Noise-induced hearing loss (NIHL) is an important problem in countries with growing industrial activity, such as India and China. NIHL is the primary occupational disease in these countries and the second most common form of sensorineural hearing loss after age-related hearing impairment (ARHI)\(^1\).

NIHL is a complex disease caused by the interaction of environmental factors and susceptibility genes, and it can significantly affect the quality of social, familial and professional life\(^2\). The responsible environmental factors involved in NIHL have been well studied. NIHL is most often caused by regular and continuous exposure to noise and repeated acoustic trauma. The noise itself exerts metabolic effects or causes mechanical damage to the inner ear\(^3\). In addition, stimulation with various noise intensities leads to unique effects: adaptation, temporary threshold shifts, and incomplete recovery, which result in permanent damage and hearing loss [i.e., permanent threshold shifts (PTS)]\(^4\).

Previous exposure to nontraumatic noise can decrease the susceptibility to noise-induced PTS\(^5,6\). In addition, the synergistic interaction of noise and chemical exposure (e.g., organic solvents and ototoxic substances, such as aminoglycosides and carbon monoxide) has also been demonstrated to contribute to NIHL\(^7\). Environmental factors, such as heat and vibration, can also influence NIHL susceptibility\(^8,9\). Furthermore, some studies have demonstrated that individual behaviors, such as smoking and medical factors (elevated cholesterol levels, high blood pressure and pigmentation) may influence the severity of NIHL\(^10-13\).

It is commonly accepted that efforts to improve the
understanding of the genetic contribution to NIHL will lead to improvement in diagnosis and prevention of the disease\textsuperscript{144}. In animal models, it has been clearly demonstrated that genetic factors alter the susceptibility to NIHL. For example, C57BL/6J mice that carry the Mobile Sites Method-derived Ahl3 gene are more resistant to noise than regular C57BL/6J mice\textsuperscript{135}. In addition, several homozygous and heterozygous knockout mice have been found to be more susceptible to NIHL than their wild-type littermates, including knockout mice for plasma membrane Ca\textsuperscript{2+}-ATPase isof orm 2 (Pmca2), Cu/Zn superoxide dismutase 1 (Sod1), Cdh23 and glutamate peroxidase 1 (Gpx1). When it comes to human, only a few association studies on related genes have been performed. Some experiments have demonstrated that heat shock proteins (HSPs) may play a role in the vulnerability to NIHL\textsuperscript{166}. Van Laer et al\textsuperscript{13} confirmed that three single-nucleotide polymorphisms (SNPs) in KCNE1 had significant associations with NIHL susceptibility. Furthermore, a positive NIHL-protective association was observed in carriers of the GSTM1 gene\textsuperscript{17, 18}. Actually, noise-induced cochlear epithelium damage is a major cause of permanent hearing loss in noise-exposed workers. Experimental systems have demonstrated that noise may damage the cochlear sensorial epithium by inducing the local release of free radicals\textsuperscript{19}. Consequently, genes involved in the regulation of reactive oxygen species, such as PON genes, are obvious NIHL candidate genes.

PONs exert antioxidant activity. The paraoxonase gene family consists of PON1, PON2 and PON3, which are all present on chromosome 7q21–q22\textsuperscript{20}. These genes possess considerable structural homology and possibly arose from the tandem duplication of a common evolutionary precursor\textsuperscript{141}. PON1 and PON3 are associated with apolipoprotein A-1 in high-density lipoprotein (HDL) and can enhance its antiatherosclerotic properties\textsuperscript{21}. In contrast to PON1, which is mainly expressed in the liver, PON2 expression is ubiquitous\textsuperscript{22}. Some investigators have found that PONs may protect against diseases, such as Alzheimer’s dementia, atherosclerosis, diabetes, and Parkinson’s disease\textsuperscript{23–30}. An analysis of PON2 polymorphisms in 94 male workers exposed to noise revealed a significant association with NIHL\textsuperscript{27}, but due to the small sample size and apparent difficulty the author had in matching cases and controls for the risk factor of smoking, these results should be interpreted with great caution\textsuperscript{27}.

To test the hypothesis that genetic variation in the PON2 gene in the inner ear might partly explain the variability in susceptibility to noise exposure, the present study analyzed several selected PON2 gene SNPs thought to play a role in the inner ear.

