Author's response to reviews

Title: A case report of Chinese brothers with inherited MECP2-containing duplication: autism and intellectual disability, but not seizures or respiratory infections

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Version: 4 Date: 6 August 2012

Author's response to reviews: see over
August 6, 2012

Dear Prof. Neri,

Enclosed please find our revised manuscript “A case report of Chinese brothers with inherited MECP2-containing duplication: autism and intellectual disability, but not seizures or respiratory infections (MS: 1977004305751863)”. We were encouraged by the positive comments from both the reviewers and from yourself, and very much appreciate the opportunity to revise our manuscript. We believe that we have fully addressed all concerns raised by the reviewers in this revised version of the manuscript. Specifically, we have provided additional patient information as requested by the reviewer and also added more details regarding the genes identified in CNVs of the patients. We have also fully revised the manuscript based on suggestions from the reviewers.

The point-by-point reply to the reviewer’s comments is provided below (reviewer’s comments in italic).

We thank the reviewers for their thoughtful and constructive comments, which we hope to have addressed sufficiently. We believe that the manuscript has been substantially improved by these revisions and hope that the revised manuscript, in its current form, can be considered suitable for publication.

Sincerely yours,

Xiang Yu, Ph.D

Point-by-point reply to reviewers

Reviewer #1:

Overall Comment

The paper by Xu et al. represents an interesting report of the first case of MECP2 duplication in the Chinese Han population. The phenotype caused by CNVs of variable size is always subjected to some degree of variability and new case reports provide more information to better define such phenotype. The case described in the manuscript is also peculiar because it involves two brothers, the deletion is inherited by the mother, who shows some borderline behavioral features, and the family pedigree is suggestive of further potential carriers, although unfortunately deceased. Moreover, the clinical presentation of the two patients lacks some of the typical
features of the MECP2 duplication syndrome, such as recurrent infections and epilepsy. For all the aforementioned reasons, I believe the paper constitutes an important contribution to the medical genetic field, but there are two major areas of the work that need some improvement: the authors should provide more detailed clinical information about the family members (i.e. for the patients percentiles of birth parameters, body measurements at the time of clinical evaluation, medical records; for the deceased relatives reason of death, medical history) and should further discuss the implications of the genes involved in the chromosomal rearrangements identified in the family members. In fact, the presence of multiple CNVs, even if inherited, can be responsible for the atypical clinical presentation and for the minor phenotypical differences between the two affected brothers.

Response: We thank the reviewer for his positive comments and detailed review of the manuscript. The revisions, as suggested by the reviewer, are detailed below.

• Major Compulsory Revisions

Abstract
1. Page 2: the authors should mention the other CNVs detected in the patients, even if they are less likely to be pathogenic than the duplication involving MECP2.

Response: All CNVs are now listed in the abstract, as suggested by the reviewer.

Background
2. Page 3, line 3: “behavior phenotypes including” should be eliminated because the three main features of autism spectrum disorders are not just related to behavior.

Response: Change made as suggested by the reviewer.

3. Page 3, line 4: “reciprocal” should be eliminated because it is redundant.

Response: Change made as suggested by the reviewer.

4. Page 3, lines 8-11: there is no reference #6 in the text and in the References List #4 and #6 are referred to the same paper. The authors should correct the list and update the numbers in the text.

Response: We apologize for the mistake, which we have corrected. We have also checked that there are no other duplications in the reference list.

5. Page 3, line 8: “led” should be “leads”.

Response: Change made as suggested by the reviewer.

6. Page 3, line 9: “progress” should be “progressive”.

Response: Change made as suggested by the reviewer.
Response: Change made as suggested by the reviewer.

7. Page 3, lines 9-11 and lines 19-21: the sentences “neurodevelopmental disorder….1:10,000 girls” and “Similarly, .... Autistic features” are redundant, the second sentence should be eliminated

Response: Change made as suggested by the reviewer.

8. Page 4, line 3: why the authors cite only reference #22 if they intend to refer to all the previously reported cases with MECP2 duplication? References #9-27 should all be cited in this case, as well as in Conclusions section (page 10, line 3).

Response: All references are now cited at the above-mentioned places, as suggested by the reviewer.

Case Presentation
9. Page 4-6, Clinical Summary: the authors should clarify if they have access to any medical record regarding the members of the family and should provide a more detailed report. Important missing information about the patients include birth parameters (only patient P01A’s birth weight is reported) and percentiles, body measurements at the time of the evaluation and percentiles, vaccinations, hospitalizations. Important missing information about other family members include parents’ medical history (developmental milestones, school, hospitalizations), reason of death for the deceased relatives and their medical history.

