

Manuscript ID: NO. 54331

Title: Identification of key genes involved in post-traumatic stress disorder: evidence from bioinformatics analysis

Dear editor,

Thank you very much for your e-mail. The comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have revised the manuscript carefully, considering the reviewers' comments. We hope it meets with approval. Should you have any questions, please contact us without hesitation. The main corrections in the paper and the response to the comments are as follows:

List of corrections

To reviewer 1

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: This is a very nice study conducted to identify genes potentially involved in PTSD using bioinformatics methods. The manuscript is well presented, however, several issues and clarifications need to be addressed.

Comment 1: The study claims to unravel potential mechanisms of PTSD using bioinformatics methods and used RTPCR to verify the level of expression of key genes of which the expression was already expected. The study should rather manipulate the level of expression of these genes and demonstrate the functional consequences in gene, protein expressions, and on behavior.

Reply 1: In the current study, we screened out a group of candidate signatures and key pathways associated with PTSD from the NCBI Gene Expression Omnibus (GEO) database by using bioinformatics analysis. And we tried to uncover the close hub genes related to the most significant pathways in an exploratory approach. Several hub genes were validated in the basic experiment. These findings might help us deepen our understanding of the underlying mechanism of PTSD at the molecular level. Our study did not mention the functional consequence in the gene, protein expression, and behavior. But your advice is a good area for our future study. Further studies are warranted to discover the functions of these genes, and the critical regulatory mechanism.

Comment 2: The data used from Muhie S. et al through the GEO2R allowing to retrieve DEGs should be further explained to show exactly the list and the number of the total genes obtained.

Reply 2: The total genes at different time points were provided. It was showed on P5, L163-166.

Comment 3: The venny method that allows the overlapping of DEGs across the groups should also be explained step by step to allow reproducibility of the data presented.

Reply 3: The venny method was presented step by step. It was showed on P5, L166-168.

Comment 4: The same remark apply the data obtained from the other tools allowing to identify the pathway enrichment, to visualize integrated networks, connections among genes and networks. The explication should be detailed so as to allow reproducibility of the data presented.

Reply 4: We tried our best to explicate in detail in the GO annotation, KEGG enrichment, and integrated network construction. It was showed on P6, L176-182; P7, L187-188.

Comment 5: How the behavioral data are linked to the main objective of the study?

Reply 5: We reported depression-like behavior because we found this interesting phenomenon.

Although PTSD and depression are two completely different conditions, they still have a wide range of connections.

A diagnosis of post-traumatic stress disorder (PTSD) and depression commonly co-occur^[1]. PTSD is characterized by symptoms of anxiety, flashbacks, and reliving traumatic experiences. The condition develops after a person experiences some sort of traumatic events such as a natural disaster, car accident, attack, abuse, or combat. On the other hand, depression is characterized by low mood, loss of interest and pleasure, and changes in energy levels.

Depression can also be a common response after a traumatic or stressful event, so it is perhaps not surprising that these two conditions can occur at the same time.

Depression is one of the most commonly co-occurring diagnoses in people with post-traumatic stress disorder. Researchers have found that among people who have (or have had) a diagnosis of PTSD, approximately 48% to 55% also experienced current or previous depression^[1].

Based on the above, we observe depression-like behavior to observe the effect of PTSD on depressive behavior and reported it.

Reference:

[1] Gros D F, Price M, Magruder K M, et al. Symptom overlap in posttraumatic stress disorder and major depression[J]. Psychiatry research, 2012, 196(2-3): 267-270.

It was showed on P13, L303-305.

Comment 6: The manuscript need to be paginated

Reply 6: We paginated the manuscript.

Comment 7: Result section, paragraph 1, line 5: "The venn diagram of upregulated and downregulated DEGs at were shown in..."should read "The venny diagram of upregulated and downregulated DEGs were shown in Figure 1."

Reply 7: We modified this sentence.

It was showed on P5, L168-169.

Comment 8: Result section, paragraph 1, line 1: The above 973 DEGs were performed using DAVID database... should better read The above 973 DEGs were assessed using DAVID database...

Reply 8: We modified this sentence.

It was showed on P6, L176-178.

Comment 9: In the list of references, the journal should be mentioned either in lowercase or capitals not both.

Reply 9: We modified the journal of references.

It was showed on P15-18, L385-502.

To reviewer 2

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The paper is well organized and of great interest. However, I have three major concerns that need to be addressed:

Comment 1: The paper needs wide grammatical and language editing. Some sentences or locutions are awkward, for example: disturbing when reminding the flashbacks, is not entirely understand.....

Reply 1: We polished our language including grammatical editing carefully.

Comment 2: You cannot formulate the prevalence of PTSD based on a single study: It's estimated that the prevalence of PTSD range from 3.1% to 61.6% including 6648 participants conducted in six countries[4] Instead, consult different and authoritative sources, and quote them duly.