Materials and Methods

Study subjects and participants

The overall participant population consisted of 1,259 noise-exposed workers (615 NIHL cases and 644 controls) from factories in the cities of Nanjing, Xuzhou, and Yizheng in Jiangsu Province, China. The workers were examined between April 2010 and May 2011. Their ages range from 21 to 59 years. These three cities were selected because of their high workforce stability. The factory working environments were similar, and the workers were commonly exposed to steady noise during their working time. In the three cities, each recruited subject was well matched with a control. In the first selection round, subjects suffering from conductive or mixed hearing loss were excluded from the study.

Subject information was gathered by a questionnaire that was administered through face-to-face interviews by trained interviewers. The questionnaire contained items concerning general health, demographic data, previous and present medical conditions, military history, hereditary factors, leisure time noise exposure, pharmaceutical preparations, smoking, drinking status and previous noise exposure during work at other factories or during military service. Subjects with a history of head injury, cardiovascular events, diabetes, hyperlipidemia, otological disease, other diseases that could affect hearing, previous or present treatment with ototoxic substances, nonmeasurable audiometric data because of poor participation by the participant or potentially harmful noise exposure during leisure or military service were excluded. In this study, workers who drank a bottle of beer or fifty grams of wine per day for at least one year were defined as ever drinkers, and the rest were defined as never drinkers. Subjects who had one cigarette per day for at least one year were defined as ever smokers, and all others were defined as never smokers.

According to the Chinese national criteria for noise in the workplace (GBZ43-2002, http://www.zybw.net), a personal sound pressure audiometer should be used in the proper place and proper time using proper methods to evaluate the real working environment. In our study, all participants underwent a tonal audiometric examination that was performed in a sound isolation cabinet by a trained technician. Both ears were evaluated at 500, 1,000, 2,000, 3,000, 4,000, 6,000 and 8,000 Hz. Noise exposure levels were assessed with a personal sound pressure audiometer (NoisePro, Quest, USA) at 10 AM, 3 PM, and 5 PM at the selected workplaces for 3 consecutive days twice per year\textsuperscript{38}. To assess the actual noise exposure level, the results were normalized to equivalent continuous A-weighted sound pressure from a nominal 8 hours
working day (Lex. 8 hours). The response rates for cases and controls were both above 85%.

The NIHL cases were selected without any restriction in age or sex. The controls, who were frequency-matched to the cases by age, exposure time, exposure level and sex, consisted of individuals from the same company who were seeking health care from the Center for Disease Control and Prevention at the same time. The definitions of NIHL cases and controls were previously described by Yang et al. In our study, due to the requirement of the Diagnostic Criteria of Occupational Noise-induced Hearing Loss (Chinese Occupational Health Standard, GBZ49-2002, http://www.zybw.net), which states that workers with low-frequency hearing loss should be transferred from the original noise-exposed environment as soon as possible, we only accepted subjects with high-frequency hearing loss. Each subject donated 5-ml venous blood samples for genomic DNA extraction.

Ethical consideration
This project was approved by the Ethics Committee of Nanjing Medical University. Written informed consent was obtained from each individual. Ethics were respected throughout the whole study period.

SNP selection and genotyping
We selected highly informative PON2 SNPs from the NCBI database (http://www.ncbi.nlm.nih.gov/) with a minor allele frequency (MAF) >0.10 in the Han Chinese population. We preferred potential functional regions to intronic regions because of their higher chances of being causative SNPs. Five PON2 SNPs (rs7493, rs12026, rs7785846, rs7786401, and rs12704796) were included in our analyses, and two of the SNPs, both resulting in non-synonymous amino acid changes, rs7493, which corresponds to a Ser→Cys amino acid substitution at codon 311, and rs12026, which corresponds to a Ala→Gly amino acid substitution at codon 148, were located in an exon. The LD values of the 5 selected SNPs were calculated and visually presented using the Haploview 4.2 software in Fig. 1. It is worth mentioning that the LD values among 4 SNPs (rs7493, rs12026, rs7785846 and rs7786401) were strong, with an $r^2$ of more than 0.90.

