Peer Review File

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Reviewer A

This paper provides evidence of two gene modules and forty-four gene associated with POAF and provides the foundation for future research in to the underlying molecular mechanisms of POAF. The manuscript could benefit from more clearly stating molecular findings and discussing their significance in relation to POAF.

Comment 1: Abstract Line 49: "..2 miRNAs, hsa-miR-19b-3p and hsa-miR-30a-5p, were closely related.." Can the authors make clear in this sentence what these changes were closely related to? Was this statistically significant?
Reply 1: Thank you for your useful comment. We intend to state the specific circRNAs and miRNAs in our circRNA-miRNA-mRNA regulatory network in this sentence. Therefore, the relationship among circRNA (hsa_circRNA_001654 and hsa_circRNA_005899), miRNA (hsa-miR-19b-3p and hsa-miR-30a-5p) and mRNA (KLF10) were based on the RNA base-pairing mechanism. To make it more comprehensible, we have modified this sentence as advised in the abstract. (see Page 2, line 62)
Changes in the text: We rewrote the sentence as "Moreover, 2 novel circRNAs, hsa_circRNA_001654 and hsa_circRNA_005899, and 2 miRNAs, hsa-miR-19b-3p and hsa-miR-30a-5p, which related with KLF10, were involved in the network."

Comment 2: Highlight Box: Key finding bullet point 1: Sentence "A circRNA-miRNA-mRNA regulatory network associated with POAF was also constructed." is a method rather than a key finding. Suggest this be removed or rephrased to include a key result.
Reply 2: Thank you for your comment. We have revised this sentence as advised. (see Page 3, Highlight BOX)
Changes in the text: We revised it as "A circRNA-miRNA-mRNA regulatory network associated with POAF was also obtained."

Comment 3: Introduction Line 68: Please provide a reference to support this statement
Reply 3: Thank you for your comment and the reference was added to support this statement. (see Page 13, line 404-405)
Changes in the text: The reference "Gaudino M, A Di Franco, LQ Rong, et al: Postoperative atrial fibrillation: from mechanisms to treatment. Eur Heart J 2023, 44:1020-1039." was added.

Comment 4: Line 81: Can the authors please elaborate how circRNA plays a role in conditions listed.
Reply 4: Thanks for your review. In this section, we aim to emphasize the circRNAs do play a role in the pathogenesis of cardiovascular diseases. However, the various

cardiovascular diseases, such as congenital heart disease, dilated cardiomyopathy, myocardial infarction, and heart failure which we mentioned in our text, may involve a large number of different circRNAs which have been supported by either bioinformatics analysis or in vivo and ex vivo experiments. Besides the specific mechanism of circRNA is still under exploring. Therefore, it cannot be elaborated how circRNAs play a role in conditions listed in the introduction part briefly.
Changes in the text: We did not make further modification in our text as described above.

Comment 5: Methods Line 98: Is this statement needed in the methods if it is to be included in the footnote?
Reply 5: We agree with your comment, and removed this statement in the methods part. (see Page 5, line 115)
Changes in the text: The sentence has been deleted from the methods part.

Comment 6: Microarray data: Can the authors please explain why two different sample types and populations were used in the study.
Reply 6: Thank you for your comment. Both of the two analyzed datasets were compared between POAF and non-POAF patients. As we mentioned in our text, due to the limited sequencing data concerning POAF, only 1 circRNA dataset (GSE97455) was available for POAF in GEO up to now. A larger sample size would have been preferable. Different sample types from different data sets might have biased our results. Therefore, more clinical samples are needed for verification, which we plan to validate with future in vivo and ex vivo experiments.
Changes in the text: We did not make further modification in our text as described above.

Comment 7: Microarray data: Please detail the method/algorithm used for background correction, log2 transformation and normalization using R.
Reply 7: Thanks for the comment. The raw data in CEL files were subjected to background correction, log2 transformation, and normalization with the “limma” package in R software. We have already mentioned it in the methods section (see Page 5, line 125-128)
Changes in the text: We did not make further modification in our text as described above.

Comment 8: Results Can the authors expand their results to mention the key genes or processes that were significant?
Reply 8: Thank you for your comment. The key genes were listed as clearly described in Figure 3C,3D. And functional enrichment analysis was further performed for better understanding the key processes based on the key genes (see Page 8, line 231-243). As we mentioned, for further expand our results more in vivo and ex vivo experiments are required for our future research (see Page 12, line 350-351).
Changes in the text: We did not make further modification in our text as described

above.

