## Original Article

# *NRN1* and *CAT* Gene Polymorphisms, Complex Noise, and Lifestyles interactively Affect the Risk of Noise-induced Hearing Loss*

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**Abstract**

**Objective** The effects of interactions between genetic and environmental factors on the noise-induced hearing loss (NIHL) are still unclear. This study aimed to assess interactions among gene polymorphisms, noise metrics, and lifestyles on the risk of NIHL.

**Methods** A case-control study was conducted using 307 patients with NIHL and 307 matched healthy individuals from five manufacturing industries. General demographic data, lifestyle details, and noise exposure levels were recorded. The Kompetitive allele-specific polymerase chain reaction (KASP) was used to analyze the genotypes of 18 SNPs.

**Results** GMDR model demonstrated a relevant interaction between *NRN1* rs3805789 and *CAT* rs7943316 ($P$ = 0.0107). Subjects with *T* allele of rs3805789 or *T* allele of rs7943316 had higher risks of NIHL than those with the SNP pair of rs3805789-CC and rs7943316-AA ($P$ < 0.05). There was an interaction among rs3805789, rs7943316, and kurtosis ($P$ = 0.0010). Subjects exposed to complex noise and carrying both rs3805789-CT and rs7943316-TT or rs3805789-CT/TT and rs7943316-AA had higher risks of NIHL than those exposed to steady noise and carrying both rs3805789-CC and rs7943316-AA ($P$ < 0.05). The best six-locus model involving *NRN1* rs3805789, *CAT* rs7943316, smoking, video volume, physical exercise, and working pressure for the risk of NIHL was found to be the interaction ($P$ = 0.0010). An interaction was also found among smoking, video volume, physical exercise, working pressure, and kurtosis ($P$ = 0.0107).

**Conclusion** Concurrence of *NRN1* and *CAT* constitutes a genetic risk factor for NIHL. Complex noise exposure significantly increases the risk of NIHL in subjects with a high genetic risk score. Interactions between genes and lifestyles as well as noise metrics and lifestyles affect the risk of NIHL.

**Key words:** Noise-induced hearing loss; Kurtosis; *CAT*; *NRN1*; Lifestyle; Interaction; Generalized multifactor dimensionality reduction

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## INTRODUCTION

Noise-induced hearing loss (NIHL) is a slowly progressive sensorineural hearing loss caused by long-term exposure to harmful levels of noise. The World Health Organization (WHO) estimates that approximately 22% of the hearing loss in adults is attributable to occupational and environmental noise exposure, and by 2030, almost 1 billion people will be at the risk of NIHL[1]. As a major occupational health risk, NIHL has become the second-largest occupational disease in China[2]. NIHL is thought to be a complex disease caused by genetic and environmental factors. The main factors include exposure to high levels of noise and individual susceptibility, such as age, gender, education level, smoking frequency, alcohol consumption, and usage practice of hearing protection devices[3-6]. Therefore, the single-locus method may not be appropriate to study common complex disorders such as NIHL.

Noise is the most common environmental factor leading to occupational hearing loss. The noise exposure metrics used in most previously published studies mainly concentrates on equivalent continuous sound level (Leq) and cumulative noise exposure (CNE). These metrics have been established based on the study of Gaussian noise and the equal-energy hypothesis (EEH), which assumes that the damage to the auditory system caused by noise exposure is proportional to the duration of exposure multiplied by the noise intensity. However, the EEH has been found unsuitable for "complex noise or non-Gaussian (non-G)" noise. Complex noise is ubiquitous in industrial and military environments. It is composed of a transient high-energy impulsive noise superimposed on stationary (Gaussian) background noise[7]. Both animal experiments and epidemiological studies have shown that the EEH underestimates the cochlear impact of complex-noise exposure. The impact of a complex noise on the auditory system was assessed using kurtosis first by Erdreich[8]. This method has simplified the time-domain variables of noise that affect hearing (e.g., pulse peak value, duration, and inter-pulse distribution) into one easy-to-calculate parameter (i.e., kurtosis), which is convenient for classifying the noise type. A high kurtosis indicates that the impulse of the complex noise was high[9]. To date, the efficacy of kurtosis in assessing complex noise has been preliminarily verified in human studies[10,11].

