Author’s response to reviews

Title: Genotype-phenotype correlation analysis of MYO15A variants in autosomal recessive non-syndromic hearing loss

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Author’s response to reviews:

3rd, Mar. 2019

Dear editor:

Thank you very much for the editing and correction you have done on our paper, we have made the formatting changes according to your comments.

Thank you very much for your attention and consideration.

Sincerely,

Qiuju on behalf of all of authors

Response to the editor:
1. Please submit your revised manuscript as a single clean copy without any tracked changes, colored or highlighted text. You may provide the manuscript with tracked changes or highlighting as a supplementary file.

Reply to the editor: Thanks for your suggestion. We have submitted a revised manuscript as a single clean copy and provide the manuscript with highlighting as a supplementary file.

2. Please include the email addresses for all authors on the title page. The corresponding author should still be indicated. Please also ensure these email addresses match the email addresses provided in the editorial manager system.

Reply to the editor: We have added the email addresses for all authors on the title page and ensured these email addresses match the email addresses provided in the editorial manager system. (Page 9, Line 36-56)

3. Rename the heading Materials and methods to "Methods".

Reply to the editor: We have renamed the heading Materials and methods to "Methods". (Page 13, Line 9)

4. Please add a separate “Conclusions” section after the “Discussion” section. This should state clearly the main conclusions of the research article and give a clear explanation of their importance and relevance.

Reply to the editor: We have added a separate “Conclusions” section after the “Discussion” section. (Page 24, Line 17)

5. Please list all abbreviations used in your manuscript under the heading "Abbreviations" after the conclusions section. If no abbreviations are used in the manuscript, please state "Not applicable" in this section.

Reply to the editor: We have listed all abbreviations used in this manuscript under the heading "Abbreviations" after the conclusions section. (Page 24, Line 45)

6. Please include a statement in the Authors' contributions section to the effect that “all authors have read and approved the manuscript”, and ensure that this is the case.

Reply to the editor: We have added a statement in the Authors' contributions section to the effect that “all authors have read and approved the manuscript”. (Page 25, Line 50)
Dear editor Gunadi and reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Genotype-phenotype correlation analysis of MYO15A variants in autosomal recessive non-syndromic hearing loss” (ID: MGTC-D-18-00494). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our paper. We have studied comments carefully and have made correction, which we hope meet with approval. The main corrections in the paper and the responds to the reviewer’s comments are as flowing.

I would like to re-submit this revised manuscript to BMC Medical Genetics, and hope it is acceptable for publication in the journal.

Thank you very much for your attention and consideration.

Sincerely,

Qiuju on behalf of all of authors

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Response to the reviewers:

Thomas Friedman, PhD/Rabia Faridi, PhD (Reviewer 1):

Background

1. Variants of HGF, CIB2, MYO7A and TMC1 are also worldwide major contributors to autosomal nonsyndromic hearing loss.

Reply to Reviewer 1: Thanks for your suggestion. We have added variants of HGF, CIB2, MYO7A and TMC1 as major contributors to autosomal nonsyndromic hearing loss. (Reference: Richard EM, Santos-Cortez RLP, Faridi R, Rehman AU, Lee K, Shahzad M, Acharya A, Khan AA, Intiaz A, Chakchouk I et al: Global genetic insight contributed by consanguineous

2. Line 56, the prevalence of MYO15A variants in the Pakistani population is 5.7% as reported in Richard et.al, 2018.

Reply to Reviewer 1: Thank you very much for your suggestions and making a great effort to improve our paper. According to the latest data (Richard et.al, 2018), we have updated the prevalence of MYO15A variants in the Pakistani population as “5.7% among Pakistani patients”. (Background: Page 11, Line 56)

3. Myosin 15 is a member of a superfamily of unconventional myosin not actin.

Reply to Reviewer 1: We have changed “a member of the unconventional actin superfamily” to “a member of the unconventional myosin superfamily”. (Background: Page 12, Line 3)

4. No need to mention phenotypes after "severe to profound congenital sensorineural deafness".

Reply to Reviewer 1: Thanks for your suggestion. We have removed the phenotypes in the revised version of the manuscript. (Background: Page 12, Line 45)

5. Background (Conclusion, last sentence), Revise the sentence "was the first study … in different populations…” Please see Supp. Table S1 of Rehman et al., 2016 where the country of origin/ethnicity was noted. Maybe this manuscript is the first to make a nice figure of these data. Is that worth boasting about?

