Peer Review File

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Reviewer A

1. Running Title: Change to 'Glucose metabolism genes in dilated cardiomyopathy'

Response: Thank you for your feedback. We have made the necessary revisions based on your comments.

27

28 **3. Running Title**

29 Glucose metabolism genes in dilated cardiomyopathy

2. Abstract: End of the first sentence is confusing: 'among other causes other factors' - please correct

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

3 **Abstract**

4 **Background:** Dilated cardiomyopathy (DCM) is a prevalent condition with diverse

5 etiologies, including viral infection, autoimmune response, and genetic factors.

6 Despite the crucial role of energy metabolism in cardiac function, therapeutic targets

7 for key genes in DCM's energy metabolism remain scarce.

3. Abstract: The information from the methods is not sufficient enough to make physicians understand what exactly was performed, based on which information the target genes were chosen, how the results were generated, and what statistics was used.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

Methods:

Our study employed the GSE79962 and GSE42955 datasets from the Gene Expression Omnibus (GEO) database for myocardial tissue sample collection and target gene identification via differential gene expression screening. Using various R packages, GSEA software, and the STRING database, we conducted data analysis, gene set enrichment, and protein-protein interaction predictions. The LASSO and Support Vector Machine (SVM) algorithms aided in feature gene selection, while the

predictive model's efficiency was evaluated via ROC curve analysis. We used the NMF method for molecular typing and the CIBERSORT algorithm for predicting immune cell infiltration.

4. Abstract: The conclusion should be phrased more restrained. Same for the last sentence of the key findings. Those results are very speculative based on calculations only.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

Results: The *DLAT* and *LDHA* genes may regulate the immune microenvironment of DCM by influencing activated dendritic cells, activated mast cells, and M0 macrophages, respectively. The *BPGM*, *DLAT*, *PGM2*, *ADH1A*, *ADH1C*, *LDHA*, and *PFKM* genes may regulate m6A methylation in dilated cardiomyopathy by affecting the *ZC3H13*, *ALKBH5*, *RBMX*, *HNRNPC*, *METTL3*, and *YTHDC1* genes. Further regulatory mechanism analysis suggested that *PFKM*, *DLAT*, *PKLR*, *PGM2*, *LDHA*, *BPGM*, *ADH1A*, and *ADH1C* could be involved in the development of cardiomyopathy by regulating the Toll-like receptor signaling pathway.

Conclusions: *PFKM*, *DLAT*, *PKLR*, *PGM2*, *LDHA*, *BPGM*, *ADH1A*, and *ADH1C* may serve as potential targets for guiding the diagnosis, treatment, and follow-up of dilated cardiomyopathy.

5. Line 70: Please reword the sentence.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

6. Line 73: Drug therapy should be adapted to: betablockers, ACE-inhibitors, mineralcorticoid receptor antagonists, and SLG2-inhibitors.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

congestive heart failure(1). It is complex and diverse, including viral infection, autoimmune inflammatory response, and genetic factors, among others(2). Currently, there is no specific therapy for DCM, and most patients are treated with beta blockers, ACE inhibitors, mineralocorticoid receptor antagonists, and SLG2 inhibitors to slow disease progression. However, the long-term prognosis remains poor with these treatments. At present, there is insufficient research into the pathogenesis of DCM, and there is also a lack of clinical indicators for the diagnosis, prevention, and monitoring of DCM. At present, there is insufficient research on the pathogenesis of

7. Many abbreviations are not explained:

7.1. Line 108: Please explain CIBERSORT.

7.2. Line 110: Please explain ASPN.

7.3. Line 110: Please explain ECM.

7.4.. Line 115 et seqq.: all genes

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

regulatory relationships in DCM(14). Study has shown that miR-129-5p may regulate DCM by targeting ASPORIN through Extracellular Matrix(ECM) signaling pathway. Macrophage infiltration may participate in ECM remodeling and eventually lead to DCM(15).

8. Last section of the introduction: Is this your data or is it cited? In case of first, results do not belong to the introduction. In case of latter, please cite.

Response: Thank you for your comments. In the last section of the introduction, we did indeed mention some research results. These results are actually a preview of our entire study, intended to guide readers to understand our upcoming methods and results sections. However, to comply with the conventions of scientific writing, we should move this content to the results section and maintain the statement of the problem and research objectives in the introduction.

