Author's response to reviews

Title: A genomic copy number variant analysis implicates the MBD5 and HNRNPU genes in Chinese children with infantile spasms and expands the clinical spectrum of 2q23.1 deletion

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Author's response to reviews: see over
Dear Editor

Thanks for the overall positive and careful comments from both reviewers. We have incorporated all comments and suggestion in the revised manuscript. The major changes are highlighted in yellow. The point to point responses are pasted below. We hope that reviewers and you will find the revised manuscript satisfactory.

Sincerely,

Yi Wang, MD, PhD.
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Point to point response:

Reviewer: Weimin Bi

Reviewer’s comment:
IS appears conserved among different ethnic backgrounds needs to be revised. For the four pathogenic CNVs, three of them actually haven’t been reported in individuals with primary diagnosis of IS as stated in the manuscript. However, it appears that the genes involved in IS and/or seizure, LIS1, HNRNPU and MBD5, are also the causative genes in Chinese IS patients. I suggest to say “Our study also supports that the molecular mechanism of infantile spasm appears conserved among different ethnic backgrounds”.

Response: We agree the reviewer’s suggestion and change is made in revised manuscript. See page 6, line 6-7

2. The duplication in Xp22.31 in S134 contains other genes in addition to the two genes, STS and VCX3A, mentioned in the manuscript. Duplication of Xp22.31 has been reported in multiple papers (Am J Med Genet A. 2012 Feb;158A(2):461-4; Eur J Med Genet. 2010 Mar-Apr;53(2):93-9; Hum Mol Genet. 2011 May 15;20(10):1975-88). There is a debate on whether this duplication is a cause of intellectual disability and/or developmental delay or a benign variant. In addition, the reference of Palka-Bayard-de-Volo et al 2012, is not an appropriate

Response: We agree the reviewer’s suggestion. We have revised the text and included these new references in revised manuscript.

3. The case S67 has a duplication at 1q21.1. This duplication is flanked by large
segmental duplications and it is a recurrent duplication that has been previously reported. The size of this duplication was smaller in Brunetti et al [23], which is due to different probe coverage in this region for the arrays used in these studies.

**Response:** We agree the reviewer’s suggestion and change is made in revised manuscript. See page 13, lines 19-20

4. The phenotype of absent hallux in patient S162 was not previously reported in 2q23.1 deletion patients. However, MBD5 mutations/ deletions have been shown to cause craniofacial abnormalities and hand/foot abnormalities in almost all the patients. It is necessary to add this information in the discussion.

**Response:** We agree the reviewer’s suggestion and change is made in revised manuscript. See page 18, lines 9-11

5. In the section of Methods, the sentence of “A DLR score above 0.20 was set as the cutoff criteria for false CNVs” is unclear. Do you mean you use a DLR score above 0.20 to indicate poor quality of array data.

**Response:** Yes, the DLR score above 0.20 indicates the poor quality of array data and increased possibility of false positive for CNV calling. Page 6, lines 18-20

5a. In Figure 1C and Figure 2C, the brain MRI images seem to be switched between S100 who has a 17p13.3 deletion and lissencephaly and S37 who has 1q44 deletion and delayed myelination.

**Response** We have double checked the radiology and the images are correct.

5b. In “Results”, section of “Genome-wide copy number variation analysis”, the CNVs were placed into 3 classes, not 4 classes.

**Response:** Thanks for the correction and we have the change in revised manuscript. Page 9, line 13.

5c. In “Results”, the proband S134 has a deletion in17p12 including PMP22. But the father carries the same duplication. Should the father carry a deletion instead of duplication as stated in the manuscript?

**Response:** Thanks for the correction and we have the change in revised manuscript. Page 15, line 15 from the bottom

5d. In Figure 1 legend, the distal breakpoint is within intron 1 of LIS1, not intron 2.

**Response:** Thanks for the correction and we have the change in revised manuscript.

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- **Minor Essential Revisions**
1. Both 17p13.3 and 17p13 are used for the deletion in individual S100. 17p13.3 needs to be consistently used throughout the manuscript.
2. Both IS and ISS are used for Infantile spasms. IS should be used all the time.
3. In abstract, section of “results”, line 8 from bottom, “for” should be used instead of “form”.
4. In “Methods”, what is the temperature used for primer extension?
5. The section of “CNVs of unknown variants of clinical significance” should be “CNVs of unknown clinical significance”. In this section, “YAP1, a protein important” should be “YAP1, a protein important”.
6. In “Discussion”, the reference “Vijav et al” should be “Tiwari et al.” and this paper actually identified CNVs in 13 trio, not 11 IS cases.
7. Figure 2 legend, the gene name of HNRNPU was misspelled.
8. In “Author’s information”, “Children’s Fudan University” should be “Children’s Hospital of Fudan University”.

