Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-704

Reviewer A

In their manuscript, the authors try to identify changes in proteins involved in atrial fibrosis caused by chronic intermittent hypoxia. To address this question, they used a rat model.

Main comments:

Comment 1: The authors address an interesting study. However, the document needs to be revised: punctuation in the abstract headings and body as well as figures and tables captions should be revised. Use the abbreviation for "CONT" the first time it is mentioned. In figure 5 B, improve the quality. In figure 6, why are there different routes with the same blue colour? Can this be changed? In table 2, the specification of abbreviations should either follow their order of appearance or be put in alphabetical order, choose one. Authors should also take care of the alignment of the content of the tables. Have the authors considered validating the selected proteins by another method of quantification such as ELISA?

Reply 1: Thanks very much for your kind suggestion. We have revised the text accordingly.

Changes in the text:

1) We have added line break before the Abstract headings of “Methods” and “Results”. Besides, punctuations in the body as well as figures and tables captions have been checked and revised.

2) The "CONT" has been defined in the "CONT" Methods section at its first place.

3) The quality of figure 5B has been improved.

4) The different nodes, lines and colors were further annotated in captions of figure 6. The

color of line is of no sense but the different types of lines (solid lines and dashed lines)

5) The specification of abbreviations was arranged in alphabetical order.

6) The alignment of the content of the tables was revised.

7) We have added the results of ELISA in the Figure 7.

Reviewer B

Zhao et al. studied the regulation of atrial expression patterns in the context of atrial fibrillation in the rat using proteome sequencing. While rationale and results are interesting, several limitations have to be considered.

Comment 1. As the results are from a rat model, they may not apply to human disease.

Reply 1: We agree with you that there are differences in the disease mechanism between Rattus norvegicus and Homo sapiens. However, rat model is a classical model for investigating the molecular mechanism of human disease and has been widely used in literatures. We also added this as a limitation in the revised manuscript. Sincerely hope it is qualified.

Changes in the text:

“This study has some limitations. First, experiments were performed in a rat model, and not in humans. Therefore, the conclusions may be not applicable in humans.”

Comment 2. As no cell sorting has been applied, it is impossible to distinguish between transcriptional regulation and altered cellular composition due to invasion and proliferation of fibroblasts and immune cells. Thus, the conclusions must be reconsidered carefully.

Reply 1: Thanks very much for your professional comments. We agree with you that our study is still preliminary and more in-depth study should be conducted for a valid conclusion.

We have added this as a limitation in the revised manuscript. Sincerely hope it is qualified.

Changes in the text:

“Third, as cell sorting was not applied, it was difficult to distinguish between transcriptional regulation and altered cellular composition due to the invasion and proliferation of fibroblasts and immune cells. Therefore, additional experiments should be conducted to obtain more robust conclusions.”

3. Physiological and pathophysiological functions of the regulated proteins should be better described aiming at a potential hypothesis for a pathophysiological mechanism.

Reply 1: Thanks very much for your professional comments. We have added more discussion about the key proteins in the discussion section of the revised manuscript. Besides, we have added a limitation for not further investigating the molecular mechanism of these key proteins.

Changes in the text:

“MYL2 is a sarcomeric protein that belongs to the EF-hand calcium-binding protein family. During early embryogenesis, MYL2 plays essential roles in maintaining cardiac morphology and in regulating cardiac contractile function (Sheikh et al., 2015; Tamamitsu et al., 2021). Moreover, MLC-2v phosphorylation has been shown to play direct roles in cross-bridge cycling kinetics, calcium-dependent cardiac muscle contraction, cardiac torsion, cardiac function, and various cardiac diseases (Weterman et al., 2013; Sheikh et al., 2015; Tamamitsu et al., 2021). Moreover, variations in MYL2 and MYL3 can cause HCM or restrictive cardiomyopathy and increase the risk of sudden cardiac death (Ingles et al., 2019; Tamamitsu et al., 2021). However, the molecular mechanism still warrants further investigation.”

“This study has some limitations. First, experiments were performed in a rat model, and not in humans. Therefore, the conclusions may be not applicable in humans. Second, the functions of the four proteins differentially expressed in AF were not explored in in vivo and in vitro experiments…”

4. English language editing should be considered. Further, false medical terminology must be corrected (e.g. “aural occlusion”; catheter ablation considered “surgery” etc.)

Reply 1: Thanks very much for your kind suggestion. We have sent our manuscript for language editing by native speakers in editage (www.editage.cn) and a certificate of editing has been obtained. Sincerely hope the language is now qualified for next review process.

Changes in the text:

“aural occlusion” has been revised to “appendage occlusion”. “Surgical methods” has been revised to “Treatment methods”.

Besides, the whole manuscript has been polished.