Reviewer's report

Title: Sequencing of NOTCH1, GATA5, TGFBRI AND TGFBRII genes in familial cases of Bicuspid Aortic Valve

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Reviewer: Shohreh Maleki

Reviewer's report:

The major new finding in this article is the identification of two new mutations in NOTCH1 gene which segregates with BAV to the offsprings. These results are interesting and presents NOTCH1 as a strong (perhaps the strongest) gene candidate associated to BAV inheritance identified in human so far. However, a recently published article, which has not been discussed by the authors, associates the NOTCH1 mutations only to a subtype of BAV patients. This has not been addressed by the authors (please see below).

1. Is the question posed by the authors well defined?
   -Yes

2. Are the methods appropriate and well described?

   The methods used are standard and appropriately chosen for the study. The only concern is that the PCR amplification is subjected to nucleotide mis-incorporation and the authors should state how many independent PCR replicates have been sequenced, particularly in the cases, where a mutation has been detected. Considering that the main core of this report is identification of the point mutations in several genes and the small number of patients (11), the possibility of nucleotide misincorporation should be excluded, even though they have 200 biological replicates (controls).

3. Are the data sound?
   -Reasonably sound.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
   -Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
   -Yes

6. Are limitations of the work clearly stated?
   -I think the authors have to mention that their sample size is very small (11 patients).

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Totally, there are four articles published on NOTCH1 mutations in patients with BAV, the authors discuss three of them, but not the most recent one (PMID: 23102684). In the most recent paper (PMID: 23102684), the authors summarize the previous data on NOTCH1 mutation in relation to patients valve calcification and aortic dilation and argue that the existing data does not establish the NOTCH1 mutations contribution to ascending aortic aneurysms in non-calcified BAVs. According to this view, there are two types of BAVs; (i) the NOTCH1 dependent BAVs, associated with stenotic, or calcified aortic valves and (ii) the NOTCH1 independent ones with non-calcified aortic valves.

Clearly, the proband P284L mentioned in the present study has had a severely calcified valve, with aneurysm development, a year later. The second patient carrying a mutation in exon 26 has also been through a valve surgery, most probably due to a dysfunctional valve. So their data seems to agree with and fit well into the proposal made in 23102684 article, and this issue should be addressed in their discussion.

8. Do the title and abstract accurately convey what has been found?
-Yes

9. Is the writing acceptable?
The English is quite fluent and understandable, I am not a native English speaker myself, but can trace a few grammatical mistakes in the text. Some editorial aid for English would improve the text. Example: Background section: remains poor investigated yet, should be changed to: remains poorly investigated.

Conclusion:
Major Compulsory Revisions:
-The information regarding replicates for PCR amplifications and sequencing should be provided.
-The authors should discuss their findings in relation to NOTCH1 possible involvement, only in a subtype of BAV patients, as is also indicated by their data.

Minor Essential Revisions
-Editing the English will improve the manuscript.

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'