Author's response to reviews

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

Authors:

Emilia Cirillo (emiliacirillo@alice.it)
Giuliana Giardino (giu.giardino@hotmail.it)
Vera Gallo (veragallo86@hotmail.com)
Pamela Puliafito (pamelapuliafito@virgilio.it)
Chiara Azzari (c.azzari@meyer.it)
Rosa Bacchetta (bacchetta.rosa@hsr.it)
Fabio Cardinale (fabiocardinale@libero.it)
Maria Pia Cicalese (mariapia.cicalese@tiscali.it)
Rita Consolini (rita.consolini@med.unipi.it)
Silvana Martino (silvana.martino@unito.it)
Baldassarre Martire (baldo.martire@gmail.com)
Cristina Molinatto (molinatto@hotmail.com)
Alessandro Plebani (plebani@med.unibs.it)
Gioacchino Scarano (gioacchino.scarano@ao-rummo.it)
Annarosa Soresina (soresina@med.unibs.it)
Caterina Cancrini (cancrini@med.uniroma2.it)
Paolo Rossi (paolo.rossi@opbg.net)
Maria Cristina Digilio (mcristina.digilio@opbg.net)
Claudio Pignata (pignata@unina.it)

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Author's response to reviews: see over
Point by point reply

Dear Editor, thank you for the comments to our manuscript, that overall we found very helpful to improve it. We did our best to answer to all the questions raised by the Reviewers.

Reviewer # 1

Major revisions

1. As observed by the Reviewer, in the last years several authors have reported on the intrafamilial clinical variability of the phenotype in 22q11.2DS. In particular, the study (McDonald-McGinn et al. 2001; Genet Med) has been quoted by the Reviewer. Aim of our study was to perform a detailed comparison of the clinical phenotype among subjects with 22q11.2DS and their affected transmitting parents. To date, a systematic and conclusive search on this topic is still missing. In particular, in our cohort of 22q11.2DS parent-child couples, the comparison has been performed for each single variable, including both major and minor phenotypic features for a total of 17 clinical hallmarks for each couple. Finally, to our knowledge, this is the largest cohort study reporting on familial cases of 22q11.2DS.

2. The Authors did not report on molecular, genetic or epigenetic study since the aim of this study was a clinical comparison of the phenotype between two generations of subjects with 22q11.2DS. The Authors only hypothesize, basing on previous reports, potential molecular mechanisms, apart from the ascertained bias, which could explain the variability of the phenotype and its worsening in the second generation. This is only a preliminary clinical study, which lays the foundation for future studies specifically aiming at the identification of the molecular mechanism responsible for the worsening of the phenotype over generations in chromosomal syndromes. Certainly, as correctly noted by the Reviewer a possible explanation for the worsening of the phenotype in the second
generation, could be an ascertained bias. However, even though this possibility is standing, this doesn’t explain the reason by which a worsening of the phenotype does occur in most subjects with this syndrome. Moreover, irrespectively of the possible ascertained bias, the clear definition of the intrafamilial variability, to our opinion, anyway deserves full consideration, since it represents an optimal background for further investigations, as below mentioned. The definition of the clinical intrafamilial variability is mandatory for the scientific community for planning additional genetic/epigenetic studies, which would not be justified per se by the general observation of clinical variability. The phenomenon of the “genetic anticipation” has traditionally been associated with triplets expansion or telomere shortening as in several autosomal dominant diseases and, obviously, never in chromosomal syndromes. Even though the intimate pathogenic mechanisms are far from being well known, genetic anticipation has recently been reported in other conditions, such as autoimmune disorders and familial hematologic malignancies (Tegg EM et al, Blood 2011). A similar mechanism for chromosomal disorders has not yet been identified, probably because of the severe impairment in reproductive fitness in other chromosomal disorders. Our data support the hypothesis of an anticipation generation by generation. Although we appreciate the observation of the Reviewer, we don’t feel that our preliminary observation regarding genetic anticipation is imprudent, since it has never been reported before. Of course, it must be confirmed on larger cohorts of patients and, to our opinion, it should be reported in the literature without further theoretic speculations. As noted by the Reviewer, we found a greater number of later onset conditions among subjects of first generation. However, even though psychiatric disorders were more represented in the first generation, as expected, the difference was not significant, thus suggesting the need of an accurate psychiatric management since childhood. The sentence “As expected, psychiatric disorders were more represented in the first generation, however, the
difference was not significant thus suggesting the need of an accurate psychiatric management since childhood” has been added in the section “Discussion and conclusions” at page 10, line 16. As for ocular defect, which was the only condition significantly more represented in the older generation, we found both congenital and progressive conditions as strabismus, abnormal retinal vessels, cataract etc. However, due to the low number of findings it seems difficult to reach a conclusive explanation. At page 4 line 17, the sentences reporting the mean age of the 26 parents has been added (Mean age +/- SD was 39.6 +/- 7.9 years (range 21-58 years).

