OPEN PEER REVIEW

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Hepatitis Monthly
Article DOI: 10.5812/hepatmon.118535
Published in: Hepatitis Monthly: 21(10); e118535

Peer Review Report for "Identification of Molecular Subgroups in Liver Cirrhosis by Gene Expression Profiles"

Author(s): Ying-Xue Zhang, Feng-Xia Sun, Xiao-Ling Li, Qing-Hua Liu, Zi-Meng Chen, Yu-Fei Guo

Review Timeline:

Submit Date: 14 Aug 2021
Revised Date: 19 Dec 2021
Accepted Date: 26 Dec 2021

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | Revision (0)

29 Aug 2021

Hello,

The authors present interesting data from their study on the gene expression profile of liver cirrhosis as a possible way to have targeted treatment and a better prognosis.
There should be some improvements to the manuscript before accepting to be published in Hepatitis monthly, both editing and structural changes.

1. The authors should improve the use of the English language in the whole manuscript. Also, the grammar and use of punctuation marks or capital letters.

2. In the Abstract, the methods should be rewritten as now they sound like instructions on how to do the study, not as the authors did. Also, there should be a presentation of the number of patients included.

3. The keywords should not include abbreviated words.

4. In the Introduction, it seems to me that info from lines 47 and 48 are repeated, and only the idea of reversibility should remain here.

5. In Materials and Methods, 2.1.1 should be rewritten, also 2.1.2.

6. In the Results, the presentation of the modules (blue, brown, green, and turquoise) should be better realized, as it is difficult to follow and to understand it as it is now.

7. In the Discussion section, the data on lines 224-229, presenting, in summary, the method may be left out as already presented in Methods.

8. In lines 243-244, "as far as the liver is concerned" is written twice.

9. The subgroup discussions should be in separate paragraphs.

10. Also, the Conclusions should be as a separate paragraph and need to be improved. The authors should discuss more the significance of these results in targeted treatment and prognosis of liver cirrhosis.

REFEREE: Associate Editor | **Revision** (0) 5 Sep 2021

Dear EIC,

The reviewer has provided constructive comments which may greatly improve the manuscript. The authors should revise the manuscript point by point before the manuscript being considered for acceptance.
Reviewer 1:
The authors present interesting data from their study on the gene expression profile of liver cirrhosis as a possible way to have targeted treatment and a better prognosis.
There should be some improvements to the manuscript before accepting to be published in Hepatitis monthly, both editing and structural changes.
1. The authors should improve the use of the English language in the whole manuscript. Also, the grammar and use of punctuation marks or capital letters.
2. In the Abstract, the methods should be rewritten as now they sound like instructions on how to do the study, not as the authors did.
3. Also, there should be a presentation of the number of patients included.
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8. In the Discussion section, the data on lines 224-229, presenting, in summary, the method

may be left out as already presented in Methods.
9. In lines 243-244, "as far as the liver is concerned" is written twice.
10. The subgroup discussions should be in separate paragraphs.
11. Also, the Conclusions should be as a separate paragraph and need to be improved.
12. The authors should discuss more the significance of these results in targeted treatment and prognosis of liver cirrhosis.

REFEREE: Reviewer | **Revision (0)** 17 Oct 2021

Dear AE,

1. The manuscript needs major revision for grammar.
2. The search terms and study path are not described clearly; a flow diagram of study is needed.
3. Materials and Methods section page 5, L 72 - 77: This does not convey useful information.
4. I suggest major re-write of the introduction. The results of genes/modules should be categorized in the following cases: biological processes (BP), cellular component (CC), and molecular function (MF).
5. The following items are needed:
a) Box plots of the gene expression data before and after normalization.
b) Heat map of down regulated deferentially expressed genes
c) Significant modules from PPI network
6. Differentially expressed genes/hub Genes obtained from microarrays analysis should be validated using a more sensitive method, such as a qRT-PCR.

REFEREE: Associate Editor | **Revision (0)** 24 Oct 2021

Comments to the Author

In this manuscript authors, analyze a gene expression data set obtained from Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo). In this data set a previous investigators performed gene expression profiling normal tissues and cirrhosis tissue chip expression profiles. The Authors used the published gene expression data and performed a series of bioinformatics analysis. The study presents with many flaws.

1- What guild line did you use, it was not important for you that the elements of study like inclusion and exclusion criteria, confounding factors and the way for controlling it, please mention those.

2- There are several Datasets when you perform a search with cirrhosis. Could some of the findings presented in this manuscript be validated in some other cohort of patients? Also, for obtaining reliable and comprehensive results, RNA-seq data should be analyzed.

