Author’s response to reviews

Title: Screening of noise-induced hearing loss (NIHL)-associated SNPs and the assessment of its genetic susceptibility

Authors:

Xuhui Zhang (994028847@qq.com)
Yaqin Ni (aileenyaqin@126.com)
Yi Liu (lyzju_2012@163.com)
Lei Zhang (719453275@qq.com)
Meibian Zhang (156890673@qq.com)
Xinyan Fang (471232298@qq.com)
Zhangping Yang (1229678689@qq.com)
Qiang Wang (hz1618@qq.com)
Hao Li (57308163@qq.com)
Yuyong Xia (1317668108@qq.com)
Yimin Zhu (zhuym@zju.edu.cn)

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Author’s response to reviews:

Reviewers’ comments and response

Reviewer #1:

This is a very interesting paper with the aim to create genetic risk evaluation and prediction for NIHL among workers. The approach is ambitious and well preformed.

However, some questions needs to be addressed and the results also have ethical considerations that have to be taken, if this kind of screening should be made general. Below please find my comments and questions.

Abstract
1. It needs to be clarified that it was 476 subjects with NIHL and 476 controls (if that was the case- see further below)

Response: done.

Introduction

2. Line 16; the National Institute…

Response: done.

3. Line 23; exposure, other risk factors and genetic risk factors. Com; Not only genetics influence the level of NIHL also other exposures like solvents, medication and vibration as well as life style factors and different diseases can influence NIHL.

Response: done.

4. Line 42, study populations were generally small ADD references to this

Response: done.

5. From Line 45 and forward; the end of the introduction is more a description of methods and results and should not be here. This part needs to be more general and end with WHY you did the study NOT how.

Response: done.

Methods

5. 2.1 - Line 35 and Figure 1; it is unclear what is meant by epidemiological examination??

Response: “epidemiological investigation” means to collect the epidemiological data such as individual life style factors (smoking and drinking status, and others), histories of occupational and non-occupational noise exposure, and other exposure to solvents, medication, and vibration as well as and different diseases.

6. 2.2 - Line 9-11. What does gender mean here? More detail is needed about how controls were selected.
Response: In the cross-sectional survey of occupational noise-exposed worker, male and female workers were included in this investigation. However, the majority of the subjects were male worker (about 91.7%), therefore, the subjects of this case-control study were restricted on male workers. This has been explained in our previous papers.

8. 2.3 - Line 48 More detail of this cohort is needed. How many in total etc… Line 1-3; The same SNPs??, which were genotyped in XXX? of the subjects in the follow-up group. Com; Were all SNPs tested of which in all subjects or some??

Response: After cross-sectional study, additional 584 subjects with normal hearing at baseline were further followed up, and the incidence of NIHL and the change of noise exposure were further collected. Only the SNPs, which were screened in the case-control study, were genotyped for these subjects.

9. 2.4 - Line 38-48 More details is needed about HOW the GRS was constructed preferably with an example for readers not familiar with the method. Especially how the SNPs that decreased the risk for NIHL were treated in this respect??

Response: the formula to calculate GRS was added in the text.

GRS was calculated as the sum of the number of risk alleles (0, 1, 2 for additive model and 0, 1 for the dominant or recessive model, dependent of the most appropriate model). If the mutant allele of a SNP decreased the risk of NIHL, then, the wild allele of this SNP was the risk allele.

Results

10. 3.1 - Line 21 CHANGE TO no statistical significant difference between NIHL subjects and controls regarding smoking….noise intensities (P > 0.05)

Response: done.

11. 3.1 Second page - Line 21-23 This sentence belong to the Method section see comment 9 above.

Response: done.

12. 3.2 I like your figures they are clear and explanatory.

Response: more detailed explanatory was added in Figure legends.
13. 3.3 second page - Line 1 and Table 3. The heading of the Table is wrong as it refers to the cohort study

Response: this is correct. Table 3 presents the odd ratios (ORs) and 95% CI of NIHL– associated SNPs, which were validated in the cohort study.

14. 3.3 second page - Line 21 Divide Table 3 into two tables as the GRS results are of different character. Com; Were the GRS in the follow-up subjects based on the same SNPs as in the cohort study, even if the associations were not the same???

Response: Table 3 presents the risk of NIHL– associated SNPs or different values of GRS in the cohort study. These associations were based on the same subjects in the cohort. Therefore, we revised the heading of the table 3 as “Odd ratios (ORs) and 95% CI of NIHL– associated SNPs or GRS in the cohort study”. The SNPs in the table 3 was the SNPs which were genotyped in the cohort study. These SNPs came from NIHL-associated SNPs screened from the case-control study.