Genomic DNA was obtained from peripheral blood samples according to standard procedures using a TianGen DNA extraction kit (Beijing, China). The genotyping of the 5 PON2 polymorphisms was performed using a TaqMan MGB probe assay from Applied Biosystems Inc. (Foster City, CA, USA). An Applied Biosystems-related company designed the primer and probe sequences for the five polymorphisms. According to the manufacturer’s instructions, amplifications and analyses were carried out using the 384-well ABI 7900HT Real-Time PCR System (Applied Biosystems). Four blank controls were included on each plate to ensure the accuracy of the genotyping. After amplification, we used the SDS 2.3 software for allelic discrimination. The analysis was simultaneously performed by two persons in a blinded fashion. More than 10% of the samples were randomly selected for repeat assays, and the results were 100% concordant.

Statistical Analysis
All of the data were entered into a computerized database. The Hardy-Weinberg equilibrium for the genotypes was tested by a goodness-of-fit $\chi^2$-test. Analyses were performed using the statistical analysis software SAS 9.1.3 (SAS Institute, Cary, NC, USA). Univariate analysis of variance and Student’s $t$-tests were used for the analysis of continuous data. Qualitative data were evaluated using Pearson $\chi^2$ contingency tables. Interactions with SNPs were tested by $\chi^2$ analysis at the genotype and haplotype level to identify differences between the NIHL patients and controls. The association of genotypes with NIHL was also evaluated by assuming dominant models. In addition, we stratified the subjects by exposure level to evaluate the association between PON2 polymor-
polymorphisms and NIHL variables. Crude and adjusted odds ratios (ORs) were determined with 95% confidence intervals (95% CIs) by multivariate logistic regression analysis to test the level of association between NIHL risk and genotype. Haplotype frequencies and association statistics for the \textit{PON2} polymorphisms were also calculated using the SAS 9.1.3 (SAS Institute, Cary, NC) software. All tests were two sided, and \( p < 0.05 \) was considered to be statistically significant.

\section*{Results}

\subsection*{Characteristics of the subjects}

Table 1 lists selected characteristics of NIHL in workers who had been exposed to occupational noise. In total, 614 subjects with hearing loss were compared with 655 subjects without hearing loss (considered to have normal hearing). There were no significant differences between NIHL and control individuals regarding age, sex, smoking status or drinking status (Table 1). In addition, the cases and controls appeared to be well matched by exposure level and exposure time (\( p = 0.717 \) and 0.314 for exposure level and exposure time, respectively). However, subjects with hearing loss were more likely to have a higher threshold value than controls (\( p < 0.001 \)).

\section*{Association of NIHL risk with \textit{PON2} SNPs}

All of the observed genotype frequencies in the controls were in agreement with the Hardy-Weinberg equilibrium (Table 2). The genotype distributions of the \textit{PON2} polymorphisms in the NIHL and control individuals are shown in Table 3. In total, 5 SNPs were analyzed in 1,259 noise-exposed laborers (614 NIHL patients and 655 controls). We listed \( p \) values for the main effect and dominant model for every SNP. As shown in Table 3, in the single SNP analysis of all five SNPs, three SNPs had a significant main effect (\( p = 0.033, 0.044 \) and 0.038 for rs7493, rs12026 and rs7785846, respectively). In addition, in the dominant model of the five polymorphisms, the rs7493 CG + GG, rs12026 CG + GG, rs7785846 CT + TT and rs7786401 GT + TT genotypes were associated with NIHL risk. To eliminate the effects of age, sex, drinking and