3- Affymetrix or Illumina gene expression data should be validated by an independent method such as real time qPCR in order to be sure of the obtained results.

4- Were the two groups really matched?

5- The hub genes should be specified and the role of the hub genes should be briefly explained.

6- Genes were clustered using a k-means clustering algorithm or using principal component analysis (PCA).

7- What's your criteria for selection of DEGs for analysis PPI and GO (in terms of Fold Change).

8- Some studies analyzed the aforementioned GSE datasets.

Seyed Moayed Alavian: EIC | **Revision** (**0**) 24 Oct 2021

Dear Authors,

Thank you for your submission. Please follow the peer-review comments and submit a revision.

Regards,
EIC

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

To Professor Seyed Moayed Alavian
Academic editor of Hepatitis Monthly

15th November 2021

Dear Professor Seyed Moayed Alavian

Revised Manuscript ID: 118535

Tittle: **Identification of molecular subgroups in liver cirrhosis by gene expression profiles** by Ying-xue Zhang et al.

We thank the reviewer for all the comments that have greatly improved the manuscript.

We would like to thank the reviewer and the editors for their very kind, too. Our responses to comments are as follows.

Comments from Reviewer 1

1. The authors should improve the use of the English language in the whole manuscript. Also, the grammar and use of punctuation marks or capital letters.

We thank the reviewer for this careful srutiny. And we apologize for our error. We have improved the use of English in manuscripts. Corrected the grammar and the use of punctuation or capital letters incorrectly.

2.In the Abstract, the methods should be rewritten as now they sound like instructions on how to do the study, not as the authors did.

We thank the reviewer for raising this important point. As requested, the method part of the abstract has been rewritten in accordance with the author's work. For the convenience of reviewers, the changes in the manuscript text are highlighted in blue (line 13-18 on page 1).

3.Also, there should be a presentation of the number of patients included.

We thank the reviewer for raising this point.The number of patients is supplemented in the method section of the abstract, and changes to the manuscript text are highlighted in blue for the convenience of reviewers (line 13 on page 1).

4.The keywords should not include abbreviated words.

We thank the reviewer for raising this point. We have modified WGCNA to weighted gene co-expression network analysis. For the convenience of reviewers, the manuscript text changes are highlighted in blue (page 2, line 31).

5.In the Introduction, it seems to me that info from lines 47 and 48 are repeated, and only the idea of reversibility should remain here.

We thank the reviewer for raising this point. Line 47 "Chronic liver disease is characterized by progressive liver fibrosis, leading to cirrhosis, HCC, and liver failure" has been deleted, only the idea of reversibility is saved (Line 45 on page 2).

6.In Materials and Methods, 2.1.1 should be rewritten, also 2.1.2.

We thank the reviewer for this careful srutiny， I am very sorry that the expressions of 2.1.1 and 2.1.2 are not clear and complete. The two paragraphs have been rearranged. For the convenience of reviewers, the changes of the manuscript text are highlighted in blue (lines 67-75 on page 3).

7.In the Results, the presentation of the modules (blue, brown, green, and turquoise) should be better realized, as it is difficult to follow and to understand it as it is now.

We thank the reviewer for raising this important point. I'm sorry, the color of the module is too similar and difficult to distinguish. It has been readjusted and the resolution has been improved. As shown in Figure 4a, the corresponding module description in the text has also been modified. For the convenience of the reviewer, the changes of the manuscript text are highlighted in blue(line 177，179，184，192，197，203，208，213，218 on page 7).

8.In the Discussion section, the data on lines 224-229, presenting, in summary, the method may be left out as already presented in Methods.

We thank the reviewer for raising this point.Because it has been introduced in the method and results section, the data in lines 224-229 (the first paragraph of the discussion) is duplicated and has been deleted.

9.In lines 243-244, "as far as the liver is concerned" is written twice.

We thank the reviewer for this careful srutiny. The one "as far as the liver is concerned" has been deleted (line 238 on page 9).

10.The subgroup discussions should be in separate paragraphs.

We thank the reviewer for raising this point. The subgroup discussions have been segmented and carried out in separate paragraphs (line 238 on page 9 - line 323 on page 11).

11.Also, the Conclusions should be as a separate paragraph and need to be improved.

We are deeply grateful to the reviewer for the suggestions that have greatly improved our manuscript. Line 324 on page 11 - line 329 on page 12 carries out word order correction and language specification. The conclusion is a separate paragraph, see line 330 on page 12 - line 337 on page 12. For the convenience of reviewers, changes to the manuscript text are highlighted in blue.

