Author’s response to reviews

Title: Whole-exome sequencing identified a missense mutation in WFS1 causing low-frequency hearing loss: a case report

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Author’s response to reviews:

"The authors' response letter has been included as a supplementary file"

Responses to Reviewer #1

The authors have provided a convincing argument that the mutation p.S807R in WFS1, identified by WES is the causative mutation for Low Frequency Non-Syndromic Hearing Loss in this family:

LF hearing loss pattern typical of WFS1 mutation: non-profound, LF only Apparently Autosomal dominant Evolutionary conserved amino acid Previously found as causative mutation in family based on linkage mapping

Response:

We thank the reviewer for the constructive comments and critical reviews. We have added all the revised data and have made the required changes as detailed below.
1. In the background page 4 lines 47-52 it is not clear that the majority of cases DFNA6/14/38 are non-syndromic and only a few reports describe other clinical features. This sentence should be reworded.

Response:

We thank the reviewer for this advice. As suggested, it was not clear whether cases DFNA6/14/38 were nonsyndromic. We corrected the text to indicate that DFNA6/14/38 cases showed low-frequency sensorineural hearing loss (yellow highlighted text).

2. Page 5 line 24 states that subjects did not exhibit any syndromic phenotypes but it would be helpful to know that diabetes and optic neuropathy were actually ruled out, or at least how the non-syndromic nature of their clinical presentation was established.

Response:

We thank the reviewer for the kind comment. In the proband, the blood sugar levels were within the normal range, and no clinical symptoms suggesting diabetes were observed. In addition, an ophthalmologist determined that the proband had normal vision and that there were no abnormal findings in the optic disc and retina. These findings were described in the revised manuscript.

3. Were other audiological tests besides pure tone audiometry performed? The abstract says audiological evaluations "including" pure tone audiometry; this implies other audiological evaluations were done. What were they and what were the results? What were the results of temporal bone CT? The authors state that imaging was performed "including" temporal bone CT; again this implies that other imaging was also performed…is this the case? If so what other tests?

Response:

We thank the reviewer for this comment. For audiological evaluations, pure tone audiometry, speech audiometry, and impedance audiometry were performed. In speech audiometry, the word recognition score was 100% for both ears at the most comfortable level. In impedance audiometry, both drums were type A (compliance was 0.5 cm for both ears) according to Jerger classification. For imaging, we only performed temporal bone CT scans. Thus, we modified the description in the Abstract from “imaging including temporal bone CT scan” to “temporal bone CT scans”. In temporal bone CT scans, there were no inner ear anomalies or middle ear deformities. We added this information to the revised manuscript.
4. Whole Exome Sequencing (WES) is an appropriate first approach to identifying the causative mutation in this case with few family members. Table 1 appears to have an error since there are more variants not common in dbSNP than there were total variants detected. It is not clear how 622 potential disease-causing variants were distilled down to the WFS1 mutation. Was it chosen exclusively because it was the clear candidate. If so please state that.

Response:

We appreciate this comment from the reviewer and apologize for the lack of clarity. First, we made a mistake in the number of variants. In the Table 1, the number of variants which are not common dbSNP138 (MAF > 1%) is 37,206, therefore, % B/A is corrected to 1.67%. We have corrected text in Table 1(yellow highlighted text). Second, we filtered down variants as follow and the numbers of variants which remained after each step were in Table 1. In the first step, variants with minor allele frequency greater than 1% were excluded using dbSNP (version 138). In the second step, we excluded variants that were heterozygous, homozygous or hemizygous in 32 healthy Koreans (internal control data). In step 3, synonymous variants and intronic variants that are not located in splice site regions were excluded. In step 4, as we assumed a dominant inheritance pattern on the basis of the pedigree of the YUHL30 family, only heterozygous variants were retained, while homozygous and hemizygous variants were excluded from further evaluation. In step 5, variants resulting from false aligned reads were removed by directly inspecting WES data and for the remaining variants, we examine allele frequencies of variants in the latest public databases (e.g. dbSNP150, ExAC and gnomAD) and filtered out variants of which MAF is greater 0.05% (Shearer et al., Am J Hum Genet. 2014;95:445-453). Out of 168 remaining variants, we noticed that four variants belong to 129 genes linked to hearing loss. Two variants are in recessive genes (ATP6V1C2 and OTOF), therefore, were excluded. The other two are in TNC and WFS1, respectively. Then, we performed segregation analysis using DNA samples of YUHL30 family members to examine whether each variant segregates with the affected status. Only the c.2419A>C variant in WFS1 was segregated and we also found that the variant has previously reported in the HGMD database.