Increasing evidence has shown the association of susceptibility genes, such as catalase (*CAT*), heat shock protein70 (*HSP70*), cadherin-23 (*CDH23*), caspase (*CASP*), and NADPH oxidase3 (*NOX3*), with the development of NIHL[12-18]. Additionally, previous studies have demonstrated that smoking, stressful lifestyle, and physical exercise are associated with hearing loss[19-21]. However, multiple genetic loci may fail to reach genome-wide significance due to the limited power in most genetic studies, and few studies have analyzed the interaction between genetic variants, noise exposure (especially kurtosis), and lifestyle factors on modulating NIHL. Previous studies have never reported multidimensional interactions involving multiple (> 7) genes (especially *NRN1* gene) and kurtosis. Therefore, the present study focused on the associations of multi-locus interactions with NIHL risk. In a case-control study with 307 NIHL patients and 307 age- and gender-matched healthy controls, a total of 18 variants (rs1049216, rs6948, rs3805789, rs2227956, rs1043618, rs2763979, rs3749930, rs12665231, rs12195525, rs3752752, rs3802711, rs1227049, rs12415607, rs1127687, rs564250, rs769214, rs769217, and rs7943316) in these 7 susceptibility genes (*CAT*, *HSP70*, *CDH23*, *CASP3*, *CASP7*, *NOX3*, and *NRN1*), three noise metrics (noise kurtosis, CNE, adj-CNE), and four lifestyle factors (smoking, video volume, physical exercise, working pressure) were included to explore the associations of gene-gene, gene-noise-metric, gene-lifestyle-factor, and noise-metric-lifestyle-factor interactions with the risk of NIHL, and identify the significant interaction model of gene-gene and gene-environment. Our results lay the foundation for a comprehensive prevention program against NIHL.

## METHODS

### *Subjects*

Subjects were continuously recruited between October 2017 and December 2018 from five manufacturing factories with high noise levels in the Zhejiang Province of East China. Inclusion criteria for the subjects were as follows: 1) individuals who had never worked in high noise-level environments from different enterprises, 2) the binaural hearing threshold difference was < 30 dB per frequency; 3) no history of military service; 4) no family history of hearing loss; 5) no history of an ear disease; 6) no history of ototoxic drugs; and 7) no history of diabetes. NIHL was diagnosed based on binaural high-frequency (3,000, 4,000,

and 6,000 Hz) average hearing threshold > 25 dB. The subjects were divided into two groups—patients with NIHL and controls with normal hearing—who were matched for gender and age (± 3 years).

***Sample Size***

Sample size was determined using the following formula for the case-control study.

$$n = \frac{2\bar{p}\bar{q}\left(z_\alpha + z_\beta\right)^2}{\left(p_1 - p_0\right)^2}$$

$\alpha = 0.05$, $z_\alpha = 1.96$; $\beta = 0.1$, $z_\beta = 1.28$; $p_0$ was the minimum allele frequency in the control group, $p_0 = 0.123$ (https://www.ncbi.nlm.nih.gov/snp/); $p_1$ was the minimum allele frequency in the case group, $p_1 = \frac{p_0 OR}{[1 + p_0(OR - 1)]} = 0.276$, $OR$ is odds ratio; $\bar{p} = \frac{p_0 + p_1}{2} = 0.200$, $\bar{q} = 1 - \bar{p} = 0.800$. A minimum of 143 samples were required for this study based on the above formula. Considering the interactions of gene-gene and gene-environment, a larger sample size was appropriate. In our study, a total of 614 subjects including 307 patients with NIHL and 307 controls with normal hearing were selected, which is sufficient based on the above calculation results for sample size.