Reply to Reviewer 1: We quite agree with you on this point. Paper “Mutational Spectrum of MYO15A and the Molecular Mechanisms of DFNB3 Human Deafness.” (Rehman et al.,2016 PMID:27375115) is the first to make a nice figure of these data and we can get lots of useful information of MYO15A from this excellent study. Therefore, we have revised the sentence to “Our work extended the MYO15A variant spectrum, enriched our knowledge of auditory phenotypes, and tried to explore genotype-phenotype correlation in different populations in order to investigate the cause of the complex MYO15A genotype-phenotype correlation.” (Background: Page 12, Line 56)

6. In mouse, the official name of the gene is Myo15 (with a superscript for the allele), not Myo15a. See the JAX entry http://www.informatics.jax.org/searchtool/Search.do?query=myo15&submit=Quick%0D%0ASe arch
Reply to Reviewer 1: The authors are grateful to the reviewer for pointing out this error. We have changed “Myo15a” as “Myo15”. (Background: Page 12, Line 14)

Methods

7. The authors indicate that they reviewed the literature up to October 22, 2018. Recently, Richards et al. 2018 reported three additional likely pathogenic variants of MYO15A segregating with deafness in Pakistani families. For completeness, the authors of MGTC-D-18-00494 might want to add these new variants to their figures 2 and 4. Additionally, please update following variants: c.9229+2T>C and c.6178-2A>G from Rehman et al, 2016.

Reply to Reviewer 1: Thanks for your suggestion. We have reviewed more references about MYO15A novel variants up to Jan 30, 2019 and added the new variants to our figures 2 and 4.

Eight recent references regarding MYO15A novel variants [1-8]:


8. Authors have mentioned that individuals ascertained for the study had mild to moderate hearing loss. Yet in Figure 2, p.Gln1510* and p.Ile1311Thr are colored brown indicating "variants without reported hearing loss".

Reply to Reviewer 1: Thanks for your suggestion. We have double-checked three related references (Rehman et.al, 2016, Sloan-Heggen et al, 2016 and Richard et al, 2019) to obtain the phenotype information of p.Gln1510* and p.Ile1311Thr, however, we did not find the exact hearing phenotype of these variants. It would be grateful if you could offer more details on hearing phenotype of these variants.

Relevant references:

p.Gln1510*:


p.Ile1311Thr:

9. In Figure 4, p.Ile1311Thr is noted below the schematic, which are "variants without known zygosity". Yet, in that manuscript, the authors show pedigrees with genotypes that indicate p.Ile1311Thr is segregating in an autosomal recessive manner.

Reply to Reviewer 1: We double-checked the Supp. Figure S2 (pedigree figure) in this paper (Rehman et.al, 2016). Variant p.Ile1311Thr of MYO15A was homozygous. We have corrected this mistake in Figure 4. (Rehman AU, Bird JE, Faridi R, Shahzad M, Shah S, Lee K, Khan SN, Imtiaz A, Ahmed ZM, Riazuddin S et al: Mutational Spectrum of MYO15A and the Molecular Mechanisms of DFNB3 Human Deafness. Human Mutation 2016, 37(10):991-1003). (Page 48, Figure 4)

10. Some variants from Rehman et.al 2016 are not written correctly in Figure 2 and in Figure 4. Please omit "V" from p. Gly1315Glu in Figure 4. The correct nomenclature for p.Gln3403delinsProThrArgValQGlyLeu in Figure 2 and 4 is p.Gln3403delinsProThrArgProValGlnLeu.

Reply to Reviewer 1: We have removed the “V” in Figure 4 and changed “p.Gln3403delinsProThrArgValQGlyLeu” to “p.Gln3403delinsProThrArgProValGlnLeu” (Page 46, Figure 2; Page 48, Figure 4)


Reply to Reviewer 1: Thanks for your suggestion. We have reviewed 8 recent publications and added total 17 MYO15A novel variants in this revised manuscript. (Please see the answer to the question 7)

12. What "genomic enrichment platform to capture exons" was used in the authors' study? Were there any common genetics variants of other genes responsible for deafness in the Han community that were pre-screened before your targeted capture?

