## Letter to the Editor

# Gene-Gene Interaction of GJB2, SOD2, and CAT on Occupational Noise-induced Hearing Loss in Chinese Han Population

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**The effects of genetic factors on the noise-induced hearing loss (NIHL) are still unclear. In the present study, eight single-nucleotide polymorphisms (SNPs) included rs1227049 and rs3802711 (CDH23), rs1695 (GSTP1), rs137852540 (GJB2), rs2289274 (PMCA2), rs4880 (SOD2), rs7943316, and rs769214 within CAT that might associated with NIHL were further validated in Chinese workers. The results showed that the carriers of the T allele (AT+TT) of rs7943316 and A allele (GA+AA) of rs769214, were significantly associated with an increased risk of NIHL compared to those with AA genotype (*P*<0.05) and GG genotype (*P*<0.05). Moreover, a significant three-locus model (*P*=0.0107) involving rs2016520, rs9794, and rs1805192 were observed that might associated with NIHL, with 53.95% of testing accuracy. Thus, our present study provided the evidence that GJB2, SOD2, and CAT genes might account for the NIHL development in independently and/or in an interactive manner.**

Noise is the most important environmental factor and the most frequent occupational hazard. Long-term exposed to loud noise or excessively loud impulse noise might damage hair cells of Corti in the inner ear, and eventually cause noise-induced hearing loss (NIHL). It has been estimated that more than 600 million workers are exposed workplace noise worldwide, especially in countries with growing industrial activity. However, susceptibility to noise damages differs remarkably among individuals, indicating that NIHL is a complex disease and induced by a combination of environmental and genetic factors[1]. The association studies on the NIHL patients indicated that oxidative stresses genes SOD2, CAT, GSTP1, and GSTT1 as putative NIHL susceptibility genes in Swedish and Chinese workers[2-3]. Additionally, genes encoding proteins involved in the potassium recycling pathway such as GJB2 and Cadherin23 (CDH23) gene, a component of inter-stereocilia links on cochlear hair cells, were significantly associated with NIHL in different studies[4-5].

Until now, the genetic factors involved in NIHL have still not been well defined and many results from the previous studies cannot be replicated[6]. In order to further validate the associations in previously identified SNPs of those candidate genes with NIHL, we analyzed the SNPs in CDH23, GSTP1, GJB2, PMCA2, SOD2, and CAT genes, and gene-gene interactions among of them that might account for NIHL development in a group of Chinese workers occupationally exposed to noise.

This study was approved by the Regional Bioethical Committee at the Soochow University. Totally, 611 Chinese male workers (466 normal hearing workers and 145 NIHL workers) were gathered from an electric power station and a glass bottle factory in Suzhou, China. All of the participants had been continuously employed in the pants for at least three years. At their workplace, they were exposed to continuous and steady-state noise at the mean equivalent level Leq=84.5±3.01 dBA. All participants underwent an audiometric examination. Individuals were excluded from the study if they had a history of head trauma, exposure to ototoxic drugs or substances, or diseases known to cause hearing impairment (such as hereditary deafness, middle ear inflammation, meningitis, mumps, other viral infections).

Eight single-nucleotide polymorphisms (SNPs) included rs1227049 and rs3802711 (CDH23), rs1695 (GSTP1), rs137852540 (GJB2), rs2289274 (PMCA2), rs4880 (SOD2), rs7943316, and rs769214 within CAT were selected presently based on known heterozygosity and a minor allele frequency (MAF)

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>0.05. SNPs genotyping was determined by using the SEQUENOM Mass ARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, San Diego, CA).

Hardy-Weinberg equilibrium was assessed within controls using the goodness-of-fit $\chi^2$ test. The distribution of the general characteristics between NIHL subjects and non-NIHL controls was compared by using two-sided Chi-square test. The significance level was set at $P<0.05$. A linear regression model was used to estimate odds ratios (ORs) and 95% confidence intervals (*CIs*) for the association between genotypes and NIHL. For quality control purpose, Hardy-Weinberg equilibrium (HWE) test was used to detect genotype typing errors by Fisher's exact test. Linkage disequilibrium (LD) between polymorphisms was estimated by using SHEsis (available online at http://analysis.bio-x.cn). Furthermore, GMDR software (version 1.0.1) was applied for further detecting gene-gene interactions associated with NIHL. The testing balanced accuracy is the measure of the degree to which the interaction accurately predicts NIHL value with scores between 0.50 (indicating that the model predicts no better than chance) and 1.00 (indicating perfect prediction). Finally, a sign test or a permutation test (providing empirical *P* values) for prediction accuracy can be used to measure the significance of an identified model.