\begin{table}[h]
\centering
\caption{Descriptive characteristics of NIHL cases and controls}
\begin{tabular}{llccc}
Variable & All \((n=1,259)\) & Cases \((n=615)\) & Controls \((n=644)\) & \(p^a\) \\
\hline
Sex  & & & & \\
Male & 1,159 & 92.1 & 568 & 92.4 & 591 & 91.8 & 0.700 \\
Female & 100 & 7.9 & 47 & 7.6 & 53 & 8.2 & \\
Age, years  & 40.4 ± 6.3 & 40.4 ± 6.3 & 40.4 ± 6.2 & 0.993 \\
< 35 & 252 & 20.0 & 123 & 20.0 & 129 & 20.0 & \\
35−45 & 754 & 59.9 & 370 & 60.2 & 384 & 59.6 & 0.973 \\
> 45 & 253 & 20.1 & 122 & 19.8 & 131 & 20.3 & \\
Exposure level, dB (A)  & 87.1 ± 7.6 & 87.1 ± 7.7 & 87.1 ± 7.5 & 0.956 \\
< 85 & 488 & 38.8 & 234 & 38.1 & 254 & 39.4 & \\
85−92 & 321 & 25.5 & 163 & 26.5 & 158 & 24.5 & 0.717 \\
>92 & 450 & 35.7 & 218 & 35.5 & 232 & 36.0 & \\
Exposure time, years  & 18.3 ± 7.5 & 18.5 ± 7.6 & 18.1 ± 7.4 & 0.318 \\
≤ 20 & 761 & 60.4 & 363 & 59.0 & 398 & 61.8 & 0.314 \\
>20 & 498 & 39.6 & 252 & 41.0 & 246 & 38.2 & \\
Smoking status  & & & & \\
Never & 521 & 41.4 & 248 & 40.3 & 273 & 42.4 & 0.457 \\
Ever & 738 & 58.6 & 367 & 59.7 & 371 & 57.6 & \\
Drinking status  & & & & \\
Never & 727 & 57.7 & 354 & 57.6 & 373 & 57.9 & 0.898 \\
Ever & 532 & 42.3 & 261 & 42.4 & 271 & 42.1 & \\
Threshold [dB (A)]  & 26.9 ± 14.7 & 37.2 ± 11.8 & 14.1 ± 4.1 & <0.001 \\
\hline
\end{tabular}
\end{table}

\(a\)Two-sided \( \chi^2 \) test for the frequency distributions of selected variables between cases and controls.
smoking, we used logistic regression analysis. After adjustment for these confounding factors, four SNP genotypes were significantly associated with hearing loss: rs7493 CG + GG (OR=1.36, 95% CI, 1.08−1.72), rs12026 CG + GG (OR=1.34, 95% CI, 1.06−1.70), rs7785846 CT + TT (OR=1.35, 95% CI, 1.07−1.71), and rs7786401 GT + TT (OR=1.32, 95% CI, 1.05−1.68). In contrast, no significantly higher risk was found for any rs12704796 genotype (p>0.05). Therefore, we calculated the main effect of the genotypes of this SNP on the entire population. However, no significant association was found, which suggests that this SNP does not have a significant effect on noise susceptibility across all exposure levels.

Furthermore, we detected the 5 SNPs versus noise exposure level interactions. Significant differences were found in the genotype distributions between NIHL patients and controls for the different noise exposure groups. These results indicate a distinguishing effect of genotype on noise susceptibility according to the noise exposure level (Fig. 2). Of the 5 SNPs, rs7493, rs12026, rs7785846, and rs7786401 were significantly associated with NIHL, while rs12704796 was not.

### Table 2. Calculation of Hardy-Weinberg equilibrium for PON2 polymorphisms in controls and cases

<table>
<thead>
<tr>
<th>SNP</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7493</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*NIHL controls</td>
<td>0.024</td>
<td>0.877</td>
</tr>
<tr>
<td>NIHL cases</td>
<td>0.793</td>
<td>0.373</td>
</tr>
<tr>
<td>rs12026</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*NIHL controls</td>
<td>0.024</td>
<td>0.877</td>
</tr>
<tr>
<td>NIHL cases</td>
<td>0.630</td>
<td>0.427</td>
</tr>
<tr>
<td>rs7785846</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*NIHL controls</td>
<td>0.557</td>
<td>0.456</td>
</tr>
<tr>
<td>NIHL cases</td>
<td>0.176</td>
<td>0.675</td>
</tr>
<tr>
<td>rs7786401</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*NIHL controls</td>
<td>0.189</td>
<td>0.664</td>
</tr>
<tr>
<td>NIHL cases</td>
<td>0.984</td>
<td>0.321</td>
</tr>
</tbody>
</table>
| *NIHL means noise-induced hearing loss.

