Responses to the Reviewers Comments

Dear editor:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Difference and clinical value of metabolites in plasma and feces of patients with alcohol-related liver cirrhosis" (Manuscript Number:84081, Observational Study). Those comments are all of great value and very helpful for us to revise and improve our paper, and also have important guiding significance for our researches. We have carefully studied the comments and made corrections, hoping meet with your approval. We have attached revised version of the manuscript for your convenience (*Revised Version*: revised portion are marked in yellow highlight font in the manuscript). Moreover, we have polished revised version of the manuscript (revised portion are also marked in yellow highlight font in the manuscript). In the following pages are our point-by-point responses to each of the comments of the reviewers.

We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in *World Journal of Gastroenterology*. We are pleasure to discuss and improve our paper if you have any questions and comments.

We look forward to hearing from you at your earliest convenience.

Yours sincerely,

Huichun Xing

E-mail: huichunxing@126.com or hchxing@ccmu.edu.cn or hchxing@sohu.com

Responses to the comments of Reviewers:

Special thanks to you for your good comments. We have studied comments carefully and have made correction which we hope meet with approval.

Next are our point-by-point responses to each of the comments of you.

Reviewer #1: In this manuscript, the authors present potentially interesting data on differences in plasma and fecal metabolomic profiles in patients with alcoholic liver cirrhosis (ALC) in comparison to healthy patients. The authors also identified specific metabolites that apparently correlate well with severity of liver disease. The manuscript also pays significant attention to changes in the intestinal microbiota in ALC patients. Although the manuscript contains some interesting findings the study design and interpretation lower significantly the enthusiasm for this work.

1.The sample size of the study was 27 ALC patients and 24 healthy controls. However, the disease patients' group is a mix of inpatients and outpatients, all males. The authors provide no indication of how many inpatient versus outpatient individuals were recruited for the study. Furthermore, little to no patient history is provided.

Response: Thanks a lot for your comments. We are very sorry for that we didn't describe clearly in the "Study design and patients" section. All patients were hospitalized patients, and patients with alcohol-related liver cirrhosis, with or without ascites, without complications of other liver diseases such as hepatic encephalopathy, and without other diseases were screened according to strict inclusion and exclusion criteria. Healthy controls are healthy people recruited from advertisements and routinely tested in outpatient settings that meet the criteria. To this end, we have reorganized "Study design and patients" section to clearly indicate the inclusion and exclusion criteria (see the *yellow highlight font* on *Page 6-7 of the revised manuscript*).

2. The authors must consider that it possible that the severity of the patients disease could be greater amongst inpatients than outpatients and that this could then create a larger standard deviation in the experimental group. This could cause a larger patient population to achieve statistical significance. It is also possible that the nature of the hospital diagnosis could also have an impact in the outcome and results presented here. For example, someone hospitalized with heart failure could have reduced blood flow to the liver, which could confound the actions of the liver to produce metabolites by a mechanism driven by ALC. If the authors had provided convincing data indicating that hospital status does not influence these endpoints measured, then that would alleviate this significant concern.

Response: Special thanks to your good suggestion. Our hospital is a key hospital for diagnosis and treatment of liver disease. The Liver Disease Center admits liver disease patients from all over the country. All patients in ALC group were hospitalized due to liver disease. And we excluded heart failure, renal failure and other disease according to clinical manifestations and laboratory testing according to inclusion and exclusion criteria (*Revised version: Page6-7 yellow highlight font*).

3. Lastly, the manuscript references constantly the changes in the intestinal microbiota in patients with ALC and how this can impact the metabolome. However, other than secondary bile acids, which are well-reported to be generated by the action of intestinal bacterial enzymes, the shifts in metabolomics cannot be attributed to changes in the gut microbiome, since the studies, as designed, were not designed to answer such question conslusively.

Response: Thank you very much. We greatly agree with your suggestions. Although we are very interested in the correlation between fecal metabolites and intestinal microbiota, we really did not explore the microbiota in this study. Therefore, we have revised the related content according to your

suggestions, focusing on the relationship between plasma and fecal metabolism (see the *yellow highlight font* on *Page 5 of the revised manuscript*). Thanks a lot.

Reviewer #2: Alcohol-related liver cirrhosis is an important clinical and social problem. A better understanding of its pathophysiological mechanisms can improve the effectiveness of diagnosis and treatment.

1. The authors processed the data with their own program using R. Is this program (script) deposited in some repository? If so, it is recommended that a link be added.

Response: Thanks a lot. Metaboanalyst is an open website based on R, all program (script) deposited in website. We can get details and download in the website of metaboanalyst (https://www.metaboanalyst.ca/MetaboAnalyst/docs/RTutorial.xhtml). And we have added this information in Statistical analysis section (see the *yellow highlight font on Page 8 of the revised manuscript*).

2. The description of the clinical data of the patients is not detailed enough. Did the patients in the healthy controls group have any medical conditions? Why were they admitted to the Liver Disease Center of Ditan Hospital? What medications were patients in the ALC group taking? Was the age of ALC diagnosis taken into account or were all patients with newly diagnosed disease? Did patients in both groups have other diseases, including colitis, pancreatitis, cholecystitis?

Response: Many thanks for your good suggestions. Sorry for insufficient description of the clinical data of the patients. Firstly, we recruited healthy controls through recruitment advertisements. At the same time, the recruited healthy controls accepted clinical assess and underwent blood and imaging tests in outpatient clinic to confirm that they met the WHO definition of a healthy person and they did not take any medications. So we have improved

the inclusion and exclusion criteria and added the detailed information about healthy volunteers. Secondly, all ALC group patients were first visit patient in our hospital. All patients in ALC group were over 40 years at the time of diagnosis of alcoholic cirrhosis, and median age of ALC group was 51 according to the statistical analysis (Details were showed clinical characterize column 'AGE' of Table 1). Besides, we excluded patients with other diseases including colitis, pancreatitis, cholecystitis, heart failure and so on. We have improved the inclusion and exclusion criteria in the revised manuscript (see the *yellow highlight font* on *Page 6-7 of the revised manuscript*).

3. Since all the patients were men, should we remove the information in the "Study design and patients" section that refers only to women?

Response: Thanks a lot for your suggestions. We have deleted information referred to women in the "Study design and patients" section (*Revised version: Page6-7 yellow highlight font*).

4.The authors conclude that individual metabolites are related to the severity of ALC. It is recommended to add clinical characteristics of ALC severity in the patients included in the study.

Response: Special thanks to you for your good suggestions. TBil, ALB, CHE, PT can reflect the severity of the disease in one way. ALC patients with higher MDF score have higher mortality risk. Thus, we analyzed the correlation between above index (MDF, TBil, ALB, CHE, PT) and plasma and fecal metabolites in the section of "***Association of amino acid or bile acid in plasma or feces with clinical features"*** in Results section (*Revised version: Page12-13 yellow highlight font*).