***Questionnaire Survey***

A questionnaire was designed for each subject based on the needs of the investigation. Collected information included the following: 1) general information (age, sex, etc.); 2) noise exposure factors (factory, work situation, duration of daily noise exposure, etc.); 3) lifestyle factors (smoking, video volume, physical exercise and working pressure). Variables were defined as follows: 1) smoking: smoking one or more cigarettes on average every day, and lasting for at least a year; 2) video volume: The 40% of the maximum volume served as the cutoff point is based on our previous study[17], high video volume is defined as higher than 40% of the maximum volume, and low video volume is defined as lower than 40% of the maximum volume; 3) regular physical exercise: physical exercise on average once a week or more, and lasting for one year or longer; 4) working pressure: a feeling of work-related hardness, frustration, distress, or tension, such as tension, anxiety, and unhappiness[22]. High working pressure is defined as very high and relatively high pressure, and low working pressure is defined as general, relatively low and very low pressure. Participants completed study questionnaires and met with trained investigators in a face-to-face interview. All the participants signed the informed consent form, and the study was approved by the Science Ethics Committee of Hangzhou Normal University (2017LL107).

***Noise Waveform Recording and Analysis***

A digital noise dosimeter (ASV5910-R, Hangzhou Aihua Instrument Co., Ltd.) that can operate continuously at a sampling rate of 48 kHz was used to record the noise for each subject for the entire shift duration. Eight-hour, continuous equivalent A-weighted sound levels ($L_{Aeq,8h}$) can be measured with a noise dosimeter, which was attached to the clothing of the participant at the shoulder by clips, with the microphone pointed up (Supplementary Material 1 available in www.besjournal.com). The measurement time was 8 h per shift. A sound level calibrator (Hangzhou Aihua Instrument, AWA6221B) was used to calibrate the noise dosimeter before and after each sampling cycle. MATLAB software (Natick, MA) was used to calculate the sampling kurtosis in a continuous 40-s window of the noise signals during the entire shift. The equation used to calculate kurtosis is shown in Formula 1.

$$\beta = \frac{m_4}{m_2} = \frac{\frac{1}{n}\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^4}{\left(\frac{1}{n}\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2\right)^2} \quad (1)$$

where, $x_i$ is the ith value, $\bar{x}$ is the sample mean, and $\beta$ is noise kurtosis. Theoretically, the kurtosis value of Gaussian noise is 3 ($\beta = 3$) and that of complex non-Gaussian noise is greater than 3. The larger the kurtosis value, the higher the impulsiveness of the complex noise. The selection of a 40-s window is acceptable for kurtosis measurement based on a 48-kHz sampling rate, as observed from previous animal data[23,24]. The median kurtosis calculated in a 40-s window was used as the kurtosis value of the entire shift time. In this study, a median kurtosis of 4 was used as the boundary value between Gaussian and complex non-Gaussian noise.

Both noise level and noise exposure time should be used to assess NIHL. Therefore, a comprehensive noise exposure metric (CNE) was used to quantify noise energy for each worker according to

Formula 2[10]:

$$CNE = L_{Aeq,8h} + 10\log T \quad (2)$$

where, $T$ is the time of noise exposure in years. CNE is measured in dB (A) per year.

To incorporate kurtosis ($\beta$) into the evaluation of complex noise environments and unify CNE calculations for epidemiologic data, including both Gaussian and complex noise, the kurtosis- adjusted CNE (adj-CNE) was calculated according to Formula 3[10]:

$$adj - CNE_{Kurtosis-adjusted} = L_{Aeq,8h} + \frac{\ln(\beta) + 1.9}{\log(2)}\log T \quad (3)$$

When Gaussian noise has a kurtosis of $\beta = 3$, the term $\left[\frac{\ln(\beta) + 1.9}{\log(2)}\right]$ becomes equal to 10. Thus, for Gaussian noise, the adj-CNE equals the unadjusted CNE. Formula (3) shows that when $L_{Aeq,8h}$ is fixed, the adj-CNE will be larger for complex noise ($\beta > 3$) than that for Gaussian noise ($\beta = 3$).