Discussion

15. Line 25; References needed

Response: done.

16. Second Page Line 23-30; This line of discussion should be more elaborated. Which ethical considerations has to be taken if this kind of screening should take place? Should workers with certain SNPs not be allowed to have certain jobs? This is not an easy question and the associations you found are interesting but by no means certain?? More studies is needed before a general screening can be suggested as prevention against NIHL!

Response: It is relatively difficult to avoid noise exposure under most occupational environments. Therefore, preventative measures for high-risk populations are essential. In this case, primary prevention (for etiological factors) is an effective and efficient measurement. Once screening and recognizing the susceptible individuals, we could take the measurements such as appropriate job selection, decreasing noise exposure, and strengthen protection (Putting on ear plugs or helmet) in noise environment in order to reduce the risk of NIHL.

17. Second Page Line 33; What did your earlier investigation show?

Response: we have added the results of this study.
18. Second Page Line 38 and following. Discussion about the potassium channel genes are interesting and could be more elaborated and clearer if the results of the cited studies were mentioned briefly.

Response: we added some results and more explanation.

19. Third Page Line 16 Suggestion NIHL after noise exposure based on multiple SNP loci. One of the strengths of the study is that our preliminary established NHL risk prediction model using 14 SNPs to screen for High risk NIHL was partly validated in the follow-up study cohort using NIHL incidence over a 5 year period. However the sample size in the prospective cohort was relatively small and the follow-up time was relatively short, thus false negative results may exists. Further studies using both men and women as well as a larger sample size should be performed to validate the results.

Response: Thank very much for your words! We have incorporated in the revised version.

Once again, thank you very much for your careful reviewing and providing relevant and positive comments and suggestion.

Reviewer #2: This study screened the noise-induced hearing loss (NIHL) associated SNPs, constructed genetic prediction models, and further validated with a prospective cohort. This study was well-designed with large sample in this filed. The findings provide new evidence of genetic susceptibility in NIHL and have significance on the prevention and control of NIHL.

1. Please describe how to select 83 candidate SNPs in this study?

Response: as described in 2.2, a total of 83 candidate SNPs in the 25 genes were selected as candidate SNPs in this study. These candidate SNPs were selected based on the HapMap database and previous reports [7,8, 11, 13]. The inclusion criteria for searching for tag SNPs were minor allele frequency (MAF) in CHB > 0.05 and a linkage disequilibrium value of r2 > 0.8.

2. Why age is not matched between cases and controls?

Response: there are two reasons: 1. hearing threshold at each frequency has been adjusted according to the age of individual subject. Therefore, hearing threshold does not effect by individual age. 2. Years of noise exposure has been regarded as a matching factor in control recruitment. There will be increasing the difficulty in control recruitment. Although there was a different distribution of age between NIHL subjects and controls, a multiple logistic regression model was used to control confounding factors.
3. In the case-control study, there are 476 NIHL cases and 476 controls. In the validation study, there are 584 subjects without NIHL at baseline. Are 476 controls in cross-sectional study part of the 584 subjects in validation cohort?

Response: No. the subjects of 584 in the cohort study were the normal hearing at baseline and recruited from the cross-sectional study. But, these subjects were different from the 476 controls in case-control study. Independent sample in the cohort study was to validate the results from the case-control study.

Reviewer #3: 1) The authors designed and conducted a matched case-control study to screen noise-induced hearing loss associated SNPs and to assess noise related genetic susceptibility and validated the results in a prospective cohort study. It is a reasonable study except the results may be limited due to not a big sample size and a few questions need to be cleared up before getting published. Age is an important risk factor for NIHL. I wonder how the variable age (for example, as a continuous or categorical variable) was adjusted in the final models. Is there any confounding residue or effect modification by age considered in this analysis? How were variables of CNE, smoking and drinking adjusted in the final models? What factors were matched in this matched case-control study?

Response: Thank you very much for your careful reviewing and positive comments. In the NIHL-associated SNP screening, a matched case-control study was used. The matching factors included gender-, intensity of noise-, and years of noise exposure. In this study, age was not regarded as the matching factor. There are two reasons: 1. hearing threshold at each frequency has been adjusted according to the age of individual subject. Therefore, hearing threshold does not effect by individual age. 2. Years of noise exposure has been regarded as a matching factor in control recruitment. There will be increasing the difficulty in control recruitment. Although there was a different distribution of age between NIHL subjects and controls, a multiple logistic regression model was used to control confounding factors.

2) Some grammar errors need to be corrected before getting published.

Response: the revision has been professional edited.