### Table 3. Distribution of PON2 polymorphisms in controls and cases and their association with NIHL

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>p*</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7493</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>382 (62.1)</td>
<td>445 (69.1)</td>
<td>1 (Ref)</td>
<td>1.36 (1.07−1.73)</td>
<td>1.36 (1.07−1.73)</td>
</tr>
<tr>
<td>CG</td>
<td>210 (34.2)</td>
<td>180 (28.0)</td>
<td>0.033</td>
<td>1.19 (0.87−1.62)</td>
<td>1.18 (0.86−1.61)</td>
</tr>
<tr>
<td>GG</td>
<td>23 (3.7)</td>
<td>19 (2.9)</td>
<td>0.099</td>
<td>1.19 (0.87−1.62)</td>
<td>1.18 (0.86−1.61)</td>
</tr>
<tr>
<td>rs12026</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>384 (62.5)</td>
<td>445 (69.1)</td>
<td>1 (Ref)</td>
<td>1.34 (1.05−1.71)</td>
<td>1.34 (1.05−1.71)</td>
</tr>
<tr>
<td>CG</td>
<td>208 (33.8)</td>
<td>180 (28.0)</td>
<td>0.044</td>
<td>1.18 (0.87−1.62)</td>
<td>1.18 (0.86−1.61)</td>
</tr>
<tr>
<td>rs7785846</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>243 (39.5)</td>
<td>256 (39.8)</td>
<td>1 (Ref)</td>
<td>1.35 (1.07−1.70)</td>
<td>1.34 (1.06−1.70)</td>
</tr>
<tr>
<td>AG</td>
<td>291 (47.3)</td>
<td>293 (45.5)</td>
<td>0.673</td>
<td>1.09 (0.64−1.27)</td>
<td>0.91 (0.64−1.28)</td>
</tr>
<tr>
<td>rs7786401</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>388 (63.1)</td>
<td>447 (69.4)</td>
<td>1 (Ref)</td>
<td>1.17 (1.07−1.74)</td>
<td>1.17 (1.07−1.74)</td>
</tr>
<tr>
<td>GT</td>
<td>205 (33.3)</td>
<td>178 (27.6)</td>
<td>0.060</td>
<td>1.13 (0.83−1.54)</td>
<td>1.13 (0.83−1.54)</td>
</tr>
<tr>
<td>TT</td>
<td>22 (3.6)</td>
<td>19 (3.0)</td>
<td>0.011</td>
<td>1.13 (0.83−1.54)</td>
<td>1.13 (0.83−1.54)</td>
</tr>
<tr>
<td>rs7786401</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>388 (63.1)</td>
<td>447 (69.4)</td>
<td>1 (Ref)</td>
<td>1.17 (1.07−1.74)</td>
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</tr>
<tr>
<td>GT</td>
<td>205 (33.3)</td>
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<td>0.060</td>
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</tr>
<tr>
<td>TT</td>
<td>22 (3.6)</td>
<td>19 (3.0)</td>
<td>0.011</td>
<td>1.13 (0.83−1.54)</td>
<td>1.13 (0.83−1.54)</td>
</tr>
</tbody>
</table>

*χ² test for the distribution of genotype frequencies. *Adjusted for age, sex, and smoking and drinking habits in the logistic regression model.
Fig. 2. Bar charts for the 5 SNPs in the Chinese population. The participants were divided into three groups according to noise exposure level (<85 dB, 85–92 dB, and >92 dB). White bars represent control subjects, and filled bars represent NIHL patients. The genotypes are indicated below each bar chart. An asterisk indicates that a significant effect (p-value <0.05) was found in that noise exposure level group. (A) rs7493, (B) rs12026, (C) rs7785846, (D) rs7786401, (E) rs12704796.
PON2 SNPs, the majority demonstrated a significant association with NIHL for both the mid-level exposure group (85–92 dB) and the high-level exposure group (>92 dB). In an analysis of the mid-level exposure group, four SNPs showed a significant difference between NIHL patients and controls: rs7493 in Fig. 2A (p=0.005), rs12026 in Fig. 2B (p=0.007), rs7785846 in Fig. 2C (p=0.007) and rs7786401 in Fig. 2D (p=0.003). In the high-level exposure group, three SNPs were found to be significantly different in frequency between the two groups studied (p=0.024, 0.034 and 0.030 for rs7493, rs12026 and rs7785846, respectively). However, in regard to rs12704796, there were no significant differences for different exposure levels.