### *Hearing Testing and Hearing Loss Diagnosis*

Experienced audiologists performed pure-tone audiometry for the left and right ears of each participant at 500, 1,000, 2,000, 3,000, 4,000, 6,000, and 8,000 Hz in a sound-insulated room with background noise < 25 dB (A) (Supplementary Material 2 available in www.besjournal.com). All the subjects were required to be outside of their daily noise environment for at least 16 h before the test. The results of the pure-tone audiometry were adjusted according to gender and age by following the ISO 1999-2013 standard Table A.3. High-frequency NIHL (hNIHL) was diagnosed based on binaural high-frequency hearing threshold levels at 3, 4, and 6 kHz ($HTL_{346}$) using Formula 4:

$$HTL_{346} = \frac{Left(HL_{3KHz} + HL_{4KHz} + HL_{6KHz}) + Right(HL_{3KHz} + HL_{4KHz} + HL_{6KHz})}{6} \quad (4)$$

A binaural threshold > 25 dB was considered binaural hNIHL[16].

### *Genomic DNA Extraction, Single Nucleotide Polymorphism (SNP) Selection, and Genotyping*

Oral mucosa cells from all the participants were collected using Yongming flocking swabs. DNA was extracted using the Tiangen Oral Mucosa Genomic DNA extraction kit (Tiangen Biotech, Beijing, China). For SNP analysis, 18 SNPs were selected from 7 genes (*CAT*, *HSP70*, *CDH23*, *CASP3*, *CASP7*, *NOX3*, and *NRN1*). The SNP selection process has previously been described[17]. The detailed information about the screened SNPs is shown in Table 1. We performed the genotyping analysis using the Kompetitive allele-specific polymerase chain reaction (KASP) method as previously described[17]. The primer and probe sequences are shown in Supplementary Tables S1 and S2 (available in www.besjournal.com). To control the quality, we randomly selected 10% of the samples and re-classified the genes; the concordance of the 18 SNPs was > 95%.

### *Statistical Analysis*

Normally distributed continuous variables are expressed as mean ± standard deviation (SD), and categorical variables are presented as percentages. Student's *t*-test and the Chi-square test were used to compare the continuous variables and categorical variables, respectively, between the cases and controls. Non-normally distributed continuous variables were expressed as median (with the lower and upper quartiles) [M (P25, P75)] and analyzed using the Mann-Whitney *U* test. The cut-off values for the CNE and adj-CNE were determined to be 97.1420 dB(A) and 96.9939 dB(A), respectively, based on the receiver operating characteristic (ROC) curve between CNE, adj-CNE, and NIHL. The Hardy-Weinberg equilibrium (HWE) was tested using the Chi-square test. The generalized multifactor dimensionality reduction (GMDR Software Beta 0.9, www.ssg.uab.edu/gmdr/) method[25] was used to examine the effects of all possible interactions. The sign test of cross-validation consistency (CVC), testing balanced accuracy (TEBA), and trained balanced accuracy (TRBA) were calculated. A multivariate logistic regression model was used for the stratified analysis of the significant interactions obtained from the GMDR. Multiple comparisons were corrected using the Benjamini-Hochberg procedure. $P < 0.05$ indicated that the differences were statistically significant (shown in bold in the following tables).

