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
Prof. Melissa Norton  
Editor-in-Chief  
BMC Medical Genetics

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 reviewers as shown below and revised the manuscript accordingly (with all changes tracked in the text).

We hope that it is now acceptable for publication in your journal.

Looking forward to hear from you soon.

Sincerely,

Qiuju Wang

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Response to Prof. Zubair Ahmed

Thank you very much for reviewing our article.

As you pointed, the study of Choi et al. in Pakistani population did not report any de novo mutation, thereby we substituted the original sentence with “Our perception is supported by two recent study that reported in Pakistani[16] and Brazilian[10] populations, in which was found a quite different mutation spectrum from those previously reported as well.” (Paragraph 1, Page 9)

Response to Prof. Thomas Friedman

Thank you very much for reviewing our article.

Our revisions are listed below according to your suggestion.

1) Abstract, 1st sentence, delete "It is well known that". Consider-“Mutations in OTOF encoding otoferlin cause DFNB9 deafness and ...”

We deleted "It is well known that" and change the 1st sentence of the abstract to "Mutations in OTOF gene, encoding otoferlin, cause DFNB9 deafness and non-syndromic auditory neuropathy (AN)." (Paragraph 1, Page 2)

2) Results, line 3 delete "firstly". If the mutation is novel it is a "first".

The 3rd paragraph in abstract, "firstly" deleted. (Paragraph 3, Page 2)

3) Discussion, the first and second sentences are difficult to understand. What is meant by "Han Chinese population with Japanese..."

The original sentence was confusing. Han Chinese in Beijing (CHB) and Japanese in Tokyo (JPT) are two populations in the international HapMap projects and both of them are from Asian ancestry and sometime be considered together. Here we just wanted to point out the uniqueness of Han Chinese population, which encouraged us to do the present study. (Paragraph 1 of Discussion, Page 8)

4) Discussion, the second paragraph is one sentence. Also, what is meant by "few related study."

We combined the first and second paragraph of discussion into one.

"Few related study" may be a little vague, so we change it into "So far, the screen of OTOF gene has been preformed on Spanish, Caucasians, Pakistani and Brazilian populations[10], however, it's not well studied on Chinese Han population." (Paragraph 1 of Discussion, Page 8)

5) Provide the accession numbers for the genomic and protein sequences used for the mutations in Table 1. Without accession numbers, the mutation numbers and codon numbers will be difficult to reconstruct by a reader.
Nomenclature of mutations is based on GenBank reference sequence accession number NM_194248.1. The nucleotide 1 is the first nucleotide of the translation initiation codon of it. Although it was pointed out in the “OTOF gene sequencing” in methods (Paragraph 1, Page 6), we restated it below the Table 1 to make it better tracked. (Table 1, Page 15)

6) Figure legend S2a, indicate how, and where in the body, the three temperatures were measured.

As your advice, we add the manner of body temperature measurement to figure legend S2a as "Axillary temperature was measured at the mean time of pure tone test producing", hopefully it will give readers a clear idea how the data of figure was acquired.
(Supplemental Data, Figure S2a, Page 3)

Response to Prof. Regie Santos-Cortez

Thank you very much for reviewing our article.

Major revision:
1) Only one copy of an OTOF variant in AN patients

As your suggestion, we stated in the RESULT section that except the TS-NSRAN patients, the other three just carry one copy of OTOF variant; (Paragraph 1 of Result, Page 6) Also, we did a little analysis based on our own understanding focusing on that. (Last Paragraph of discussion, Page 10)

2) Mutation spectrum

According to your advice, we pointed out that "Han Chinese seems have a lower mutation spectrum coverage of OTOF gene on AN patients than other populations" in the body of text. (Last Paragraph, Page 8; First Paragraph, Page 9; Last Paragraph of discussion, Page 10)

3) Polyphen prediction

Even though Polyphen predicts p.D398E and p.E594K to be benign, besides our study, there are still pathogenic variants published on previous study showing benign in the software as well, like p.E1733K, p.R1856Q (Choi BY, et al. 2009) and p.N1929H (Jihane Romanos, et al. 2009). Here we just link the variants and disease together, to truly identify the causative relationship of them, functional research may be required. Also, to make our work more strict, we change the "pathogenic variants" to "possibly pathogenic variants".

Discretionary revisions:
1) Confidence interval of prevalence rates between populations.
Here we collected population based AN study and calculated confidence interval, showed in table below. However, we found it may inappropriate to estimate those numbers in that way.

Because first of all, those studies focused on familial or sporadic cases or both, in which familial cases obviously will show a higher mutation frequency than sporadic ones, no wonder those who linked familial cases with OTOF and then performed a screen. Simply compile those mutation incidence together would be biased.

Additionally, there are some studies based on NSHL, like Choi et al, 2009. Without performing otoacoustic emission testing, they cannot distinguish AN with other NSHL, which make the mutation incidence incompatible with AN based study, such as ours.

Finally, most of population based AN studies have a small size of sample (less than 100), which may insufficient to epidemiological counting, and the inequality of sample size between different study makes the calculation result less significant as well.

Our study introduces the OTOF screen result based on Chinese population. To estimate the mutation prevalence difference may require further epidemiological research.

<table>
<thead>
<tr>
<th>Study</th>
<th>Familial cases</th>
<th>Isolated cases</th>
<th>AN patient number in total</th>
<th>OTOF mutation incidence</th>
<th>Country</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jihane Romanos et al, 2009</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>Brazilian</td>
<td>7/11 (63.6%)</td>
</tr>
<tr>
<td>Montserrat Rodriguez-Ballesteros et al, 2008</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>13</td>
<td>Spanish, Argentinean, Austrian, French, German, Italian, Lebanese, Libyan</td>
<td>11/20 (55%)</td>
</tr>
<tr>
<td>R Varga et al, 2006</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>American, Spanish, Turk, English, Cuban</td>
<td>5/9 (55.6%)</td>
</tr>
<tr>
<td>R Varga et al, 2003</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>Chinese Han</td>
<td>3/4 (75%)</td>
</tr>
<tr>
<td>Our study</td>
<td>1</td>
<td>72</td>
<td>73</td>
<td>4</td>
<td>Chinese Han</td>
<td>4/73 (5.5%)</td>
</tr>
</tbody>
</table>

Confidence interval (our study included, 6 study in total):  
Mean  56.900%±27.956%  
95% CI  27.562% to 86.238%

Confidence interval (our study excluded, 5 study in total):  
Mean  67.180%±13.578%  
95% CI  50.321% to 84.039%

2) Age of onset
We provided that information of entire case group in Supplemental data (Fig S1), and as your suggestion, we also gave the age-of-onset of the patients carrying an OTOF pathogenic variants, added in Table 1. (Supplemental Data, Figure S1, Page 4; Table 1, Page 15)