Author’s response to reviews

Title: A Novel Pathogenic Variant in OSBPL2 Linked to Hereditary Late-onset Deafness in a Mongolian Family

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Author’s response to reviews:

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BMC Medical Genetics

Dear editor,

Thank you for taking the time to consider our paper for publication.

We have enclosed a substantially revised version of our manuscript “A Novel Mutation in OSBPL2 Linked to Hereditary Delayed Deafness in a Mongolian Family ” (MGTC-D-18-00356), carefully incorporating the comments from the three reviewers. This includes the discussion that the new mutation we identified will result in an abnormal protein, modified figures and a
point-to-point answer to each reviewer's comments detailing the changes to this manuscript. An overview of the revisions to the paper can be found below.

1. We have stated the new frameshift mutation of OSBPL2 gene we identified, and discussed the impact of the mutation on the protein in discussion.

2. The original five figures had been changed to three figures, according to reviewers' suggestions: the original Figure 3 was deleted, the original Figure 4 and 5 were combined into a new Figure 3. Each figure was explained in detail below.

3. According to reviewers' suggestions, the original Table 1 was deleted. Relevant contents were added to original Table 2 (new Table 1). SPSS software is used for difference analysis.

4. All comments raised by the three reviewers were responded one by one. Point to point modification in response to reviewers’ comments and suggestions was shown in main text.

5. References are increased from 13 to 17.

6. We added supplementary materials.

There is no question that the modification has been greatly improved our manuscript compared to our initial submission. We would like to thank the editing team and reviewers for their expertise advice throughout this process. We remain convinced that the frameshift mutation of OSBPL2 gene meaningfully contributes to our research into deafness genes and will be a valuable resource to your readership.

Sincerely,

Qizhu Wu, Prof.

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Reviewers' Comments:

Thank you for careful review of our manuscript, many constructive suggestions which helped to improve this paper throughout the entire process. We highlight the comments and suggestions of the three reviewers in different colors of the article (yellow for Reviewer #1, green for Reviewer #2, and pink for Reviewer #3).

Re-re-review of Wu et al. "A Novel Pathogenic Variant in OSBPL2 Linked to Hereditary Late-onset Deafness in a Mongolian Family"

Reviewer #1:

Remarks to the Author:

The authors need to state in the discussion (at least) that the new mutation they identified is leading to the same abnormal protein than a previously reported mutation (Xing et al, 2015). This is an important fact, which is strengthening the pathogenicity of their own mutation, and needs to be discussed. One or two sentences about the impact of the mutation on the protein (abnormal sequence for one specific domain for example) will also beneficiate to the discussion.

Response:Thank you very much for your comments and suggestions. We have stated the new frameshift mutation (c.158_159delAA) of OSBPL2 results in abnormal OSBPL2 protein product with amino acid change starting from 53rd amino acid and right before the sterol binding pocket on OSBP-related domain (aa75-470, http://pfam.xfam.org/protein/Q9H1P3), completely abolishing the binding capacity of mutant protein to sterol(Line 196-200).
Figure 1: it would be more informative to add the segregation data to the pedigree. Consider adding a legend to the figure where the arrow would be explained.

Response: In the pedigree, we have tested and verified that members of the genotype have added wild type (+/+), heterozygous (+/−) and proband (V-9) in Figure 1 (Line 78-80).

The authors stated that they have performed whole genome sequencing to identify the mutation however there is no details about the reason why they used whole genome sequencing vs. whole exome sequencing. In addition, the authors may consider adding more details about the whole genome sequencing protocol.

Response: We knew that whole exome sequencing is more straight forward than whole-genome resequencing to identify mutation in the coding area. Actually, in this study, we first tested a panel of 127 genes (Supplementary table1) related to hereditary deafness on the proband of this family by exome capture sequencing to identify the possible mutation. However, no pathogenic mutations were found in the pilot study. We realized the Mongolian population has lots of unique polymorphic based on our previous study (Bai H et al. Nat Genet. 2018 Dec;50(12):1696-1704. doi: 10.1038/s41588-018-0250-5, PMID: 30397334). As part of the Mongolian Disease Genome Project, we want to learn more comprehensive genetic information of hereditary deafness. Considering that whole-genome sequencing can detect mutations in all genomic regions, not limiting to protein-coding regions, we adopt the whole genome sequencing. We added details about the whole genome sequencing protocol in the Supplementary materials (SupplementaryTable1-2).

