Peer Review File

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Reviewer A

In this paper, authors identified gut microbiota and metabolite biomarkers of IgAN by analyzing microbiomes and metabolomes of fecal and serum samples of IgAN patients and healthy controls using 16s ribosomal RNA gene sequencing and liquid chromatography-tandem mass spectrometry, and bioinformatics approaches.

The paper is interesting, however there are some points that may be clarified before the publication.

1) Microbiota depends on and is affected by diet and lifestyle factors, and these factors can be very different between ethnic populations. Therefore, authors should highlight that their findings regard the Chinese population (or perhaps a subgroup of Chinese population). This should be reported also in the title and in the abstract.

Reply: Thanks for your suggestion. As mentioned in the discussion, accurately assessing fecal metabolites, most of which are affected by diet and lifestyle factors, and distinguishing host-derived metabolites from microbially generated metabolites is extremely challenging (see Page 17, line 343-345). To reduce the interference of external factors on the results, we recruit IgAN patients and healthy controls from southern China as research objects. All subjects were Han Chinese with comparable eating habits and lifestyles.

Change in the text: We have modified our text as advised (see page 1, lines 2-3; page 2,lines 29-30; page 4, lines 82-83).

2) In the microbiome analysis an appropriate control group (for example membranous glomerulonephritis) is missing. Without this kind of control group we can not define whether the changes in gut microbiota that authors found are typical of IgA nephropathy or if they were typical of a glomerulonephritis in general. This should be specified in the discussion.

Reply: It is really true as you suggested that different types of glomerulonephritis should be further investigated. Our findings reveal a significant alteration in the gut microbiota and metabolic phenotypes of IgAN patients. However, to improve the accuracy and specificity of molecular diagnoses and to identify molecular biomarkers, in-depth research on the characteristics of the gut microbiota and relative metabolites associated with different types of glomerulonephritis will be necessary. In order to give readers a better understanding of our results, we have stated the limitations of our article in the discussion.

Changes in the text: We have added this part in the discussion section (see page 17,

lines 345-350).

3) For the same reason authors can not assert in the conclusions that differential metabolites, could serve as significant biomarkers for IgAN. Further study including several control groups will be necessary.

Reply: Thank you for your correction again. In our study, the metabolic network map between the intestine and blood circulation reflects the metabolic patterns of IgAN. These significant metabolites we identified are primarily used for the identification of metabolic characteristics of IgAN. Although we still cannot tell the difference between IgAN and the other types of glomerulonephritides using these metabolites, we believe that these metabolites are core metabolites closely related to IgAN. We will continue to conduct further in-depth study in the next work. To avoid misunderstanding, we decide to substitute "core metabolites" for "biomarkers".
Changes in the text: We have modified our text as advised (see page 2, lines 27-28; page 11, lines 220, 225-226; page 18, lines 359-363) and added this part in the discussion section (see page 17, lines 345-350).

4) Authors at page 11 in the results named Figure 1F and 1G as 2F and 2G, respectively. Moreover, at page 12 they named Figure 2 (Figure 2A, 2B, etc.) instead of Figure 3.

Reply: We are very sorry for these writing errors and would like to thank you again for your careful review and comments.
Changes in the text: We have modified our text as advised (see page 10, lines 192 and 199; page 11, lines 220, 222, 225, 227, 231, and 232).

5) Correlationship between the Oxford classification of IgA nephropathy and the hub metabolites is interesting. Can authors try to correlate also relative abundances of bacteria with Oxford classification in subgroups of IgAN patients?

Reply: As reported in our text, *Streptococcus, Bacteroides, and Enterococcus* showed significant variation in IgAN. Therefore, we mainly correlate the relative abundances of these three pivotal gut bacteria with Oxford classification. The results showed that no significant correlation was found between the Oxford classification of IgA nephropathy and the core gut microbes *Streptococcus, Bacteroides, and Enterococcus* (see figure 5B). As described in the text, the effects of the microbiome on IgAN are likely associated with holistic dysbiosis rather than specific bacterial species. (page 14, line 287-289).
Changes in the text: We have added Figure 5B and relative results in our text (see figure 5B; page 13, lines 253-255; page 27, lines 559-561).

6) Authors should correct the size of figure 4.

Reply: The size of figure 4 has been corrected according to the standards for image requirements.
Changes in the text: We have modified figure 4 as advised (see figure 4)

Reviewer B

Nice effort to identify a potential correlation between gut microbiota, its metabolites and IgAN pathogenesis. Experimental procedures and interpretations of results are reasonable and informative.