## RESULTS

### *General Characteristics of the Subjects*

A total of 614 participants (474 males and 140

females), including 307 NIHL patients and 307 controls, were selected (Table 2). The median age of the subjects was 35 years. 307 patients with NIHL and 307 controls were from packing worker (5.2% and 6.0%, respectively), coating worker (2.1% and 1.0%, respectively), electric welder (3.1% and 2.4%, respectively), fitter (6.8% and 7.0%, respectively), machinist (7.8% and 8.6%, respectively), wire drawing worker (2.3% and 1.8%, respectively), cold heading workers (4.6% and 8.3%, respectively), silk knead worker (3.6% and 4.6%, respectively), grinder (2.0% and 2.4%, respectively), Heat-treater (1.5% and 1.3%, respectively), punching worker (1.6% and 2.3%, respectively), and others (9.5% and 4.2%, respectively). The median kurtosis in the NIHL group was 7.25 (4.63–14.30), which was significantly higher than that in the control group [5.85 (4.06–12.51); $P$ = 0.006]. The proportion of the subjects exposed to complex noise ($\beta \geq 4$) was significantly greater in the NIHL group than that in the control group ($P$ = 0.038). The median $HTL_{346}$ in the NIHL group was 36.83 (29.83–49.83) dB, which was significantly higher than that in the control group [17.83 (14.17–21.00) dB; $P < 0.001$). The proportion of the subjects with high CNE (≥ 97.1420), high adj-CNE (≥ 96.9939), smoking habit, propensity to watch videos at high volume, or sedentary lifestyle was significantly greater in the NIHL group than in the control group ($P < 0.05$). However, there was no significant difference in education level or working pressure between the two groups ($P > 0.05$).

### *Associations of the Gene-Gene Interactions with the Risk of NIHL*

The genotype frequencies among the cases and controls did not deviate from the HWE for any of the 18 SNPs ($P > 0.05$, Table 3). We assessed for NIHL-related interactions among the 18 genetic variants by using GMDR. Consequently, 18 models were generated from the 18 SNPs (Table 4). After adjusting age, gender, education level, years of noise exposure, kurtosis, CNE, adj-CNE, smoking, video volume, physical exercise, and working pressure, a significant two-locus model ($P$ = 0.0107) involving *NRN1* rs3805789 and *CAT* rs7943316 was found (Table 4, Supplementary Figure S1 available in www.besjournal.com). The CVC of this two-locus model was 10/10, and the TEBA was 0.5768. We then conducted a stratified

**Table 1.** Basic information for 18 SNPs

| SNP ID | Gene | Chromosome | Position | Function | Alleles |
|---|---|---|---|---|---|
| rs1049216 | *CASP3* | 4 | 184628935 | 3 Prime UTR Variant | G/A |
| rs6948 | *CASP3* | 4 | 184627976 | 3 Prime UTR Variant | T/G |
| rs3805789 | *NRN1* | 6 | 6003752 | Intron Variant, 5 Prime UTR Variant | C/T |
| rs2227956 | *HSPA1L* | 6 | 31810495 | Missense Variant | G/A, C, T |
| rs1043618 | *HSPA1A*/*HSPA1L* | 6 | 31815730 | 5 Prime UTR Variant, 2KB Upstream Variant | G/A, C, T |
| rs2763979 | *HSPA1B* | 6 | 31826815 | 2KB Upstream Variant | C/T |
| rs3749930 | *NOX3* | 6 | 155440112 | Missense Variant | G/T |
| rs12665231 | *NOX3* | 6 | 155395463 | 3 Prime UTR Variant | T/C |
| rs12195525 | *NOX3* | 6 | 155454846 | Stop Gained, Synonymous Variant | G/A, T |
| rs3752752 | *CDH23* | 10 | 71695444 | Synonymous Variant | T/C |
| rs3802711 | *CDH23* | 10 | 71784329 | Missense Variant | G/A |
| rs1227049 | *CDH23* | 10 | 71675131 | Missense Variant | G/A, C, T |
| rs12415607 | *CASP7* | 10 | 113678445 | 2KB Upstream Variant | C/A |
| rs1127687 | *CASP7* | 10 | 113730350 | 3 Prime UTR Variant | G/A |
| rs564250 | *CAT* | 11 | 34437314 | 2KB Upstream Variant | T/A, C |
| rs769214 | *CAT* | 11 | 34438170 | 2KB Upstream Variant | G/A |
| rs769217 | *CAT* | 11 | 34461361 | Synonymous Variant | C/T |
| rs7943316 | *CAT* | 11 | 34438925 | 2KB Upstream Variant | A/T |

***Note.*** *CASP3*: Caspase3; *NRN1*: Neuritin1; *HSP*: Heat shock protein; *NOX3*: NADPH Oxidase3; *CDH23*: Cadherin-23; *CASP7*: Caspase7; *CAT*: Catalase.