Reply to Reviewer 1: Thanks for your comments, for the genomic enrichment platform in this study, we used the SureSelect Biotinylated RNA Library (BAITS) for enrichment.

We did not perform common genes variants screening, which were covered in our panel. In addition, since the progress of the next generation sequencing (NGS) technology, it is much more economical to perform NGS directly.

13. Spell out BWA and GATK?

Reply to Reviewer 1: We have spelled out the BWA (Burrows-Wheeler Aligner) and GATK (Genome Analysis Toolkit) in the manuscript. (Methods: Page 14, Line 33, 36)
Mutation analysis and control screening

14. Line 56, "PolyPhen-2 analyses (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org) were performed". Omit "analyses" after PolyPhen-2 and move to the end of the sentence.

Reply to Reviewer 1: Thank you very much for your suggestion. We have rewritten the sentence to “PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org) analyses were performed. (Methods: Page 14, Line 56)

15. Line 15, "whether the potential mutations in pathogenic genes co-segregated with the disease phenotype in these families". Genes are not pathogenic, but variants of genes can be.

Reply to Reviewer 1: The authors are grateful to the reviewer for pointing out this error. We have changed this sentence to “Sequence analysis was performed using ABI 3730 Genetic Analyzer for both affected cases and normal controls to identify whether the potential pathogenic variants in genes co-segregated with the disease phenotype in these families.” (Methods: Page 15, Line 17)

16. Line 26, Consider "ethnically matched controls" instead of "local controls".

Reply to Reviewer 1: Thanks for your suggestion. We have changed “local controls” to “ethnically matched controls”. (Methods: Page 15, Line 31)

Results

17. Line 56, "potentially pathogenic variants of MYO15A variants". Delete the second "variant" in this sentence.

Reply to Reviewer 1: The second "variant" has been removed. (Results: Page 16, Line 1)

18. What is meant by "Subsequent Sanger sequencing"? Never heard of the "subsequent" version of Sanger sequencing.

Reply to Reviewer 1: The “Subsequent” has been removed. (Results: Page 16, Line 33)

19. There is a Section 3.2 and a Section 3.3 but no Section 3.1.

Reply to Reviewer 1: We have removed the caption of “Section 3.2” and “Section 3.3”. (Results: Page 17, Line 17; Page 18, Line 20)

20. End of Section 3.2, remove the extra period after "audiometry".
Reply to Reviewer 1: The extra period after "audiometry" has been removed. (Results: Page18, Line 12)

21. Section 3.3. The authors state that "There were 136 reported MYO15A variants in individuals from the Middle East, which is the greatest number of MYO15A variants in all populations". Add a reference to support this statement.

Reply to Reviewer 1: Thanks for your suggestion. We have double-checked the reported MYO15A variants from all include references in our review. Then, we concluded that the reported number of MYO15A variants in individuals from Middle East, Southeast Asia, South Asia, Europe, South America and North America was 99, 92, 42, 23, 7 and 2, respectively (Figure 5). Therefore, based on our review, the reported number of MYO15A variants from Middle Eastern was largest. To make it clear, we have changed this sentence to “In our review, the reported number of MYO15A variants in individuals from Middle East, Southeast Asia, South Asia, Europe, South America and North America was 99, 92, 42, 23, 7 and 2, respectively, and the reported number of MYO15A variants from Middle Eastern was largest.” (Results: Page 18, Line 56)

22. Discussion, Human and mouse MYO15A/Myo15 have 67 exons not 66 exons. See Rehman et al., 2016. An additional protein coding, alternatively spliced exon was reported in Rehman et al., and it is located between the giant exons 2 and exon 3.

Reply to Reviewer 1: We have corrected the exons number of MYO15A/Myo15 in the manuscript. (Discussion: Page 19, Line 55)

23. At least on function of the large N-terminus of myosin 15 was reported in Fang et al., 2016, eLife PMID: 26974472. The 133-kDa N-terminal domain enables myosin 15 to maintain mechanotransducing stereocilia and is essential for hearing.

