Reviewer’s report

Title: Use of a Targeted, Combinatorial Next-Generation Sequencing Approach for the Study of Bicuspid Aortic Valve

Version: 2 Date: 7 July 2014

Reviewer: Gordon Huggins

Reviewer’s report:

Bonachea present their study in which targeted capture of candidate genes followed by next-generation sequencing was applied to a case-only cohort with bicuspid aortic valve compared with control sequence data from publicly accessible databases. This study demonstrates that mutations in genes involved in the Wnt pathway were identified as being over represented in this BAV cohort. Further, the approach of sequencing pools of genomic DNA was tested. Here are compulsory revisions:

1. Two individuals were described as carrying de novo mutations. Does this mean the mutations had not previously been reported? Or that the mutations were present but not found in the parental DNA? Please clarify. Table 1 indicates that the two de novo mutations actually rs ID numbers and were identified in 1000G and EVS.

2. The cohort was for the most part non-Hispanic white in racial and ethnic background. Were the few non-whites more likely to be found to have a mutation? The concern is that the few non-whites might carry gene variants that are related to their ancestry and not related to having BAV.

3. The basis for selecting the candidate genes for sequencing is not described. Perhaps the identification of Wnt pathway genes carrying mutations is because the gene selection process was biased towards the Wnt pathway?

4. The mutation discovery approach is called “gene” sequencing; however, it is not clear whether the sequencing approach is inclusive of introns and promoter regions or whether it is restricted to just the exons of the candidate genes. If just the exons were sequenced then that point needs to be made much clearer to the reader.

5. Would this approach be likely to discover a copy-number variation within the candidate genes?

6. The methods section indicates that parental DNA was also available- was the parental DNA also screened? Or was a mutation found in a proband also confirmed in the parents?

7. References are used inconsistently. For example the first sentence of the Results: “We studied a previously described cohort of 78…” that sentence must reference the manuscript that first reported the cohort in order for the reader to then go and learn more about the cohort. Please review closely and provide references throughout the manuscript where appropriate.
8. Since there is no replication cohort these findings should be clearly described as “hypothesis generating”

9. A table describing the clinical characteristics of the patients found to carry a mutation would add value. Currently there is no description of the cohort within this manuscript.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I have no competing interests.