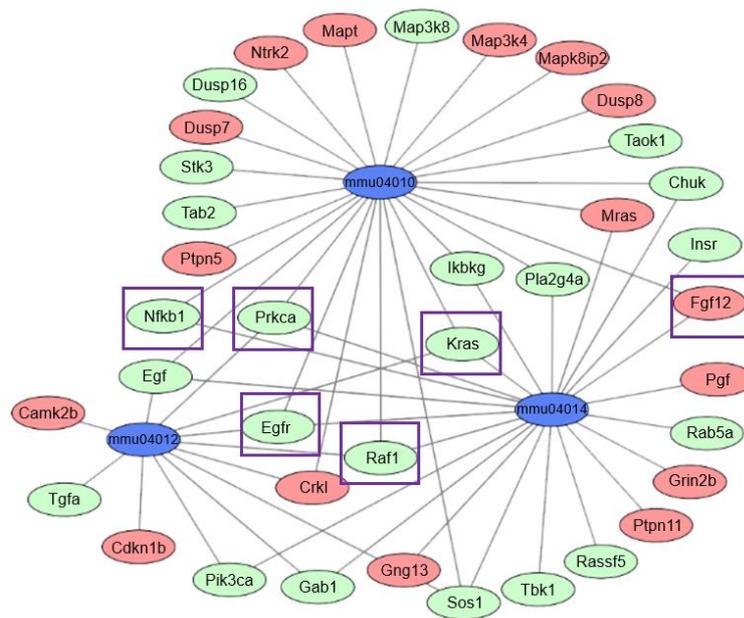
Reply 2: Thanks for the good advice, we searched recent and authoritative publications and quote them duly.

It was showed on P2, L51-55.

Comment 3: You have studied several genes related to PTSD. There is still a confusion hereby; probably you should explain why you've been concentrating your research on these genes. Other authors include / mention other genes as well; for example, recently The South East European (SEE) - PTSD Study (DOI: 10.24869/psyd.2019.210) has published in Psychiatria Danubina a set of papers. In these studies, other genes have been scrutinized. Please include such a perspective, at least theoretically, at least in the discussion. Why this set of genes in a study, and another one elsewhere?

Reply 3: It might due to the different sample sources. The South East European (SEE) PTSD studies used the EDTA blood from humans, while our studies used the hemibrain tissues from mice. In the current study, the altered genes were enriched in the most 3 significant pathways (the co-expression pattern, Fig. 3). That is, most of the genes we selected were related to the 3 enriched pathways (MAPK, Ras, and ErbB signaling pathway). The critical nodes connected to these pathways were presented in a more intuitive way as below (marked with purple square). To discover the critical roles of these genes via the associated pathways, we tried to uncover the closely related genes related to the most significant pathways in an exploratory approach. The genes in the central roles connected to the three enriched pathways were considered as hub genes. And several hub genes were selected based on the literature due to their neurobiology assumption to be related to PTSD. However, all the enriched genes we identified should be further validated for their genetic impact on PTSD. This will be our future study.

It was showed on P9, L217-220; P12, L282-284.



To Editorial office's comments

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

Comment 1: Scientific quality: The manuscript describes a basic study of the identification of key genes in PTSD. The topic is within the scope of the WJP. (1) Classification: Grade C and Grade C; (2) Summary of the Peer-Review Report: This is a very nice study conducted to identify genes potentially involved in PTSD using bioinformatics methods. The manuscript is well presented, however several issue and clarifications need to be addressed. The data used from Muhie et al through the GEO2R allowing to retrieve DEGs should be further explained to show exactly the list and the number of the total genes obtained. The paper needs wide grammatical and language editing. The questions raised by the reviewers should be answered; and (3) Format: There are 3 tables and 6 figures. A total of 35 references are cited, including 9 references published in the last 3 years. There are no self-citations.

Reply 1: We revised the manuscript carefully according to the reviewer's advice.

Comment 2: Language evaluation: Classification: Grade B and Grade B. A language editing certificate issued by ShineWrite was provided.

Reply 2: We polished our language in the manuscript carefully.

Comment 3: Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License

Agreement, and the Institutional Animal Care and Use Committee Approval Form. The authors need to fill out The ARRIVE Guidelines with page numbers. No academic misconduct was found in the CrossCheck detection and Bing search.

Reply 3: We provided ARRIVE Guidelines with page numbers through the system.

Comment 4: Supplementary comments: This is an unsolicited manuscript. The study was performed with 8 financial supports. The topic has not previously been published in the WJP. The corresponding author has not published articles in the BPG.

Reply 4: We added them to the footnotes of the manuscript.

It was showed on P13-14, L312-330.

Comment 5: Issues raised: I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s).

Reply 5: The approved grant documents were provided through the system.

Comment 6: I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

Reply 6: The decomposable figures were provided through the system.

Comment 7: I found the authors did not add the PMID and DOI in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout.

Reply 7: We added the PMID and DOI in the reference list and listed all authors of the references.

It was showed on P15-18, L385-502.

Comment 8: I found the authors did not write the “article highlight” section. Please write the “article highlights” section at the end of the main text.

Reply 8: We added the “article highlight” section at the end of the main text.

It was showed on P14-15, L339-382.

Comment 9: please don't include any *, #, †, §, ‡, ¥, @....in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as aP < 0.05, bP < 0.01 (P > 0.05 usually does not need to be denoted). If there are other series of P values, cP < 0.05 and

dP < 0.01 are used, and a third series of P values is expressed as eP < 0.05 and fP < 0.01. 6 Re-Review: Required. 7 Recommendation: Conditionally accepted.

Reply 9: We modified by using the superscript numbers for illustration and superscript letters for statistical significance.

It was showed on P10, L237-238; P11, L249-250.

(2) Editorial office director: I have checked the comments written by the science editor.

Reply 1:We revised the manuscript carefully according to the science editor's comments.

(3) Company editor-in-chief: I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements.

Reply 2:We revised the manuscript carefully according to the reviewers' comments.