Figure 2: the authors may consider adding a legend on the audiograms with the units (thresholds dB, frequency Hz). They may want to add the age of the individuals on the panel or adding it to the legend. Symbols used (O, <, > and X) are not explained neither in the figure nor the legend. "Severe" is misspelled in the legend.

Response: (1) We added a legend on the audiograms with the units (Y: thresholds dB, X:frequency Hz). (2) We added the age of the individuals in the Figure. (3) Symbols used (O, <, > and X) are explained in the legend. (4) The spelling mistake "Severe" has been corrected (Line 123-128).
In section 2.2 of the text (Whole genome sequencing results), the authors stated that they have used "a variety of databases". It would be more informative to give some examples with their appropriate reference/website.

Response: In section 2.2 of the text (Whole genome sequencing results), "a variety of databases" changed to a variety of databases including Single Nucleotide Polymorphism database (dbSNP), Exome Aggregation Consortium (ExAC), Functional annotation of genetic variants from high-throughput sequencing data (esp6500siv2_all), ALL variants dataset of 1000 Genomes Project released in Aug 2015 (1000g2015aug_all)(Line 140-145).

Figure 3: it may help the reader to point the OSBPL2 locus with an arrow.

Response: Figure 3: we have pointed the OSBPL2 locus with an arrow and also display in combination with other related panels(Line 154-161).

Table 1: I would suggest to revise the title of the different cells of the table (nucleotide change, amino acid change for example)

Response: Table 1 was deleted as suggested by other reviewers, and then detailed in the relevant content.

Figure 4 is missing a negative control.

Response: The electrophoresis results (original figure 4) did not show a negative control, even though each experiment we had a negative control without DNA polymerase and another control without DNA template. Original figure 4 was deleted as suggested by other reviewers.

Figure 5: I would suggest to replace "normal" to "non affected" or "control" in (a) and "c.158_159delAA" with "affected" or "carrier"
Response: Figure of Sanger sequencing (original figure 5): We replaced "normal" to "non affected" and "c.158_159delAA" with "affected" (Line 186-190).

Table2: the authors may consider adding the units for the different measures

Response: We added the units for the different measures.

In the discussion, the authors stated that OSBP/ORP family members have been shown to cause diseases. Even if the references are noted, the authors might consider adding the name of some of the disease in parenthesis in the main text.

Response: In the discussion, we added the name of some of the disease in parenthesis in the main text. For example, dyslipidemia and cardiovascular disease were added and references are noted (Line 203-204).

Reviewer #2:
Remarks to the Author:

Abstract: clarify the last sentence of the "objective" sub-section "…to provide a new candidate gene for the early genetic screening and diagnosis of this disease."

Response: Thank you for your suggestion. We added “and to provide evidence for the early genetic screening and diagnosis of this disease.”, and it's highlighted in green (Line 19-21).

Introduction: I encourage the authors to use a different sentence that describes the number of genes that have been identified using next generation sequencing, as the original 230 rare disease genes discovered by next generation sequencing is from Boycott et al., 2014, which is a bit
Response: In recent years, the application of next-generation sequencing technology has greatly accelerated the identification of new rare disease genes. We highlighted that this is the third family reported with hearing loss due to OSBPL2 frameshift variants in the text (Line 61-63).

Methods: (1) what was the type of genome sequencing kit used, what other details about sequencing can be provided, which sequencer was used for sequencing? (2) What other databases and bioinformatics tools were used for variant prioritization; (3) How were the data filtered and what conventions were used for variant prioritization? (4) Were there any other candidate variants identified? (5) What was the average coverage of the genome data and of the variant? (6) Which family members were involved in co-segregation testing or was it only limited to the five that were also genome sequenced? This will help understand the extent of the co-segregation analysis that was performed. (7) Add the ref seq NM_144498.2 in the text (Line 76-77) (7) (Line 193-196).