**Table 2.** General characteristics of subjects between NIHL and control groups

| Characteristics | Total (*n* = 614) | NIHL (*n* = 307) | Control (*n* = 307) | $\chi^2/z$ | *P* |
|---|---|---|---|---|---|
| Sex, *n* (%) | | | | | |
| Male | 474 (77.2) | 237 (38.6) | 237 (38.6) | 0.000 | 1.000 |
| Female | 140 (22.8) | 70 (11.4) | 70 (11.4) | | |
| Age, y | 35 (30–43) | 36 (30–43) | 34 (30–42) | −1.959 | 0.050 |
| Education, *n* (%) | | | | | |
| High school and above | 347 (56.5) | 168 (27.4) | 179 (29.2) | 0.802 | 0.371 |
| Junior high school and below | 267 (43.5) | 139 (22.6) | 128 (20.8) | | |
| Type of work, *n* (%) | | | | | |
| Packing worker/ Coating worker/ Electric welder | 69 (11.2)/ 19 (3.1)/ 34 (5.5) | 32 (5.2)/ 13 (2.1)/19 (3.1) | 37 (6.0)/ 6 (1.0)/15 (2.4) | – | – |
| Fitter/Machinist | 85 (13.8)/101 (16.4) | 42 (6.8)/48 (7.8) | 43 (7.0)/53 (8.6) | | |
| Wire drawing worker/ Cold heading workers/ Silk knead worker | 25 (4.1)/ 79 (12.9)/50 (8.2) | 14 (2.3)/ 28 (4.6)/22 (3.6) | 11 (1.8)/ 51 (8.3)/28 (4.6) | | |
| Grinder/Heat-treater/ Punching worker | 27(4.4)/ 17 (2.8)/24 (3.9) | 12 (2.0)/ 9 (1.5)/10 (1.6) | 15 (2.4)/ 8 (1.3)/14 (2.3) | | |
| Others | 84 (13.7) | 58 (9.5) | 26 (4.2) | | |
| Years of noise exposure, y | 3.00 (1.43–6.00) | 3.00 (1.20–6.00) | 3.00 (2.00–6.00) | −1.002 | 0.317 |
| $HTL_{346}$, dB | 25.00 (17.83–36.96) | 36.83 (29.83–49.83) | 17.83 (14.17–21.00) | −21.442 | **< 0.001** |
| Kurtosis, median ($P_{25}$–$P_{75}$) | 6.62 (4.28–13.15) | 7.25 (4.63–14.30) | 5.85 (4.06–12.51) | −2.755 | **0.006** |
| < 4 | 114 (18.6) | 47 (7.7) | 67 (10.9) | 4.039 | **0.038** |
| ≥ 4 | 500 (81.4) | 260 (42.3) | 240 (39.1) | | |
| CNE, median ($P_{25}$–$P_{75}$), dB(A) | 93.69 (89.48–97.57) | 93.73 (89.67–98.24) | 93.62 (89.07–96.88) | −1.23 | 0.219 |
| < 97.1420 | 449 (73.1) | 213 (34.7) | 236 (38.4) | 4.384 | **0.036** |
| ≥ 97.1420 | 165 (26.9) | 94 (15.3) | 71 (11.6) | | |
| Adj-CNE, median ($P_{25}$–$P_{75}$), dB(A) | 94.89 (90.02–99.23) | 95.34 (90.20–99.61) | 94.61 (89.75–98.59) | −1.564 | 0.118 |
| < 96.9939 | 387 (63.0) | 178 (29.0) | 209 (34.0) | 6.717 | **0.010** |
| ≥ 96.9939 | 227 (37.0) | 129 (21.0) | 98 (16.0) | | |
| Smoking, *n* (%) | | | | | |
| No | 325 (52.9) | 150 (24.4) | 175 (28.5) | 4.086 | **0.043** |
| Yes | 289 (47.1) | 157 (25.6) | 132 (21.5) | | |
| Video volume, *n* (%) | | | | | |
| Low | 149 (24.3) | 60 (9.8) | 89 (14.5) | 7.453 | **0.006** |
| High | 465 (75.7) | 247 (40.2) | 218 (35.5) | | |
| Physical exercise, *n* (%) | | | | | |
| Never | 418 (68.1) | 224 (36.5) | 194 (31.6) | 6.745 | **0.009** |
| Regular | 196 (31.9) | 83 (13.5) | 113 (18.4) | | |
| Working pressure, *n* (%) | | | | | |
| Low | 113 (18.4) | 49 (8.0) | 64 (10.4) | 2.440 | 0.118 |
| High | 501 (81.6) | 258 (42.0) | 243 (39.6) | | |