3. Only in 1 case, a few features were previously objective of scientific analysis. This case has been noted in the supplementary table.

4. We share the Reviewer criticism since the term proband may be confusing in families in which two or more subjects with 22q11.2 have been identified in the second generation. So we replaced it in the text with the term “affected subject”. The analysis has been performed considering siblings as separate affected subjects and, thus, double counting the affected parents. Table 2 and Figure 1 are in accordance with this finding. We prefer not exclude siblings since aim of the study was to focus on subjects with inherited 22q11.2 deletion.

5. We agree with the Reviewer and the sentence “Neuropsychiatric evaluation has been performed by skilled clinicians using the Schedule For Affective Disorders and Schizophrenia for School-aged Children, Present and Lifetime (K-SADS-PL). Affected subjects older than 18 years and their parents were interviewed with the Structured Clinical Interview for Axis I DSM IV Disorders (SCID). Data were collected by medical records” has now been added on Page 5, line 13-17.

6. A new section has been added in the discussion reporting the limitations of the study “This is the largest cohort of subjects affected with familial 22q11.2DS. A detailed characterization of the clinical features of such subjects has been performed. Certainly,
an ascertained bias related to the milder phenotype of adults who get married could partially explain the observation that the phenotype worsens in the second generation. Another possible explanation could be the co-inheritance of a further genetic defect from the non-affected parent. This seems unlikely since this co-inheritance should have occurred in all cases exhibiting the worsening of the phenotype. As for the CHD, it should be noted that none of the non-affected parents had a CHD, thus excluding, at least for this feature, this hypothesis.”

7. We agree with the Reviewer and the conclusion in the abstract has been modified. The sentence “Subjects with inherited deletion showed a more complex phenotype in comparison to their affected parents. In addition, the identification of adults with a milder phenotype deserves careful attention because the early recognition of disorders could benefit from an early treatment and genetic counseling.”

Minor essential revision

1. Unfortunately, detailed data on this are not available because in our record we only used for IQ the cut off upper/lower 70. Further studies are necessary to carry out a complete comparison of the intellectual disabilities and neuropsychiatric profile of parent-child couples affected with 22q11.2DS.

2. As noted by the Reviewer, in 5 families at least 2 siblings with 22q11.2 DS were present. At page 4, line 17 the sentence “…In 5 families, 2 or more affected siblings were detected… has been replaced by …” in 4 families, 2 affected siblings were diagnosed and in one further family, 3 subjects were identified.” In addition, in order to clarify the clinical data we have added this information in the Table 2.

3. In the fifth paragraph of the Results, we defined behavioral abnormalities as any activity judged to be outside the normal behavioural pattern for a subject of that particular class and age, associated to social isolation and rejection, impairment in social and daily living skills and low self-esteem according to Doron Gothelf et al. Dev Disabil Res Rev 2008.
Now on Page 8, line 4 the sentence “represented by a trend to social isolation and rejection, impairment in social and daily living skills and low self-esteem” has been added. The two psychotic disorders reported in the text meet the DSM-IV criteria for psychotic disorder “not otherwise specified” (NOS). According to this, the sentence on Page 8, line 3 has been modified accordingly. Phobia has been included, since about 23–61% of children and adolescent with 22q11.2 DS is affected with specific and social phobias, thus representing a frequent condition among these subjects. Furthermore, it requires medical intervention.

4. The long sentence in the last paragraph of the paper has been changed in “Even though our observation may be partially explained by a bias related to the low rate of reproductive fitness of adults with a more severe phenotype, as matter of fact, in our child-parent couples with 22q11.2DS, a worsening of the clinical phenotype was clearly observed in the last generation. If such observation will be confirmed in larger cohorts of patients, this model might be useful to approach pathogenetic studies aimed at explaining the increased complexity of the phenotype of chromosomal syndromes over generations”.