Comment 9: Line 181: I suggest that it is made clear throughout what the sample type is for each population (tissue vs plasma). How comparable are these sample types? Can the authors make comment on this in the paper?
Reply 9: Thanks for your valuable comments. For the sample type of different datasets, we had described it clearly in the Methods part (see Page 5, line 122; Page 5, line 125). We apply WGCNA analysis to find key genes and modules of POAF on the basis of tissue samples by GSE143924 dataset. One on hand, due to the limited sequencing data concerning POAF, only 1 circRNA dataset (GSE97455) based on plasma samples was available for POAF in GEO up to now. On the other hand, circRNAs are more abundant in exosomes than tissue cells. Hence the differentially expressed circRNAs (DECs) were obtained through the analysis of GSE97455 dataset. As we described in the methods part, to construct the circRNA-miRNA-mRNA regulatory network, the target miRNAs and mRNAs based on the DECs were based on the computer prediction. The candidate mRNAs for network construction were selected from the overlap between the predicted target mRNAs and the differentially expressed genes (DEGs) from GSE143924 dataset. Those two different sample types could not be compared directly for analysis. And we utilized the possible characteristics of circRNAs as exomes in plasma for further combined network analysis by virtue of the limited available datasets in GEO database. Besides, we also mentioned that different sample types from different data sets might have biased our results in our discussion part. (see Page 12, line 347-349)
Changes in the text: We did not make further modification in our text as described above.

Comment 10: Line 203: Can the authors please detail why only the cluster with the highest score was chosen, and specifically state what it is chosen for. This is not clear.
Reply 10: Thank you for your comment. In the cluster analysis, clusters with higher scores indicate closer gene interactions and are more likely to be core genes within the module. Therefore, we selected the cluster with the highest score for further analysis.
Changes in the text: We did not make further modification in our text as described above.

Comment 11: Line 206-208: Suggest adding this detail to the methods
Reply 11: We have added the relevant content to the Methods section as advised (see Page 6, line 154-156)
Changes in the text: More details as advised have been added into the Methods section.

Comment 12: Line 212: Please elaborate further on the statement that "Key genes in the brown module were generally related to...". What makes then "generally" related? Is there any stats here?
Reply 12: Thank you for your useful comment. In this section we only try to list the key processes which were derived from the Gene Ontology analysis. There were not

any stats here. Therefore, we changed "were generally related to" to "were found to be related to" (see Page 8, line 231-232).
Changes in the text: The sentence has been modified as advised accurately.

Comment 13: Line 213-224: Suggest a tabulated form of these results to improve ease of understanding. It is suggested that a more detailed description of significant findings for specific genes and pathways is included.
Reply 13: Thank you for your comment. We have illustrated the corresponding results in Figure 4. Currently, we have decided not to supplement the table. We hope for your understanding and approval.
Changes in the text: We did not make further modification in our text as described above.

Comment 14: Line 238-244: as above, suggest to tabulate these findings
Reply 14: Thank you for your comment. We have organized the corresponding results in Figure S2. Currently, we have decided not to supplement the table. We hope for your understanding and approval.
Changes in the text: We did not make further modification in our text as described above.

Comment 15: Discussion Line 259: "POAF is likely caused by vulnerable triggers and existing substrate" What is meant by vulnerable triggers and existing substrate? Can this be expanded on?
Reply 15: Currently, the underlying mechanisms for POAF are incompletely defined. The vulnerable triggers were known as intraoperative and postoperative phenomena, such as inflammation, sympathetic activation and cardiac ischemia. And the existing substrate were known as the atrial substrate that were apt to the induction and maintenance of AF under the superimposition of those triggers.
Changes in the text: We did not make further modification in our text as described above.

Comment 16: Line 293: How was it decided that KLF10 "may" figure prominently in POAF? Can the authors please elaborate on this?
Reply 16: Thank you for your comment. Based on the bioinformatic analysis, we constructed a circRNA-miRNA-mRNA regulatory network related to POAF. KLF10 is the only mRNA in our network which combined two miRNAs and circRNAs. Therefore, we conclude that KLF10 may figure prominently in POAF.
Changes in the text: We did not make further modification in our text as described above.

