Author’s response to reviews

Title: Screening mutations of the Otoferlin gene (OTOF) in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy

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Author’s response to reviews: see over
RE: Manuscript ID 2031313112327804 entitled "Screening mutations of the Otoferlin gene (OTOF) in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy"

Dear Professor Norton,

Thank you for reviewing our manuscript. We are grateful to the reviewers’ helpful comments.

As suggested, we have addressed all the questions raised by the reviewer as shown below and revised the manuscript accordingly (with all changes tracked in the text).

We felt that this revised manuscript is much improved and hope that it is now acceptable for publication in your journal.

We look forward to hearing from you soon.

Sincerely,

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Response to Prof. Regie Santos-Cortez

Major compulsory revisions

1. **The age-of-onset distribution of entire sample**
   A: Please see our supplemental data (Figure S1)

2. **A description of the speech and language abilities of the patients**
   A: That means the language communication ability of patients loss is much severe than his or her performance on pure tone test.

3. **The influenced by the selection of patients with a later age of onset of disease**
   A: First of all, with knowing the various causes of AN, we have precluded those cases caused by other than genetic reasons as far as we can at the beginning when we were collecting our sample pool. Besides, we were not subjectively selecting cases with a later age of onset of cases. On the opposite, some patients older than 25 year were closed out from our study, with considering the etiology of AN might be complicated exponentially with the growth of age.

Minor essential revisions:

1. **Figure 2**
   A: We have revised our legend according to your advice, which started as “we found two heterozygous mutations…”

2. **Discretionary revisions**
   A: We have added SNP ID to table S2.

Response to Prof. Muhammad Hassan

1. **Method section**
   A: Table of PCR primers is replaced by clinical detail of NSRAN patient

2. **Results section**
   Instead of showing 15 variants together, we presented 5 pathogenic mutations first and then non-pathogenic sequence variants. (Page 7 the first paragraph of result)
   Sequencing chromatograms of each novel pathogenic variant with control and carrier sequence have been showed in figure 2.

3. **Figure 2**
   A: We re-corrected our legend about Fig.2a (the original figure 2) and also make it colored, which will be recognized better. Moreover, according to your suggestion, we added the sequencing chromatograms of the other 3 novel pathogenic variants as Fig.2b.

4. **Table of non-pathologic variants**
   We have substituted “mutations” with “variants” in instruction of table S2

5. **Chinese Han population information**
   A: please see the first two paragraphs of discussion
Response to Prof. Thomas Friedman

Major issues:
1. If recessive mutations of OTOF are responsible for AN, what are the possible reason for finding only one mutant allele of OTOF in the three AN subjects
   A: It is a good question. As we know, among the recessive hereditary hearing loss genes, the high prevalence genes of GJB2 or SLC26A4 also can be found only one mutant allele in the congenital hearing loss or enlarged vestibular aqueduct syndrome patients. We assumed that many reasons can understand the only one mutant allele of gene to the patients, may be digenic or methylation et al.
2. Provide supporting clinical data to convince a reader that the compound heterozygous mutation carrier is NSRAN
   A: please see figure 1 and supplemental data table S1

Minor issues:
1. OTOF wasn’t mapped to chromosome 2.
   A: we have make correction in the last paragraph of page 4
2. How would the identification of more alleles of OTOF provide greater understanding of the “molecular pathways for this complex neurological disorder”?
   A: After identifying more alleles of OTOF gene, we might be able to find that pathologic mutations are typically concentrated on a specific region, which will be powerful evidence to support the importance of that region to OTOF function.
3. Figure 1b “intercepted ClustalW alignment”
   A: The “intercepted ClustalW” means partial result, because that we can’t show the alignment result of the whole OTOF gene due to space limitation.
4. Figure 1 could be improved
   A: Please see our updated figure 1.

Response to Prof. Zubair Ahmed

1. Compound heterozygous variants of NSRAN patient
   A: For the 3 allelic variants found in the NSRAN patient, we consider p.A53T to be non-pathological, because pA53V is a published non-pathogenic variant. However, the other two, c.2975_2978delAG and p.R1607W, are thought to be pathological. Because the parents of patient, each of whom contribute one mutated allele to their son, are showing normal hearing. With missing the wild type allele, the 2 inherited mutant alleles can not complement each other any more, thereby caused the NSRAN phenotype of that patient.

   Additionally, we have screened all the exons and their flanking regions of OTOF gene on both directions, as a result, we believe our study covered the entire region above and detected all the variants of our samples, if there is any.
2. **Conclusion readdress**
   A: We have substituted our conclusion with “Screening revealed that mutations in the OTOF gene account for AN in 4 of 73 (5.4%) sporadic AN patients, which shows a lower genetic load of that gene in contrast to the previous studies based on other populations.”

3. **Title**
   Both *OTOF* and Otoferlin are the approved gene name of that gene (http://www.genenames.org/data/hgnc_data.php?hgnc_id=8515), and *OTOF* is the gene symbol. However, according to your advice, we substitute our title with “Screening mutations of *OTOF* gene in Chinese patients with auditory neuropathy, including a familial case of temperature-sensitive auditory neuropathy”.

4. **Background**
   A: We corrected the corresponding part into “The *OTOF* gene has been reported to be responsible for 2 to 3% of NSHL cases.”

5. **Result**
   A: We substituted primer table into audiometric data of NSRAN case.

6. **Figure 1**
   A: According to your advice, we add drosophila to our aliment result. Different from other vibrate species that have multiple Ferlin-like proteins (otoferlin is one of them), drosophila only has one, misfire, which was used for our alignment showed in figure 1[1] and Choi et al’s alignment result[2].


7. **Figure 2**
   A: We substituted figure 2 with a colored one, showing the variant names with a both cDNA and protein change nomenclature format. Considering the consistency of figures, we didn’t change the bases after framshift variants with “NNNNNs”, we believe that with colorful chromatograms everything states from themselves clearly. Additionally, we also added the chromatograms of the other 3 novel pathologic variants identified in this work showing as figure 2b.