Reviewer's report

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

Version: 2 Date: 5 September 2013

Reviewer: Gregory Costain

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This manuscript by Cirillo et al. reports on the phenotype in children with inherited 22q11.2 deletions and their parents with the deletion. The main findings highlighted by the authors are: (i) that congenital/developmental conditions associated with 22q11.2 deletion syndrome (22q11.2DS) were more common in individuals ascertained on the basis of clinical features of 22q11.2DS than in their transmitting parents (who were all tested and diagnosed only after the birth of their more severely affected child); and (ii) that later-onset phenotypes were less common in a group of children (mean age 10.4 years) with 22q11.2DS than in their affected (adult) parents.

Strengths of this study include the size of the cohort (n=26 probands, n=6 siblings, and n=26 parents with 22q11.2 deletions), and the use of a standard evaluation protocol leading to the apparent availability of detailed cardiac, endocrine, speech, psychiatric, cognitive, and other phenotype data for every participant. Fathers with 22q11.2DS are relatively uncommon, and the authors have data on n=9. The research topic would likely be of some interest within the 22q11.2DS research community and, as with most research on this representative genomic disorder, the approach and overall findings could be generalizable to other large, rare, recurrent copy number variants of clinical significance. The co-senior author, Dr. MC Digilio, has expertise in 22q11.2DS. The results could be seen as replicating select findings in a (possibly new) cohort.

There are several critical aspects of the manuscript where clarification is needed, however, particularly with respect to the novelty of the findings, interpretation of the results, and statement of study limitations.

Major Compulsory Revisions

1. It is not clear how the results significantly advance our understanding of the variability of the 22q11.2DS phenotype within families. If the goal of these authors was to conduct the first “extensive and conclusive intergenerational and intrafamilial comparison”, more justification is needed for how these results significantly extend those of the previous reports referenced in this manuscript (e.g., McDonald-McGinn et al., 2001, Genet Med).

2. The authors’ proposal that “both genetic and epigenetic mechanisms may be involved in the phenotypic variability” could very well be true, but (i) these data do
not appear to directly support that interpretation (no molecular genetic or epigenetic studies were performed, aside from confirming the 22q11.2 deletions), and (ii) a well-recognized and simpler explanation for their findings is an ascertainment bias. It would seem that each proband was ascertained because he or she had clinical features of 22q11.2DS which brought them to medical attention and led to (in n=25/26 cases, targeted) testing for a 22q11.2 deletion. All of the transmitting parents were only ascertained as a result of having such an affected child. It stands to reason, then, that the transmitting parents would on average have fewer classic features of 22q11.2DS than their offspring (or else they would have been diagnosed earlier in life). Additional clarification of why their finding is notable is therefore needed. The implicit suggestion that they have evidence for genetic anticipation in 22q11.2DS is imprudent.

3. Similarly, as briefly acknowledged by the authors in the Discussion, the finding of a greater number of (generally) later-onset conditions in a group of adults compared to in a group of children seems expected. Additional explanation of why this is an important finding is needed. On a related note, the mean age of the n=26 parents is not reported.

4. The authors should state if there is any known (or suspected) overlap of their study participants with previously published cohorts. In particular, is there any overlap with the n=15 familial cases reported by Digilio et al. in 2003 in Clinical Genetics?

5. Use of the term ‘proband’ to refer to each of two or more siblings in n=5 different families is confusing. If the standard definition of ‘proband’ is applied ("first affected individual in the family to seek medical attention for the genetic condition"), there would typically be n=26 probands (not n=32) for the n=26 families. Considering siblings as separate probands leads to double counting of their affected parent in some instances, for example in Table 2 and Figure 1. The analyses should somehow take into account the fact that some in the ‘proband’ group have the same affect parent [i.e., a unique proband in each family should be identified (my preference), or an alternate approach should be used and justified].

6. Details are needed regarding how all study participants were assessed for psychiatric conditions. In the Methods (first paragraph of ‘Patients’ section), there is a mention of ‘neuropsychiatric evaluation’ but only methods for determining IQ are reported.

7. Limitations of the study should be carefully and extensively considered in a dedicated section of the Discussion.

8. The conclusion in the abstract states “We highlight that the identification of adults with a milder phenotype deserves careful attention because the early recognition of disorders could benefit of an early treatment.” While I agree with the sentiment of this (sole) conclusion, why this is seen as the primary conclusion stemming from their results is not clear.