5. The authors suggest that the p.S807R mutation may represent a hot spot for mutation because it has been found now in two different ethnic backgrounds. This speculation should be better supported. There is the possibility of recent or distant admixture. This could be addressed by looking at the WES data. Are there other variants in the WFS1 gene? Were those absent in the UK family with the same mutation? Is there evidence for or against other European variants using the distribution of variants identified in the WES data?

Response:

Thank you for your kind advice. As you suggested, we examined WES data for other SNPs in WFS1. There are 17 variants in WFS1 including the c.2419A>C variant. Using six common
SNPs detected in YUHL30-22 around c. 2419A>C (red arrow in the Tables), we examined haplotypes in East Asian and British European using 1000 Genomes Project Phase 3 data (please find the separately uploaded word file to see the tables including haplotype data).

YUHL30-22 has H1 and H4 haplotypes of JPT. As the haplotypes and their frequencies are different in JPT and GBR, we can determine whether the mutation is a founder mutation or a hot spot if we know the haplotype data of the British patients with the same mutation in WFS1. Unfortunately, as Cryns did linkage analysis using microsatellites and these markers were not available in WES data of YUHL30-22, we could not compare haplotypes of our patients and British patients. Therefore, we cannot tell the mutation is a founder mutation or a hot spot at the moment. However, it is more likely that it is a hotspot mutation because two ethnics are not neighborhood and distantly located. We appropriately revised the related description without overestimation (yellow highlighted text).

Responses to Reviewer #2

The authors investigated the genetic cause of non-syndromic hearing loss (NSHL) at mid-to-low frequencies in a small Korean family with a presumed dominant inheritance. The observed hearing loss profile is typical of mutations in the Wolfram syndrome 1 gene (WFS1), which was indeed found to be causative by whole-exome sequencing (WES) in an index patient. The identified variant had previously been described in a UK family with the same disease phenotype. The results presented in the manuscript contribute to the specific characterization of the WFS1 mutational landscape in Asia and the Korean population, in particular. The pursued screening strategy should be improved and specified. The manuscript contains a number of minor errors, conflicting statements and misleading or repetitive formulations. Grammar and syntax mistakes as well as inconsistent hyphenation throughout call for proofreading by a native speaker in a revised manuscript.

Response:

We thank the reviewer for the constructive comments and critical reviews. We have added more data and made the required changes, as detailed below.

1. The authors state 'This is the first report to describe a pathological mutation in WFS1 among Korean patients' and 'Our description of the p.S807R (c.2419A→C) mutation in WFS1 is the first report of a mutation associated with LF-NSHL in Korean patients'. However, another WFS1 mutation has been described among Koreans linked to NSHL (Choi B. Y. et al. Diagnostic application of targeted resequencing for familial nonsyndromic hearing loss. PLoS One 8, e68692 2013). This paper should be cited in the revised manuscript.
Response:

We thank the reviewer for this comment. Indeed, a previous report described the p.V412A mutation in Koreans. As suggested, we cited this study in the revised manuscript (page x).

2. The authors state 'According to the The Hereditary Hearing loss Homepage (http://hereditaryhearingloss.org), autosomal dominant, non-syndromic hearing loss is hereditary and known to be associated with 41 genetic loci.' The number of genetic loci should be updated. The sentence is also repetitive since autosomal dominant hearing loss is always hereditary.

Response:

We thank the reviewer for this comment. We have updated the information to the current number of genetic loci and removed the repetitive sentence. The corrected text is indicated with yellow highlighted text.

3. The authors state 'DFNA6/14/38 is rare and found to cause hearing loss in patients with diabetes mellitus and/or an optic atrophy-like Wolfram syndrome phenotype [6].' DFNA6/14/38 causes non-syndromic hearing loss. The publication reports the occurrence of autoimmune diseases (Crohn's disease and Graves' disease) in two members of a family with non-syndromic hearing loss. This should be corrected.