Response: The whole genome sequencing of this project was completed by Novogene Bioinformatics Institute, Beijing, China. Questions (1) to (5) were described in the Supplementary materials (SupplementaryTable1-2). (6) 13 of family members were involved in co-segregation testing (VI-6, IV-9, IV-14, V-6, VI-5, V-9, VI-1, V-11, V-7, VI-4, IV-2, IV-12, V-13), and were also marked in the pedigree. (7) We added the ref seq NM_144498.2 in the text (Line 76-77) (7) (Line 193-196).

Results: (1) Was there any evidence of incomplete penetrance? (2) What was the age of onset for each respective individual from whom audiological measurements were obtained? Also, what was the age at testing of each individual in Figure 2? Are serial audiograms available from individuals? This would be very helpful to gain insight into the rate of progression. (3) What were the results of tympanometry? (4) I noticed an air-bone gap in Figure 2B. Was this testing repeated in the individual? Is there any explanation for this or is this also an aspect hearing loss due to OSBPL2? (5) The first mention of the c. position of the frameshift should also include the p. position details.
Response: (1) Clinical phenotype and gene test results showed no incomplete penetrance. (2) The age of onset and the age at testing were shown in the table 1. Serial audiograms were available from individuals. (3) The results of tympanometry were normal(Supplementary figure1).(4) After consultation with clinical experts, it was determined that this phenomenon was caused by individual response errors to sound(1)(Line 130-131) (2)(Line 186).

Discussion : Much of the first paragraph is more suitable to an introduction to the topic. Consider moving some of the text to the introduction.

Response: We have moved some of the text to the introduction(Line 41-63).

Figure 1: I would recommend integrating the segregation results into this figure, either via genotype or +/- (for heterozygous) and +/- for wild type result directly below the pedigree symbol for each respective individual. This helps to clarify who was recruited/tested.

Response: The genotypes of all tested individuals were directly marked in the pedigree, for example, V-9 is the proband, +/- for heterozygous and +/+ for wild type(Line 78-80).

Figure 2: Describe A, B, C, D in the legend.

Response: Figure 2 is revised and describe A, B, C, D in the legend(Line 121-122).

Figure 4: It is not necessary for this figure to be presented in the manuscript. It could either be removed entirely or moved to a supplementary section (Additional files). If this figure is moved, how long was the amplicon?

Response: We removed the original figure 4. The target fragment spanning the frameshift mutation (c.158_159delAA) was amplified by PCR and purified with agarose gel electrophoresis (426 bp) (Line 165-167).
Figure 5: consider moving this figure to a subfigure of Figure 1 (Figure 1A could be the pedigree, Figure 1B could be the Sanger sequencing validation).

Response: Original Figure 5 was combined with others in Figure 3.

Table 1: include the reference for the transcript (NM_) here and add the human genome build information.

Response: We deleted Table 1 and discussed the content in the text as other reviewers suggested.

Minor points for revision:

English improvements:

Abstract:(Line 18-38)

Results sub-section: (Line 112-190)

...19 of them were diagnosed with post lingual deafness with the age of onset between 10 and 40 years...(Line 27-29)

Patients with hearing loss showed bilateral symmetry and mild to severe sensorineural deafness(Line 30-31).

Whole genome sequencing identified a novel pathogenic frameshift mutation (c.158_159delAA) in the gene OSBPL2...(Line 31-34)

Conclusion sub-section: (Line 234-238)

Our finding expands the mutational spectrum of…(Line 235-238)

Introduction:(Line 42-63)

Deafness is a defect of the human auditory system…(Line 42-43)

…with genetic factors contributing to the majority…(Line 45-46)

…50% to 60% of hearing loss is inherited…(Line 46-47)

Check the word "posterior"
…we applied whole genome sequencing…(Line 61-63)

Materials and Methods:

extremely severe could be revised to profound

Results:(Line 113-174)

All affected subjects had no history of ototoxic drug use…(Line 115-117)

Hearing tests were diagnosed as bilaterally symmetric…(Line 117-118)

We next sought to confirm the frameshift…associated with the hearing loss in this family.(Line 165-166)

Discussion:(Line 193-232)

Check the word "posterior" at the end of the first paragraph.

