Reviewer's report

Title: Sequencing of NOTCH1, GATA5, TGFBR1 AND TGFBR2 genes in familial cases of Bicuspid Aortic Valve

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Reviewer: Gregor Andelfinger

Reviewer's report:

In this report, Foffa et al undertake directed sequencing of candidate genes in a cohort of Italian patients with familial bicuspid aortic valve (BAV). They sequenced NOTCH1, GATA5, as well as TGFBR1 and TGFBR2 and find two novel variants not previously reported in NOTCH1 to be likely causal alleles.

Strengths of the study include the analysis of a familial cohort, which should be enriched for causal alleles co-segregating with disease. Also, the authors dispose of a phenotyped, ethnically matched control cohort with 100 participants.

Major Compulsory Revisions

- it is mandatory that the nomenclature of mutations follow closely the recommendations of the HGVS (http://www.hgvs.org/mutnomen/). It is crucial to give full info (position on genomic DNA, on cDNA) and to let the reader know which assembly and which databases were used.

- Study population: The authors state that familial BAV was defined when '2 or more affected relatives had presumptive or proven BAV'. This wording is easy to misunderstand. Were any families defined as multiplex families in the absence of imaging data? The word 'presumptive' means 'not proven'.

- The family pedigrees A and I should give the full segregation pattern of the NOTCH mutants. If possible, the authors should extend familial screening by imaging all living first-degree relatives of the affected parent. Were there any other offspring in those families, or miscarriages? The authors need to give a full account of the phenotype of all family members by echocardiography (or other imaging method) and need to depict the allele status for every family member. It would be of great interest to learn whether, for example,

- Genetic analysis of GATA5: I did not find the Thr67Pro variant in the exome server, as stated by the authors. It is important to note that the frequencies given by the authors for the control population are heavily skewed through a dataset from the Maslen lab, which seems to have submitted sequencing data from a congenital heart disease dataset. The authors do not give the frequency of this variant in their ethnically matched controls and should absolutely do so. Of great interest, Padang seems to have identified the same variant in their BAV patients.

- the meaning of the word 'strumental" is unknown.

Minor essential revisions
A thorough linguistic review is needed in order to enhance readability of the manuscript.

**Level of interest:** An article of importance in its field

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

No conflicts.