Reviewer’s report

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

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Reviewer: Kim McBride

Reviewer’s report:

This report by Foffa et al is a small study of 11 patients with BAV, including 2 with coarctation of the aorta, where sequencing of NOTCH1, GATA5, TGFBR1 and TGFBR2 was performed. They identify 2 new mutations in NOTCH1 which appear to be pathogenic (p.P248L and p.Y1619X). They identify polymorphisms but no pathogenic changes in the other genes sequenced.

Major points:

Background: The paper background should be expanded, as the cases described, BAV and CoA, represent part of the spectrum of left ventricular outflow tract malformations that include congenital aortic valve stenosis and hypoplastic left heart syndrome. NOTCH1 mutations have been noted in individuals with those phenotypes by our group (McBride et al Hum Mol Genet. 2008; Riley, McBride, Cole Biochem Biophys Acta 2011) and others (Iascone et al Clin Genet 2012), and were also seen in the original report by Garg et al. There is some suggestion that NOTCH1 mutations may contribute to calcific aortic valve disease, but may play less of a role in BAV with aortic dilation (Kent et al J Thorac Cardiovasc Surg 2012). GATA5 has recently been implicated in both mouse and human studies as a cause of BAV, but has not been described in other LVOT malformations.

Methods: It is unclear in the methods section if the families include 2 or 3 total affected individuals. How was the diagnosis of BAV confirmed in each family member? I am not certain what a presumptive diagnosis of BAV might mean.

Results: The score or specific result from the PolyPhen2 or SIFT analysis should be stated. It would be helpful to indicate the phenotypes on the pedigree figure for each individual. The authors mix allele frequency and genotype frequency when commenting on the GATA5 variant rs6142775 (p.Thr67Pro): the allele frequency is 0.22 in their cohort. They do not state what the frequency of this variant was in their own control group. The frequency of this variant is in fact present in dbSNP, where data from the 1000 Genomes Project shows a minor allele frequency of 0.15 in Europeans and 0.17 in the Toscani Italian subpopulation. Thus this variant is NOT a very rare allele, but a common polymorphism.

Discussion: I agree with the findings of the NOTCH1 mutations they found, but more information is required on the bioinformatics analyses they performed. I disagree that NOTCH1 mutations are responsible for a large percentage of BAV
cases; their study is very small, and when combined with other studies suggest a role for NOTCH1 mutations in a few percent of individuals with BAV. I disagree with their discussion of GATA5 regarding the rs6142775 variant. There is no data to back their claim it is a modifier. They have misread the paper by Panang et al; the variant was not reported as being absent in controls, but that it was not assessed in their control group. In addition, the frequency of the variant in their cohort does not appear to be different from other European populations, and they do not provide a frequency of this variant in their own control cohort. The current discussion and conclusions for this variant should be removed, and the variant should be described as simply a variant of no significance.

Additional comments:

Please review the paper for proper use of gene and protein names. All human genes should be italicized and all caps, proteins should be all caps and not italicized. Rodent genes are italicized and have the first letter capitalized, with proteins not italicized. The proper abbreviations is TGFBR1 and TGFBR2 not TGFBRI and II. The attached file contains some track changes for English wording suggestions.

**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 no competing interests