DCM(19).

In light of the aforementioned gaps in our understanding of DCM and the potential of glycolytic genes as molecular markers, this study aims to investigate the role of glycolysis in the occurrence and development of DCM and its underlying molecular regulatory mechanisms. Specifically, we will focus on eight signature glycolytic genes (PFKM, DLAT, PKLR, PGM2, LDHA, BPGM, ADH1A, and ADH1C) to explore their potential clinical significance in predicting DCM prognosis and guiding treatment strategies.

By employing a combination of bioinformatics technology and machine algorithms, our study seeks to contribute to a better understanding of DCM pathogenesis and provide insights into the development of effective diagnostic and therapeutic approaches.

9. Often, there is more than one space between two words.
Response: Thank you for your comments. Sometimes these issues can be caused by layout issues, and I will make the necessary adjustments.

10. Data sources in line 126 et seqq.: Gave patients informed for contribution to the databases?
Response: Thank you for your comments. We haven't informed patients about this yet.

11. Methods Section: Too many abbreviations not explained.
Response: Thank you for your comments. We have made the necessary revisions based on your comments.

Reviewer B

The paper titled “Analysis of the role of glucose metabolism-related genes in dilated cardiomyopathy based on bioinformatics” is interesting. PFKM, DLAT, PKLR, PGM2, LDHA, BPGM, ADH1A, and ADH1C can be used as new targets to guide the diagnosis, treatment, and follow-up of dilated cardiomyopathy. However, there are several minor issues that if addressed

would significantly improve the manuscript.

1) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

#Introduction

Dilated cardiomyopathy (DCM) is an idiopathic primary myocardial disease. It is characterized by the enlargement of the left or right ventricle or both, accompanied by impaired ventricular systolic function. It may or may not be accompanied by congestive heart failure(1). Despite extensive research efforts, there are still significant knowledge gaps and limitations in our understanding of DCM, particularly regarding its underlying mechanisms and the lack of clinical indicators for diagnosis, prevention, and monitoring(2). Currently, there is no specific therapy for DCM, and most patients are treated with beta blockers, ACE inhibitors, mineralocorticoid receptor antagonists, and SLG2 inhibitors to slow disease progression. However, the long-term prognosis remains poor with these treatments. At present, there is insufficient research into the pathogenesis of DCM, and there is also a lack of clinical indicators for the diagnosis, prevention, and monitoring of DCM. At present, there is insufficient research on the pathogenesis of DCM, and there is also a lack of clinical indicators for the diagnosis, prevention, and monitoring of DCM(3). Therefore, there is an urgent need to explore novel approaches to improve our understanding of DCM pathogenesis and identify potential molecular markers that can aid in diagnosis and guide effective treatment strategies.

In light of the aforementioned gaps in our understanding of DCM and the potential of glycolytic genes as molecular markers, this study aims to investigate the role of glycolysis in the occurrence and development of DCM and its underlying molecular regulatory mechanisms. Specifically, we will focus on eight signature glycolytic genes (PFKM, DLAT, PKLR, PGM2, LDHA, BPGM, ADH1A, and ADH1C) to explore their potential clinical significance in predicting DCM prognosis and guiding treatment strategies.

By employing a combination of bioinformatics technology and machine algorithms, our study seeks to contribute to a better understanding of DCM pathogenesis and provide insights into the development of effective diagnostic and therapeutic approaches.

2) How to use bioinformatics to mine the core genes of dilated cardiomyopathy and analyze the survival prognosis of patients? It is recommended to add the content of the discussion.

Response: Thank you for your valuable comments. Regarding your concern about how to use bioinformatics to mine the core genes of dilated cardiomyopathy and analyze the survival prognosis of patients, we fully understand your concern. However, we currently do not have sufficient data to perform survival analysis. Although we did not conduct survival analysis, we believe that the results of this study are valuable for understanding the pathogenesis of dilated cardiomyopathy and providing a foundation for future research. We will emphasize this point in the discussion section to address your concern. Thank you again for your valuable comments.

3) It is recommended to increase functional research on key genes in this study.

Response: Thank you for your comment. We will incorporate this aspect into our follow-up research.