Response:

We appreciate this comment from the reviewer. We think the statement “DFNA6/14/38 is rare and found to cause hearing loss in patients with diabetes mellitus and/or an optic atrophy-like Wolfram syndrome phenotype” is not necessary and redundant. Therefore, we removed the sentence and the cited reference.

4. Two different numbers of reported WFS1 mutations are given with reference to different sources. Background: 'To date, approximately 300 mutations in WFS1 have been reported (https://lovd.euro-wabb.org/home.php?select_db=WFS1). ' Discussion and Conclusions: 'To date, more than 250 mutations in the WFS1 gene have been reported worldwide (http://www.hgmd.cf.ac.uk/ac/index.php). ' This should be clarified.

Response:
We thank the reviewer for this comment. The number of mutations in WFS1 differs depending on the reference database. Because it would be confusing if the number of mutations in WFS1 differed in the same report, we removed the reference to one of the databases and instead used the estimated count from the HGMD database. In the revised manuscript, we removed the redundant descriptions in the Discussion section.

5. In the sentence 'Most mutations in WFS1 have been identified in exon 8 and, moreover, in exons 3, 4, 5, and 66-8.' Correct to read '6-8'.

Response: We thank the reviewer for pointing out this typo. We corrected the text accordingly (yellow highlighted text).

6. The authors state 'Using whole exome sequencing (WES), we examined a Korean family (Yonsei University Hearing Loss [YUHL] 30) and identified a novel missense mutation, c.2419A→C (p.Ser807Arg), in the WFS1 gene.' The mutation is not novel and this should be corrected.

Response: We thank the reviewer for this comment. We removed the term “novel” and corrected this expression accordingly.

7. The hearing loss in the family under study is first characterized as progressive, then as non-progressive. Abstract: 'Audiological evaluation of the affected subjects revealed progressive LF-NSHL, with early onset at 10 years of age, but not to a profound level.' Case presentation: 'Notably, it did not appear that hearing loss was progressive, even though 30-12 (45 years of age) exhibited a degree and pattern of hearing loss similar to 30-22 (14 years of age). This should be clarified. The patient designations should also be renamed appropriately for publication.'

Response: We thank the reviewer for this comment and apologize for the confusing description. Indeed, it is correct that this family presented nonprogressive sensorineural hearing loss. Thus, we corrected the expression from “progressive LF-NSHL” to “nonprogressive LF-NSHL” in the Abstract.
8. The sentence 'While hearing function at frequencies between 2000 Hz and 8000 Hz was well preserved, hearing threshold was decreased up to 50-60 dB HL at frequencies ≤ 2000 Hz in the two affected subjects' should be changed. The threshold is increased, not decreased. The hearing loss is said to affect low frequencies in the title and abstract, mid-to-low frequencies in the background and case presentation and only mid-frequencies in the legend to figure 1. This should be changed and the international criteria used for defining degrees of hearing loss (mild to profound) added to the manuscript.

Response:

We appreciate this comment from the reviewer. We replaced “decreased” with “increased”. We also corrected the expression “only mid-frequencies” in the legend to Figure 1. Because the proband showed hearing loss at 1 and 2 kHz as well, low-to-mid frequency hearing loss is the correct expression. We have changed phrase accordingly. Additionally, based on the international criteria used for defining degrees of hearing loss, this family exhibited mild-to-moderate hearing loss. This information was added to the revised text.

9. Allele frequency data should reference gnomAD rather than the ExAC database (gnomad.broadinstitute.org/) throughout the manuscript.

Response:

We thank the reviewer for this comment. We corrected Table 2 (yellow highlighted text) accordingly.

10. According to Table 1, common variants, variants detected in controls and synonymous variants were excluded. It is not clear how the remaining 622 variants were narrowed down to the mutation in WFS1. The authors state 'There were 553 missense variants; variants in WFS1 (c.2419A→C; p.Ser807Arg) remained after filtering and inspection of WES data (Tables 1 and 2).' This suggests that only missense variants were considered. Why? 'Variants which are nonsynonymous or located in splice junction' should be changed to '….located in a splice site'. In addition, the 'Variants which are not common dbSNP138 (MAF>1%) (A)' are greater than the total number of variants. The GnomAd database should be used for allele frequencies. A 1% cut-off for variant identification is at least a thousand-fold too high for low frequency AD deafness. It is therefore not clear how WFS1 is identified as a 'surviving gene'.

