Reviewer's report

Title: Detecting 22q11.2 deletion in Chinese children with conotruncal heart defects and discovery of single nucleotide polymorphism in the haploid TBX1 locus in cases of 22q11.2 deletion

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BMC Medical Genetics

Xu et al enrolled 212 patients with conotruncal heart defects (CTD) at the Shanghai Children’s Medical Center. The authors performed karyotypic analysis, multiple ligation-dependent probe amplification, and TBX1 polymorphism discovery among all cases. Among the 212 patients, only 13 were found to have the 22q11.2 deletion syndrome (6.13%).

The manuscript's main purpose is to establish the prevalence of del22q11.2 among Chinese CTD patients, which preliminary data from the same group of investigators suggested was lower than previously reported among European-descent populations. The authors seem to reach their stated goal, but they also seem to overreach based on the data collected on these patients. There are several concerns from this Reviewer that should be addressed for this to be a useful report in this journal.

Major Compulsory Revisions

1. The authors mention that a previous study conducted by the same authors found a lower prevalence of del22q11.2 in 24 Chinese CTD patients. Were these same 24 patients analyzed as part of the 212 patients reported in the manuscript? Either way, the authors should be explicit that the 212 either included or did not include the 24 previously examine patients.

2. Under the subsection titled “Gene PCR and sequence”, several details are missing. For example, how were the polymorphisms identified (what software was used and with what settings?)? For the controls, were any quality control metrics employed such as tests of Hardy Weinberg Equilibrium, etc? And, who are the healthy controls? No demographic data (sex, age, race/ethnicity, etc) is provided for these 139 dizygous healthy controls. Perhaps the demographic data for the controls can be provided in a table with the cases. And, why do the authors state that the healthy controls are needed to “confirm the results of the patients.” This statement does not make sense.

3. In the same subsection, the authors state that “it may, therefore, be implied that the sister chromatid which contains the 933G is predisposed to be deleted.” This conclusion and statement is too strong based on the weak evidence of
association (p=0.046). And, many tests of association were performed without correction or acknowledgement of multiple testing, so this is likely a false positive result.

4. In the same subsection, the authors also state that “it is, therefore, possible that fetuses with del22q11 who carry the A allele in the rs41298838 locus were spontaneously aborted due to a flawed development of the heart.” Again, this statement is too strong given the very small number and weak statistical evidence.

5. In the Discussion section, the authors state that “this may be due to the small size of our group investigated...”. Please include “(n=4)” in this statement to show the readers how small the sample size is.

6. There are at least two statements in the Discussion section that are too strong based on the data collected and described here. The first is, “It, therefore, seems that either the sister chromatid, which contains the 933G allele in the locus, is predisposed to be deleted, or the fetuses carrying the G allele were miscarried during pregnancy due to causes unknown.” The second is, “It, consequently, is possible that del22q11 fatuses [sic] with the A allele in the rs41298838 locus were spontaneously aborted due to a faulty development of the heart or other relevant organs.” Also, the last two sentences of the section “Conclusions” are over-reaching given the small sample sizes and lack of power in this study.

7. As mentioned above, the authors should include additional demographic data for the 139 control samples included in the study. Perhaps a new “Table 1” for all 212 patients and 139 controls detailing demographic data such as age, sex, etc could be included in the manuscript.

8. Table IV only displays the data for one SNP because it was significantly different in frequency between deleted patients and controls at p=0.046. Table IV should be expanded to include ALL tests of association so that readers are clear as to how many statistical tests were performed. The same comment applies to Table V: please show all the data for all tests of association performed.

Minor Essential Revisions

9. In the Results section and subsection titled “Multiplex ligation-dependent probe amplification”, the authors state that the incidence is “lower than the potential microdeletions reported by authors in the West.” What are the references to these reported by “authors in the West”?

10. In the Results section and subsection titled “Gene PCR and sequence”, the authors state that “all of the substitutions had a frequency of p>0.05…” This does not make sense—perhaps the p (a symbol for p-value) is misplaced here?

11. The x-axis of Figure 3B is too small to read.

12. How do the control sample frequencies compare with the International HapMap data?

13. There are several minor typos throughout the manuscript. For example, there is a misplaced common in the Introduction. The authors sometimes italicize gene names, and they sometimes do not. “Chromarid” is used instead of “chromatid” in
the Discussion section.

**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.