4) How can the results of this study help to develop therapeutic strategies against dilated cardiomyopathy? It is recommended to add relevant content.

Response: Thank you for your valuable comments. Regarding your concern about how the results of this study can help develop therapeutic strategies against dilated cardiomyopathy, we will add relevant content to the discussion section.

Second, our study analyzed the immune infiltration pattern of dilated cardiomyopathy. By utilizing bioinformatics tools such as CIBERSORT, we assessed the relative proportions of different types of immune cells in the myocardial tissue of dilated cardiomyopathy patients. We found that immune factors may be involved in the occurrence and development of DCM and DLAT and LDHA may affect the course of DCM by regulating the distribution of immune cells.

By analyzing the immune infiltration characteristics of dilated cardiomyopathy, we can gain a deeper understanding of the disease's pathogenesis and progression. This information may help guide future research and development of immunotherapeutic strategies for dilated cardiomyopathy. For instance, modulating immune system activity or designing drugs targeting specific immune cell types may bring new breakthroughs in the treatment of dilated cardiomyopathy.

5) Suggest citing relevant literature “Integrative bioinformatics analysis of potential therapeutic targets and immune infiltration characteristics in dilated cardiomyopathy, PMID: 35433958”.

Response: Thank you for your suggestion. We have cited the recommended literature in the literature review section: "Integrative bioinformatics analysis of potential therapeutic targets and immune infiltration characteristics in dilated cardiomyopathy, PMID: 35433958".

15. Yang Y, Liu P, Teng R, et al. Integrative bioinformatics analysis of potential therapeutic targets and immune infiltration characteristics in dilated cardiomyopathy. Ann Transl Med 2022;10:348.

6) It is recommended to add in vivo and in vitro experimental validation of the results of this study.

Response: Thank you for your comment. We will incorporate this aspect into our follow-up research.

7) How to analyze the immune infiltration pattern of dilated cardiomyopathy? It is recommended to add relevant content.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

DCM.

Second, our study analyzed the immune infiltration pattern of dilated cardiomyopathy. By utilizing bioinformatics tools such as CIBERSORT, we assessed the relative proportions of different types of immune cells in the myocardial tissue of dilated cardiomyopathy patients. Immune cell infiltration has significant implications in disease progression and treatment. We found that in patients with dilated cardiomyopathy, the infiltration of certain immune cell types. These findings suggest that the immune system may play a crucial role in the pathogenesis of dilated cardiomyopathy, and therapeutic strategies targeting these immune cells may be beneficial for improving the course of the disease. By analyzing the immune infiltration characteristics of dilated cardiomyopathy, we can gain a deeper understanding of the disease's pathogenesis and progression. This information may help guide future research and development of immunotherapeutic strategies for dilated cardiomyopathy. For instance, modulating immune system activity or designing drugs targeting specific immune cell types may bring new breakthroughs in the treatment of dilated cardiomyopathy.

Reviewer C

1. Table S1

Please provide the table header

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

2. Table S2

Please provide the table header

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

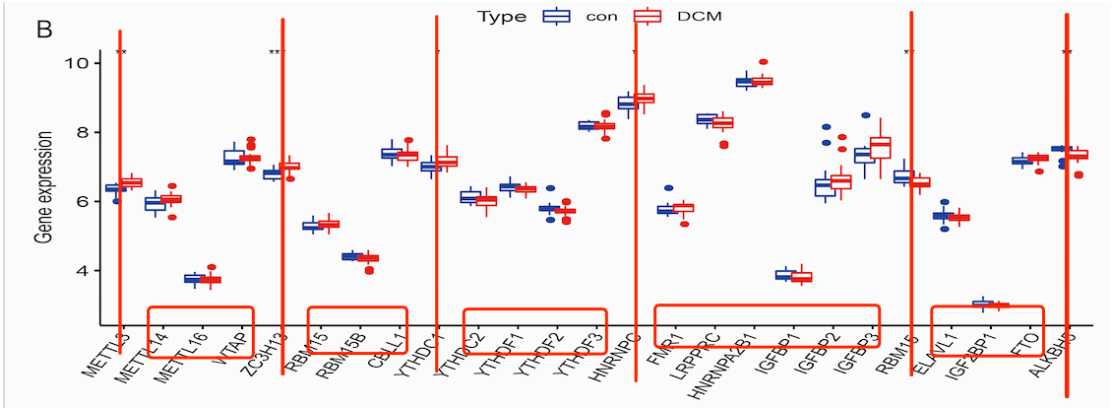
3. Please check all abbreviations in the figures, such as GSEA; GEO; PPI, et al. in Figure 1. Abbreviated terms should be full when they first appear.

protein interactions. (D) Analysis of glycolytic core genes in the PPI network. GSEA: XXX; GEO: XXX; PPI: XXX.

