Author’s response to reviews

Title: Associations of genetic variation in CASP3 gene with noise-induced hearing loss in a Chinese population: a case-control study

Authors:

Yinyin Wu (48037663@qq.com)
Juntao Ni (674457518@qq.com)
Mingjian Qi (1007990279@qq.com)
Chengjian Cao (2504493086@qq.com)
Yuxian Shao (hyde.hishi@163.com)
Liangwen Xu (13588118965@163.com)
Haiyan Ma (mhynym@163.com; hyde.hishi@163.com)
Lei Yang (yanglei62@hznu.edu.cn)

Version: 1  Date: 09 Mar 2017

Author’s response to reviews:

Dear David Ozonoff,

Thanks for the copy-editing of our manuscript entitled "Associations of genetic variation in CASP3 gene with noise-induced hearing loss in a Chinese population: a case-control study" (ENHE-D-16-00364). We truly appreciate the in-depth criticisms and constructive comments from the reviewers. Based on reviewers’ comments, the revised manuscript has been carefully proofread. The attached please find a detailed reply to reviewers’ comments. And changes to the original manuscript are marked with 'tracked changes'. If you need any more information concerning the manuscript please do not hesitate to contact us.

We are looking forward to hearing from you.
With best regards,
Sincerely yours,
Yinyin Wu

Following are our replies to the queries.

Reviewer #1:

Comment 1: I suggest to check plagiarism and revise the sentences in all the text, especially in the abstract, method and conclusion.

Answer: We have followed the suggestion and revised the sentence in all the text. Please see line 2–4, paragraph 1, line 1, 2, 5–8, paragraph 2, line 1–6, paragraph 3, line 2, paragraph 4 of the abstract section, line 2, 5–6, 8–13, paragraph 1, line 5–7, paragraph 2, line 1–3, paragraph 3 of the participants section, line 1–6, paragraph 1 of the physical examination and epidemiological investigation section, line 1, 5–7, paragraph 1 of the statistical analysis section, and line 2, paragraph 1 of the conclusion section.

Comment 2: Please specify if there is a quality criterion for genotyping error rate per patient and per SNP?

Answer: We have described the quality criterion in the method section. Please see line 10–11, paragraph 3 of the SNP selection and genotyping section.

Comment 3: Some remarks to subjects recruitment. As usually, the noise-exposed subjects with average of binaural HL in high frequency > 40dB indicates hearing loss, which is a precondition of diagnosis of occupational noise-induced hearing deafness. So, why this study use the definitions of case as "the average of binaural HL in high frequency > 25dB"?

Answer: We adopted this definition of cases based on some previous studies (e.g. 1. Prevalence of hearing loss and differences by demographic characteristics among US adults: data from the
Comment 4: Control have normal hearing. I suppose, that low frequency hearing loss in study group was excluded? I suppose that non-occupational reasons of hearing loss (mainly in high frequency) were also excluded (e.g. tumors, autoimmunological diseases and others). But the author take some non-occupational reasons (listening to music, telephone using and sleeping later) as the risk factors of NIHL, it is not reasonable. Please explain it.

Answer: The main objectives of our study was to explore the association between the genetic factors and NIHL. And hearing loss in high frequency is an early indicator for NIHL. Thus, those with high frequency hearing loss were selected as cases, regardless of whether or not they have low frequency hearing loss. Besides, those non-occupational reasons we excluded (e.g. tumors, autoimmunological diseases and others) are the causes of hearing loss. Some studies indicated that the other non-occupational reasons (e.g. listening to music, telephone using and sleeping later) may be the risk factors of NIHL. They could affect the association between the genetic variations and NIHL, and that’s why we included those factors in our study.

Comment 5: The Chinese Diagnostic Criteria of Occupational NIHL (GBZ49-2007) was abolished and the new set is GBZ49-2014. Please note it.

Answer: We did use the GBZ49-2014, and made a mistake in our manuscript. This mistake has been corrected. Please see line 4, paragraph 1 of the audiological status assessment and environmental noise measurement section.

Comment 6: In the part "SNP selection and genotyping", the author selected SNPs according to "reported in previous studies", please cited the references.
Comment 7: The author was suggested to performed Bonferroni correction to control for multiple testing.

Answer: Bonferroni correction has been performed for multiple hypothesis testing. The results have been added, and the sentences have been corrected. Please see line 1, paragraph 3 of the abstract section, line 15, paragraph 1 of the statistical analysis section, line 3, 4, 6, 10–12, paragraph 2, line 4, paragraph 4 of the results section, and line 3 of the footnote of table 4.

