Reviewer's report

Title: ALDH1A2 (RALDH2) genetic variation in human congenital heart disease

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Reviewer: Judith Goodship

Reviewer's report:

This manuscript addresses the hypothesis that rare or common variants in RALDH2 contribute to cardiovascular malformation. The rationale underlying the hypothesis is that aldehyde dehydrogenase, the enzyme encoded by this gene, converts retinaldehyde to retinoic acid and that retinoic acid is required for normal cardiac development.

I do not have expertise to comment on the structural analyses (methods, results and interpretation, Figure 2 and Fig 5) or on the mRNA secondary structure analyses (Figures labelled 6 and 7 but referred as supplementary Figure 2 in text) and therefore limit my comments to the patient phenotypes, mutation analysis, splicing assay and association study. The structural studies are an important component in deciding whether the manuscript should be published hence I have checked the 'unable to decide on publication' box.

Summarising the study:
1. Search for and investigation of rare variants.

The first step of the study is mutation screening in 83 cases with various congenital heart defects (including 33 tetralogy of Fallot cases). One known SNP was detected and six intronic changes.

The authors went on to screen for mutations in 50 tetralogy of Fallot cases. In this group they detected 2 missense changes, both inherited from a normal parent but not present in 100 normal control chromosomes. The obvious next questions are whether these changes are polymorphisms or rare variants and if a rare variants whether pathogenic. Whilst finding a de novo change increases the likelihood that it contributes to the phenotype the majority of changes described in CHD candidate genes to date have been inherited even when in vitro studies support their involvement. However more ethnically matched controls have to be analysed.

What is the evidence for the Ala151Ser being pathogenic:

Sequence conserved across species - yes

Splicing assays – the in vitro splicing assay indicates that this change does not diminish splicing efficiency. No aberrant transcripts observed.

Thus the case for pathogenicity hinges on the modelling – I am not able to comment on how robust these data are – if robust it is surprising that the authors do not refer to them in the abstract
What is the evidence for the Ile157Thr being pathogenic:
Sequence conserved across species - no
Again the case for pathogenicity hinges on the modelling – I am not able to comment on how robust these data are – if robust it is surprising that the authors do not refer to them in the abstract.

Compulsory revisions:
Currently the methods section of the abstract is blank.

When describing DNA changes HGVS nomenclature (http://www.hgvs.org) must be followed with a reference sequence so that readers are able to identify precisely which base is changed. This applies to intronic changes as well as exonic changes. I suggest that in the text the authors state that in the screen on the 83 CHD patients they detected a known polymorphism and six intronic changes with a comment whether these changes occurred within the KOZAK sequence.

Splicing assay and Figure 3
The lanes in panels D and E must be labelled and the sizes of bands or markers indicated. It is misleading to show the insert in panel C giving rise to RNA lacking the RALDH2 exon as it gives rise to two species of RNA.

Figure 3 legend states that HIV exons 1 and 2 flanking aldh1a2 exon 4 is a 250bp PCR product whilst only HIV exons 1 and 2 gives a product of 380bp – one of these must be incorrect. The legend states there is a significant decrease in splicing strength with the A453G transition and in the text relating to this splicing assay the authors state that the polymorphism is associated with reduced capacity to splice exon 4 p=0.36 – p=0.36 does not indicate statistically significant reduction in exon 4 splicing.

Minor essential revisions:

Abstract:
The patient numbers do not always add up: the methods ‘patient’ section states samples were collected from 101 trios. The abstract refers to 234 congenital heart disease patients being studied. The mutation screening was done in 83 cases in the first instance and fifty Fallot’s, were these another 50 Fallot’s or did this include the 33 Fallot’s cases in the first 83 cases? These figures must be clarified so there is consistency through the manuscript.

Also in the abstract ‘we determined that exon 4 alterations impact on splicing’ is an overstatement.

Conclusion of abstract – use of the term mutation implies pathogenicity to many clinical readers even though it is a neutral term. Unless the modelling data is very strong the case is not proven. The authors are circumspect in their title and
should be equally circumspect in the abstract and discussion.

100 control chromosomes (50 individuals) is not sufficient – see Mitchell A et al. Genome Research 15:960-966 2005, On the probability that a novel variant is a disease causing mutation.

Clinical descriptions of the two patients with missense changes:
Should include a statement that the patients have been checked and do not have a chromosome 22q11 deletion.

The clinical descriptions are given for their presentation as babies but these are young adults. Are the patients of normal intelligence, normal height now? If giving heights and weights the centile should be given to save the reader having to look these up.

The patient with the Ile 157Thr change had Wilms tumour as well as tetralogy of Fallot. There are a number of syndrome featuring childhood tumours and congenital heart disease – Beckwith-Wiedemann and the conditions associated with RAS/MAP kinase pathway mutations. It is clearly important to know that this individual has been assessed and does not have a syndromic explanation for his TOF. These possibilities should be included in the section of the discussion relating to the Wilms tumour.

Re: association studies. A power calculation should be included to demonstrate what size of effect could be detected with these sample sizes.

Discretionary revisions:
p3 locus heterogeneity is more specific than genetic heterogeneity in the context used in this section.
p4 end of bracket is ommitted after (reviewed in [18]

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

Quality of written English: Acceptable

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