Response: Thank you for your comments. We have made the necessary revisions based on your comments.

4. Figure 6B

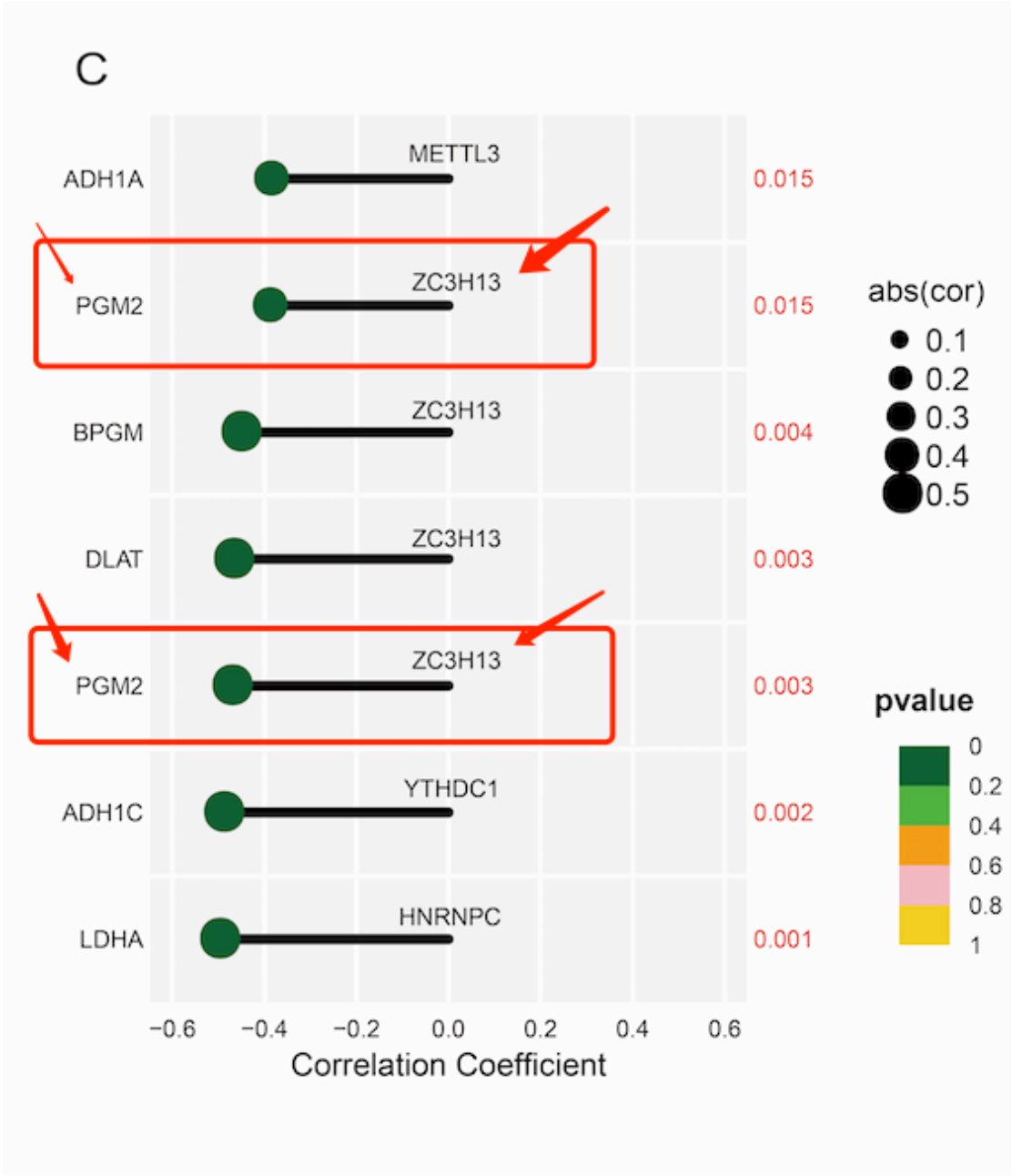
Did the content in the red boxes have P values? Please check and confirm.