Minor Essential Revisions

9. Why is IQ data collection described in the absence of presentation of these
data in some form?

10. In the Methods (last paragraph of ‘Patients’ section), the authors state that, “Since in 5 families more than 1 proband was detected, the phenotype was analyzed in a total number of 32 couples in 26 families.” For the numbers to work, this implies that there were n=2 affected in the proband’s sibship in 4 families and n=3 affected in 1 family. If correct, please state this directly in the text.

11. In the fifth paragraph of the Results, please define ‘behavioral anomalies’/’behavioral abnormalities’ (and use consistent term) and specify the two ‘psychotic disorders’ (which were apparently neither mood disorders nor schizophrenia?). Unless severe, I question whether the ‘phobia’ should be included.

12. The second-to-last paragraph of the paper is one long sentence, the logic of which is not comprehensible to me. Stated in full, the sentence reads: “Even though our observation may be due to a bias related to the low rate of reproductive fitness of adults with a more severe phenotype, as matter of fact, the child parent couples with 22q11.2DS, in which almost invariably the clinical expression worsens in the last generation, represent a valuable clinical model to approach pathogenetic studies aimed at explaining the increased complexity of the phenotype of chromosomal syndromes over generations.” I agree that decreased reproductive fitness (secondary to decreased survival, as with serious congenital heart disease in past generations, and/or secondary to decreased mating success, as with schizophrenia and intellectual disability) is a compelling explanation for some of their findings. What is the meaning of the second half of this sentence, and how does it relate to the first half?

13. In the concluding paragraph, I cannot understand the phrase: “…we found a not casual intrafamilial clinical phenotypic variability, not related to the variable penetrance of the syndrome.” Please rewrite.

14. Table 1 is confusing in the absence of footnotes explaining what each group of conditions entailed. For example, are the authors reporting in the “Infections” row that only 3/26 adult parents had ever in their life had an infection? Are ‘facial anomalies’ restricted to those that are characteristic of 22q11.2DS? Were some children too young to be adequately assessed for certain conditions (e.g., dental anomalies, learning difficulties)? Etc.

15. The effect of proband and parental sex on the findings should be explored and discussed.

16. There are typos (e.g., “troncus arteriosus”), grammatical mistakes (“could benefit of an early treatment”), and several phrases I cannot understand (e.g., those noted at other points in this review). Additional proofreading is needed.

Discretionary Revisions

17. If IQ data from the WISC or WAIS were available for every participant, then reporting this (including mean Full Scale IQ, Verbal IQ, and Performance IQ) for probands and parents would be definite added value. And similarly for laboratory values.
18. How many health issues were newly diagnosed in the adult parents as a result of your targeted investigations following diagnosis of a 22q11.2 deletion?

19. Much of the first paragraph of the Results is repetitive of what is in (or should be in) Table 1, and could be cut.

20. In the second-to-last paragraph of the Results, the finding that n=2 of 6 partners with data available had cognitive/behavioral problems (incl. one with IQ=68) is striking to me and consistent with other data I have seen. This raises important considerations with respect to assortative mating. Additional attempts to phenotype ‘unaffected’ partners of transmitting parents could potentially yield significant dividends.

21. Reporting the length of the 22q11.2 deletions would be of interest given the conflicting reports about the smaller nested (~1.5Mb) deletions being more common in familial cases, although I appreciate that this information is likely not available.

22. A major strength of a related study (McDonald-McGinn et al., 2001, Genet Med) was the publication of the pedigrees for all participating families (including all pregnancies/offspring of transmitting parents). This manuscript may greatly benefit from a similar approach, which would also increase transparency with respect to the data and transmission patterns. Such a figure, if it included major phenotypes, would render Figure 1 and Table 2 unnecessary.

23. Of interest to me is whether the authors did or did not find evidence of preferential transmission of 22q11.2 deletions from fathers to their sons (cf. their daughters) (Costain et al., 2011, J Med Genet).

24. The importance of Figure 1 is questioned. It distracts from, rather than clarifies, the presentation of the data. If the above recommendation (#22) is not accepted, then this data may be better presented in table format.

25. A basic table summarizing demographic features for the probands and parents may be helpful.

26. In Table 1, the denominators in each column are the same in every row (/32 and /26). These could therefore be stated only once, at the top of the column, to limit clutter within the table. Also, significant p values could be bolded.

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

**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.