**Table 3.** The genotype and allele frequencies of 18 SNPs in NIHL cases and control subjects

| SNP ID | Gene | Group | Genotype (frequency %) | | | Allele (frequency %) | | *P* | *OR* (95% *CI*) | MAF[a] | MAF[b] | HWE *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | AA | AG | GG | A | G | | | | | |
| rs1049216 | *CASP3* | Case | 10 (3.3) | 105 (34.2) | 192 (62.5) | 125 (20.4) | 489 (79.6) | 0.046 | 1.046 (1.005–1.802) | 0.403 | 0.160 | 0.616 |
| | | Control | 9 (2.9) | 80 (26.1) | 218 (71.0) | 98 (16.0) | 516 (84.0) | | | | | |
| | | | GG | GT | TT | G | T | | | | | |
| rs6948 | *CASP3* | Case | 10 (3.3) | 92 (30.0) | 205 (66.8) | 112 (18.2) | 502 (81.8) | 0.064 | 1.334 (0.982–1.809) | 0.426 | 0.143 | 0.431 |
| | | Control | 8 (2.6) | 72 (23.5) | 227 (73.9) | 88 (14.3) | 526 (85.7) | | | | | |
| | | | CC | CT | TT | C | T | | | | | |
| rs3805789 | *NRN1* | Case | 84 (27.4) | 163 (53.1) | 60 (19.5) | 331 (53.9) | 283 (46.1) | 0.864 | 1.020 (0.815–1.276) | 0.300 | 0.466 | 0.216 |
| | | Control | 93 (30.3) | 142 (46.3) | 72 (23.5) | 328 (53.4) | 286 (46.6) | | | | | |
| | | | CC | CT | TT | C | T | | | | | |
| rs2227956 | *HSPA1L* | Case | 15 (4.9) | 86 (28.0) | 206 (67.1) | 116 (18.9) | 498 (81.1) | 0.942 | 0.989 (0.744–1.316) | 0.123 | 0.191 | 0.671 |
| | | Control | 10 (3.3) | 97 (31.6) | 200 (65.1) | 117 (19.1) | 497 (80.9) | | | | | |
| | | | CC | CG | GG | C | G | | | | | |
| rs1043618 | *HSPA1A/ HSPA1L* | Case | 42 (13.7) | 118 (38.4) | 147 (47.9) | 202 (32.9) | 412 (67.1) | 0.903 | 0.985 (0.777–1.250) | 0.481 | 0.332 | 0.587 |
| | | Control | 36 (11.7) | 132 (43.0) | 139 (45.3) | 204 (33.2) | 410 (66.8) | | | | | |
| | | | CC | CT | TT | C | T | | | | | |
| rs2763979 | *HSPA1B* | Case | 168 (54.7) | 102 (33.2) | 37 (12.1) | 438 (71.3) | 176 (28.7) | 0.453 | 1.098 (0.860–1.403) | 0.448 | 0.306 | 0.387 |
| | | Control | 151 (49.2) | 124 (40.4) | 32 (10.4) | 426 (69.4) | 188 (30.6) | | | | | |
| | | | GG | GT | TT | G | T | | | | | |
| rs3749930 | *NOX3* | Case | 86 (28.0) | 148 (48.2) | 73 (23.8) | 320 (52.1) | 294 (47.9) | 0.123 | 1.192 (0.953–1.492) | 0.197 | 0.477 | 0.662 |
| | | Control | 68 (22.1) | 157 (51.1) | 82 (26.7) | 293 (47.7) | 321 (52.3) | | | | | |
