Author’s response to reviews

Title: Effect of GRM7 Polymorphisms on The Development of Noise-Induced Hearing Loss in Chinese Han workers: A Nested Case-Control Study

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Author’s response to reviews:

Dear editors and reviewers,

Thank you very much for offering us an opportunity to revise and improve our manuscript. The comments and suggestions on this article are very good and we have learnt a lot in the process of revision. In addition, some opinions also gave us a new inspiration not only for this paper but also for the research design of our subject. We have taken every question in this paper seriously and try our best to perfect this article. There might still be some places where we still have not completed well and we hope that you can give us more advice. We cherish this chance that you have given us very much, but at the same time, we sincerely hope that we have not trouble you too much. If you have other suggestions on this article, please do not hesitate to mention them to us. We are very happy to communicate with you and improve our work. And now, we resubmit our manuscript entitled Effect of GRM7 Polymorphisms on The Development of Noise-Induced Hearing Loss in Chinese Han workers: A Nested Case-Control Study for your vetting.
Yours sincerely,

Shanfa Yu

The responses are as follows:

Isabelle Schrauwen (Reviewer 1): This study explores the effect of metabolic glutamate receptor7 gene (GRM7) polymorphisms on the susceptibility to noise-induced hearing loss (NIHL). Though the study lacks in genetic methodology (only 5 SNPs were analyzed in one candidate gene, and no population stratification seems to have been assessed), the population and longitudinal data collected is very interesting.

I have a few specific comments:

- 'In the process of selecting cases and controls, subjects were excluded if they had a history of ear disease, ear trauma, ototoxic drug use or feigned deafness, exaggerated deafness, toxic deafness and so on.' Please specify all information gathered from study participants relevant to hearing in a supplementary table. Especially all exclusion criteria that were used to reduce confounding.

Response:

Thank you very much for your good comments. Due to the issue of copyright, we could not offer all hearing information of the study participants in an attached supplementary table, but on the other hand we also think it is necessary to provide relevant data. Therefore, we make a summary of the information used as exclusion criteria and add to this paper. The corresponding information is as follows:

“In the cohort, there were 9 individuals with a history of being an airman, 76 with a history of being an artillerist, 53 with a history of head trauma, 3 with a history of blast exposure hearing damage, 10 with a history of eardrum perforation, 1 with a history of taking ototoxic drugs, 32 with a familial history of deafness, 15 with a history of rubella, 4 with a history of Meniere's syndrome, all of which were excluded in the case and control selecting.”

- 'The inclusion of cases was that binaural average hearing threshold levels (HTLs) in high frequencies (3kHz, 4kHz, 6kHz) ≥ 40 dB(A).' As multiple hearing evaluations were done, please specify if the most recent measurement was used or not. Is it possible to analyze the progression of hearing loss in the 'case' and 'control' groups and add this information to the paper?

Response:
Thank you very much for your comments. Firstly, this study was conducted after the most recent hearing measurement finished. As for the comment of analyzing the progression of hearing loss in the case and control groups and adding this information to the paper, we have done a detailed analysis in another paper. Therefore, it may not be appropriate to do the same work here.

- ‘Fig.2 Best model gained by the analysis of GMDR. The left bars represent the sum of scores in case and the right represent the control. High risk cells are expressed by black shadow, low risk cells by light shadow and empty cells by no shadow.’ Is it possible to add an interpretation of the high risk cells for a more lay audience?

Response:

Thank you very much for your good suggestion and the interpretations about the high risk cells, low risk cells and the empty cells have been added in the paper in the legend of figure 2.

- Was anything done to assess population stratification between case and control groups?

Response:

Thank you very much for your good suggestion. The evaluation of the population stratification between the case and control groups has been done in the matching process of the case and control groups and the detailed information about the assessment can be seen in table 1. We have evaluated the age in different stages, gender, working years, cumulative noise exposure of individuals, binaural average hearing threshold level in high frequencies, environment noise exposure, smoking, drinking and hypertension between the case and control groups.

- Why was only GRM7 considered for analysis? If you are considering a candidate gene approach there are many genes suggested as NIHL candidates.

