Author’s response to reviews

Title: Whole-exome Sequencing Identifies a Novel Missense Variant within LOXHD1 Causing Rare Hearing Loss in a Chinese Family

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Author’s response to reviews:

1. Severity of the sensorineural hearing loss observed in the proband can be classified based on the WHO criteria in the paper, as the reviewer suggested.

Response: Thanks for your suggestion. We have corrected the severity of hearing loss of this proband as “profound sensorineural hearing loss”.

2. In the abstract, the mutation information of LOXHD1 only needs to show once, not necessary twice.

Response: Thanks for your suggestion. We have revised our abstract and showed the mutation information of LOXHD1 once.

3. In Background, third paragraph, at least 60 different mutations of the LOXHD1 gene have been documented in the HGMD. Based on the database, it is more informative to introduce the details of mutation spectrum, including Fuchs corneal dystrophy and hearing loss.

Response: Thanks for your suggestion. According to the HGMD database (http://www.hgmd.cf.ac.uk/docs/login.html, professional 2018.3 version), there are 47 missenses/nonsenses, 5 splicing variants, 5 small deletions, 1 small insertion, 1 small indel and 1 gross deletion identified in the LOXHD1 gene. In these variants, 47 variants are associated with
hearing loss and 16 variants are associated with late-onset Fuchs corneal dystrophy (FCD, MIM #136800). We have added this content in the Background section.

4. Page 6, 2nd paragraph: If the WES and associated bioinformatics analysis were conducted in-house, please provide details of the methods as supplemental information and references.

Response: Thanks for your question. The WES was performed by the Illumina HiSeq X10 platform (San Diego, CA, USA). First, raw data was evaluated by fastp, then filtered clean reads was aligned to the reference genome (UCSC hg19, 2009) using the BWA-MEM, and followed by excluding PCR duplicates using SAMBLASTER and correcting reads using GATK. Second, variants including single-nucleotide polymorphisms (SNPs) and indels were identified by SAMtools and VarScan 2, and further annotated by ANNOVAR. A promising candidate gene was considered that a variant met all the following criteria: (1) nonsense, missense, frame shift, or splice site variants; (2) absent in the repeat region; (2) absent or frequency < 1% in all the population databases, including dbSNP, gnomAD, ExAC, 1000genomes and ESP; (3) predicted as “pathogenic or damaging” in at least one softwares, such as SIFT, Polyphen2 and MutationTaster; and (4) novo variants (heterozygous variants in the proband that absent in her parents) or shared variants (homozygous variants in the proband that heterozygous in her parents). We have added this content and references in the supplementary materials.

5. Last sentence of the paragraph: “Several variants …”: this is not a precise statement. Again, check the HGMD for more accurate description.

Response: Thanks for your suggestion. We have revised this sentence as “Nowadays there have been 47 variants within LOXHD1 associated with hearing impairment according to HGMD database, but c.5948C>T (p.S1983F) was not reported previously”.

6. Page 7, the 2nd to the last paragraph, a genotype-phenotype correlation of the LOXHD1 gene can be discussed in more details.

Response: Thanks for your suggestion. We have revised this as “In the past ten years, about 60 variants within this gene were identified in NSHL cases. They showed different auditory characteristics, varying from stable to progressive and from mild to profound HL. The limited variant spectrum of LOXHD1 strongly requires more studies to fill in gaps in the genotype-phenotype correlations of DFNB77”.

7. Table 1-3 should be included as supplemental data.
Response: Thanks for your suggestion. We have included Table 1-3 as supplementary table S1-S3.

8. Please consider a comparison in terms of genotype-phenotype correlation of the LOXHD1 gene in a Table. A decent discussion is necessary.

Response: Thanks for your suggestion. We have summarized the published genotype-phenotype correlations of DFNB77 confirmed by segregation analysis in Table 1, and added related discussion in Discussion section.

9. Provide the primers used for validation the mutations in LOXHD in Sanger sequencing.

Response: Thanks for your suggestion. The primer sequences (5’→3’) were: forward-p, ATCGTGGTGCTTTTAACCTGC; reverse-p, GGGTGCTTGCACAGGATTG. We have provided the primers in the third paragraph pf “Case presentation” section.

Reviewer reports:

Jianjun Xiong (Reviewer 1): The authors identified a novel missense variant at LOXHD1 gene exome associated with a rare NSHL from a Chinese family. The results demonstrate the effectiveness of whole-exome sequencing for molecular diagnosis of rare diseases, and expand the genotypic spectrum of DFNB77. The manuscript is well written and recommended to publish.

Suggestion: please list the primers for validation the variant in LOXHD.

Response: Thanks for your suggestion. The primer sequences (5’→3’) were: forward-p, ATCGTGGTGCTTTTAACCTGC; reverse-p, GGGTGCTTGCACAGGATTG. We have provided the primers in the third paragraph pf “Case presentation” section.
Yuan Li (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

The article is well written.

1. Her mutation, analysed by pedigree and software, suggests a pathogenic mutation, but the pathogenicity of this missense mutation is still lacking in animal models or in the verification of other deaf persons. Although these deficiencies exist, it is generally possible due to the presence of rare genes.

Response: Thanks for your comment. Indeed, our identified missense variant is lacking in animal models or in the verification of other deaf persons. We have added this limitation in our discussion.

2. According to the WHO criteria for hearing classification, the proband in this study should be profound sensorineural hearing loss.

Response: Thanks for your suggestion. We have corrected the severity of hearing loss of this proband as “profound sensorineural hearing loss”.