Response: We thank the reviewer for this comment and have tried our best to address it. Additional information regarding hospitalizations and general health of P01A and P01B has been added. We have also added information regarding the deceased relatives and the pregnancy history of P01D. Unfortunately we were not able to obtain all the requested information due to limitations in our ability to access the patients’ medical records.

Family P01 is from Xiapu County of Ningde City in Fujian Province, which is in a rural region of China, over 5 hours by train from Shanghai. The family first visited the Department of Child Healthcare of the Children’s Hospital of Fudan University on April 5th, 2007. P01A was diagnosed with autism using DSM-IV during the visit. On April 13th, 2011, all 4 members of the family visited the hospital again to give blood samples for genetic studies. When we identified the inherited MECP2 duplication in the family, there were no longer willing to return to Shanghai for further examinations, including ADOS evaluations. Thus, we went to Ningde City in Fujian Province to carry out the ADOS, Bayley, Weschler and SCL90 evaluations. Since we were working in borrowed space of the local hospital, we were not able to take the patients’ body measurements at the time of examination.
Regarding detailed medical history of the family, including deceased members, unfortunately, in rural China, the patients, rather than the hospitals, are responsible for keeping all medical records. Thus, it is not possible to obtain the medical records of patients from the local hospital. Furthermore, when individuals die at home, usually no postmortem is carried out to determine the cause of death. Thus, the only information we can obtain is from patient family members. We apologies for not being able to provide all additional information requested. We have really tried our best to obtain as much information as possible.

10. Page 4, lines 22-23: the sentence “Both the boy…the mother (P01D)” should be eliminated because it is just repeating what assessed at the end of the previous paragraph and is not pertinent with the context of the clinical summary.

Response: Change made as suggested by the reviewer.

11. Page 7, lines 2-12: the authors should provide more details about the genes involved in the reported CNVs: function, pathway, tissue expression pattern, possible interaction with MECP2 or other genes associated with autism and/or intellectual disability.

Response: We thank the reviewer for this comment. Since we already provided more information on IRAKI and SLC6A8 in the Discussion section, in order to not repeat ourselves, we provided additional information on the other genes, including Gabra3, GABRQ, L1CAM, PDZD4, and PLXNB3 also in the Discussion section (last paragraph of P9 and beginning of P10). We also added a sentence in the Case Presentation section to direct the readers to the Discussion section for information on these genes.

12. Page 7, lines 12-14: the statements “the other CNVs... can be ruled out as disease causing” is too strong, considering that the authors based their assessment only on the literature. Also, the patients, especially P01B, carry multiple CNVs, that may have a cumulative effect not observable in the parents carrying just one (father) or two (mother) of such rearrangements. The authors should thoroughly discuss the implications of multiple CNVs in the patients and the potential role in determining the phenotype differences between the two brothers.

Response: We thank the reviewer for this comment. Based on the reviewer’s suggestions, we have changed the corresponding section to the following: “Since, based on the existing literature, the other CNVs found in Patients P01A and P01B are unlikely to be the main cause of the patient phenotypes, our whole genome CNV results provided further evidence that the main genetic abnormality in patients P01A and P01B is their MECP2-containing duplication, inherited from the mother. The other CNVs could also, in principle, contribute to the patient phenotype in the background of the MECP2-containing duplication.”
Discussion
13. Page 8, line 14: the word “patient” should be eliminated because redundant and the author should provide at the end of the sentence the size range for the deletions reported in the literature and the corresponding references.

Response: Change made as suggested by the reviewer. The size range for deletions reported in the literature is added together with the corresponding references.

14. Page 9, lines 17-27: the whole paragraph just repeats the results and presents no discussion. Since the clinical features are already repeated at the beginning of the Discussion section, and in the following Conclusions, the authors should either discuss the findings or eliminate the paragraph (except for lines 15-17).

Response: The paragraph was eliminated as suggested by the reviewer.

Supplemental Table
The genes must be in italics.

Response: Change made as suggested by the reviewer.

• Minor Essential Revisions

Abstract
1. Page 2, lines 2-3: “developmental neurological” should be replaced with “neurodevelopmental”, as well as in the Background section (page 3, line 2).

Response: Change made as suggested by the reviewer.

2. Page 2, line 4: “this disorder” should be replaced with “these disorders”, since the spectrum includes more than one condition.

Response: Change made as suggested by the reviewer.

3. Page 2, line 5: “loss-of-function” should be replaced with either “loss of function” or “loss-of-function mutations”, as well as in the Background section (page 3, line 8 and line 19).

Response: loss-of-function was replaced by loss of function throughout the text.

Background
4. Page 4, line 1: “duplication in MECP2” should be replaced with “a duplication encompassing the MECP2 gene”.

