figure2. In this way, it is much intuitive to show heterogeneity among SNPs. Moreover, we also update all the figures with higher resolution(300 dpi) so as to make them clear to read.

5. **Emphasize the broader implications, potential limitations, and future research directions in the discussion section.**

   Answer: Thank you for the comment and we make revision of the manuscript especially the discussion section by emphasizing the limitations about potential bias introduced by population choice, confliction of hypothesis and unavoidable confounders together with the limitation about generalizability and applicability of the findings. Moreover, broader implications are also presented in the discussion section, especially about potential clues for drug repurposing targeting bradykinin receptors. Further research direction about evaluating immune-inflammation interaction in neurodevelopmental disease is also highlighted discussion and conclusion section.

Your feedback is invaluable to us, and your insights will undoubtedly contribute to the refinement of our manuscript. We have addressed these points in our revision to enhance the quality and comprehensibility of our work.