12. The authors should discuss more the significance of these results in targeted treatment and prognosis of liver cirrhosis.

We are grateful to the reviewer by this great suggestion. The significance of these results on the targeted treatment and prognosis of liver cirrhosis has been further discussed in the conclusion. For the convenience of reviewers, the changes of the manuscript text are highlighted in blue (lines 330-337 on page 12).

Comments from Reviewer 1

1. What guild line did you use, it was not important for you that the elements of study like inclusion and exclusion criteria, confounding factors and the way for controlling it, please mention those.

We thank the reviewer for raising this important point.I'm very sorry, the inclusion and exclusion criteria have been supplemented and improved. For the convenience of reviewers, the changes in the manuscript text are highlighted in blue (page 3, lines 76-81).

2. There are several Datasets when you perform a search with cirrhosis. Could some of the findings presented in this manuscript be validated in some other cohort of patients? Also, for obtaining reliable and comperhensive results, RNA-seq data should be analysed.

We are grateful to the reviewer for the comment.The findings in this manuscript are based on previous studies and preliminary discussions on molecular subtypes of liver cirrhosis. They have not been verified in other patient cohorts. In the future, we look forward to further prospective verification in the population, and RNA-seq Data is analyzed. It is hoped that this study will inspire future research: Similar to the transcriptional composition in cancer, future liver cirrhosis studies should also introduce multi-omics data to reveal more precise molecular subgroups of liver cirrhosis.

3. Affymetrix or Illumina gene expression data should be validated by an independent method such as real time qPCR in order to be sure of the obtained results.

We are grateful to the reviewer by this great suggestion. This study is a preliminary discussion on the subgroups of liver cirrhosis, focusing on the differences between patients with different subgroups of liver cirrhosis. Although our findings indicate that different subgroups of cirrhosis cases may have different expression patterns, they are based on previous studies. , Related research lacks relevant information on clinical characteristics, and requires rigorous analysis methods and prospective verification by a larger population. However, considering the issue of funding and time, I am very sorry that there is no verification yet. I hope this research can inspire follow-up research. I hope that I will have the opportunity to verify in the future. Please be considerate and thank you very much.

4.Were the two groups really matched?

We thank the reviewer for raising this point. This study is to explore the differences between the subgroups of liver cirrhosis, focusing on the differences between patients with different subgroups of liver cirrhosis, which belongs to the analysis within the group.Unlike previous studies, the cirrhosis and normal groups are studied, so the matching of the two groups is not involved.

5. The hub genes should be specified and the role of the hub genes should be briefly explained.

We are deeply grateful to the reviewer for the suggestions that have greatly improved our manuscript. The hub genes have been supplemented in the text. For the convenience of reviewers, the changes in the manuscript text are highlighted in blue (lines 240-255 on page 9, 287 on page 10 - 303 on page 11, 314-316 and 320-321 on page 11). Some references have been added to the manuscript. For the convenience of reviewers, references added to the manuscript text are highlighted in orange.

6.Genes were clustered using a k-means clustering algorithm or using principal component analysis (PCA).

We thank the reviewer for raising this point.In this study, the included liver cirrhosis samples were divided into different subgroups according to the closeness of internal connections, and the K-means algorithm was used for clustering. WGCNA is used to cluster the specific up-regulated genes in each subgroup. WGCNA has been proven to be an effective method for detecting multiple co-expression modules and can be used to find clusters (modules) of highly related genes. The optimal Power value was found through the Power value scatter plot, and the distance between genes was calculated. In addition, the average method and the dynamic method were used for hierarchical clustering analysis, the clustering diagram and the module classification of genes were, respectively,established, and similar modules were merged. We finally determined 6 functional modules.

7.What’s your criteria for selection of DEGs for analysis PPI and GO (in terms of Fold Change).

We thank the reviewer for raising this point.This study did not involve PPI analysis. The GO and KEGG analysis was to use the "limma" package to compare cases in a specific subgroup of liver cirrhosis with cases in other subgroups to determine the specific upregulated genes of each subgroup.The Wilcoxon’s-sum rank test was used to test the differential expression, the corrected P value threshold was <0.05, and the absolute value of the mean difference was >0.2.