Comment 17: Line 293: Please define TIEG1.
Reply 17: We have modified our text as advised (see Page 11, line 312-313).
Changes in the text: TIEG1 was defined as “TGF-beta inducible early gene 1(TIEG1)” as modified in our text.

Comment 18: Line 294: TGF-B already defined earlier in text.
Reply 18: We have modified our text as advised (see Page 11, line 314).
Changes in the text: The corresponding text has been deleted.

Comment 19: Line 299: What is PTEN/AKT signaling? Can this be briefly explained?
Reply 19: The PTEN/AKT signaling was derived from the study of Cen's et al which titled as as "TIEG1 deficiency confers enhanced myocardial protection in the infarcted heart by mediating the Pten/Akt signalling pathway". In their study, they observed the altered expression of phosphatase and tensin homolog (Pten) and Akt in TIEG1 KO mice. And as summarized, KLF10 was reported to induce apoptosis and inhibit proliferation through PTEN/AKT signaling in cardiac myocytes.
Changes in the text: We did not make further modification in our text as described above.

Comment 20: Line 306: What is Pitx2?
Reply 20: Thank you for your comment. PITX2 is stand for Paired-like homeodomain transcription factor 2 which was modified our text (see Page 11, line 326-327).
Changes in the text: The content " Paired-like homeodomain transcription factor 2 (PITX2)" has been added.

Comment 21: Line 308-309: "In our results, miR-19b was downregulated by circRNA_001654" Please include these types of explanations throughout the results to improve the readability of the manuscript
Reply 21: Thanks for the comment. Previous study by Wang et al. confirmed that miR-19b could inhibit the predisposition to AF. In our results, hsa_circRNA_001654 was screened as the DECs in Figure 5B which was significantly upregulated in the condition of POAF. KLF10 was also significantly upregulated in the condition of POAF as shown in Figure 6A. In Figure 6B, hsa_circRNA_001654-miR-19b-KLF10 was predicted to participate in the circRNA-miRNA-mRNA network. Therefore, our above results may hint that the downregulation of miR-19b could promote the occurrence of POAF, which was in line with the results by Wang et al. According to this comment, the sentence was rewritten to improve the readability of the manuscript (see Page 11, line 329-335).
Changes in the text: The sentences were rewritten as "In our results, hsa_circRNA_001654 was screened as the DECs (Figure 5B) which was significantly upregulated in the condition of POAF. KLF10 was also significantly upregulated in the condition of POAF as shown in Figure 6A. In Figure 6B, hsa_circRNA_001654-miR-19b-KLF10 was predicted to participate in the circRNA-miRNA-mRNA network. Therefore, those findings may hint that the downregulation of miR-19b could promote the occurrence of POAF, which was in line with the results by Wang".

Comment 22: Line 309-310: It is not clear if this sentence is related to the results from this manuscript or if this has been reported elsewhere.
Reply 22: Thank you for your comment. This is a part of our findings, and we have

made the necessary modifications (see Page 11, line 336-339).
Changes in the text: We modified as “Furthermore, as depicted in Figure 6B, miR30a was found to be associated with POAF. Besides, miR-30a was also reported to induce myocardial fibrosis and promote AF via targeting the Snail 1 protein.”

Comment 23: Line 311: Please define TBX5
Reply 23: We have modified our text as advised (see Page 11, line 339).
Changes in the text: The content “T-box transcription factor 5 (TBX5)" has been added.

Comment 24: Line 313-315: Can authors please elaborate how a zebrafish model can provide evidence or relatable models for research in to POAF?
Reply 24: Thanks for your valuable comment. The zebrafish model we cited here provide specific mechanism of miR-30a in AF occurrence through the regulation of TBX5 expression. POAF is a type of secondary AF. The sentence should be modified accurately (see Page 11, line 342).
Changes in the text: We modified the sentence as “Their zebrafish model assays demonstrated the interaction of miR-30a and TBX5 3'-UTR, which also support the role of miR-30a in AF.”

Comment 25: Conclusion Line 329: PKB/AKT already defined earlier.
Reply 25: We have modified our text as advised (see Page 13, line 358).
Changes in the text: The corresponding text has been deleted.

Comment 26: Ethical Statement Line 351-352: Already stated that the study has been conducted in accordance with the declaration of Helsinki.
Reply 26: We did not deleted the description og Ethical Statement as theEditor of JTD suggested
Changes in the text: There is no change in the text.

