Reviewer's report

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

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**Reviewer:** Carol J Liang

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In this study the authors separated hepatitis B-caused cirrhosis patients into two groups, dampness-heat accumulation syndrome (DHAS) group and liver depression and spleen deficiency syndrome (LDSDS) group based on the Zheng diagnosis by traditional Chinese medicine theory. RNA were isolated from leukocytes of the patients and microarray gene analysis was performed. The authors showed that the gene expression profiles were different between these two groups of patients. Among the differentially expressed genes, three (PNP, AQP7, and PSMD2) were identified, and verified by real-time PCR with more patient samples. It was a good approach to establish some biomarkers for the “Zheng” diagnosis in traditional Chinese medicine. However, there are some concerns regarding the study design and data analysis.

**Major Compulsory Revisions**

1. Patient demographic information should be included.
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.
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.
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.

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

Minor Essential Revisions

1. Figure 1. Figure legend mentioned figure 1A. There was no labeling in figure itself.
2. Figure 3. More details are needed for the figure legend.
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.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests