Peer Review File

Article information: https://dx.doi.org/10.21037/tp-23-201

Reviewer A

In this manuscript Shanshan Zhan et. al. used the published transcriptomics dataset of 20 T1DM and 20 control PBMCs samples and by employing WGCNA they have identified plausible key pathogenic genes of T1DM in children e.g., CCL25 and EGFR. I found their results and conclusions are interesting. I have following comments.

1) Authors need to mention that do their differential expression analysis is in agreements with the original research paper (PMID: 35058920) who first analyse and published this dataset. If possible, add short summary for the clinical characteristic of control and T1DM individuals who have participated in the study.

Reply: Thank you for your comment. We analyzed the results of this article (PMID: 35058920) during the discussion. Because the data was reused and the patient's clinical baseline data was not directly obtained. We apologize and regret that we were unable to include a brief summary of clinical features in the study.

Changes in the text: Paragraph 6 / Discussion

2) Authors need to mention the name of the genes in the key module and possibly their gene network and pathway analysis.

Reply: Thank you for your comment. Your approach is very correct. This analysis can uncover potential pathogenic mechanisms and key diagnostic factors. In terms of gene network construction, we screened key genes by constructing a co expression network, and then constructed a gene network again. The work is repeated, and they will show a strong correlation, otherwise they will not be assigned to the same module. In terms of pathway analysis, due to the small number of genes, we are unable to obtain meaningful results.

Changes in the text: None

3) Authors need to elaborate the discussion that if CCL25 and EGFR are reported in the other autoimmune diseases or other form of diabetes and their gene-network and possible link with the pancreas, if any.

Reply: Thank you for your comment. We added relevant content in the discussion.

Changes in the text: Paragraph 6 / Discussion

Reviewer B

The paper titled “Screening of key pathogenic genes of type 1 diabetes in children” is interesting. WGCNA was used to identify the key pathogenic genes of T1DM in children,

including CCL25 and EGFR, which have good diagnostic efficacy for T1DM in children. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The description of some methods in this study is too simplistic, please describe in detail.

Reply: Thank you for your comment. We added relevant content in the methods.

Changes in the text: Paragraph 1,2,3,5 / Methods

2) The figures in the results are too scattered, and it is recommended to partially integrate them according to the magazine's requirements.

Reply: Thank you for your comment. We integrated Figure 10 and 11.

Changes in the text: Figure 10 and 11

3) It is suggested to add further experiments to study the detailed molecular mechanism and biological function of the key genes of T1DM in children.

Reply: Thank you for your comment. Our research is limited to bioinformatics analysis and lacks further exploration of mechanisms, which is a limitation of our research. We have elaborated on this in the discussion.

Changes in the text: Paragraph 7 / Discussion

4) The background did not indicate the clinical needs for this research focus, and needs further revisions.

Reply: Thank you for your comment. We have revised the background.

Changes in the text: Paragraph 2 / Background

5) This study is only the result of bioinformatics analysis and requires experimental validation with a larger sample size.

Reply: Thank you for your comment. Our research is limited to bioinformatics analysis and lacks experimental validation, which is a limitation of our research. We have elaborated on this during the discussion.

Changes in the text: Paragraph 7 / Discussion

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Bioinformatic gene analysis for potential biomarkers and therapeutic targets of diabetic nephropathy associated renal cell carcinoma, PMID: 33457229”. It is recommended to quote the article.

Reply: Thank you for your comment. We have revised the background.

Changes in the text: Paragraph 2 / Background

7) How to judge the prognostic characteristics of T1DM in children based on the results of this study? How to provide candidate targets for the treatment of T1DM in children? It is recommended to include relevant descriptions in the discussion.

Reply: Thank you for your comment. We lack clinical information on prognosis and are unable to determine the prognostic characteristics of T1DM based on the results of this study. The purpose of this study is to screen diagnostic biomarkers. We added content on candidate targets in the discussion.

Changes in the text: Paragraph 6,7 / Discussion

8) The bioinformatics analysis in this study is too simplistic. It is recommended to conduct an integrative bioinformatics analysis of the data, which may be more meaningful.

Reply: Thank you for your comment. Our study used co expression networks to screen disease-related genes, but further research is still needed to confirm. We elaborated on the shortcomings

of this study in the discussion.
Changes in the text: Paragraph 7 / Discussion

Reviewer C

1. Abstract:
Please indicate the full name of "PBMCs" and "WGCNA" in the abstract. All abbreviated terms should be full when they first appear.

33 **Methods:** The transcriptome sequencing results of PBMCs of children with T1DM
34 (GSE156035) were downloaded from the Gene Expression Omnibus (GEO) database.

62 **Conclusions:** WGCNA was used to identify the key pathogenic genes of T1DM in
63 children, including *CCL25* and *EGFR*, which have good diagnostic efficacy for T1DM

Reply: Thank you for your comment. We have added them.

2. Please check if any more references need to be added in the below 2 sentences since you mentioned "Studies", but only one reference was cited. If not, "studies" should be changed to "a study/a previous study".

80 pregnancy and the neonatal period to reduce T1DM risk (8,9). Other studies have
81 reported that CD3 monoclonal antibody injection could delay the onset of T1DM in
82 children (10). Accurate prevention and treatment of T1DM requires the identification

238 Recent studies have explored key genes involved in the development of T1DM. One
239 study used polymerase chain reaction (PCR) and western blot techniques to examine
240 the caspases-3 messenger RNA (mRNA) and protein levels in PBMCs of T1DM
241 patients (15), and found that the caspases-3 levels were reduced both in mRNA and
242 protein expressions (15). The deficit of caspases-3 expression and function was related

Reply: Thank you for your comment. We have revised them.

3. Figure 8-9:
The main text below don't match with your Figure 8-9. Figure 8 is brown module and Figure 9 is pink module. Please note that Figures should be cited consecutively in the text.

214 the pink module contained 9 hub genes (*Figure 8*) and the brown module contained 52
215 hub genes (*Figure 9*). The intersection of the hub genes and DEGs included 2 genes,

Reply: Thank you for your comment. We have revised them.

4. Figure 10:
Please provide a summarised legend for Figure 10.
The format of legend should be like: Figure 1. Xxxxx (summarised legend). (A)xxxx. (B)xxxx.

Reply: Thank you for your comment. We have revised it.