Author’s response to reviews

Title: Clinical Characteristics and Mutation Spectrum of Neurofibromin 1 in 12 Chinese Families with Orbital/Periorbital Plexiform Neurofibromatosis Type 1

Authors:

Peiwei Chai (chaipeiwei123@sjtu.edu.cn)
Chuandi Zhou (smile_mapletree@sjtu.edu.cn)
Yefei Wang (paper34@163.com)
Xianqun Fan (fanxq@sjtu.edu.cn)
Renbing Jia (renbingjia@sjtu.edu.cn)

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Author’s response to reviews:

Response letter

Dear editors,

We would like to thank the reviewers for their thorough and thoughtful comments. As suggested, we have revised the manuscript carefully and performed additional experiments, which are described in new figures and in the revised text (in red).

We believe that the revised manuscript has addressed all of the reviewers’ questions, and we hope that our manuscript is now suitable for publication in the BMC medical genetics.

Our point-by-point responses are included below.

MGTC-D-19-00108R1

Reviewer reports:

Ashwin Dalal (Reviewer 1): Title:

Clinical Characteristics and Mutation Spectrum of NF1 in 12 Chinese Families with 3 Orbital/Periorbital Plexiform Neurofibromatosis Type 1
The authors have carried out a retrospective genetic analysis in 12 Neurofibromatosis families of Chinese origin with 26 family members, indicating earlier age at onset in successive generations.

The study revealed fourteen different NF1 mutations were identified. Eleven (91.7%) OPPN families presented NF1 mutations, and 7 identified mutations were novel. Nine (64.3%) out of the fourteen identified mutations were distributed in exons, three (21.4%) were found on splicing sites and one (7%) was found in an intron.

Comments:

1. Although there is nothing new about mutation analysis in NF1, it is interesting to see the clinical characterization of a subtype of NF1.

2. There are many grammatical errors. Please check for the same.

Thanks for your correction. We have consulted English language editing service in American Journal Experts (http://bit.ly/AJE_BS) as suggested.

3. Correct the grammar of this sentence on line number 19 in Discussion section"This is might due to the difference of age" - change to This might be due to the difference of age.

Thank you. We have revised the sentence ”This is might due to the difference of age" to “This might be due to the difference of age" in Page 9, Para. 2.

4. The conclusion regarding anticipation is far fetched and it is better if the authors do not draw such a conclusion without enough proof. Did the authors find any particular mutation which contributed more towards the anticipation or severity of the disease in successive generation?

Thanks. We have noticed the anticipation might be due to the enhanced awareness of the elder generation, which may lead to the exaggerating of “anticipation”. We have revised “anticipation” to the description of the successive generation of OPPNs patients presented the onset at an earlier age and exhibited more severe ocular signs with their parents or grandparents. We have revised our discussion section in Page 9, Para. 2.

4. Any genotype-phenotype correlation possible with available data.

Thanks for your comment. We didn’t observe any genotype-phenotype correlation in these OPPN patients. Also, no obvious genotype-phenotype correlation was identified in Neurofibromatosis. Of note, although OPPN patients in one family share a common NF1 mutation, the symptoms might present differently. This is might due to the difference of age, gender and other epigenetic factors. We have discussed this issue in Page 8, Para. 3.
5. Data needs to be provided about presence or absence of same/different mutation in parents along with zygosity.

Thanks, we have collected at least two affected patient’s genomic DNA sample in each family (24 patients in 12 families and 2 unaffected volunteers were chosen as control). The affected patients shared a same NF1 mutation spot which was absent in the unaffected patients. For example, in 3rd OPPN family, 3-2-1 was the unaffected father while 3-2-2 and 3-3 were the OPPN patients. The affected patients shared the mutation while the unaffected patient presented with “wild-type”.

<table>
<thead>
<tr>
<th>AAChange.refgene</th>
<th>ExonicFunc.refgene</th>
<th>3-2-1</th>
<th>3-2-2</th>
<th>3-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1:NM_000267:exon17:c.C1919T</td>
<td>nonsynonymous SNV</td>
<td>C/C</td>
<td>C/T</td>
<td>C/T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ratio=47.35</td>
<td>Ratio=45.17</td>
</tr>
</tbody>
</table>

We have revised the text in Page 5, Para. 2. The original sequencing data was uploaded in the revised supplementary table 1.

6. Better resolution figures should be provided

Done.

PVGK Sarma, Ph.D (Reviewer 2):

1. The article appears to interesting

2. The authors needs to submit the novel mutations to Genbank as these sequences are novel and the Genbank accession number need to be included in the manuscript

Thanks, we have uploaded these novel NF1 mutations to ClinVar database (Submission Number: SUB5888032 and SUB5888038). We will provide the accession number immediately after confirming the information by the NCBI staff. Notably, we have uploaded all sequencing microarray data in Supplementary table 1. The fastq or bam files could be requested by contacting with chaipeiwei123@sjtu.edu.cn. We will share our sequencing data if required.

3. The PCR gel images, and Sanger chromatogram indicating the novel base mutations needs to be shown and these shoul be inculded in the revised manuscript

The PCR gel images, and Sanger chromatogram indicating the novel base mutations was shown in Figure 3. The Primers of each mutation site were listed in Supplementary Table 2. We have also revised the method section in Page 6, para. 2 and result section in Page 7, para. 1.