Comment 27: Table 1 Please define which datasets this is related to perhaps in a footnote in the table
Reply 27: A footnote was added as suggested (see Page 19, line 600-601).
Changes in the text: We added the footnote “The circRNAs were acquired from the differential expression analysis of GSE97455 dataset” under the table.

Comment 28: Figures are quite small and hard to read on paper.
Reply 28: All the figures have been enlarged. (see Page 19-31)
Changes in the text: We enlarge the figures in our text.

Comment 29: Figure 2 B: suggest to bold significant results D: is the Y-axis representative of p-values? please make this clear E: please make clear in graph titles which graph corresponds to which module
Reply 29: B: We added the asterisk for the significant results which the p value shown in the bracket lower than 0.05.

D：The Y-axis represents mean gene significance across genes associated with POAF in the module.
E：The gene significance in the brown and magenta module have been clearly depicted by the legend below the X-axis, which is automatically created by R program.
Changes in the text: We have modified figure 2 and its figure-legend as suggested. (see Page 20; Page 21, line 616, 619-621)

Comment 30: Figure 3 C&D: Italicize genes in box
Reply 30: We have italicized genes in box.
Changes in the text: We have modified figure 3. (see Page 22,)

Comment 31: Figure 5 Can this image be split in to no-POAF and POAF to improve readability **Reply 31:** Thank you for your suggestion. Figure 5 is titled as "Volcano plots and heatmap plots of DECs." The DECs (Differentially Expressed CircRNAs) were obtained when comparing non-POAF to POAF samples. Therefore, it cannot be split.
Changes in the text: There is no change in the text.

Comment 32: Table S2 Suggest to add these values to the corresponding module network images so that reader does not have to refer back
Reply 32: Thanks for your suggestion. For the WGCNA analysis, the gene count usually will not be labeled in the module network images. Therefore, to describe our results more clearly, we tabulate the gene count for each module in Table S2.
Changes in the text: There is no change in the text.

Reviewer B

The paper titled “Gene modules and genes associated with postoperative atrial fibrillation: weighted gene co-expression network analysis and circRNA-miRNA-mRNA regulatory network analysis” is interesting. The study provides foundational expression profiles following POAF based on WGCNA. The circRNA-miRNA-mRNA network offers insights into the biological processes and underlying mechanisms of POAF. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 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.
Reply 1: Thanks for your valuable suggestions. In recent years, the management and prognosis of patients with POAF has been increasingly paid. However, the pathogenesis of POAF has not been elucidated yet. Currently, there is a paucity of data utilizing WGCNA to identify key modules or genes related to POAF. And few studies have explored the role of circRNA-miRNA-mRNA networks in POAF. Accordingly, we

perform this study to identify key gene modules and genes and to conduct a circRNA-miRNA-mRNA regulatory network analysis of POAF on the basis of bioinformatic analysis. And our results may provide candidate targets for understanding the underlying mechanism and subsequent treatment of POAF. Therefore, the limitations and clinical significance had already been mentioned in the introduction. (see Page 4, line 84-861; Page 4, line 92-93; Page 4, line 102-106)
Changes in the text: We did not make further modification in our text as described above.

Comment 2: It may be more meaningful to add functional research on key ceRNAs and key genes of key modules.
Reply 2: We appreciate for your critical suggestion. We have already stated this limitation in our discussion part (see Page 12, line 350-351). Importantly, we have already planned further research to validate our findings through in vivo and ex vivo experiments and the experimental data will exhibit in our next study.
Changes in the text: We did not make further modification in our text as we have already planned further research to validate our findings and the experimental data will exhibit in our next study.

Comment 3: Figures 1,2,4,5 and S1 are not clear enough. It is recommended to provide clearer figures again.
Reply 3: The figures have been enlarged to be clearly read. (see Page 19, 20, 24, 27, 30)
Changes in the text: The figures have been enlarged to be clearly read.