Comment 8: Table 1. Noise intensity means noise Lex8h measured during the experiment? Dose CNE was calculated using this value and years of employment? But if the worker was employed at several workplaces which varied with the exposure during the whole-life? It is not clear to the reader how cumulative noise exposure is defined, please add the calculation method of CNE in the method.

Answer: Noise exposure was evaluated with continuous dB(A)-weighted sound pressure levels (Lex.8h) according to the National Criteria of Measurement of Noise in the Workplace (GBZ/T189.8-2007) (China, 2007). Cumulative noise exposure (CNE) was calculated as CNE=Lex.8h+10logT, where T means years of noise exposure. The calculation method of CNE has been added in the method. Besides, the main objectives of our study was to explore the association between the genetic variations and NIHL, and the cases we have included are those with normal hearing before exposure. Considering the fact that most of the participants of our study have not changed the workplace, and it was difficult to investigate the noise exposure history during the whole-life of the participants, thus we used the information of noise exposure of their current working place. Please see line 4–6, paragraph 2 of the audiological status assessment and environmental noise measurement section.

Comment 9: For noise is the most frequent cause of NIHL, I suggest to conduct stratified analysis by noise intensity and CNE.
Answer: We have conducted stratified analysis by noise intensity and CNE, and the results have been added. Please see line 9–10, paragraph 1 of the statistical analysis section, and line 1–9, paragraph 3 of the results section.

Comment 10: In order to further analyze, the authors are suggested to analyze the effects of haplotypes of associated SNPs on NIHL and/or the interactions between SNPs.

Answer: Considering that we have chosen different SNPs in different genes in this study, we adopted the multifactor dimensionality reduction (MDR) analysis to explore potential gene-gene interaction. And the results have been added. Please see line 10–13, paragraph 1 of the statistical analysis section, and line 11–12, paragraph 4 of the results section.

Comment 11: The discussion is too long. Particularly, the paragraphs on the function of the candidate genes should be shortened, or at least move parts of it to the introduction.

Answer: The paragraphs on the function of the candidate genes has been shortened according to the advice by the reviewer. Please see line 1–5, paragraph 2 of the discussion section.

Comment 12: Please explain all abbreviations in the text and in the abstract.

Answer: According to the advice by the reviewer, all abbreviations in the text and in the abstract have been explained. Please see line 1–5, paragraph 1 of the abbreviations section.

Comment 13: Replication of the results is needed and could be included in this section.

Answer: We have followed the suggestion and revised the sentences. Please see line 3–4, paragraph 1 of the conclusion section.
Reviewer #2:

Comment 1: The author should induce which people were defined as "noise exposure workers"? Noise exposure level of 8h/day or 40h/week no less than 80 dB(A) and so on.

Answer: The definition of noise exposure workers has been added according to the advice by the reviewer. Please see line 7–8, paragraph 1 of the participants section.

Comment 2: Whether the noise exposure workers exposed to other occupational hazards such as heat, CO…, the author should add these information.

Answer: None of the workers was exposed to other occupational hazards. And the sentence has been corrected according to the advice by the reviewer. Please see line 5–6, paragraph 1 of the participants section.

Comment 3: Whether these subjects enrolled had a normal hearing in the pre occupational health surveillance?

Answer: Our participants enrolled had a normal hearing before exposure based on results of their pre occupational health examination. Please see line 2, paragraph 2 of the participants section.

Comment 4: The cases in this study were defined as hearing threshold worse than 25 dB at high frequency, however, whether the hearing threshold at low frequency (500, 1000, 2000Hz) worse than 25dB? Or whether these hearing loss workers were all induced by noise?

Answer: The main objectives of our study was to explore the association between the genetic variations and NIHL. And hearing loss in high frequency is an early indicator for NIHL. Thus, those with high frequency hearing loss were selected as cases, regardless of whether or not they have low frequency hearing loss.
Comment 5: In table 2, the noise intensity exposure of the cases was "83.65(80.00-87.40)", there may be many cases exposed to the noise intensity exposure below 80 dB(A). However, the noise exposure environment was defined as the noise intensity exposure more than 80 dB(A) (GBZ/T224-2010).

Answer: As mentioned in the discussion section, we use factory, fixed-point measurement of noise level for individual noise exposure values due to limited conditions. Thus, there may be cases who were exposed to the noise intensity exposure below 80 dB(A). And this is also one limitation of our study which may have affected the accuracy of the results.

Comment 6: The unit of the hearing threshold is dB, not dB(A). The author should revised these units in the manuscript.

Answer: We have followed the suggestion and revised these units in the manuscript. Please see line 3, 5, paragraph 2 of the participants section.