In this study, a new frameshift mutation…in OSBPL2 was found in a family with hereditary delayed…(Line 193-196)

OSBPL2 encodes a receptor…, which play an important…(Line 201-203)

However, in this study we did not detect any blood lipid abnormalities in individuals with hearing loss, suggesting the…

OSBPL2 is highly expressed in the inner and outer hair cells of the mouse cochlea(Line226).

However, this hypothesis needs to be tested…in the cochlea by studying mice with OSBPL2 deficiency(Line 230-232).

Response: Thank you for carefully reading and suggestion to improve our paper. The text has been modified and highlighted in green.

Reviewer #3:

Remarks to the Author:

The term ‘mutation’ should be applied with caution according ACMG 2015 guidelines (Richards et al, Genetics in Medicine 2015); it is better to use 'pathogenic variant'.
Response: Thank you for carefully reading our paper, we changed 'mutation' to 'pathogenic variant' throughout the manuscript (highlighted in pink).

It seems that the term 'late-onset deafness' will be better than 'delayed deafness' used in title and in the text of manuscript.

Response: Thank you for carefully reading our paper. The 'late-onset deafness' were changed to replace 'delayed deafness' in title and in the text of manuscript (Line 2, 20, 39, 61, 78, 236).

Page 2, line 41: 'X-linkage' -> 'X-linked'.

Response: Thank you for carefully reading our paper. The mistake has been corrected (Line 48, 54).

Page 4, line 1: '1.2 Method' -> '1.2 Methods'

Response: Thank you for carefully reading our paper. The mistake has been corrected (Line 65).

Page 4, lines 11-27:

Response: The mistake has been corrected (Line 87-93).
- Frequency ranges are also needed to verify, according to Mazzoli et al 2003:

Frequency ranges:
Low frequencies: 0.5 kHz
Mid frequencies: 0.5 kHz - 2 kHz
High frequencies: 2 kHz - 8 kHz
Extended high frequencies: 8 kHz

Response: According to Mazzoli et al 2003, the frequency range has been revalidated (Line 90-93).

Page 4, lines 41-45: OSBPL2 upstream primers -> OSBPL2 forward primer; OSBPL2 downstream primers -> OSBPL2 reverse primer.

Response: The mistake has been corrected (Line 99-101).

Page 4, line 56: 2 RESULT2 -> 2 RESULTS

Response: The mistake has been corrected (Line 112).

Page 5, lines 47-51: Please, specify all individuals who were subjected to the whole genome sequencing.

Response: We conducted whole genome sequencing of 5 family members, including 3 affected subjects (IV-2, IV-9, and V-9) and 2 normal controls (IV-14 and V-6) (Line 133-135).
Page 6, lines 1-13: Please, specify (in more detail) how a candidate gene OSBPL2 was identified from the list of candidate genes in four linkage candidate regions (chromosomes 1, 10, 11, 20).

The data on variant c.158_159delAA (Table 1, only one line) is no need to present as Table.

Response: Through genome sequencing and bioinformatics analysis of five members of the family, we detected four candidate genes for SNP and three candidate genes for indel in the family. However, only OSBPL2 is on four linkage candidate loci (Figure 3). We deleted the original table 1.

Page 8, lines 10-15: 'The results showed that the individuals with hearing loss have similar serum lipid level as compared to normal individuals, suggesting the redundancy of other OSBP proteins in the control blood lipid metabolism (Table 2)' - this conclusion needs to be supported by appropriate statistics.

Response: In this study, blood lipid examination found that both non affected and affected were within the normal range. There was no significant difference between patients and normal subjects (P>0.05) (Table1) (Line 186)