Figure 2(A) shows that in the mid-level (85−92 dB) and high-level (>92 dB) exposure groups, NIHL patients were more likely to carry the CG or GG genotype for rs7493, whereas in controls, the CC genotype dominated. This pattern was also noted for three other SNPs. For rs12026, the resistant workers were more likely to carry the CC genotype in the mid- and high-level exposure groups, whereas heterozygous workers (CG) and mutant homozygous workers (GG) were more likely to be susceptible [Fig. 2(B)]. For rs7785846, the CT or TT genotype prevailed among NIHL cases, while workers with the CC genotype were more often resistant to noise [Fig. 2(C)]. In regard to rs7786401, workers with the GT or TT genotype in the mid- and high-level exposure groups were more likely to be sensitive, whereas susceptible workers more often carried the GG genotype [Fig. 2(D)]. Unfortunately, we did not discover any obvious dominant genotypes in the low-level exposure group (<85 dB).

Table 4. Associations between risk of NIHL and frequencies of inferred haplotypes by the observed genotypes in NIHL cases and controls

<table>
<thead>
<tr>
<th>Haplotypes \a</th>
<th>Cases</th>
<th>Controls</th>
<th>Adjusted OR (95% CI)</th>
<th>\b</th>
<th>p \b</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCGC</td>
<td>971</td>
<td>1,066</td>
<td>78.9</td>
<td>82.8</td>
<td>1</td>
</tr>
<tr>
<td>GGTT</td>
<td>247</td>
<td>214</td>
<td>20.1</td>
<td>16.6</td>
<td>1.27 (1.03–1.55)</td>
</tr>
<tr>
<td>Others \c</td>
<td>12</td>
<td>8</td>
<td>1.0</td>
<td>0.6</td>
<td>1.67 (0.68–4.11)</td>
</tr>
</tbody>
</table>

\aThe alleles of haplotypes were arrayed in the order of the location of the SNPs in PON2 from 5’ to 3’ (e.g., CCGC denotes Crs12026-Crs7493-Grs7786401-Crs7785846).  
\bAdjusted for age, sex, and smoking and drinking habits.  
\cHaplotypes with a frequency<0.05 were pooled into a mixed group.

Haplotype analysis

By considering both D’ and r² of linkage disequilibrium (LD), we analyzed two different haplotypes as presented in the Table 4 (CCGC and GGTT). Haplotypes with a frequency<0.05 were pooled into a mixed group. After adjusting for age, sex, and smoking and drinking habits, we found that the frequencies of the GGTT haplotype were significantly greater in the NIHL group than in the normal one (p=0.023). Compared with the CCGC haplotype, carriers of the GGTT haplotype had increased odds of developing NIHL after noise exposure (adjusted OR=1.27 and 95% CI, 1.03–1.55). An analysis of the GGTT haplotype in the Chinese sample found a significant interaction, indicating a significant difference in haplotype distribution between NIHL cases and controls.

Discussion

Temporary or permanent hearing loss in both humans and animals can result from excessive exposure to noise. The etiology of NIHL, which remains an unavoidable problem in occupational health, involves an interaction between environmental and genetic factors. Currently, only a limited number of studies have been performed on the genetic factors involved in NIHL. Studies in animal models suggest that genetic factors may affect individual susceptibility to noise. Mice in which genes such as cadherin23, PMCA2 or glutathione peroxidase1 have been knocked out have been reported to be more sensitive to NIHL. Unfortunately, certain evidence for the involvement of genetic factors in human NIHL is still scarce. A possible association between the absence of GSTM1 and NIHL was studied, but the results of that study were not in agreement with another recent study on double deletion polymorphisms in the GSTM1 and GSTT1 genes from 3 distinct workplaces in a Caucasian population. Another survey suggested that SOD2, PON1 and PON2 polymorphisms could predispose humans to NIHL.

Previous studies on the PON2 gene have found positive associations in several NIHL populations in Italy. To the best of our knowledge, no studies on the PON2 gene and NIHL have been performed.
in Chinese patients. The present study investigated the association between PON2 gene polymorphisms and susceptibility to NIHL in a Chinese population stratified according to noise exposure levels. Our findings in this Chinese population agree with the results of a previous analysis of PON2 gene polymorphisms and NIHL, which confirmed positive associations in a case-control study on 28 patients and 61 controls, and the allele frequencies of rs7493 and rs12026 observed in our controls are similar to those observed in other Chinese studies. The association between PON2 SNPs and NIHL in the Chinese population may indicate that the PON2 gene plays an important role and that other gene-environment interactions are involved in NIHL pathogenesis.