Response: Change made as suggested by the reviewer.
Case Presentation
5. Page 4, lines 9-10: can the authors provide further information about this cohort of 53 ASD patients? For example, age range, number of males and females, tools used to make the diagnosis. In Table 1 and later in the text the authors mention the ADOS questionnaire: they should clarify if that was used in all these 53 patients.

Response: All patients in the cohort were male, the average age at first diagnosis was 4.15 ± 0.27 years, and diagnosis was made using DSM-IV. This information has been added to the Case Presentation section.

6. Page 4, line 10: after “boy” the authors should add “(P01A)”, since later in the section they start address the other members of the family with similar codes. In order to be consistent, the author should also replace “Patient 1A” and “Patient 1B” with “Patient P01A” and “Patient P01B”, respectively on page 5 (lines 3, 23, and 24) and 7 (lines 2, 6, and 11).

Response: Changes made as suggested by the reviewer.

7. Page 6, lines 16-17: the sentence “mother more apparently so” is not very clear, the authors should rephrase it and explain if the mother is closer to a pathologic score than the father.

Response: The sentence has been changed to “…with the mother scoring further away from the cutoff score than the father”.

8. Page 6, line 28 and page 7, line 1: “between the brothers and with the mother” should be replaced with “in the brothers and the mother”.

Response: Change made as suggested by the reviewer.

9. Page 7, line 28: the authors use the words “very approximate”, implying the analysis is not very precise. How actually reliable is the analysis?

Response: The words “very approximate” have been removed. It is an unbiased analysis based on the published literature. We present our results and let the readers decide on the quality of our analyses and interpretations.

Discussion
10. Page 8, lines 15-16: the sentence “making it likely…duplicated” is not very clear and should be re-phrased. A possible alternative is “suggesting a potential duplication of a functional copy of the GABRA3 gene”.

Response: The sentence has been rephrased as suggested by the reviewer.
11. Page 1, line 3: the authors should provide the web address for the Primer3 software.

Response: The requested information was added.

• Discretionary Revisions

Background

1. Page 3, line 16: “whose that have found” is not very clear, it should be re-phrased.

Response: This sentence was changed to: “when the examinations were carried out, autistic phenotypes were prominent among patients with MECP2 duplication”.

2. Page 3, line 22: “deletion or duplication can both result in autistic phenotypes in patients” could be replaced with “both deletion and duplication can generate autistic phenotypes”.

Response: Change made as suggested by reviewer.

Case Presentation

3. Page 7, lines 6-7: the sentence “that contained no known genes” should be between comas, like the following sentence “containing GDLD4”, in order to avoid confusion.

Response: Change made as suggested by reviewer.

Discussion

4. Page 8, line 2: the authors should add “Han” after “Chinese” to better specify the ethnicity of the reported family.

Response: Change made as suggested by reviewer.

5. Page 8, line 4: the authors should add “in size” after “2.22 Mb”

Response: Change made as suggested by reviewer.

Reviewer #2:

Overall Comment

I am not sure that there is truly a clinical difference between these 2 brothers and other patients previously reported with dup MECP2. This difference might be by chance; there is no clinical sign that HAS to be present in any genetic disorder and any difference can be explained by variable expressivity. Also the respiratory infections may occur later in life.... Also, if these two brothers have dysmorphic
features of some relevance, a picture of them should be published. I agree that this might be the first report of MECP2 duplication in Chinese patients but I do not think this deserves too much emphasis: I mean that the manuscript should be dramatically shortened.

Response: We thank the reviewer for her constructive comments. We agree with the reviewer that differences observed between different patients are most likely correlations, rather than causal relationships, given the complexity of genetic and environmental influences on human development. We state explicitly in the Abstract: “Further cases are required to determine if the above described clinical differences are due to individual variations or related to genetic background of the patients.” We do not attempt to make our cases special, but rather just to start describing patients from another part of the world and a different ethnicity. We also agree with the reviewer that respiratory infections may occur later in life, although, P01B was already 18 years old at the time of examination. Many of the MECP2 patients reported to have respiratory infections were much younger than our patients. For example, in Ramocki et al., 2009, where all patients described were younger than P01B, 7 out of 9 boys were reported to have repeated respiratory infections. Again, we only intend to report our observations, to be available to others studying or interested in this disorder.

Regarding pictures of the patients, unfortunately, we were not able to obtain permission from the parents to publish the pictures. It is very, very difficult to obtain such permissions from patients in China for cultural reasons.

With regard to the length of the manuscript, the Clinical Summary in the Case Presentation is slightly shortened to reduce repetitions. The Discussion section of the manuscript is also significantly shortened (especially the section regarding clinical features of P01A and P01B) as suggested by the reviewer.

Once again, we thank both reviewers for their detailed review of our manuscripts and constructive comments. We believe that these comments have helped us to substantially improve this manuscript.