Response: Thanks for reviewer’s careful reading and corrections and we have the change in revised manuscript

- Discretionary Revisions

2. The Xq22.1 duplication in the male patient S34 is inherited from the mother. Checking for this duplication in maternal male relatives may help determine the clinical significance of this duplication.

Response: This is a good suggestion but there is no maternal male relative available for the study.

3. Suppl. Table S1, there is one column on “Dev at Last Review”. It is necessary to indicate the patients’ ages for the last reviews.

Response: We appreciate reviewer’s suggestion. Because these patients are very young, we feel that adding the age to this will not have much the impact.

4. In section of “Materials and Methods”, DNA from patients and controls was digested. It is unclear which controls were used for array CGH. It is better to provide website or citation on how to access the published database for the CNVs from ~1000 Han Chinese individuals.

Response: The control DNA is the reference DNA provided by Agilent. We have changed the “control” to “reference”. The database for 1000 Han Chinese individual is an internal database that has not been published and not yet opened to public access.

5. Figures 1D and 2D is better to placed right behind Figures 1A and 2A, respectively, to be consistent with the order in Figure 3. The texts for the colored bars need to be consistent between Figure 1D and figure 2D.

Response: We have made some changes.
Reviewer: Joseph Glessner

1. 14 CNVs in 47 IS cases is very low in the era of denser arrays CNVs should be detected in all samples.

Response: Thanks for the comments. We have detected a total of 364 CNVs in 47 IS. We have included this information in revised manuscript. The 14 CNVs were determined to be clinical relevant. Page 5, lines 4-6

4. Absence in DGV is also important: “The medical annotation was performed by reviewing the clinical reports related to each of the CNVs in PubMed, the candidate gene(s) in CNVs relevant to the OMIM entries, and the function of these genes in the evidence provided by other model organisms or experimental systems. Through these analyses, we determined the pathogenicity of the CNVs in IS based on a comprehensive assessment of the existing clinical data, the size of the CNV, the gene content of the CNV, and the inheritance of the CNV.”

Response: We have added this information in the revised manuscript. Page 10, Line 5

5. Pathogenic CNVs section descriptions are very long.

Response: We have shortened this section.

8. “conserved molecular mechanism leading to pathogenic CNVs across different ethnic backgrounds” is not clear as the molecular mechanism of most CNVs is non-allelic homologous recombination for both pathogenic and neutral CNVs.

Response: We agree the comment and have revised the statement. Page 6, lines 6-7

10. The tables are good. Although Table 2 is unclear why 3 papers are being represented.

Response: These three papers are chosen because it included more than 95% of cases with 2q23.1 deletion and MBD5 in literatures. Others are case reports.

11. “distinct and more severe neurological presentations than other cases with similar sized deletions [20, 41, 42](Table 2).” May suggest additional variants play a role in this more complex phenotype.

Response: We agree reviewer’s comment and have made the change in revised manuscript accordingly. Page 18, lines 6-7

Minor Essential Revisions

12. Abstract “We report herein the first genome-wide CNV analysis,” should be
We report herein the first genome-wide CNV analysis in children with ISS,
13. “In particularly” should be “In particular”
14. “IS have been shown” should be “IS has been shown”
15. “20.2+9.7” should be “20.2+-9.7”
16. “However, no significant sequence variants were identified in the all coding
exons of MBD5 and ORC4 genes.” Should be “in all”
17. “There are numerous reports suggesting that NRG3 contributes to the
susceptibility of schizophrenia and other neuropsychiatric disorders [29, 36, 37].
In our case, because the duplication is inherited from the healthy parent, the role
of this duplication in proband related to IS could not be determined with
confidence.” should be “could not be”
18. “In the case S34, a 396 kb duplication in X chromosome containing
NGFRAP1(NADE) gene in boy was inherited from the healthy mother. The
similar duplication has not been reported before associated with disease in
humans. The function of NGFRAP1 related to TSC1 suggested that the
NGFRAP1 may be risk factor for the IS.” Should be “been reported before
associated” and “may be a risk factor for IS.”
19. “Therefore, it is less likely that PMP22 related CNV found in proband is a risk
factor for IS and additional investigation is warranted to search for other possible
cause in this case.” Should be “Therefore, it is less likely that the PMP22 related
CNV found in proband is a risk factor for IS and additional investigation is
warranted to search for another possible cause in this case.”
20. “The finding of CNVs at 1q44, 2q23.1, and 1q21 in IS is novel and first time.”
Should be “and reported for the first time.”
21. “These may simply due to the clinical information in these reports were
typically extracted from limited medical records for the subjects in the studies.”
Should be “These may simply be due to the”
22. “These results indicate that Mbd5 is important for brain development but the
exact role of Mbd5 remains defined.” Should be “remains to be defined.”

Response: Thanks reviewer’s careful reading and corrections. We have made all the
changes in revised manuscript.