Table 3 lists some of the PON2 SNPs associated with NIHL (p=0.033, 0.044 and 0.038, for rs7493, rs12026 and rs7785846, respectively). PON2 acts as an antioxidant enzyme, and its overproduction is capable of lowering the oxidative state of cells stimulated with hydrogen peroxide. In this paper, we demonstrate that the dominant models of rs7493, rs12026, rs7785846 and rs7786401 are associated with NIHL, regardless of age, sex, and drinking and smoking habits. Workers with rs7493 CG + GG genotypes are more susceptible compared with workers with a CC genotype (OR=1.36, 95% CI, 1.08−1.72), and similar results were found for workers with rs12026 CG + GG genotypes compared with the CC genotype (OR=1.34, 95% CI, 1.06−1.70), rs7785846 CT + TT genotypes compared with the CC genotype (OR=1.35, 95% CI, 1.07−1.71), and rs7786401 GT + TT genotypes compared with the GG genotype (OR=1.32, 95% CI, 1.05−1.68). These results suggest a genetic predisposition to NIHL. The pathogenesis of NIHL may include the release of oxygen species induced by long-term exposure to high-decibel sounds. This release could damage Corti’s organ. The local release of free radicals in humans may lead to damage to ciliated cells of Corti’s organ, which can lead to neurosensorial hearing loss. In addition, in animal models, the concentrations of superoxide radicals in the cochlear fluid increase when animals are exposed to noise. This result is similar to the association between a PON2 polymorphism (rs7493) and Alzheimer’s disease, one kind of neurodegenerative disease in which oxidative stress may play a crucial role in pathogenesis.

Overall, after grouping the participants by noise exposure level and testing for association in separate noise exposure groups, there was a significant association of NIHL with the majority of PON2 SNPs in the mid-level exposure group (85−92 dB) and high-level exposure group (>92 dB). These results indicate that polymorphisms in PON2 have a larger effect when laborers are exposed to higher levels of noise. This is not unexpected because higher noise levels are more harmful, and therefore, a larger significant effect would be expected.

No significant main effect was observed for SNP rs12704796 in the different noise exposure level groups. This may be a result of the small sample sizes of the respective noise exposure level groups. Power calculations for the Chinese population were based on a selection derived from 1,259 samples, but when this population is separated into three noise exposure groups, it is possible that the size of the individual noise exposure groups is too small to be able to detect a significant effect of rs12704796. Alternatively, other unknown environmental factors may explain the lack of difference. Therefore, it remains to be elucidated whether this SNP contributes to NIHL susceptibility. On the other hand, in order to ensure the association of a gene with a disease, it is not necessary that all the identical SNPs are associated in a population under study. Different SNPs associated with different populations but within the same gene can be regarded as replication.

These results reveal the complexity of NIHL and indicate that analyzing effects by exposure levels is an interesting option for prospective research that aims to study the genetic factors related to NIHL. Therefore, there is a chance that after accounting for exposure levels, effects similar to the effects of PON2 polymorphisms on NIHL susceptibility might also be observed with other genes.

When performing genetic association studies, haplotype-based linkage disequilibrium mapping is a cost-effective and powerful method, particularly in identifying genes that contribute to causing complex diseases. Our haplotype analysis revealed that the GGTGT haplotype had a negative effect in Chinese sample sets, resulting in increased odds of developing NIHL (adjusted OR=1.27 and 95% CI=1.03−1.55). This result suggests that individuals carrying the GGTG haplotype are more prone to NIHL when exposed to noise. The mechanisms through which these PON2 haplotypes are associated with the development of NIHL remain unknown but clearly warrant further investigation.

In conclusion, several associations were found between PON2 polymorphisms and NIHL susceptibility in independent noise-exposed Chinese populations. In particular, we observed significant interactions between genotypic variations and noise exposure levels and their effect on NIHL, particularly in the mid- and high-level noise exposure groups. We note that these SNPs may be responsible for the association with NIHL. Further genetic and functional studies focused on identification of the underlying mecha-
nism of NIHL should be performed.

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