Rajesh R Tampi, Science Editor, Company Editor-in-Chief, Editorial Office

World Journal of Psychiatry

Re: **Manuscript NO: 67401**

July 27, 2021

Dear Editor-in-Chief,

We have attached a copy of our revised manuscript entitled " **CPEB1, a novel risk gene in Recent-Onset schizophrenia, contributes to mitochondrial complex I defect caused by a defective provirus ERVWE1 (Manuscript NO: 67401, Basic Study)** ". We thank you and the reviewers for carefully reading the manuscript and the helpful comments.

As suggestion, we have revised the paper. We have carefully addressed the reviewer's questions and the editorial office's comments point-by-point (please see the following pages for the details).

We believe that the revised manuscript has been substantially improved and hope it meets the standard for publication in the World Journal of Psychiatry. We look forward to hearing from you.

By the way, I did not receive the processing email about the manuscript (Manuscript NO: 67401) in my hotmail address (zhufan@hotmail.com). I do not what is the problem. All e-mails are transferred from my co-authors. So please also send it to my other email (fanzhu@whu.edu.cn) for the next time.

Sincerely yours,

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RESPONSES TO THE REFEREE'S COMMENTS (blue texts are the original comments)

Many thanks for the valuable comments on the manuscript. We have made a substantial revision to the manuscript. Please see below for details of our responses to your comments.

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Summary: The authors have looked at CPEB1, NDUFV2 and NDUFVP1 as potential biomarkers for schizophrenia. The in vitro experiments are well designed that showed that ERVWE1 regulates CPEB1 proteins, which regulates NDUFV2 levels, which affects the mitochondrial complex, which causes abnormal mitochondrial functioning. This mechanism affects neurons and eventually leads to schizophrenia. I believe the manuscript may benefit from the suggestion given below.

Response: Thanks.

Major points: 1. Did the authors try the knockdown and over expression of CPEB1, ERVWE1 or NDUFVP1 in primary neurons, since authors have used SHSY5Y. Although SHSY5Y is used routinely, the metabolism by mitochondria may not be close that in normal neuronal cells.

Response: It is a good suggestion. In fact, we once detected NDUFV2 mRNA expression after overexpression of CPEB1 in rat primary neurons. The result indicated that CPEB1 overexpression didn't affect NDUFV2 mRNA expression in rat primary neurons (data showed in Figure 1). This result was similar with that in SH-SY5Y cells. The reason that we did not continue to try the knockdown and over expression of CPEB1, ERVWE1 or NDUFVP1 in rat primary neurons is: rat NDUFV2P1 is not exit

in the National Center for Biotechnology Information (NCBI) site (http://www.ncbi.nlm.nih.gov). By the way, the homolog gene of ERVWE1 in rat is Syncytin-A, which sequence is quite different from ERVWE1. So we did not use rat primary neurons to continue.

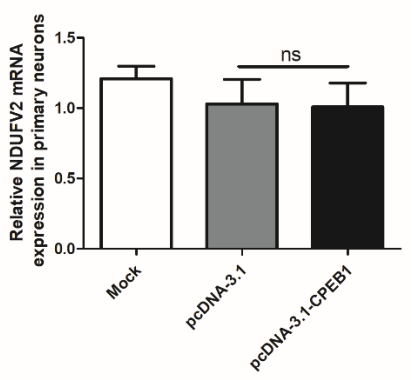

Figure 1 The mRNA levels of NDUFV2 after overexpression of CPEB1 in rat primary neurons using qPCR

2. The potential biomarkers were looked in the blood samples of schizophrenia patients, can a confirmatory staining be done using some archived schizophrenia brain tissue samples, so that biomarkers are linked with the disease directly.

Response: Good suggestion. If using some archived schizophrenia brain tissue samples, the results will be more convinced. But as you know, because of medical ethics and other reasons, we cannot get the schizophrenia brain tissue samples.

3. The discussion section of article, should ideally refraining from staining the results again. However, the current discussion section has extensively reiterated the results again. The result interpretation would be emphasized here.

Response: Thank you for your kind reminder. As suggested, we revised the discussion section by emphasizing on result interpretation and removing the sentences which reiterated the result.

4. Authors should discuss or speculate on the following points • Schizophrenia mostly sets in after the age of 20s, can the current mechanism explain this onset.

Response: Many thanks for reminding us of that. We have added explanation regarding this issue to the discussion section of the revised manuscript (Page19, paragraph 1).

The modifications are as follows: “Schizophrenia mostly sets in after the age of 20s. Mitochondrial energy metabolism dysfunctions have been implicated in the etiology of schizophrenia in early adulthood from age of 20-30s. Research from our and other groups suggests that environmental factors, including viral infection, drug stimulation, and genetic variation, can cause an abnormal expression of ERVWE1. In this study, we demonstrated that increased ERVWE1 induces mitochondrial metabolism deficits, which ultimately leads to the pathogenesis of schizophrenia.”

• Which other factors regulate the expression of NDUFV2 other than ERVWE1 which might have impact on the current results

Response: Thank you for reminding us of that. To our knowledge, there were several factors, such as Sp1 and MeCP2, fluoride, A. angustifolia extract, and mitochondrial Src tyrosine kinase, could regulate the expression of NDUFV2 other than ERVWE1. Other researchers in our group found that ERVWE1 could regulate Sp1 and tyrosine kinase (data not published). However, the relationship between ERVWE1 and these factors is still unclear, which will be assessed in our subsequent studies.