Reply to Reviewer 1: We thank reviewer the constructive comments and suggestions. The function of the large N-terminus of myosin 15 has been added in discussion section. (Page 20, Line 58)

24. Line 56, "FERM" not "ERM".

Reply to Reviewer 1: We have corrected it.” (Page 20, Line 6)

25. What is ADNSL? Spell out and correct it.

Reply to Reviewer 1: ADNSHL means autosomal dominant non-syndromic hearing loss. We have corrected it in the manuscript. (Page 23, Line 14)

26."IQ" not "IQ3. Correct in the text and figures

Reply to Reviewer 1: We have corrected "IQ3 to "IQ" in the text and figures. (Page 20, Line 1; Page 22, Line 36; Figure 2, Page 46; Figure 4, Page 48 ; Table 2, Page 43 line 38)
27. In the section on "Availability of data and material" Who decides if a request is "reasonable"? Where can one find the written guidelines used to establish a request as "reasonable" or "unreasonable"? Are there guidelines for sharing that authors must abide by if you publish in BMC Medical Genetics? I suggest deleting the word "reasonable".

Reply to Reviewer 1: We have removed the word "reasonable". (Page 25, Line 36)

28. The Acknowledgement section of the manuscript is duplicated (i.e there are two copies).
Reply to Reviewer 1: We have deleted the duplicated acknowledgement section. (Page 24, Line 1)

29. Many of the references are not formatted correctly for BMC Medical Genetics. The first letter of each word of a journal name is capitalized.

Reply to Reviewer 1: We have corrected the format of references in the revised version of the manuscript. (Page 26-38)

30. The font size for words and variants in the pedigree figure are way too tiny to read. There is plenty of room to use a much larger font size.

Reply to Reviewer 1: We have revised the font size for words and variants in the pedigree figure. (Page 50, Figure S1)

31. Figure 2b, The word "Exon" on the left side above the schematic drawing doesn't make sense. Put it either above exon 1 or remove it.

Reply to Reviewer 1: We have remove the word "Exon" in Figure 2b. (Page 46)

32. Figure 2c; correct spelling of "phenotype" not "pheontype".

Reply to Reviewer 1: We have corrected the spelling of "phenotype" in figure 2c. (Page 46)

33. Figure 3, State in the legend the meaning of the numbers after the name of each country.

Reply to Reviewer 1: We have stated the meaning of the numbers after the name of each country in Figure 3 legend. The meaning of the numbers after the name of each country is the number of reported MYO15A variants with a milder auditory phenotype of each country in different periods. (Page 40, line 20)

34. Figure 3 legend "The number of previously reported MYO15A variants with a milder auditory phenotype in four periods" which is referred to as less serious in the figure.

Reply to Reviewer 1: Thanks for your question. Generally, serious auditory phenotype of MYO15A variant is bilateral congenital or pre-lingual severe to profound hearing loss in whole frequencies, whereas several auditory phenotypes are considered as the milder auditory phenotype, such as mild /moderate /moderate-to-severe hearing loss, progressive post-lingual
deafness and severe hearing loss with a typical slope toward high frequencies (residual hearing at low frequencies). (Page 40, line 14)

35. Figure 4 legend, for "variants without known zygosity", consider "variants of unknown zygosity".

Reply to Reviewer 1: We have changed "variants without known zygosity" to "variants of unknown zygosity". (Page 40, line 49)

Xiaodong Jiao (Reviewer 2):

1. In this study the authors assess the Genotype-phenotype correlation analysis of MYO15A variants in autosomal recessive non-syndromic hearing loss. The project is well conceived, the discussion is complete and concise. Specific comment follow:

from prediction information the missense variant p. Arg2924His seems a polymorphism, even this variant was absent in 200 normal Chinese controls, it is still probably not a disease-causing mutation, the authors need carefully checking the genotyping if there is another variant causing disease to meet compound heterozygotes mutations in the family1507361.

Reply to Reviewer 2: Thanks for your suggestion and making a great effort to improve our paper. We have double checked the raw data of the genotyping in the family 1507361, and there was no other variant in the gene MYO15A. Based on the current data, we have not enough evidence whether the missence variant p. Arg2924His is pathogenic or not, further evidence such as functional study or another patient with this variant is necessary for the description of this variant.