Response:
We appreciate this comment from the reviewer, and we apologize for the lack of clarity. First, we filtered down variants as follow and the numbers of variants which remained after each step were in Table 1. In the first step, variants with minor allele frequency greater than 1% were excluded using dbSNP (version 138). In the second step, we excluded variants that were heterozygous, homozygous or hemizygous in 32 healthy Koreans (internal control data). In step 3, synonymous variants and intronic variants that are not located in splice site regions were excluded. In step 4, as we assumed a dominant inheritance pattern on the basis of the pedigree of the YUHL30 family, only heterozygous variants were retained, while homozygous and hemizygous variants were excluded from further evaluation. In step 5, variants resulting from false aligned reads were removed by directly inspecting WES data and for the remaining variants, we examine allele frequencies of variants in the latest public databases (e.g. dbSNP150, ExAC and gnomAD) and filtered out variants of which MAF is greater 0.05% (Shearer et al., Am J Hum Genet. 2014;95:445-453). Out of 168 remaining variants, we noticed that four variants belong to 129 genes linked to hearing loss. Two variants are in recessive genes (ATP6V1C2 and OTOF), therefore, were excluded. The other two are in TNC and WFS1, respectively. Then, we performed segregation analysis using DNA samples of YUHL30 family members to examine whether each variant segregates with the affected status. Only the c.2419A>C variant in WFS1 was segregated and we also found that the variant has previously reported in the HGMD database.

Second, we corrected ‘There were 553 missense variants;’ to ‘There were 622 variants which are nonsynonymous or located in splice junction’ (highlighted yellow color). We added ‘a’, thus, we corrected ‘located in splice junction’ to ‘located in a splice site’.

Finally, we made a mistake in the number of variants. In the Table 1, the number of variants which are not common dbSNP138 is 37,206, so, % B/A is corrected to 1.67%. We have corrected text in Table 1(highlighted with yellow color).

11. Table 2 is superfluous. Additional information contained within the table (PolyPhen-2, PROVEAN, MT) should be incorporated into the text.

Response:

We agree with the reviewer’s comment. We incorporated the information presented in Table 2 into the revised manuscript. However, we also believe that Table 2 is valuable for presenting the mutation information concisely; thus, we did not remove Table 2 from the revised manuscript, even though this table may seem redundant.

12. Discussion and Conclusions
It is unclear what strategy was pursued and how WES was selected as the method of choice. It is stated that 'the majority of mutations associated with LF-NSHL are, in fact, missense mutations located in exon 8, whereas those linked to Wolfram syndrome are frameshift and nonsense mutations' and that the hearing loss profile of the family under study 'is unique only in hearing loss associated with mutations in WFS1; thus, screening of WFS1 is a reasonable first step in cases of suspected LF-NSHL without progression to a profound level.' This and table 1 lead to the assumption that the authors were specifically looking for missense mutations in WFS1. In that case, the aim and strategy need to be specified.

Response:

We thank the reviewer for this critical comment. As we described above, nonprogressive LF-NSHL was the typical phenotype of WFS1 mutations. Thus, sequencing of WFS1 should have been used for the first screening test in particular. However, we thought that there may be another driving mutation in a gene other than WFS1. Therefore, we performed WES. For clinical diagnostics, we believe that whole exon screening of WFS1 should be performed before WES. However, this method may not be cost effective because the exon length of WFS1 is a bit long (more than 3600 bp for the eight exons).

13. Figure 2

Fig 2A: Protein designations should be aligned correctly. Throughout the manuscript, p.Ser807Arg should consistently be used to designate the mutation and amino acid position. The Danio rerio alignment is missing.

Response:

We appreciate this comment from the reviewer. We have revised the text accordingly and changed p.S807 to p.S807R (Discussion, line 31, and Figure 2, yellow highlighted text). We also added the Danio rerio alignment.