Major

Comment 1. Authors described that subjects recruited for the study were not treated with any drugs or nutritional supplements for at least two months. Does this mean treatment-naïve IgAN patients?

Reply: Some drugs used to treat IgAN including glucocorticoids and immunosuppressants may affect gut flora in composition and abundance. In order to exclude the interference of relative drugs on our results, only the treatment-naïve IgAN patients are selected as research objects. Additionally, all subjects had not taken any drugs (e.g., antibiotics or aspirin) or nutritional supplements for at least two months. We are sorry that this part was not clear in the original manuscript and we have revised the contents of this part.
Changes in the text: We have modified our text as advised (see page 4, lines 81-82).

Comment 2. No information on clinical and/or histological grades in IgAN patients.

Reply: We are sorry for not providing information of histological grades in IgAN patients in the previous text. Please check clinical characteristics of IgAN patients and healthy controls in Supplementary Table S1. Please check the histological grades of IgAN in Supplementary Table S2.
Changes in the text: We have added information of histological grades in IgAN patients (see Supplementary Table S2) and modified our text (page 9, lines 172-173).

Comment 3. According to your findings and previous evidence, please discuss translations outlook and perspectives for therapeutic approach.

Reply: Thanks for your advice. At present, systemic corticosteroids and immunosuppressive drugs are the main treatments for IgAN. However, side effects of these drugs and high recurrence rates should be emphasized. The complexity of the etiology of IgAN requires comprehensive treatment and management. Our findings provide a new therapeutic approach involving reconstruction of the intestinal microenvironment and maintenance of metabolic balance. Fecal microbiota transplantation (FMT) is rapidly emerging due to its safety and stability (40). As mentioned above, Bacteroidetes, one of the earliest colonizing and most abundant constituents of the intestinal flora that may induce an anti-inflammatory milieu (41), were significantly lower in IgAN patients. Food supplements, pharmaceutical products,

and FMT may be promising therapeutic approaches to re-establish a stable abundance of Bacteroidetes. In addition, fat and protein metabolism disorders in IgAN patients, especially polyunsaturated fatty acid deficiency, should be considered. Maintaining metabolic balance and homeostasis is a top therapeutic priority. Previous studies have reported that n-3 and n-6 long-chain polyunsaturated fatty acid supplements had a positive therapeutic effect in type 2 diabetes mellitus and coronary heart disease patients (42, 43). A reasonable diet and wise supplementation of polyunsaturated fatty acids are likely to be essential in the prevention and treatment of IgAN.

References:

40. Vindigni SM, Surawicz CM. Fecal Microbiota Transplantation. Gastroenterol Clin North Am. 2017;46(1):171-185.

41. Xu M, Xu X, Li J, Li F. Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front Psychiatry. 2019;10:473

42. Forouhi NG, Imamura F, Sharp SJ, et al. Association of Plasma Phospholipid n-3 and n-6 Polyunsaturated Fatty Acids with Type 2 Diabetes: The EPIC-InterAct Case-Cohort Study. PLoS Med. 2016;13(7):e1002094.

43. Bird JK, Calder PC, Eggersdorfer M. The Role of n-3 Long Chain Polyunsaturated Fatty Acids in Cardiovascular Disease Prevention, and Interactions with Statins. Nutrients. 2018;10(6):775.

Changes in the text: We have added the discussion of the translational outlook and perspectives for therapeutic approach in our text as advised (see pages 16-17, lines 326-342; page 24, lines 490-497).

Minor

Comment 1. Introduction: Please correct '{Li, 2004 #10;D'Amico, 2004 #11;D'Amico, 2004#11}'.

Reply: We are sorry for the format error. The error has been corrected.

Changes in the text: We have modified our text as advised (see page 3, line 46)

Comment 2. Methods: Please correct '22–56'.

Reply: The format error has been corrected.

Changes in the text: We have modified our text as advised (see page 4, line 75)

Comment 3. Methods: Please correct '–C', '–' showing primer sequence, and "2 –L'.

Reply: The format error has been corrected.

Changes in the text: We have modified our text as advised (see page 5, line 103-104)

Comment 4. Results: Please correct 'Fig. 2F' and 'Fig. 2G'. They may be 'Fig. 1F' and 'Fig. 1G'.

Reply: We are sorry for these writing errors and would like to thank you again for your careful review and comments.
Changes in the text: We have modified our text as advised (see page 10, lines 192 and 199; page 11, lines 220, 222, 225, 227, 231, and 232).

Comment 5. Figure 4: Reviewer cannot see the full view.

Reply: The format of the main text, figures and tables have been reedited. Please check it.