Author's response to reviews

**Title:** Study of ZHENG Differentiation in Hepatitis B-caused Cirrhosis: a Transcriptional Profiling Analysis

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**Author's response to reviews:** see over
Dear editor,
Thank you very much for the comments and suggestions.
We have revised the manuscript according to the comments and suggestions of reviewers, and highlighted the amendments in the revised manuscript. The respond is as listed below.
With kindest regards,
Yi-Yu Lu, Shi-Bing Su

Replies to Reviewer #1:
1. Patient demographic information should be included.
   Answer: Thanks for the suggestions. We have added patient demographic information to the Table 1 (Page 20).

2. Missing normal group. I noticed that normal group (n=3?) was in array analysis, but there was no normal control for RT verification. Without normal, the comparison between two disease groups was much less powerful and meaningful. I assume that both samples from DHAS and LDSDS patients were compared with normal controls and then compared to each other for the array analysis. Authors should give detail statement if the disease groups and normal group were run for microarray at same time and how the samples were compared.
   Answer: Thanks for the suggestions. We carried the study as the reviewer said that both samples from DHAS and LDSDS patients were compared with normal controls (n=3) and then compared to each other for the array analysis. We have added the detail statement of how the samples were compared (Page 8 line 20-22; Page 9 line 1-4).

3. Authors stated that “DEGs were defined as |fold change|>2, p<0.05” and did not indicate which genes were up regulated and which genes were down regulated in the each disease group compared to that in the normal group. It is necessary to distinguish the up-regulated genes and down-regulated genes in each disease group.
   Answer: Thanks for the suggestions. We have added the up-regulated genes and down-regulated genes to the manuscript (Page 9, line 6).

4. The outcome of this study can be great if up- and down-regulated genes are able to differentiate these two groups. For example, genes a, b and c are up-regulated, while genes x, y, and z down-regulated in one group; and genes a, b and c are down-regulated, while genes x, y, and z up-regulated in another, when compared with a normal. This expression pattern can be used to differentiate these two groups. When a patient with hepatitis B-caused cirrhosis is presented, RT-PCR analysis of these gene expression patterns can be used to classify the individual patient. With current analysis presented in the manuscript, three genes were further verified with 15 more samples from each group, but the comparison was performed between two disease groups. Although the difference of the genes was shown between those two groups, these genes have no differential diagnostic value when next patient is
presented.

**Answer:** We completely agree with this valuable suggestion by the reviewer. Although the outcome can be great if up- and down-regulated genes are able to completely differentiate these two groups, this study limited to the two groups, and lack of the differential value for others. It needs to find more diagnostic value genes and/or their combination for the differentiation of multiple ZHENGs in further researches. To interpreting the role of genes combination in ZHENGs differentiation, we applied the a stepwise logistic regression model to combine the three genes named PAP panel (Page 2, line 20-22; Page 6, line 20-24; Page 7, line 1-3; Page 10, line 18-24; Page 13, line 12-14; Figure 5-B ). The result showed the PAP panel had higher accuracy (AUC=0.818) in discriminating DHAS from LDSDS.

5. In figure 3 it shows that there was higher expression level of all three genes, PNP, AQP, and PSMD2 in the LDSDS group, but they were in different patterns in figure 4. PNP expression was higher in group A and AQP7 and PSWD2 were higher in group B. The authors need to explain this result.

**Answer:** Thanks for the suggestions. The figure 3 was not a diagram of gene expression. It showed the degree centrality difference which was a network property of the above two GeneRelNets, indicated a possibility of distinguishing DHAS and LDSDS groups by the degree centrality difference of multiple DEGs (Page 13, line 7-9). The figure 4 showed the mRNA expressions. PNP expression was higher in DHAS group and AQP7 and PSWD2 were higher in LDSDS group, indicated a possibility of distinguishing DHAS and LDSDS groups by the expressions of genes. In addition, the expressions of the three genes were consist to the results from microarrays.

**Minor Essential Revisions**

1. **Figure 1.** Figure legend mentioned figure 1A. There was no labeling in figure itself.

**Answer:** Thanks for the suggestions. It was a typo. It should be “Figure 1” instead of “Figure 1A”. It has been corrected. We have added the labeling of “DHAS” and “LDSDS” in the figure.

2. **Figure 3.** More details are needed for the figure legend.

**Answer:** Thanks for the suggestions. We have added more details for the figure legend (Page 8, line 4-7).

3. **Figure 4.** More details are needed for the figure legend. There is no indication in figure legend for figure 4 and readers cannot figure out which group is A and which group is B in the graph.

**Answer:** Thanks for the suggestions. We have replaced the “DHAS” for “A” and “LDSDS” for “B” in figure 4.

Replies to Reviewer #2:
Major comments:

1. As we all know, dampness-heat accumulation syndrome (DHAS) and the liver depression and spleen deficiency syndrome (LDSDS) are two main types of syndrome, can we distinguish other type syndrome by transcriptional Profiles?

Answer: Thanks for the suggestions. As we mentioned in the Introduction part, the advent of high-throughput technologies, including the transcriptional Profiles may provide us a new approach to investigate the TCM syndrome (ZHENG) differentiation. In this study, we only demonstrated the distinguishing of DHAS and LDSDS by the transcriptional approach. In the future, we would expand other type ZHENGs and the sample size of discovery set and validation set to explore possibilities of further distinguishing ZHENGs in Hepatitis B-caused cirrhosis and other diseases. Therefore, we added this valuable suggestion to our conclusion for the future prospects.