Of 611 male workers recruited in the present study, 350 subjects were selected from an electric power station and 82 persons were diagnosed as NIHL, 261 participants came from a glass bottle factory, and 63 of them were diagnosed as NIHL. No significant difference was observed between the persons from electric power station and glass bottle factory. Moreover, no significant differences of age (27.60±5.67 *vs.* 27.23±5.49 yrs), exposure time to noise (4.59±3.66 *vs.* 4.54±4.11 yrs) and exposure levels of noise (83.7±4.01 *vs.* 84.8±3.27 dBA) were observed between the subjects with or without NIHL.

As shown in Table 1, there was no significant difference of the SNPs distributions between NIHL persons and normal control. After adjustment for age, exposure noise level and exposure time to noise,

**Table 1.** Distributions of the Eight SNPs Genotypes in NIHL Persons and Normal Control

| Variabilities | | Individuals with Normal Hearing (*n*=456) | Individuals with NIHL (*n*=145) | *P* Value |
|---|---|---|---|---|
| | rs1227049 | | | 0.51 |
| CDH23 | GG/GC/CC | 220/200/36 | 62/71/12 | |
| | C (%) | 7.9 | 8.3 | |
| | rs3802711 | | | 0.81 |
| CDH23 | CC/CT/TT | 276/158/22 | 92/47/6 | |
| | T (%) | 4.8 | 4.1 | |
| | rs1695 | | | 0.15 |
| GSTP1 | AA/AG/GG | 293/153/10 | 92/45/8 | |
| | G (%) | 2.2 | 5.5 | |
| | rs137852540 | | | |
| GJB2 | AA/AG/GG | 256/166/34 | 84/54/7 | 0.55 |
| | G (%) | 7.5 | 4.8 | |
| | rs2289274 | | | 0.97 |
| PMCA2 | GG/GA/AA | 234/181/41 | 74/57/14 | |
| | A (%) | 9 | 9.7 | |
| | rs4880 | | | 0.78 |
| SOD2 | TT/TC/CC | 330/120/6 | 106/36/3 | |
| | C (%) | 1.3 | 2.1 | |
| | rs7943316 | | | 0.05 |
| CAT | AA/AT/TT | 223/197/36 | 57/69/19 | |
| | T (%) | 7.9 | 13.1 | |
| | rs769214 | | | 0.06 |
| CAT | GG/GA/AA | 223/196/37 | 57/69/19 | |
| | A (%) | 8.1 | 13.1 | |

the linear regression analysis results showed that the carriers of the T allele (AT+TT, dominant model) of rs7943316 and A allele (GA+AA) of rs769214, were significantly associated with an increased risk of NIHL compared to the carriers of the AA genotype (mean difference=1.48, 95% CI=1.01-2.16, *P*=0.04) and the carriers of the GG genotype (mean difference=1.48, 95% CI=1.01-2.16, *P*=0.04), respectively (Table 2). These results were similar to the previous studies. The findings from two large independent populations from Sweden and Poland reported that the polymorphisms of CAT gene are NIHL susceptibility genes and their effects can be detected if noise exposure levels are taken into account[7]. However, we were not able to validate the relationships between the risks of NIHL and the other six SNPs that previously published. Therefore, investigate replication is still needed to investigated the genetic associations with NIHL and extend them to different population.

NIHL is well known as a multi-factorial disease thus probably involving multiple SNPs in a variety of genes. The investigation of gene-gene interaction surely will expand our current understanding of gene-tics effects on NIHL. Therefore, we employed the GMDR analysis in the present study to assess the impact of the interaction among the eight SNPs, after adjustment for covariates including age, exposure noise level, and exposure time to noise. As the Table 3 shown, there was a significant three-locus model (*P*=0.0107) including rs137852540, rs4880, and rs769214, with the highest level of testing accuracy (53.95%) and showed a better cross-validation consistency (6/10). Thus, our results suggested that rs769214 of CAT, rs4880 of SOD2 and rs137852540 of GJB2 gene, might account for the increased risks of NIHL in Chinese male workers in an interactive manner.