• Does the abnormal mitochondrial activity proposed by the authors also affect the muscle cells of the schizophrenia patients

Response: Schizophrenia is a neurological disease. So we devote our study in nerve cells and haven’t performed this experiment in muscle cells. As a matter of experience, we speculate that abnormal mitochondrial activity may also affect the muscle cells because muscle cells have high energy requirements and are very sensitive to mitochondrial defects.

• Can the schizophrenia patients have dysregulated NDUFV2P1 independently of CPEB1?

Response: To our knowledge, there are only 2 research papers about NDUFV2P1 (see in Reference 1 and Reference 2), and no regulatory factor is reported to dysregulate NDUFV2P1. Up to now, we are the first to reveal the regulatory factor of NDUFV2P1. We have added a discussion of this issue in the discussion section of the revised manuscript (Page18 and paragraph 1).

The modifications are as follows: "Up till to now, there have no published data on the mechanisms that regulate the NDUFV2P1. Our finding might be the first report of regulating NDUFV2P1 in schizophrenia."

Reference 1: Bergman O, Karry R, Milhem J, Ben-Shachar D. NDUFV2 pseudogene (NDUFV2P1) contributes to mitochondrial complex I deficits in schizophrenia. Mol Psychiatry 2020; 25: 805-820 [PMID: 30531937 DOI: 10.1038/s41380-018-0309-9];

Reference 2: de Coo R, Buddiger P, Smeets H, Geurts V K A, Morgan-Hughes J, Weghuis D O, Overhauser J, van Oost B. Molecular cloning and characterization of the active human mitochondrial NADH:ubiquinone oxidoreductase 24-kDa gene (NDUFV2) and its pseudogene. Genomics 1995; 26: 461-6 [PMID: 7607668 DOI: 10.1016/0888-7543(95)80163-g].

Minor points: Language editing: Although the manuscript is well written there are few places were correction would help. Several key sentences seem incomplete and need revision to make them clear for example: • To address novel potential risk factors and underlie the mechanisms of mitochondrial complex I deficiency caused by ERVWE1 in schizophrenia. • Recent studies have shown that human endogenous retroviruses (HERVs), making up about 8% of the human genome, is acted as a novel risk factor for schizophrenia

Response: Thank you for your kind reminder. As suggested, we have revised this sentence. The detailed change is shown in the revised manuscript

The modifications are as follows: "We aimed to address novel potential risk factors and underlie the mechanisms of mitochondrial complex I deficiency caused by ERVWE1 in schizophrenia." (page 3, paragraph 2)

“Recent studies have shown that human endogenous retroviruses (HERVs), making up about 8% of the human genome, emerge as novel risk factors for schizophrenia.” (page 5, paragraph 3)

We have also revised others in our manuscript.

EDITORIAL OFFICE'S COMMENTS

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

Science editor:

1 Scientific quality: The manuscript describes a review of the anorexia nervosa. The topic is within the scope of the WJP. (1) Classification: Grade B; (2) Summary of the Peer-Review Report: This review comprehensively summarized all aspects of anorexia nervosa, which is the most common eating disorders with poor prognosis. The questions raised by the reviewers should be answered;

Response: Thank you for your kind reminder. We have revised the manuscript according to the reviewer and given a point-by-point answer to all questions. We hope that the revised paper meets the standard for publication.

By the way, our manuscript is not a review of the anorexia nervosa but a basic study of the schizophrenia.

(3) Format: There is 1 table and 1 figure;

Response: There are 2 tables and 7 figures in revised manuscript.

(4) References: A total of 158 references are cited, including 66 references published in the last 3 years; (5) Self-cited references: There are 2 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations (i.e. those that are most closely related to the topic of the manuscript) and remove all other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated;

Response: There are 53 references are cited, including 18 references published in the last 3 years in our revised manuscript. There are 5 self-cited references (reference 18, reference 19, reference 38, reference 39 and reference 49). The self-referencing rates are 9.4%, which is less than 10% and meets the magazine's standards.

(6) References recommendations: The authors have the right to refuse to cite improper references recommended by the peer reviewer(s), especially references published by the peer reviewer(s) him/herself (themselves). If the authors find the peer reviewer(s) request for the authors to cite improper references published by him/herself (themselves), please send the peer reviewer's ID number to editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately.

Response: Thank you.

2 Language evaluation: Classification: Grade A. A language editing certificate issued by English Science Editing was provided. 3 Academic norms and rules: No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. No financial support was obtained for the study. The topic has not previously been published in the WJP.

Response: Thanks. Our study was supported by the National Natural Science Foundation of China (81971943, 81772196, 31470264, 81271820, 30870789, and 30300117), the Stanley Foundation of United States (No. 06R-1366) for Dr. F Zhu, and the Medical Science Advancement Program (Basic Medical Sciences) of Wuhan University (Grant No. TFJC 2018002). We have also uploaded the approved grant application forms attached to the revised manuscript.

5 Issues raised: (1) The "Author Contributions" section is missing. Please provide the author contributions;

Response: Thank you for your suggestion. We have provided the author contribution in the revised manuscript (page 1, paragraph 6).

(2) The authors did not provide original pictures. 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; and (3) If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted,

the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights. For example, “Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]”. And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

Response: We have provided original pictures using PowerPoint to ensure all graphs or arrows or text portions can be reprocessed by the editor. All the figures haven’t been published or presented elsewhere.

6 Re-Review: Not required. 7 Recommendation: Conditional acceptance.

Response: Thank you very much for your help.

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 of the World Journal of Psychiatry, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision by Authors. Before its final acceptance, please upload the primary version (PDF) of the Institutional Review Board’s official approval in official language of the authors’ country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the

document, and authors from the United States and the United Kingdom should upload the English version of the document, etc.

Response: Thank you very much for your help.