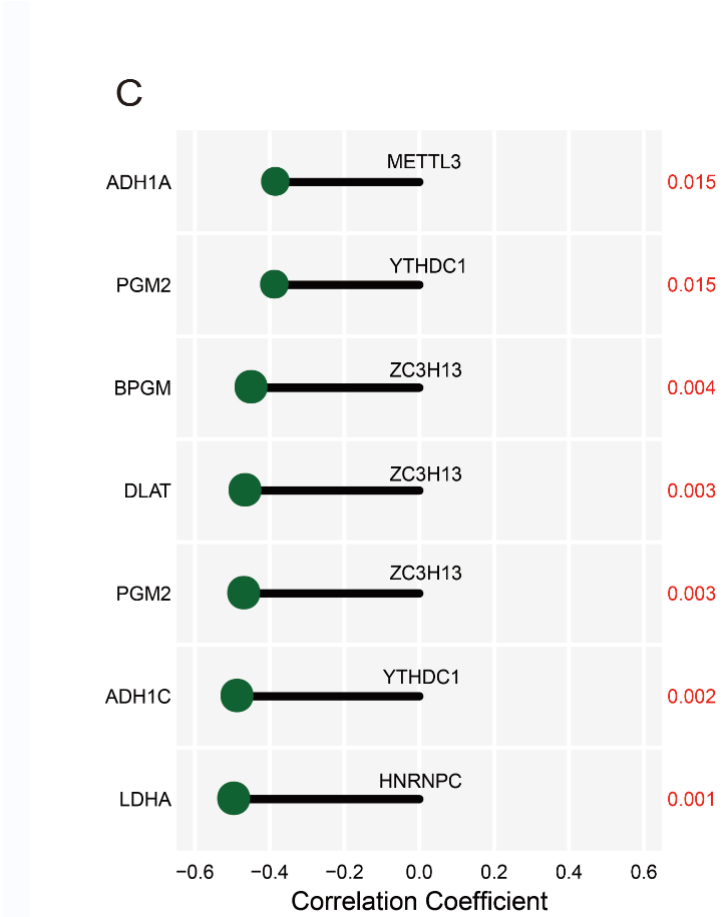
Response: Thank you for your comments. We have made the necessary revisions based on your comments.

5. Figure 6C

The content in the red boxes are the same. Is it correct? Please check and confirm.

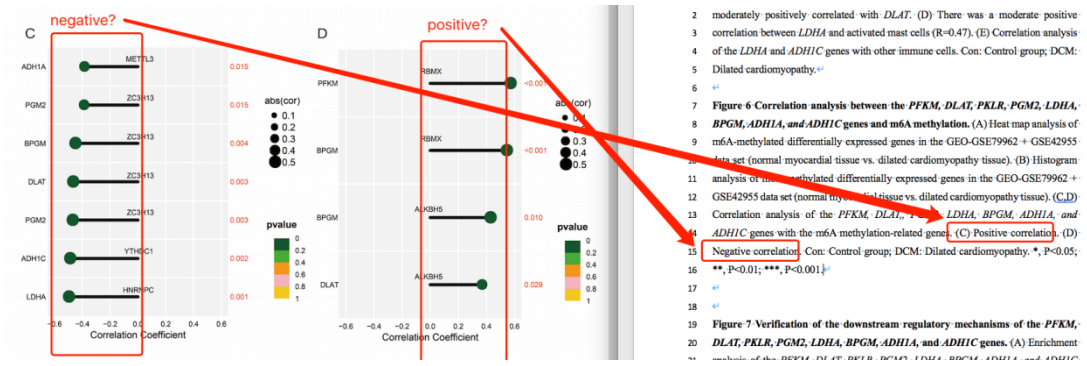


Response: Thank you for your comments. We have made the necessary revisions based on your comments.



6. Figure 6C and 6D

Please check the legend for Figure 6C and 6D.



Response: Thank you for your comments. We have made the necessary revisions based on your comments.