Reviewer's report

Title: A unique phenotype in a patient with a rare triplication of the 22q11.2 region, and new clinical insights of the 22q11.2 deletion and microduplication syndromes: a report of four cases

Version: 2 Date: 5 January 2015

Reviewer: Heather McDermid

Reviewer's report:

This manuscript gives the clinical phenotype of 4 previously reported patients from the Azores (Pires et al, 2014). An MLPA screen of 87 patients with heart defects revealed 4 unrelated patients with 22q11.2 CNVs – 2 deletions, 1 duplication and one triplication. The previous work characterized the CNVs with MLPA, FISH, and array-CGH and showed slightly varying breakpoints. Brief clinical descriptions were also given. The new manuscript expands on these clinical descriptions.

The patient with a triplication of 22q11.2 is of great interest, since this is only the second reported case.

The patient with the duplication is also of interest, although there have been many cases reported. The fact that the patient had a monozygotic twin with a different heart defect is also interesting (the same duplication is assumed but not confirmed).

- Major Compulsory Revisions

1) I feel there is no utility in reporting the expanded clinical features of the two patients with 22q11.2 deletions, since this is a well reported and common microdeletion syndrome. At this point, a unique feature is likely to be unrelated to the deletion, if never seen in previous cases. Also, the unique features in the second case could easily be due to medication taken very early in the pregnancy – there is no way to sort this out, so the features as part of the deletion syndrome are too suspect to warrant further reporting.

I therefore suggest that the manuscript focus on the triplication case, with an additional case of duplication described, and remove all data concerning the 2 deletion cases.

2) I think it’s important to connect the patients’ original designations in Pires at al (2014) to the new manuscript. Currently Patient A is Case 2, Patient B is Case 4, etc. For continuity I suggest keeping the original designations from Pires et al, to help the future reader compile the data. Also, you should give more information on the duplication/triplication (breakpoints) and reference Pires et al each time information from that paper is used (especially in the case presentations).
3) To put the 2 duplication patients into context, it is imperative that Table 1 be restructured. The two deletion patients can be removed. There should be four new columns: one that summarizes the features of all previously reported patients with a 22q11.2 microduplication syndrome to compare to Case 3/Patient C, one that gives the features of the first case of triplication (Yobb et al) to compare to Case 4/Patient B and columns for the duplication parents of the children with triplications. Putting all this in table form will make it much easier for comparison to future cases, plus put the cases with duplications into better context. Please note that in the first patient with a triplication, the Table in the Yobb et al paper indicates that the hearing defect is probably secondary to otitis media.

4) One of the main conclusions of this paper is that Case 4/Patient B with a triplication has a more severe phenotype than the first reported patient with a 22q11.2 triplication, “and this genetic alteration could be responsible for a variation of the 22q11.2 microduplication syndrome, with aggravated phenotype due to the major dosage of implicated genes”. Since there are only two patients with triplications in the literature, one mild and one more severe, the evidence does not support this conclusion and other hypotheses must be discussed. Multiple cases of 22q11.2 duplication syndrome have been found using techniques that should pick up a triplication, and there is no evidence that the more severe cases are triplications. The first patient showed a mild phenotype with no heart defect, and was ascertained based on behavioral problems. The second more severe patient was ascertained due to the heart defect, which highlights ascertainment bias for this syndrome. Unless there is a very broad molecular population screen performed, this syndrome will be plagued by bias and low numbers, so it’s dangerous to hypothesize that the triplication is worse than the duplication, especially based on 2 patients. The phenotype of this more severe patient easily fits within the duplication spectrum and the patient from Yobb et al is at the milder end of the duplication spectrum. I think that genetic background is much more likely to account for these large differences.

- Minor Essential Revisions

5) Case 4 – Are you suggesting that the Sturge-Weber syndrome is related to the presence of the triplication? If not, you should state this clearly, and in the table there should be an asterisk next to all the Sturge-Weber symptoms, with a footnote indicating they are likely unrelated to the triplication. Also, is there any other additional phenotypic information about the patient’s affected father?

6) The monozygotic twin of case 4 with a different cardiac defect is very interesting. It would be helpful to have more information on previous cases of MZ twins with the same syndrome but difference cardiac defects – is this common?

7) The abstract refers to DiGeorge syndrome but not VSFS. 22q11.2 deletion syndrome should be referred to as DGS/VCFS here and later in the paper, rather than just DiGeorge Syndrome. The description of the phenotype should include palatal defects and velopharyngeal insufficiency.
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