Reviewer's report

Title: Increased expression of NF-AT4 and NF-AT3 in the atria correlates with procollagen I carboxyl terminal peptide and TGF-beta1 levels in serum of patients with atrial fibrillation

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Reviewer: Guangliang Wang

Reviewer's report:

Atrial fibrillation (AF) is the most common abnormal heart rhythm that is often associated with palpitations, fainting, or heart failure. Many medical conditions, particularly the mitral valve disease (MVD), increase the risk of AF. Characterizing the mechanism of AF development may help prevent the disease. In addition, markers that are associated with AF can help for diagnosis of AF.

In this manuscript, Zhao et al characterized the expression of NF-AT3/4, pro-collagen I/III, and TGF-beta1 in AF patients in both mRNA and protein levels. By carefully analysis, they investigated associations of all these markers to persistent AF (PeAF), paroxysmal AF (PaAF), and sinus rhythm (SR). In the end they concluded that these genes can be used as biomarkers for AF diagnosis. Overall this study is of interest to the readers of BMC Cardiovascular Disorders because it provides useful expression information of important genes on the valvular heart disease. However, the paper is not organized well. There are many confusing parts including figures and tables. Revisions are needed to warrant acceptance of this manuscript for publication.

Major comments:

1. The authors talked a lot about the NF-AT3/4, but there not background introduction of these two important genes. They mentioned a little bit about their roles in the calcineurin signaling pathway. Actually both are important transcription factors that regulate cytokine genes in T cells (nuclear factor of activated T cells), as well as cardiac genes. I would suggest the authors give a little bit more background about these genes, and what we already know their roles in cardiac disease.

2. P13: a little background of PeAF, PaAF and SR would help readers understand all association assays. (e.g. clinical symptom: PeAF>PaAF>SR). In addition, it would be nice to show atrial images of each category so that the differences in atrial sizes can be appreciated.

3. Table 1 has a lot of value information that can be talked in more details in the result (e.g. disease condition, drug taken, NYHA class etc.) or in the method. It would be worthwhile to study the correlation between these conditions to AF gene expression too.

4. P14: Line10-12 and line13-16 are repetitive. They just said the same result
twice. Actually there is so much information in the figures 3-5, but they were not presented properly in the text. Maybe the author need describe in more detail on the comparison. Additionally, how was the nuclear NF-AT3/4 expression assay performed? I didn’t read anything about the assay in the method.

5. Regarding to the correlation analysis, I feel figures 6-8 are not very necessary. To be honest, the information I only looked was the R and p value. I would suggest that tables are good enough. Also, what is the summary for each correlation analysis? They seem all correlate with each other, then what’s the point?

6. P17: My same concern is about the last NF-AT3/4 expression essay in different valvular disease. The author simply listed all comparisons. Then what’s the point? What messages do they want to deliver?

7. P18-19: The first two paragraphs in discussion are just background. They can be put in the introduction. The discussion should be talking about the discovery and the interpretations, the potential mechanisms, and the clinic applications.

8. Table 3 and figures 3-4 are repetitive. Table 4 and figure 5 are repetitive. Figures should be good enough.

9. Table 6 and 8 doesn’t have any data.

10. It seems there some format issues on all figures. There are 15 pages of figure, but figures are only numbered to 8. Some pages should be combined.

11. In the end, personally, I would argue the semi-qRT-PCR is not very reliable for mRNA quantification. A Real-time PCR would be the best way for future mRNA quantification studies.

Minor comments:

1. P1: title: “Increased expression of NF-AT4 and NF-AT3 in the atria correlates…” should be “Increased expression of NF-AT3 and NF-AT4 in the atria correlates…”

2. P2: Line 9: “Atrial fibrillation is the most common cardiac arrhythmia…” should be “Atrial fibrillation (AF) is the most common cardiac arrhythmia…” It the first time to define AF.

3. TGF-#1. TGF-beta1, TGF-# should all be unified to the same format.

4. P6: line 13: “We excluded four categories of patients from this study”. I only see three categories.

5. P7: line 21: 4% paraformaldehyde?

6. P9: line 14: which lysis buffer did you use?

7. P10: line 10: “the” should be “then”

8. P13: line 10: “Right atrial diameters were not significantly different among the three VHD groups.” It is repetitive with the prior sentence.

9. P14: line 16: FigureS3B should be Figure3B.

10. P21: line 14: “Further” should be “Furthermore”.
11. For all tables, I'm confused by the small superscript characters. What do they really mean?
12. In figure 1 and 2, a sale bar is need.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests