Reviewer's report

Title: Submicroscopic aberrations on 1q21.1 in patients with congenital heart defects and velocardiofacial-like features

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Submicroscopic aberrations on 1q21.1 in patients with congenital heart defects and velocardiofacial-like features by Bruneti et al.

This manuscript reports a small study of CNVs in a cohort of patients presenting with congenital heart defects. In an initial discovery cohort of 18 cases, the authors use array CGH genome-wide, and detect one case with a duplication reciprocal to the TAR syndrome deletion on chromosome 1, in addition to a number of other inherited CNVs. They then study a second replication cohort using MLPA with probes in 1q21.1 and identify one further CHD patient with an apparently identical duplication of this same TAR locus.

Overall the manuscript is well written, and the conclusions, with some exceptions, are generally valid. The possible association of this 1q21.1 duplication with heart defects is novel and worthy of publication. However, this study does not have sufficient power to determine if this CNV really is a cause of CHD, or if the identification of these two cases in their CHD patients is merely chance, making its impact questionable.

I have the following comments:

In the abstract the authors state that “We have analyzed 18 patients with VCFS-like features by array comparative genomic hybridisation (aCGH). We performed a sandwich-like hybridization of the sample and reference DNAs onto two different arrays: a whole genome and a chromosome 22-specific BAC array.” The phrase “sandwich-like hybridisation” is not by any means a standard phrase and stated in the abstract in this way is rather opaque. On further reading the manuscript it becomes clear that the authors have in fact used a novel method to hybridise two microarrays simultaneously face-to-face using a single mix of labelled genomic DNA in between. I find this novel method of practical interest that I believe is worthy of additional explanation, as it may be of use to other investigators. I think that if the authors were to expand on their description of this technique, adding some technical details and a figure, it would add a new dimension to the manuscript, making it of broader appeal to readers. Can the authors comment on technical performance of this methodology compared to performing single hybridisations? For example it would be nice to know if data reproducability is comparable to single hybs, and do the authors make any
technical modifications, eg. to the amount of DNA used, the amount of COT DNA added, and the practical details of how they construct the ‘hybridisation chamber’. Such details would be a useful addition to this manuscript.

The authors should make it clear that their case of 1q21.1 rearrangement was previously published by Mefford et al. NEJM 2008.

I find the discussion of the additional CNVs detected in some patients a little misleading. While it is possible that some of these CNVs contribute towards the phenotype of the patients, at least one of them is a frequently observed variation reported in many studies of normal individuals, making it highly unlikely that it is related to heart defects. Further the somewhat detailed discussions of potential candidate genes that are located in these CNVs (page 12 and 13) is over-interpreting the data, as the authors have no data to say if these genes (or exons) are even included in the CNV detected.

Table 1 presents a list of CNVs identified by BAC array CGH in their initial discovery cohort. For this list of CNVs, the authors state the coordinates of the BAC clone that was variant, accompanied by a list of genes within that BAC. However, BAC array CGH is notorious for over-estimating the size of CNVs, and it should be made clear that because of this it is not possible to state if these CNVs include all of the genes listed or just a subset of them. Furthermore, because only BAC array CGH was used, the size estimates given for these CNVs are VERY approximate, which again should be made clear. This point is particularly relevant to a statement on page12 of the manuscript: ‘Finally, a gain of BAC RP11-138G4, located on 22q13.2, which was maternally inherited, was also identified with this chromosome-specific array. This region has not been reported in the literature as a CNV and spans the SLC25A17 gene that encodes a peroxisomal membrane protein belonging to the family of mitochondrial solute carriers [OMIM *606795].’ Without breakpoint characterisation, given that the CNV is defined by only a single BAC clone, I do not think that the authors can know whether this gene is actually included in the CNV identified or not.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.