8. Some studies analysed the afformentioned GSE datasets.

We thank the reviewer for this careful srutiny.With the cost reduction of high-throughput sequencing technology and the development of bioinformatics, increasing researchers will use high-throughput technology and bioinformatics to reveal the pathogenesis of cirrhosis. Most studies only focus on the differences between patients with cirrhosis and normal controls, but little attention is paid to the differences between patients with cirrhosis. We provide a valid explanation of how our study is different from already published ones, as follows:

1.Unlike previous studies, which only studied the gene expression profiles of patients with cirrhosis or compared with the gene expression profiles of normal controls. We have classified the cases of cirrhosis into subgroups according to the relevant gene expression profiles, and analyzed them through a series of bioinformatics methods, annotating the corresponding co-expression function modules to reveal the characteristics of each subgroup.

2.The subtypes of cirrhosis have improved our understanding of the pathogenesis of the

disease.Through the research results of cirrhosis subtypes, we have speculated the possible pathogenesis of each subgroup, and the conclusions have certain guiding significance for clinical treatment, new drug development and prognosis judgment.

Finally, We would like to thank the reviewer again for taking the time to review our manuscript. We are looking forward to the manuscript being approved by reviewers and editors. We also expect the manuscript to be accepted.

Note: Black represents the reviewer's suggestions, green represents our answers to the reviewer's suggestions, blue represents the changes made in the manuscript according to the reviewer's comments, and orange represents the references added in the manuscript according to the reviewer's comments.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Associate Editor | **Revision** (1) 28 Nov 2021

Dear Authors

I thank the authors for special attention to all the reviewers comments that have remarkably improved the manuscript.
However, comment of reviewer 2 (comment 5): The hub genes should be specified and the role of the hub genes should be briefly explained. Your reported hub genes is not acceptable.

Please note that the hub genes extracted from topological analysis. I strongly recommended that "hub genes" should be reported. Topological network analysis have performed for PPI networks and the most important hub genes can be identified. The network analyzer plugin of Cytoscape can used to analyze topological properties of the networks. Among several parameters, degree, betweenness centrality and closeness centrality had better outcomes and can used for topological analysis of the PPI networks. Degree measure refers to the number of neighbors (edges) of a node and the nodes with higher degree centrality are considered biologically important hub genes within biological networks.

Best regards

Seyed Moayed Alavian: EIC | **Revision** (1) 28 Nov 2021

Dear Authors,

Please follow the peer-review comments and submit a revision.

Regards,
EIC

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

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You can read the authors' responses to the reviewers' comments in the next page.

To Professor Seyed Moayed Alavian
Academic editor of Hepatitis Monthly

17th December 2021

Dear Professor Seyed Moayed Alavian

Revised Manuscript ID: 118535

Tittle: **Identification of molecular subgroups in liver cirrhosis by gene expression profiles** by Ying-xue Zhang et al.

We thank the reviewer for all the comments that have greatly improved the manuscript.

We would like to thank the reviewer and the editors for their very kind, too. Our responses to comments are as follows.

Comments from Reviewer 1

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We are deeply grateful to the reviewer for the suggestions that have greatly improved our manuscript. The hub genes have been supplemented in the text. I have imported the subgroup difference genes into the STRING database to construct a PPI network. Import the PPI network into Cytoscape 3.8.0 software, and arrange the nodes in an orderly manner according to the MCC value. The top 10 targets of each subgroup are shown in Figure 3. It is speculated that these targets are the hub genes of the subgroup. I added the corresponding methods and results,and briefly explained the role of the central gene.For the convenience of reviewers, the changes in the manuscript text are highlighted in blue (lines 102-107 on page 4,lines 168-173 on page 6,lines 247-257 on page 9, 294 - 302 on page 11, 314-327 and 334-338 on page 12). Some references have been added to the manuscript. For the convenience of reviewers, references added to the manuscript text are highlighted in orange.

Finally, We would like to thank the reviewer again for taking the time to review our manuscript. We are looking forward to the manuscript being approved by reviewers and editors. We also expect the manuscript to be accepted.

Note: Black represents the reviewer's suggestions, green represents our answers to the reviewer's suggestions, blue represents the changes made in the manuscript according to the reviewer's comments, and orange represents the references added in the manuscript according to the reviewer's comments.

OPEN PEER REVIEW

Revision (2)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Associate Editor | **Revision** (2) 26 Dec 2021

Thank you so much for revised version. I have read considered the revised version. I am positive with this version. The authors have clarified several of the questions I raised in my previous review. The major problems have been addressed by this revision. The results are clearly presented. The presented tables and figures is informative. My decision is Accept in the current form.

Seyed Moayed Alavian: EIC | **Revision** (2) 26 Dec 2021

Dear Authors,

I am delighted to inform that your manuscript is accepted for publication in Hepatitis Monthly journal.

Regards,
EIC