5. The sentence on Page 12, lines 16, “…we found a not casual intrafamilial clinical phenotypic variability, not related to the variable penetrance of the syndrome… now reads …”We found an intrafamilial phenotypic variability, characterized by worsening of the clinical manifestations in the second generation, due to a still unexplained molecular mechanism.”

6. In order to improve the quality of the table, footnotes have been added. “Only severe infections (sepsis, pneumonia), requiring hospitalization, or history of recurrent infections were considered”.

Of course, since we compared children with adults, the possibility that a few clinical features appear later is possible. If this would appear, the phenotype of subjects of the
second generation would be, therefore, ever more severe, thus further confirming our observation.

7. Recently, the female sex has emerged as a significant positive predictor of fitness. The sentence “We found a preferential maternal transmission, in keeping with the recent observation that female sex represents a significant positive predictor of fitness:” has been added on page 4 line 21.

8. Typos and grammatical mistakes have been corrected.

Discretionary revisions

1. Unfortunately, detailed data on this are not available because. In our records we only used for IQ the cut off upper/lower 70 (see answer 1, Minor revisions). As for the laboratory values, in the Case Report Form they have been marked as normal abnormal for age.

2. After the identification of the deletion in the parents, approximately 20 new abnormal conditions have been diagnosed. In particular, echocardiography revealed the two minor congenital hearth defect (PDA and DAA), a mitralic regurgitation, not reported in the present study since it cannot be considered CHD. The only adult with calcium phosphorus abnormalities was diagnosed after the evaluation of serum levels of calcium. Other clinical conditions diagnosed during the study include psychiatric disorders (mood disorder, anxiety disorders), skeletal abnormalities (cyphosis, cyphoscoliosis), ocular abnormalities, autoimmune thyroiditis. The sentence “About 15% of the clinical disorders were diagnosed during the study.” has been added in the text on page 7 line 1.

3. We agree with the Reviewer and the first paragraph of the Results has been cut.

4. As observed by the Reviewer, a possible explanation for the phenotype worsening in the second generation could be the co-inheritance of a genetic defect from the non affected parents or an environmental effect. Even though not conclusive data could be provided, due to the small number of unaffected partners of the transmitting parent analysed, none
of them have a congenital hearth defect, despite 2 affected sons, thus excluding this hypothesis for the cardiac phenotype. As for mental retardation the only subject with an IQ of 68 was a mother with very poor school education, with no learning difficulties and no psychosocial limitations. Thus, no clear evidence in supporting the role of the unaffected partner in determining the clinical phenotype in subjects of the second generation is available. These data have been discussed in the section “Advantages and limitations of the study”.

5. No further information is available on the length of the deletion. However, even though a smaller nested deletion has been reported as more common in familial cases, nowadays data are not conclusive. We are planning a further study, in collaboration with geneticists of several Centres. Even though we have preliminary data, we prefer not to include this information in the present study.

6. Due to the usual limitation in space, the Authors would prefer not to publish 26 pedigrees since all the fundamental information is anyway available in the data shown.

7. We didn’t find preferential transmission of 22q11.2DS from fathers to sons.

8. The Authors would prefer to keep the Figure 1, since it highlights the higher number of disorders observed in the second generation.

9. A supplementary table summarizing demographic features of each couple has been added.

10. In Table 1 significant p values are now in bold and the denominators have been eliminated as required.

Reviewer #2

1. As appropriately suggested by the Reviewer a mosaic status in the carrier parent could be the explanation of the variability and of the more benign phenotype, even though this is really a very rare condition. This hypothesis has been reported by the authors in the section “Background” and a new sentence has been added in the Discussion.
2. We agree with the Reviewer on the opportunity to perform array-CGH in each family in order to evaluate additional CNV. However, aim of this study was limited to the clinical comparison of the phenotype among subjects with 22q11.2DS and their affected transmitting parents. Further studies are necessary for the identification of the possible mechanism of the phenotype worsening in family with 22q11.2DS.

3. As suggested by the Reviewer the term mental retardation has been replaced throughout the paper with the term Intellectual disability.