Comment 4: Different sample types from different datasets may cause bias in the results, and it is recommended to use clinical samples for validation.
Reply 4: Thank you for your useful comment. To minimize the bias from different datasets, raw data from different datasets were subjected to background correction, log2 transformation, and normalization with the "limma" package in R software. However, there is certain bias for the sample types we utilized which we also mentioned that different sample types from different data sets might have biased our results in our discussion (see Page 11, line 340-342). One on hand, due to the limited sequencing data concerning POAF, only 1 circRNA dataset (GSE97455) based on plasma samples was available for POAF in GEO up to now. On the other hand, circRNAs are more abundant in exosomes than tissue cells. Hence, we apply WGCNA analysis to find key genes and modules of POAF on the basis of tissue samples by GSE143924 dataset. And the differentially expressed circRNAs (DECs) were obtained through the analysis of GSE97455 dataset (plasma samples). As we described in the methods part, to construct the circRNA-miRNA-mRNA regulatory network, the target miRNAs and mRNAs based on the DECs were based on the computer prediction. The candidate mRNAs for network construction were selected from the overlap between the predicted target mRNAs and the differentially expressed genes (DEGs) from GSE143924 dataset. Those two different sample types could not be compared directly for analysis. Therefore, we

have tried our best to minimize the bias as we had stated in our methods part and discussed in the discussion section. And we also plan to conduct further in-depth research in this regard in the future (see Page 12, line 350-351).
Changes in the text: We did not make further modification in our text as described above.

Comment 5: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Development and validation of a diagnostic model based on left atrial diameter to predict postoperative atrial fibrillation after off-pump coronary artery bypass grafting, J Thorac Dis, PMID: 37559620". It is recommended to quote the articles.
Reply 5: We appreciate for your suggestion. The related reference has already been cited in the introduction. (see Page 13, line 406-409)
Changes in the text: We added the advised reference in the introduction.

Comment 6: How to judge the prognostic characteristics of POAF based on the results of this study? How to provide candidate targets for the treatment of POAF? It is recommended to include relevant descriptions in the discussion.
Reply 6: Thank you for your critical comment. Our results provide a circRNA-miRNA-mRNA network which those two circRNAs may appear as prognostic maker in plasma. Consequently， patients who have a higher number of those prognostic makers are more easier to suffer from POAF. Besides, those patients need to pay more attention on the electrocardiograph monitoring after surgery as most of POAF event is transient. In addition, our results in key gene or target in WGCNA and circRNA-miRNA-mRNA network analysis may act as the upstream therapy in the treatment of POAF. However, as we mentioned in our discussion, our findings should be validated with future in vivo and ex vivo experiments (see Page 12, line 350-351). Therefore, we did not make any relevant descriptions in the discussion. And we will discuss it in our next study for further validation.
Changes in the text: We did not make further modification in our text as described above.

Reviewer C

1. **Figure 4B**

Please revise the X-axis.

Reply: We have revised the X-axis of figure 4B in our text. Please find the attachment

2. **Table 1**

Please check if “11” is correct, as there are only 10 miRNAs in table 1.

264 S1). Ultimately 11 overlapping target miRNAs were identified (Table 1, Figure S1).

265 To predict the mRNAs, the aforementioned 11 miRNAs were searched for in the

Reply: Thank you for your review. The “11” is correct. And we revised table 1in our text.

3. Figure S2

There is no ‘miRNA’ in figure or figure legend, but you indicate its full term. Please check and revise.

730 Figure S2 Functional enrichment analysis of predicted mRNAs. (A) Top 10 GO terms

731 of the predicted mRNAs classified by BP, CC, and MF. (B) Top 10 KEGG pathways

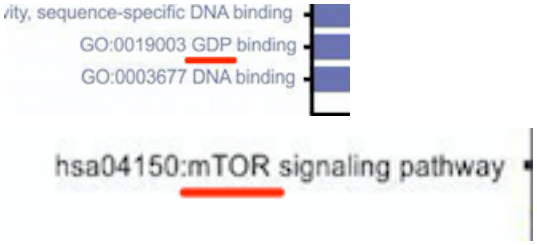
732 of the predicted mRNAs. BP, biological process; CC, cellular component; MF,

733 molecular function; miRNA, microRNA; circRNA, circulating RNA; mRNA,

734 messenger RNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and

Reply: Thank you for your critical review. We have deleted this description in the figure legend of Figure S2 in our text.

4. **When using abbreviations** in table/figure or table/figure description, please mention the entire expression in a footnote below the corresponding table/figure. Please check and revise. Such as: mTOR, GDP, (in figure S2).



Reply: Thank you for your reminder. We have supplemented the footnotes accordingly.