Response:

Thank you very much for your good suggestion. Our research group has done a series of work to detect the genes which may be associated with noise-induced hearing loss by candidate gene approach and many genetic discoveries have already been published. However, the founding about the association of the GRM7 and the susceptibility to noise-induced hearing loss is a part of them and researches on this hand are rare. That’s why we only considered GRM7 for analysis. In addition, we have conducted a comprehensive analysis on the association of candidate genes and the development of NIHL through the candidate gene approach in another paper.

Have any other genes been analyzed in the past in this same set? If yes, please add that to this manuscript to give an overview of what has been found.

Response:
Thank you very much for your good suggestion. Other genes, like heart shock protein (HSP70), eye absent homolog 4 (EYA4), POU-domain transcription POU4F3 and Grainyhead-like2 (GRHL2) had been analyzed in the same set before and the information of these genes has been added in this manuscript.

I would recommend to review the English language of this manuscript to improve its grammar and fluency.

Thank you very much for your kind suggestion. We have revised the language in this manuscript carefully.

Daniela Zanetti (Reviewer 2): Comments to the Author

The authors present an interesting study about the effect of metabolic glutamate receptor7 gene polymorphisms (GRM7) on the susceptibility to noise-induced hearing loss (NIHL). The results suggest that the CC genotype of rs1485175 in GRM7 may reduce the susceptibility of individuals to NIHL in Chinese Han population.

The analysis is generally well-conceived and executed, and the paper is well written and easy to follow. However, the sample size for the association analyses is very limited (292 cases and 584 controls) and the study needs of additional analysis in order to validate the results.

Major comment.

¥ pag 9 The continuous variables were implemented tests of normality and all of them were not in accordance with normal distribution, hence they were expressed by the median (range) and the differences between groups were analysed by Wilcoxon rank sum test. Did the authors consider to rank-transform the variables to normality? The authors could use the rntransform function in R and after that, rerun all the analysis and compare the results.

Response:

Thank you sincerely for your good suggestion. The rank-transform function had been considered in the process of data analysis when the continuous variables were not in normal distribution. However the purpose of variable analysis in this stage was to verify the matching effect of this nested case-control study and the difference between the case and control groups was not statistically significant which meant that the matching was successful. What’s more, the logistic regression analysis, the main analysis method of this study, does not have strict requirements on variables and the basic variables were used as covariance correction in the process of analyzing the association of GRM7 polymorphisms and the susceptibility to NIHL. Hence, we did not convert the continuous variables which didn’t fit the normal distribution to normality.

¥ Considering the 10 years of follow-up (January 1, 2006 - December 31, 2015) it would be interesting to run time-to-even association analysis (Cox's proportional hazards model) in order to analyze the association of the incidences events during the 10 years of follow-up.
Thank you very much for your good comments. The threshold of p value after Bonferroni correction has been clarified in the annotations under the table 3 and table 4 where the multiple comparing by pairs was conducted and the adjusted p values (pbon) have also been corrected. Specifically, in the effect analysis of the genetic models and the risk of developing NIHL, the p values are adjusted by means of 0.05/5 taking the 5 SNPs into consideration; in the process of exploring the relationship between rs1485175 and NIHL after stratified by CNE with a cut-off point of 97 dB (A), the significant p value are corrected by 0.05/2.

Minor comments:

Abstract: The background section is actually an aims section. The author should summarize important results outlined by others in the same field, critically evaluating existing knowledge. They should also identify gaps that this paper is intended to fill.

Response:

Thank you very much for your good advice. The content in the background of the abstract has been supplemented according to your suggestion.
The authors should revise carefully the language, for example:

Pag 2: Bacakground

Pag 8: locis

Response:

Thank you very much for your good suggestion. We have seriously revised the language of this article all over again.

In Table 2, MAF and genotype data comes from NCBI dbSNP and 1000 Genomes Browser (CHB). It would be interesting to provide the same information using their dataset of Chinese Han population.

Response:

Thank you very much for your good suggestion. The data of Allele in table 2 derives from Han Chinese in Beijing (CHB) in 1000 Genomes Browser which presents the general level in Chinese Han population. What’s more, smaller allele frequencies of the alleles are the minor allele frequencies (MAFs) in Chinese Han population. The information of MAF in table 2 comes from NCBI dbSNP which presents the general level of minor allele frequencies in population. Therefore, we can also see the difference of the MAF in Chinese Han population and in population.