Reviewer’s report

Title: Intergenerational and Intrafamilial Phenotypic Variability in 22q11.2 Deletion syndrome Subjects

Version: 3 Date: 10 November 2013

Reviewer: Gregory Costain

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Cirillo and colleagues have resubmitted an improved manuscript. The data presented by this group warrant publication in some form. Several issues remain:

Major Compulsory Revisions

1. The authors state in their cover letter that, "...we don't feel that our preliminary observation regarding genetic anticipation is imprudent, since it has never been reported before." If the authors are referring to their finding that the phenotype in 22q11.2DS may often appear to worsen over generations, this seems false (see McDonald-McGinn et al. 2001, Digilio et al. 2003, and others). If the authors are referring to their conjecture that a molecular mechanism leading to true genetic anticipation may underlie the finding, this also seems false (see Digilio et al. 2003: "Furthermore, the extension of an unstable mutation at the 22q11.2 locus could also be responsible for intrafamilial variability (12, 26, 27)." and references therein, and others).

The authors have modified their manuscript, but continue to simultaneously (i) speculate about possible underlying genetic mechanisms that could account for their findings in the absence of any molecular data or novel epidemiologic data, and (ii) downplay or ignore alternative simple partial or complete explanations for their findings (as listed in my previous review and below). I agree with the authors that the former (i.e., genetic/epigenetic mechanisms) is a definite possibility deserving of discussion within this manuscript and further study. However, these data and the associated discussion have not convincingly refuted the latter possibility (i.e., ascertainment biases), which I argue should be seen as the 'null hypothesis'. A more balanced presentation is encouraged.

2. There remains little to no evidence that the authors have considered how the ascertainment of the probands may or may not have influenced their results. These 26 individuals were apparently ascertained on the basis of clinical features of 22q11.2DS. Therefore, it stands to reason that they were a priori less likely to have mild/atypical phenotypes. This should be acknowledged by the authors.

The authors have included in their revised manuscript new data that would allow them to comment to some degree on this potential ascertainment bias. By my count (Table 2), for the 5 families with multiple affected children, the 5 probands who brought the family to medical attention had a total of 16 core features (mean 3.2) while their 6 siblings had a total of 11 core features (mean 1.8; t-test
p=0.03). While a t-test would not be the most appropriate test in this setting, my point is that these data suggest that some of the authors’ findings (though certainly not all, as the 5 parents had a total of 2 such features) could be attributed to this ascertainment bias rather than to a true worsening of the phenotype over generations caused by yet-to-be-determined molecular genetic/epigenetic mechanism. [On the other hand, if the total number of features are considered (using the data in Figure 1) then there appears to be less of a difference between probands and siblings.] Evidence in the text that the authors have at least considered such basic partial explanations for their findings is warranted.

3. That the authors appear to be missing basic demographic data (age) for 9 of the 26 parents (and none of the children - see Supplemental Table) suggests that the rigor and comprehensiveness of the phenotyping may not have been equal between the two groups of participants. This possibility and its likely impact on the results should be considered by the authors.

4. That the authors appear to be missing the 'age at diagnosis' variable for 17 of the study participants (see Supplemental Table), as well as 'age' for 9 parents (see above), suggests that there may be missing data with respect to the other variables considered in this study. Can the authors please clarify in the text if there were or were not additional missing data? Are there any data points that might be better considered as 'not (properly) assessed' rather than 'absent'?

Minor Essential Revisions

5. On page 6, the authors state: "Conversely, ocular disorders, were more frequent in the parents than in their affected sons (3.1 vs 23%, P = 0.037)." Why were only sons considered here and in another statement on page 11? Also, the extra comma can be removed.

6. Various terms are used to refer to the primary group of 32 subjects with inherited 22q11.2 deletions: "first group", "last generation", "second generation", "subjects", etc. Consistency may improve clarity of presentation.

7. The legend for Figure 1 should include the same information as Table 2 regarding (i) which subjects were siblings and (ii) which sibling in each family was the true proband.

Discretionary Revisions

8. "Features" (or "features of 22q11.2DS") may be preferred to "disorders" throughout.

9. There remain grammatical mistakes and sentences that are worded in a confusing manner. The manuscript would benefit from additional, careful proofreading.

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 that I have no competing interests.