Reviewer’s report

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

Version: 1 Date: 28 April 2015

Reviewer: Michael D Weston

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Major Compulsory Comments

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.

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.”

#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).

#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).”

#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.

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

#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.

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

#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.

#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.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.

Sincerely,

Michael D. Weston