Author's response to reviews

**Title:** Targeted exome sequencing reveals novel USH2A mutations in Chinese patients with simplex Usher syndrome

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**Author's response to reviews:** see over
Dear Dr. Tim,

Thank you very much for your letter and advice. We have revised the manuscript, and would like to re-submit it for your consideration. We have carefully considered the reviewers’ excellent suggestions and critiques, both of which have significantly improved the quality of our manuscript.

Point-to-point responses to all comments and critiques provided by the reviewer are listed below this letter. We hope the revised manuscript is deemed publishable.

For your convenience, we have indicated our revisions in BLUE.

Again, thank you for your consideration. We are looking forward to hearing from you at your earliest convenience.

With best wishes,

Yours sincerely,

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Response to Reviewers:

We would like to express our sincere thanks to the reviewers and the editor for the positive and constructive comments.

Reviewer #1:
This manuscript details the use of targeted exome sequencing of 199 genes using the Illumina HiSeq platform to identify both known and novel USH2A mutations segregating 2 families where probands have been diagnosed with Usher syndrome Type II. The study’s stated goal was to “…evaluate this [sequencing] method as a routine diagnostic tool for USH patients. The writing is clear and the manuscript’s organization is coherent, including the methods and presentation of the data in the tables and figures. This article would be of specific interest to clinicians and geneticists who diagnose and counsel patients/families with hearing loss and/ or vision loss. It also would be of potential interest to researchers who study protein structure/folding/stability because the authors report a novel missense mutation in a common protein motif (FNIII) of the usherin protein.

RE: Thank you very much for your kind words about our study. In the following sections, you will find our responses to each of your points and suggestions. We hope our revisions meet your approval. Thank you once again for your thorough review and valuable suggestion.

Major Compulsory Comments
None.

Minor Essential Revisions.

#1. Results line #122. The following sentence “Analysis of pure tone audiogram testing showed bilateral moderately deteriorating hearing loss.” is somewhat problematic. After reading this description of the patient’s hearing loss I expected to see serial audiograms over a period of time that showed hearing acuity deteriorating in the patient. Instead the single audiogram show a moderate to severe hearing loss,
moderate at low frequencies sloping to severe at the highest frequencies tested. This is a classic Type II hearing loss description, which definitely fits the audiogram in Figure 1. In my opinion, a “deteriorating” hearing loss versus “sloping” hearing loss describe two very different types of hearing status in patients. I suggest the authors consider changing the sentence to read “...bilateral moderate to severe hearing loss, moderate at low sound frequencies sloping to severe at higher tested frequencies.”

RE: We thank the reviewer for the suggestion and revised this sentence.

#2. Figure 2 line 277. From “…moderate hearing loss in the patient…” to “…moderate to severe hearing loss in one patient...”. This change would be consistent with the above requested change (#1) and for the following requested change (#3).

RE: We have revised this sentence.

#3. Results line 130. Clinical data in this report are specific to only the proband in Family F1. For clarity, the end of the second to last sentence should include a “...(data not shown).”

RE: Thanks. This has been included.

#4. Results line 150. The sentence would be improved with the addition of the phrase: “In family F1, we confirmed…” so it is consistent with the next sentence.

RE: Revised as suggested.

#5. Results line 154. The affected proband that harbors the compound mutations in family F2 needs to be changed from “…F1-1-1…” to “…F2-II-6...”.

RE: Thanks. We have revised this sentence.

#6. Results line 163. The phrase “speculated by prediction software to effect protein functions” offers the reader no specifics with respect to what protein functions the prediction software suggests/speculates may be affected. I think more work needs to
be done here to convince the reader that this mutation, in this protein, in this location is a causative mutation. The fact that the isoleucine is evolutionarily conserved is very strong evidence. The missense mutation is in the 29th of 34 Fibronectin type III domains (FNIII). Both isoleucine and phenylalanine are nonpolar but the aromatic side chain may affect protein folding or impart incompatible stacking interactions with other residues in the predicted beta sheet sandwich structure.

