Author's response to reviews

Title: Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach

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Author's response to reviews:

Dear Sir/Madam:

Enclosed please find the new version of our manuscript entitled “Abnormal fecal microbiota community and functions in patients with hepatitis B liver cirrhosis as revealed by a metagenomic approach” (MS: 5904524771074849). We have modified the revised version of the manuscript based on the comments raised by the reviewing editors. A point-by-point response to the reviewers’ comments is appended below.

We hope that the new version of the manuscript is now acceptable for publication in the ‘BMC Gastroenterology’. For any additional information concerning this paper, do not hesitate to contact us.

Looking forward to hearing from you soon. Thank you.

Sincerely yours,

Jing Yuan,

Institute of Disease Control and Prevention,

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No. 20 Dongda street, Fengtai District,
Point-by-point response to reviewers’ comments

Reviewer’s comments

Reviewer: Isabella Inden

Major Compulsory Revisions:

1. How many HBV-DNA copies have the patients in the different CTP-grading groups? Is there a correlation between viral load and microbiota community/functions?

Authors’ response:

We have supplemented the data of HBV-DNA copies in patients with different CTP-grading in Supplementary Table 1 according to the reviewer’s opinion. We further analyzed the correlation between viral load and microbiota community, and found that the correlation index between viral load and Bacteroides (R = -0.08), Veillonella (R = -0.17), and Escherichia (R = -0.16) were not statistically significant. Also, we analyzed the correlation between viral load and microbiota metabolism at the KEGG pathway level, and the correlation index between viral load and glutathione metabolism (map00480, R = 0.02), propanoate metabolism (map00640, R = 0.01), valine, leucine and isoleucine degradation (map00280, R = 0.10) and synthesis (map00290, R = -0.07) were not statistically significant.

In brief, the data showed that there is no correlation between viral load and microbiota community and functions.

Supplementary Table 1 Number of patients with different HBV-DNA copies

<table>
<thead>
<tr>
<th>HBV-DNA (copies/ml)</th>
<th>Number of HBLC patients*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP-A#</td>
<td>CTP-B#</td>
</tr>
<tr>
<td>&lt;10³</td>
<td>15</td>
</tr>
<tr>
<td>10³-10⁴</td>
<td>16</td>
</tr>
<tr>
<td>10⁴-10⁵</td>
<td>8</td>
</tr>
<tr>
<td>10⁵-10⁶</td>
<td>0</td>
</tr>
<tr>
<td>10⁶-10⁷</td>
<td>1</td>
</tr>
</tbody>
</table>
Total 40 40 40

*Total number of HBLC patients is 120.

#Total number of patients with CTP score of A is 40, with CTP score of B is 40, and with CTP score of C is 40.

The correlation between viral load and microbiota community and functions were analyzed. The correlation index between viral load and Bacteroides (R = -0.08), Veillonella (R = -0.17), and Escherichia (R = -0.16) were not statistically significant. Also, the correlation index between viral load and glutathione metabolism (map00480, R = 0.02), propanoate metabolism (map00640, R = 0.01), valine, leucine and isoleucine degradation (map00280, R = 0.10) and synthesis (map00290, R = -0.07) were not statistically significant. In brief, the data showed that there is no correlation between viral load and microbiota community and functions.

2. Is there a difference in microbiota community and functions between HBV-treated patients/non-treated patients and resolver?

Authors’ response:

In this study, the resolvers were not been enrolled. Among the 120 patients, 74 were HBV-treated and 46 were non-treated. Abundance of genes annotated to bacterium and KEGG pathways was not significantly different between HBV-treated patients and non-treated patients.

A follow-up study is now being conducted to determine the changes of fecal microbiota community and functions of patients along with the course of diseases and treatment until they are resolved.

3. Is the control cohort also Anti-HBV negative or only HBV-DNA negative?

Authors’ response:

The control group was individuals with only HBV-DNA negative. Among the 120 controls, 32 with HBsAg positive, and 88 with HBsAg negative. All healthy individuals had normal liver biochemistry test. Abundance of genes annotated to bacterium and KEGG pathways was not significantly different between HBsAg positive and negative patients. So the abnormal fecal microbiota community and functions in cirrhotic patients were not related to chronic hepatitis B infection.

Minor Essential revisions:

1. Some spelling mistakes, for example "negtive" in Table 1, or "possessd" in DISCUSSION.

Authors’ response:
Sorry, this is our mistake.

We have revised the word "negtive" as "negative" in Table 1.

We have reworded the sentence "So we inferred that the patients' microbiota possessd more bacteria which were fastidious, requiring more nutrients in the external environment for survival and growth." as "Therefore, we inferred that the microbiota from HBLC patients harbored more fastidious bacteria that required more nutrients in the external environment for survival and growth."

Point-by-point response to reviewers' comments

Reviewer's comments

Reviewer: Matthias Zilbauer

In their study Wei and colleagues have examined composition of gut microbiota in healthy controls and patients with Hepatitis B liver cirrhosis (HBLC). Specifically, they have extracted bacterial DNA from stool samples and used next generation sequencing to assess composition and partly function of gut bacteria. Their results demonstrate clear differences in the composition and function of gut microbiota between controls and HBLC patients. Overall the manuscript is well written, data presented in a concise way and methodologies as well as statistical analysis seem sound.

Major criticism

1) Lack of novelty, i.e. what is new compared to the data published by Chen et al in 2011 (Hepatology. 2011 Aug;54(2): Characterization of fecal microbial communities in patients with liver cirrhosis)

Authors’ response:

Chen and coworkers did conduct a similar study on fecal microbial communities in patients with liver cirrhosis. However in our study, high-throughput Solexa sequencing of the complete metagenomic DNA and bioinformatic analysis were conducted, which provides deeper insights into the abnormal fecal microbiota community and its functions in patients with hepatitis B liver cirrhosis (HBLC) were provided. First, our data clearly showed the significantly differential bacteria at the genus level and species level between the controls and HBLC patients, whereas, Chen et al. defined the significantly differential bacteria only at the family level. At the same time, we confirmed our results by real-time PCR. Second, we have carried out the functional characterization of HBLC intestinal microbiota at the most general hierarchal level and also at the pathway level for carbohydrate metabolism, lipid metabolism, amino acid metabolism and others, which have not been covered in previous studies. For these reasons, we believe that the present study provides additional important information that cannot be overlooked.
2) What is the relevance to disease - e.g. chronic hepatitis B infection? How can this data be used to improve treatment/management, use as biomarker?

Authors’ response:

It is generally believed that the abnormal fecal microbiota community and functions in cirrhotic patients were related more to liver cirrhosis than to chronic hepatitis B infection. Previous studies found no statistical differences between HBV-related and alcohol-related cirrhosis.

The significantly differential species and metabolisms of the fecal microbiota detected in the present study could be seen as candidate biomarkers that might guide the regulation of the fecal microbiota community and its functions to improve the prognosis of cirrhotic patients.

3) One of the main and most important questions that remain is what is cause and consequence? Most likely, changes in gut microbiota are the consequence of cirrhosis; what would be interesting to know is in what way changes in the gut microbiota influence disease progression.

Authors’ response:

Yes, this is a very good question, we agree with you. Because the patients with HBLC and normal individuals were enrolled simultaneously, making it a case-control study, we are unable to distinguish cause from consequence. To address this question, a cohort study may be conducted in future studies.