Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-1078

Reviewer A

As the authors are well aware, there are large bodies of literature documenting the role of inflammation in the etiology of both Crohn's disease and atrial fibrillation. Here, the authors seek to assess the intersection of studies for which the same GSE datasets have already been separately evaluated (AF: doi: 10.1186/s12920-020-00754-5; CD: doi: 10.3389/fimmu.2022.1074271). The methods used in this paper nearly completely overlap with those in the CD manuscript cited. Thus, the level of creativity in this paper is very low. When one looks for overlap between two inflammatory disease, one expects to find overlap. The clinical and scientific significance of this report is minimal. The authors state that their study "represents a pioneering effort in utilizing bioinformatics tools to comprehensively investigate the genetic link between atrial fibrillation (AF) and Crohn's disease (CD)." This is inaccurate. The authors have evaluated the overlap of differentially expressed genes between the two diseases. No genes, genotypes or genetic loci have been evaluated or reported. Overall, the abstract and conclusions greatly oversell the significance of this study.

Reply: ***Thank you for your comments and feedback on our manuscript. We appreciate your input and would like to address your concerns.***

We acknowledge that there is existing literature documenting the role of inflammation in both Crohn's disease and atrial fibrillation (AF). Our study aimed to assess the intersection of gene expression studies for these two conditions using the same GSE datasets. While the methods used in our study may overlap with the CD manuscript you cited, we believe that our analysis provides valuable insights into the shared genes, pathways, and immune cells between AF and CD.

We apologize if our statement about pioneering efforts in utilizing bioinformatics tools was misleading. Our intention was to highlight the application of bioinformatics methodologies in exploring the genetic link between AF and CD. We focused on identifying shared genes and conducting pathway enrichment analysis, rather than evaluating specific genes, genotypes, or genetic loci. We agree that further investigations into these aspects are warranted and could provide additional insights. We appreciate your feedback regarding the abstract and conclusions. We will carefully revise the text to ensure that the significance of our study is appropriately represented. We understand that the clinical and scientific implications of our findings may be limited, and we will make sure to provide a balanced and accurate assessment of our results.

Changes in the text: we have modified our abstract and conclusion as advised (see

line 35-41, 385-388)

Reviewer B

Certainly, the work is very well done. It revealed the underlying immune relationship between AF and CD, as well as identified CXCL16 and HLA-DPB1 as the potential connection genes between these diseases. However, some questions as follows remain to be addressed.

Reply: Thank you for your positive feedback on our work and for raising important questions and concerns. We appreciate your thorough review of the manuscript. We will address each of your points below.

1. In Fig 5, what model was used to compute the ROC curves? Also, some of the results seem like don't have high True Positives, the range is very large, such as GSE79768, GSE14975. Could you discuss the reason?

Reply: In Fig 5, we used the R package pROC to calculate the ROC values in different datasets. The p-ROC package is developed for visualizing, smoothing and comparing receiver operating characteristic (ROC curves). Area under the curve (AUC) can be compared with statistical tests based on U-statistics or bootstrap. Confidence intervals can be computed for (p)AUC or ROC curves. The large range of True Positives observed in some datasets, such as GSE79768 and GSE14975, may be attributed to the heterogeneity of the samples in those datasets. The variations in the gene expression profiles could additionally contribute to the wide range of True Positives. The low patients number in both datasets were another potential reason. Overall, we established a threshold of an area under the curve (AUC) value greater than 0.7 to determine biomarkers with substantial diagnostic effects. Based on this criterion, we identified CXCL16 and HLA-DPB1 as potential biomarkers that hold promise for diagnosing both atrial fibrillation (AF) and Crohn's disease (CD).

2. From the GO enrichment analysis in Fig 3, it showed MHC class 2 is more relative to these two diseases. Please discuss why the infiltration of immune cells in AF in Fig 7, CD8+ cells showed more significance.

Reply: The GO enrichment analysis in Fig 3 indicated a significant enrichment of pathways related to MHC and antigen processing. Regarding the infiltration of immune cells in AF shown in Fig 7, the higher significance of CD8+ cells could be attributed to the specific immune response in the atrial tissue during AF. CD8+ T cells are known to play a crucial role in cytotoxic immune responses and may be involved in the pathogenesis of AF. The activation and infiltration of CD8 cells into the atrial tissue can lead to the release of pro-inflammatory cytokines and chemokines, promoting atrial remodeling and electrical disturbances. This immune response mediated by CD8 cells may contribute to the perpetuation of AF and its progression to a more chronic and persistent state [1,2].

1. Friebel J, Witkowski M, Wegner M, Blöbaum L, Lammel S, Schencke PA, Jakobs

K, Puccini M, Reißner D, Steffens D, Moos V, Schutheiss HP, Landmesser U, Rauch U. Cytotoxic CD8+ T Cells Are Involved in the Thrombo-Inflammatory Response during First-Diagnosed Atrial Fibrillation. Cells. 2022 Dec 29;12(1):141.
2. Chang G, Chen Y, Liu Z, Wang Y, Ge W, Kang Y, Guo S. The PD-1 with PD-L1 Axis Is Pertinent with the Immune Modulation of Atrial Fibrillation by Regulating T Cell Excitation and Promoting the Secretion of Inflammatory Factors. J Immunol Res. 2022 May 12;2022:3647817.

Changes in the text: we have added this content in the discussion part as advised (see line 364-368)

3. Certainly, mononuclear macrophage infiltration is the pathological feature of chronic atrial inflammation, could you discuss why the heatmap result of AF in Fig 7, macrophage shows no significance?

Reply: Thanks for your comment. We totally agree that macrophage plays an important role in inflammatory disease. While mononuclear macrophage infiltration is a pathological feature of chronic atrial inflammation, the heatmap result of AF in Fig 7 might indicate that macrophages do not exhibit a significant difference in infiltration levels between AF and control samples in the datasets analyzed. This could be due to the specific characteristics of the datasets used in our study or the complexity of macrophage involvement in AF, which may require further investigation. Besides, the quantification of macrophage in bulk RNA sequencing in this study was performed using the single-sample gene set enrichment analysis (ssGSEA) method, which relies on the expression of classical macrophage markers.. To gain a clearer sight of the role of macrophage in AF, we suppose that further experimental study such as IHC and flowcytometry is needed. These techniques would provide a more detailed and direct assessment of macrophage presence and function within the atrial tissue, thus enhancing our knowledge of their involvement in AF pathogenesis.

4. In Fig 4E, the number of genes analyzed by LASS is different from that described on page 8, line 219-223.

Reply: Thanks for your comment and we have corrected this mistake in our manuscript.

5. Some figures were not clear, such as Fig2A-C, please improve the resolution. Especially in Fig3 and Fig6, it is hard to identify what each term is. These affected the review of the results.

Reply: Thanks for your comment. We acknowledge the issue regarding the clarity of some figures, particularly Fig 2A-C, Fig 3, and Fig 6. We will improve the resolution of these figures to ensure better readability and facilitate the review of the results. We apologize for any inconvenience caused.

6. There are several writing errors, that should be carefully checked and corrected by

the author. Such as the soft threshold in WGCN should be sFigure 2A and 2B on page 7, line 187. And on page 8, line 235, it should be GSE14975, not GSE14965. Please unify the font and spacing.

Reply: Thank you for pointing out the writing errors. We will carefully review and correct them, including the reference to the soft threshold in WGCNA as sFigure 2A and 2B on page 7, line 171, and the correction of GSE14975 on page 8, line 235. We will also ensure consistent font and spacing throughout the manuscript.