Manuscript NO: 63979

Title: Dysregulation of gut microbiota and altered profiles of fecal bile acids correlate with inflammatory responses in patients with ulcerative colitis

Thank you very much for the reviewer's comments concerning our manuscript. These comments are all valuable and very helpful for revising and improving our paper. All the authors have studied the comments carefully and revised the manuscript based on them. According to the suggestions of the company editor-in-chief, we have adjusted the title of the article to "Altered profiles of fecal bile acids correlate with gut microbiota and inflammatory responses in patients with ulcerative colitis".

Answering Reviewer 05174548

Thank you very much for your comments and suggestions, which are very valuable for us to improve and revise our paper. We have made revisions or explanations point by point. Revised portions are marked in yellow in the revised version of the manuscript.

Specific comments:

1.To evaluate the pathogenetic role of factors such as intestinal microbiota and faecal bile acids, samples should be prospectively collected in a cohort before and after the diagnosis of ulcerative colitis. Otherwise, it is difficult to establish the causal relationship between the described changes and the onset of IBD.

Answer: Thank you very much for reminding us of this crucial issue. Our results only showed fecal bile acid metabolism correlated with gut microbiota and inflammatory responses in patients with UC, there is no evidence that they have participated in the pathogenesis of UC. Since previous animal and vitro studies showed that fecal bile acid metabolites affect colon inflammatory responses. There is an interaction between fecal metabolism and human body. It is possible that UC induced the changes of gut microbiota and fecal bile acids. Our hypothesis that altered profiles of fecal bile acids might play a role in the pathogenesis of UC is not exact. Such cross-sectional studies do not provide information about the timing of dysbiosis relative to disease onset and, therefore, should be interpreted with caution particularly with regards to cause–effect relationships[1]. First, we corrected the relevant details in the manuscript (abstract and discussion sections). Second, the discussion on this issue has been further elaborated in

the limitations of the study in the 1st paragraph on P 18, highlighted in yellow. Third, the conclusions based on this observational study prevent us from determining the causal relationships. We will later conduct intervention studies and animal experiments to verify their relationship.

2.In this cohort the disease duration varies greatly among patients, and it is unclear how the changes described may evolve over time. In particular, the time of active disease is not specified. Furthermore, patients had different grades of activity, and no patients in remission were enrolled.

Answer: Thank you for pointing out this important problem we neglected to illustrate in our manuscript. On the one hand, in order to prevent initiation of medical treatment from changing the composition of the intestinal flora of UC patients, we collected stool samples as soon as possible within 1 day of UC patients' visit to ensure that the clinical symptoms are significant at the study entry. Except for 5 patients who took mesalazine for a short period of time, the remaining active (relapse) patients included in our study did not receive any treatment before collecting stool and serum samples. Although the time of active disease is not specified, the Mayo score of disease activity of the enrolled patients was required to be 4-12. We added some details in describing the method of collecting stool samples from subjects in the 3rd paragraph on P 7 and the 2nd paragraph on P 8, highlighted in yellow. On the other hand, previous studies have shown that there are differences in the intestinal flora of UC patients during active and remission periods[2, 3]. Longitudinal analyses revealed reduced temporal microbiota stability in UC, particularly in patients with changes in disease activity[4, 5]. Subgroup analyses of the disease severity provide the indication that distinct pathogenic UC gut microbiotas exist and are associated with baseline disease severity[6]. However, due to the relatively small sample size in this study, subgroup analysis of the microbiota composition and bile acid profiles with different disease activities and stages of UC patients has not been performed yet. Therefore, the discussion on this issue has been further elaborated in the limitations of the study in the 3rd paragraph on P 17, highlighted in yellow. As the number of subjects increases, we will collect stool samples from a large population of patients with UC at different time points during periods of active (relapse) and inactive

(remission) disease and rank the contribution of variables to microbiota composition and fecal bile acid profiles.

3.UC patients may have different factors affecting the composition of the bacterial flora, such as accelerated intestinal transit time and the use of antibiotics/probiotics. Even if this last was considered an exclusion criterion, the effects of a prolonged antibiotic therapy may last for more than two weeks.

Answer: Thank you very much for reminding us of this very important issue. I'm sorry we didn't exactly describe this in the method of exclusion criteria for UC patients. Subjects who took probiotics or prokinetics, antispasmodics and analgesics within 2 weeks, or antibiotics, corticosteroids, immunosuppressants, BA sequestrants, and lipid-lowering agents within 3 months before the study were excluded. In the revised manuscript, we described the method of exclusion criteria for UC patients in more detail in 4th paragraph on P 7.

4.Diet heterogeneity can be a major bias.

Answer: For this vital question, first, considering that the short-term modification of a diet can rapidly disturb the gut microbiota[7], all subjects were required to maintain their daily dietary habits at least 1 wk before the collection of the stool samples. we added dietary details in describing the method of collecting stool samples from subjects in the 2nd paragraph on P 8, highlighted in yellow. Second, dietary constituents have been shown to affect the immune response and the inflammatory status, in great part mediated through the modulation of the microbiota[8, 9], it is better to supply a standardized diet for subjects. However, the difference between the standardized diet and the usual dietary habits of patients may cause perturbation to the gut microbiota, masking the gut microbiota under usual dietary habits. Therefore, the measures of gut microbiota and BAs before and after a standardized diet combined with a detailed assessment of the usual dietary habits of patients are necessary for a future study. The discussion on this issue has been further elaborated in the limitations of the study in the 1st paragraph on P 18, highlighted in yellow.

References:

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