Reviewer's report

Title: OTOF mutation screening in Japanese severe to profound recessive hearing loss patients

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Reviewer: Ignacio del Castillo

Reviewer's report:

This manuscript reports on the results of screening a cohort of 160 Japanese patients with autosomal recessive non-syndromic hearing impairment for mutations in OTOF, the gene encoding otoferlin. Results are sound, and globally the manuscript is written clearly, but I have some comments on the interpretation of the results.

Major Compulsory Revisions

1) Patients of this cohort were recruited on the basis of having ARNSHL (no bias to collect specifically ANSD cases). Nothing is said about the clinical re-evaluation of those cases shown to carry OTOF mutations, which allowed the authors to establish the ANSD phenotype reported in Table 3.

2) In this manuscript, comments on the pathogenicity of the four novel missense mutations are restricted to the Results section. This point should be discussed in more detail. In my opinion, pathogenicity of p.R1583H is proven by several facts: it was found in compound heterozygosity with p.R1939Q, it was absent in 366 controls, it affects a C2 domain, and the scores provided by prediction programs also agree with this conclusion. The pathogenic potential of the three other variants is not so clear. It is true that they affect C2 domains, and that the scores of the prediction programs would support their classification as pathogenic variants. However, note that all these three variants have been found in the heterozygous state with no accompanying mutation in the other allele, and that p.D450E was found in controls. Moreover, none of the patients carrying these mutations presents with ANSD (this could be due to a loss of OAEs, but it could also mean that the cause of deafness are mutations in another gene). In Table 3, it is not reported whether the case is familial (multiplex) or sporadic (simplex). This could be of help to determine the pathogenicity of some sequence variants: familial cases could be genotyped for genetic markers close to the OTOF gene and a haplotype analysis could exclude linkage in some of them.

3) Results of this work also cast doubts about the pathogenicity of other previously reported variants. I appreciate the conclusion that p.R1676C is a polymorphism. It had been reported previously in the heterozygous state with no accompanying mutation in the other allele. Note that this was also the case for p.D398E and p.N727S, and that in this work they are found again in the same state and both have been found in controls. This point should be also discussed in the text (and note its implications regarding major comment 2).
4) Discussion, paragraph 2. Although heterozygous cases without a second mutation have been reported in different screenings, their numbers are not so high if we exclude those cases with variants of uncertain pathogenicity (see major comment 3). This point should be considered in the text. Note also that among the possible explanations for unelucidated heterozygotes you have not mentioned that the cause of their hearing impairment could be mutations in another gene, and that they might be just coincidental carriers.

Minor Essential Revisions

1) Background, paragraph 2, line 6. AUNX1 should not be included here as responsible for non-syndromic ANSD, since it was reported in subjects with early-onset ANSD plus a late-onset peripheral sensory neuropathy.

2) Results, line 12. The frequency in the control population and the results of the prediction software for p.R1676C are not shown in Table 1. The frequency in controls is shown in Table 2, but the most relevant data from the prediction programs should be added to the main text.

3) Discussion, first line. Reference 14 is not appropriate for a comprehensive list of OTOF mutations. There are more recent works (e.g. ref 12).

4) Discussion, paragraph 2, line 17. In fact, large deletions of OTOF have been reported (Zadro et al., Int J Pediatr Otorhinolaryngol 2010; 74:494-8), and they should be mentioned at this point of the Discussion.

5) Discussion, paragraph 4, line 2. The first report of good outcomes of cochlear implants in patients with mutations in OTOF was that of Rodriguez-Ballesteros et al., Hum Mutat 2003; 22:451-6.

6) All abbreviations in Table 1 should be explained in the table footnotes. Data in this table should be carefully checked. I find it bizarre that you obtain SIFT scores for nonsense mutations (p.Y474X, p.W717X, p.S1318X). In fact, NA (I suppose it means "not applicable") is assigned to the first two mutations. For p.S1381X, it is assigned a "T" (tolerated?)(!).

7) Minor issues not for publication (some typos):
   a) "isofrom" (background, paragraph 2, line 11)
   b) "encoding for" ("coding for" or "encoding" would be correct; background, paragraph 2, line 12)
   c) "program" (it should read "programs"; results, line 5)
   d) "p.1778I" (A "V" is missing; results, line 6)
   e) "D. renio" ("D. rerio", Figure 1)
   f) "Migliogi" ("Migliosi", Table 2)
   g) "Rodriguez" ("Rodriguez-Ballesteros", Table 2).
   h) Please check citation #14, the reference is not complete.
   i) Tables 1 and 2. References should be cited by using the numbers in the reference list. Note that there are two articles that could be Wang et al, 2010 (refs 17 and 27).
Level of interest: An article whose findings are important to those with closely related research interests

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