It is well known that genes involved in regulating the reactive oxygen species manganese-SOD2 and CAT could influence cochlea vulnerability to noise, because noise induces the release of free radicals and may damage the cochlear sensorial epithelium[8-9]. In the present study, our data firstly suggested that GJB2 polymorphisms might account for the NIHL in an interactive manner. Usually, the mutations of GJB2 are generally recognized as risks for the individuals with human hereditary hearing loss

**Table 2.** Logistic Analysis for the Associations between SNPs and NIHL

| Genes and SNPs | Genotype | Individuals with Normal Hearing (*n*=456) | Individuals with NIHL (*n*=145) | *OR* (95%CI) | *P* Value* |
|---|---|---|---|---|---|
| CDH23 | | | | | |
| rs1227049 | GG | 220 | 62 | 1 | |
| | GC+CC | 236 | 83 | 1.00 (0.86-1.82) | 0.25 |
| rs3802711 | CC | 276 | 92 | 1 | |
| | CT+TT | 180 | 53 | 0.88 (0.60-1.30) | 0.53 |
| GSTP1 | | | | | |
| rs1695 | AA | 293 | 92 | 1 | |
| | AG+GG | 163 | 53 | 1.04 (0.70-1.53) | 0.86 |
| GJB2 | | | | | |
| rs137852540 | AA | 256 | 84 | 1 | |
| | AG+GG | 200 | 61 | 0.93 (0.64-1.36) | 0.7 |
| PMCA2 | | | | | |
| rs2289274 | GG | 234 | 74 | 1 | |
| | GA+AA | 222 | 71 | 1.01 (0.70-1.47) | 0.95 |
| SOD2 | | | | | |
| rs4880 | TT | 330 | 106 | 1 | |
| | TC+CC | 126 | 39 | 0.96 (0.63-1.47) | 0.86 |
| CAT | | | | | |
| rs7943316 | AA | 223 | 57 | 1 | |
| | AT+TT | 233 | 88 | 1.48 (1.01-2.16) | **0.04** |
| rs769214 | GG | 223 | 57 | 1 | |
| | GA+AA | 233 | 88 | 1.48 (1.01-2.16) | **0.04** |

***Note.*** *Adjusted for gender, age, exposure noise level, and exposure time to noise.

**Table 3.** Best Gene-Gene Interaction Models Identified by the GMDR

| Locus No. | Best Combination | Cross-validation Consistency | Testing Accuracy | *P* Value* |
|---|---|---|---|---|
| 2 | rs1227049, rs769214 | 4/10 | 0.5075 | 0.623 |
| 3 | rs4880, rs137852540, rs769214 | 6/10 | 0.5395 | 0.0107 |
| 4 | rs1227049, rs137852540, rs4880, rs769214 | 4/10 | 0.4891 | 0.9453 |
| 5 | rs3802711, rs1695, rs137852540, rs2289274, rs769214 | 4/10 | 0.4858 | 0.623 |
| 6 | rs3802711, rs1695, rs137852540, rs2289274, rs4880, rs769214 | 6/10 | 0.5118 | 0.1719 |
| 7 | rs3802711, rs1227049, rs1695, rs137852540, rs2289274, rs4880, rs769214 | 9/10 | 0.4870 | 0.623 |
| 8 | rs3802711, rs1227049, rs1695, rs137852540, rs2289274, rs4880, rs769214, rs7943316 | 10/10 | 0.4767 | 0.623 |

***Note.*** *Adjusted for gender, age, exposure noise level, and exposure time to noise.

but not for the hearing loss induced by noise[10]. Therefore, more investigations should be performed to identify the interaction among susceptibility genes for the pathogenesis of NIHL in future.

The limitations of this study should be considered. Similar to the previous studies, our findings might not be generalizable to other populations because of the racial specificity. Moreover, the present sample size was modest, although the number of study participants met the requirement for analysis. Independent replications in larger Chinese workers occupationally exposed noise are required to confirm the role of the gene-gene interaction detected in present study for NIHL.

In conclusion, the significant associations were validated between two CAT polymorphisms and NIHL susceptibility in Chinese male workers occupationally exposed noise. Additionally, our results firstly indicated that significant gene-gene interactions among GJB2, SOD2, and CAT that might account for the NIHL development. In order to understand the questions behind the observed associations, further genetic and functional studies focused on identification of the underlying mechanism of NIHL should be performed in future.

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