

Response letter

Dear Editor,

We would like to thank you and the reviewer for the precious time and constructive comments regarding our manuscript entitled 'Integrative transcriptomic and proteomic analysis reveals that SERPING1 inhibits neuronal proliferation via the CaMKII-CREB-BDNF pathway in schizophrenia' (ID: 100214). We have carefully read through the comments and revised our manuscript accordingly. Below, we provide a comprehensive point-by-point response to the issues raised in the peer-review report. We highly appreciate your efforts to improve our manuscript and for the consideration of publication.

Yours Sincerely,

Dr. Xiao-bin Wei

On behalf of all authors

Reviewer #1

Q1: 1. In the first part of this paper, SERPING1 is identified as a potential biomarker in PBMCs, which is interesting. However, SERPING1 is merely a marker in PBMCs, and its expression pattern in the brain is unknown. Therefore, the rationale for this study using rat neurons in the latter part of the paper is a bit unclear. In recent years with the advent of induced pluripotent stem (iPS) cell technology, it is recommended to utilize neurons derived from iPS cells for pathological research of psychiatric disorders. Considering the author's workload, the overall completion of the experiment is still acceptable. If the above requirements can be met, the logic will be more complete.

Reply: Thank you for your question. As we all know, before the development of induced pluripotent stem (iPS) cell technology, analyzing cells such as fibroblasts or blood cells from patients have been considered very useful. In addition, as blood samples are relatively easy for us clinical doctors to obtain, we planned to use patient blood samples in the initial stage of our experimental design. For the iPS you mentioned, our next plan is to use neurons derived from induced pluripotent stem (iPS) cells for pathological research on mental disorders, to validate our current research findings. We greatly appreciate your suggestion.

Q2: 2. - Clarify if the WB results shown in Figure 6 are derived from the same samples as those in Figure 4 or if they are independent.

Reply: Thank you for your question. Figure 6: Expression of SERPING1 was detected by western blot (n=150, including three samples of the results shown in Figure 4a; Here only a subset' WB images are shown in Figure 6). The issue you mentioned has been clearly explained in the diagram in Figure 6. Figure 4 is a small sample experiment we conducted in the early stage, while Figure 6 can be considered as a large sample validation in the later stage.

Q3: 3.- Indicate explicitly in the legend that only some samples' WB images are shown in Figure. If showing only a subset, consider adding images of all samples in the supplementary materials for greater credibility. Use "Normal" or "Disease" labels instead of in-house IDs for clarity.

Reply: Thank you for your suggestion. The modified parts have been highlighted in yellow (Figure 6a). We have added images of all samples in the supplementary materials, but due to the large number of images, we believe that the numbering method is relatively clear (SZ group numbered 1-150, HC group numbered 301-450) . Please refer to the attached materials for details.

Q4: 4.- You have selectively examined the relationship between caMKII, CREB, and BDNF. Please

explain why these particular molecules were of interest.

Reply: Thank you for your question. Differential gene bioinformatics analysis suggested that the neuroactive ligand-receptor interaction pathway may play a crucial role in the progression of SZ (Figure 3d). So, for the next possible mechanism verification, we selected the neuro-associated conduction protein pathway for related research, these molecules are important molecules on this pathway.

Q5: 5.-Discussion

- Consider discussing how events in the blood link to those in the brain. You can add some citations of high scoring literature

Reply: Thank you for your suggestion. we have added discussions on the connection between the two. The modified parts have been highlighted in yellow (the part of Discussion).

Reviewer #2

Q1: ### 1 - Clarify the specific criteria used to select "The ten most significant DEGs." It is presumed to be fold change.

Reply: Thank you for your question. In section 3.1 of the results, we have clearly described it: The top 10 genes with the most significant statistical differences in gene expression are presented in Figure 1a, with their details provided in Table 4. We sort and screen differentially expressed genes based on statistical differences in gene expression levels.

Q2: ### 2 - Why are neural pathways emerging in blood sample analysis?

Reply: Thank you for your question. Peripheral blood mononuclear cells (PBMCs), recognized for their high sensitivity and specificity as biomarkers for various diseases, are extensively used in SZ research. Relevant studies have shown that peripheral blood mononuclear cells and brain tissues have similar expression patterns in some signal transduction and metabolic pathways. We have already elaborated on it in the introduction and discussion section. Furthermore, we obtained possible pathways through bioinformatics analysis of the differentially expressed genes.

Q3: ### 3 - The rich factor and the software used for analysis should be described in the methods.

Reply: Thank you for your suggestion. We have added some description of the analysis software. The modified parts have been highlighted in yellow (method 2.2.3 and 2.2.4).

Q4: ##4 All the cases included in the study were patients with recurrent schizophrenia who had received long-term antipsychotic treatment. This may introduce confounding factors, affecting the accuracy and universality of the results of peripheral blood transcriptomics and proteomics analysis, and thus cannot well represent the entire schizophrenia patient population, especially the situation of first-episode patients or untreated patients.

Reply: Thank you for your suggestion. Our study were patients with schizophrenia who had received long-term antipsychotic treatment, thus cannot well represent the entire schizophrenia patient population, especially the situation of first-episode patients or untreated patients. There are indeed difficulties in collecting a sufficient number of cases for first-time or untreated patients, and we have discussed the potential impact on this study in the discussion section.

Q5: ##5 The study mainly focuses on the exploration of the basic mechanism. Although it has been found that the SERPING1 gene may be a potential therapeutic target, there is a lack of discussion on how to translate these basic research achievements into clinical applications. For example, it does not mention whether new diagnostic methods or therapeutic drugs can be developed based on the results of this study, as well as the possible challenges and solutions in clinical practice.

Reply: Thank you for your suggestion. The suggestions you mentioned are exactly the direction we will be studying next. We believe that it is too early to consider clinical translation and target drug research at present. Our next plan is to use neurons derived from induced pluripotent stem (iPS) cells for pathological research on mental disorders, to validate our current research findings.

Q6: ##6 Future studies may consider incorporating other sample types, such as cerebrospinal fluid or post-mortem brain tissue samples (if feasible), to explore the pathogenesis of schizophrenia from different perspectives more comprehensively and verify the reliability and relevance of the PBMCs research results.

Reply: Thank you for your suggestion. Our next plan is to use neurons derived from induced pluripotent stem (iPS) cells for pathological research on mental disorders, to validate our current research findings. Of course, the cerebrospinal fluid samples you mentioned are also an interesting research direction. There is still considerable resistance to autopsy brain tissue samples in China at present.

Q7: ##7 During the process of integrative analysis of transcriptomics and proteomics data, relying solely on correlation analysis of gene expression and protein expression (such as Spearman correlation coefficient analysis) may not fully reveal the complex regulatory relationships between genes and proteins. In addition, for the differentially expressed genes and proteins screened out, there may be some false positive or false negative results, and the study did not conduct more in-depth verification and screening optimization. Suggestion: Adopt multivariate statistical analysis methods, such as principal component analysis (PCA) or partial least squares discriminant analysis (PLS - DA), to further explore the hidden information in the data and improve the accuracy of analyzing the regulatory relationships between genes and proteins.

Reply: Thank you for your suggestion. In the comprehensive analysis of transcriptomic and proteomic data, we conducted multi-level analysis. However, due to limited space, we only selected the most important part for presentation. As we conduct correlation analysis on all quantitative proteins and genes, significant differential proteins and genes, proteins and mRNA with similar expression trends, and calculate their correlation coefficients. If you need this data, I can further present it, but we don't think it's a good idea to include it in the main text. We acknowledge that our ability to mine data needs to be further strengthened.