Reviewer #4: Comments to the Author:

Based on the continuation of two previous studies based on a cross-sectional investigation (Zhang et al. 2014; Zhang et al. 2015), This work reported the screening of noise-induced hearing loss (NIHL)-associated SNPs and the assessment of its genetic susceptibility. The authors found that Seven SNPs were significantly associated with the risk of NIHL, and positive correlation between GRS values and odds ratio (OR) for NIHL was also observed. Subjects with higher GRS showed a higher risk of NIHL incidence. Furthermore, two SNPs, rs212769 and
rs7910544, were validated in the cohort study. The topic is of interest since NIHL is a worldwide occupational health risk and a common sensorineural hearing loss, and genetic susceptibility plays important roles but its mechanism remains unclear. However, the following should be addressed:

Major Comments

1. The research progress is not adequately described in the section of Introduction, making it difficult for readers to understand the progression of research on SNPs in NIHL. Besides, the references are dated. The authors need to read more advanced related articles and make a good summarization.

2. The authors state that subjects with 476 NIHL and matched controls were recruited from a cross sectional survey on NIHL in China. Please provide more information about inclusion criteria of the two groups.

Response: In case-control study, the subjects including NIHL cases and controls were recruited from a cross-sectional study. NIHL was defined using the following criteria: the workers with normal hearing before exposure, >1 year of occupational noise exposure, and a HTHF > 40 dB of the hearing level (HL). To exclude workers whose hearing loss was caused by factors other than noise, the worker was excluded from the study when the difference of HTHF between the left and right ears was > 35 dB (HL).

Healthy controls were gender-, intensity of noise-, and years of noise exposure-matched with NIHL cases and expose at the same noise environment.

3. Is there any difference between occupational and non-occupational NIHL, and how to make sure there is no detrimental effects form occupational risks in the workplace to the subjects with NIHL.

Response: There may be overlapped and different genetic susceptibility between occupational and non-occupational NIHL. This study mainly focused on occupational NIHL. In this study, we have investigated the exposure status of other detrimental factors with questionnaire–based interview and excluded the subjects with these exposure.

3. Detailed information on the occupation health conditions in the workplace should be provided in the section of Methods.

Response: Annual health examinations, including routine physical examination, pure tone audiometry (PTA), and epidemiological investigation were administered for each subjects in the
cross-sectional study. The hearing capacity was assessed according to PTA. The prevalence of other chronic diseases was also collected with questionnaire-based interview.

5. Given that NIHL is the second most frequent form of sensorineural hearing loss, after age-related hearing impairment (ARHI). In the present study, the mean age of the NIHL subjects was significantly older than the control individuals. Is there the possibility that age participates in the hearing impairment?

Response: The controls in this case-control study were recruited with matching to NIHL cases according to the factors of gender, intensity of noise, and years of noise exposure. In this study, age was not regarded as the matching factor. There are two reasons: 1. hearing threshold at each frequency has been adjusted according to the age of individual subject. Therefore, hearing threshold does not effect by individual age. 2. Years of noise exposure has been regarded as a matching factor in control recruitment. There will be increasing the difficulty in control recruitment. Although there was a different distribution of age between NIHL subjects and controls, a multiple logistic regression model was used to control confounding factors.

Minor Comments

1. Remove the repeated words in Acknowledgments 'This study was supported by … supported this study'.

Response: done.

2. In the cross-sectional study, a total of 476 occupational workers were recruited, while 565 subjects were followed in the cohort study. What is the relationship of the subject between cross-sectional and cohort study?

Response: Case-control study included 476 NIHL cases and 476 control were included in case-control study while 486 subjects with normal hearing were included in cohort study. Both of these subjects did not overlapped and recruited from the cross-sectional study. The flow chart of subject recruitment was presented in figure 1.

3. Is there any confounding factors that may interfere the following results of hearing loss in the cohort study?

Response: Not only genetics influence the level of NIHL also as life style factors (smoking and drinking status, etc.) and other exposures like solvents, medication and vibration and different diseases (such as ear diseases) can influence NIHL.
In this study, in order to control the effect by other ear diseases, the worker was excluded from the study when the difference of HTHF between the left and right ears was > 35 dB (HL). Age was controlled with age-adjusted Hearing threshold. Matched case-control study was to control the potential confounding bias by gender, intensity of noise, years to noise exposure. Furthermore, multiple logistic regression was used to control the confounding bias by age, smoking and drinking status, and cumulative noise exposure (CNE).

However, the limitation is no adjustment to the disease histories of hyperglycemia and dyslipidemia. Both of the abnormality were recently found to be associated with the hearing capacity.

Thanks again for all the reviewers and your excellent work.