RE: We are grateful to the Reviewer for this excellent suggestion. In this study, the pathogenicity of missense was then evaluated by four algorithms, PolyPhen2, SIFT, PANTHER and Pmut. All these four algorithms showed the p.I4386F was damaging. We added the information to this paragraph.

#7. Results 164. “..UAH2A..” change to ”..USH2A..”.

RE: Thanks. It has been corrected.

#8. Discussion line 179 - 180. The sentence about the reported mutations “..were ultimately confirmed to be disease causing” is somewhat of an overstatement. I think the fact that all of the mutations were not found in the general population, 2 of the 3 were identified in other USH2A patients, and there were no other candidate sequence changes in the other 198 screened genes is evidence that the mutations are very likely to disrupt usherin mRNA processing or protein structure/stability/function. To say that they have been “confirmed to be disease causing” requires some kind of complementation experiment, such as demonstrating and correcting protein misfolding/binding or mRNA stability.

RE: We agree with the reviewer. This sentence has been revised as “…were identified to be possibly disease-causing”.

#9. Discussion line 180. “..SNP..” change to “..SNV..”. This would match the “SNV” in the list of abbreviations (line 200). Polymorphisms are defined as being >=1% in the population.

RE: Revised as suggested.
#10. Figure 3. The second and third electropherogram are mislabeled. The second electropherogram is c.13156A>T. The third electropherogram is “IVS47+1G>A”. The “c.9570+1G>A” isn’t accurate since the actual mutation doesn’t involve the cDNA sequence, it is the 1st base of the 47th intron of the gene. I suggest the “c.9570+1G>A” be changed throughout the manuscript. Even if it was reported in reference #8 as c.9570+1G>A, this isn’t what the mutation nomenclature should be, to the best of my knowledge.

RE: Thanks very much and we agree with the reviewer. Figure 3 was corrected and the “c.9570+1G>A” was changed to “IVS47+1G>A” throughout the manuscript.

Reviewer #2:

General Review: The authors provide an example of the application of targeted exome sequencing to a disease (Usher Syndrome) with extensive genetic heterogeneity. The only novel information presented in the paper is one of the mutations—there have been multiple other papers using exome sequencing to identify mutations in patients with Usher Syndrome.

RE: Thank you very much for your kind words about our study. In the following sections, you will find our responses to each of your points and suggestions. We hope our revisions meet your approval. Thank you once again for your thorough review and valuable suggestion.

Minor Essential Revisions:

1. Background:

Line 50: the hearing is patients with Usher Syn II is generally described as congenital hearing loss that is mild-moderate in low tones and moderate to severe at higher frequencies.

RE: Thanks. We have revised this sentence.

Line 52: regarding Usher III—should remove the word “also” not born deaf.
Line 60: there are 12 Usher Syndrome genes: CDH23, CEP250, CIB2, CLRN1, DFNB31, GPR98, HARS, MYO7A, PCDH15, USH1C, USH1G, USH2A.

RE: Thanks. It has been corrected.

2. Results

Candidate mutations: Line 143: more specific information should be provided about a list of candidate gene mutations. Were there any other possible Usher Syndrome genes with mutations?

RE: Thanks very much and we have revised this sentence. In this study, by employing this stepwise analyses approach, there were only one homozygous and two heterozygous variants in USH2A in these two pedigrees, respectively. There were not any other candidate variants after our filtering pipeline.

Analysis of USH2A mutations: Line 163: more specific information about the prediction software used and what the results were should be provided. As written it is very vague.

RE: We are grateful to the Reviewer for this excellent suggestion. In this study, the pathogenicity of missense was then evaluated by four algorithms, PolyPhen2, SIFT, PANTHER and Pmut. All these four algorithms showed the p.I4386F was damaging. We added the information to this paragraph.

Discretionary Revisions

1. Results-clinical findings: It would be much more useful to other researchers if additional information was provided about clinical features, such as age of onset of disease. Moreover, virtually no information about the phenotype of the proband in F2 is given.

RE: Thanks. The on-set age of both probands are about fifteen years old. This information has been added to the Line 119-120. Considering the proband in F2
displayed the typical phenotype of Usher Syn II, the clinical information was not shown in this study.