| | | | CC | CT | TT | C | T | | | | | |
| rs12665231 | *NOX3* | Case | 23 (7.5) | 100 (32.6) | 184 (59.9) | 146 (23.8) | 468 (76.2) | 0.841 | 0.974 (0.749–1.265) | 0.188 | 0.243 | 0.126 |
| | | Control | 23 (7.5) | 103 (33.6) | 181 (59.0) | 149 (24.3) | 465 (75.7) | | | | | |
| | | | GG | TG | TT | G | T | | | | | |
| rs12195525 | *NOX3* | Case | 267 (87.0) | 35 (11.4) | 5 (1.6) | 569 (92.7) | 45 (7.3) | 0.057 | 1.471 (0.987–2.193) | 0.129 | 0.104 | 0.103 |
| | | Control | 249 (81.1) | 52 (16.9) | 6 (2.0) | 550 (89.6) | 64 (10.4) | | | | | |
| | | | CC | TC | TT | C | T | | | | | |
| rs3752752 | *CDH23* | Case | 84 (27.4) | 142 (46.3) | 81 (26.4) | 310 (50.5) | 304 (49.5) | 0.954 | 0.994 (0.794–1.243) | 0.445 | 0.493 | 0.154 |
| | | Control | 85 (27.7) | 141 (45.9) | 81 (26.4) | 311 (50.7) | 303 (49.3) | | | | | |
| | | | AA | CA | CC | A | C | | | | | |
| rs3802711 | *CDH23* | Case | 19 (6.2) | 107 (34.9) | 181 (59.0) | 145 (23.6) | 469 (76.4) | 0.305 | 1.151 (0.880–1.506) | 0.139 | 0.212 | 0.345 |
| | | Control | 11 (3.6) | 108 (35.2) | 188 (61.2) | 130 (21.2) | 484 (78.8) | | | | | |
| | | | CC | CG | GG | C | G | | | | | |
| rs1227049 | *CDH23* | Case | 37 (12.1) | 127 (41.4) | 143 (46.6) | 201 (32.7) | 413 (67.3) | 0.325 | 1.129 (0.887–1.436) | 0.185 | 0.301 | 0.612 |
| | | Control | 26 (8.5) | 133 (43.3) | 148 (48.2) | 185 (30.1) | 429 (69.9) | | | | | |
| | | | AA | CA | CC | A | C | | | | | |
| rs12415607 | *CASP7* | Case | 51 (16.6) | 155 (50.5) | 101 (32.9) | 257 (41.90 | 357 (58.1) | **0.035** | 1.280 (1.017–1.611) | 0.268 | 0.360 | 0.237 |
| | | Control | 35 (11.4) | 151 (49.2) | 121 (39.4) | 221 (36.0) | 393 (64.0) | | | | | |

Continued

| SNP ID | Gene | Group | Genotype (frequency %) | | | Allele (frequency %) | | *P* | *OR* (95% *CI*) | MAF[a] | MAF[b] | HWE *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | AA | GA | GG | A | G | | | | | |
| rs1127687 | *CASP7* | Case | 17 (5.5) | 94 (30.6) | 196 (63.8) | 128 (20.8) | 486 (79.2) | 0.103 | 0.801 (0.613–1.046) | 0.230 | 0.248 | 0.955 |
| | | Control | 19 (5.5) | 114 (37.1) | 174 (56.7) | 152 (24.8) | 462 (75.2) | | | | | |
| | | | CC | TC | TT | C | T | | | | | |
| rs564250 | *CAT* | Case | 195 (63.5) | 92 (30.0) | 20 (6.5) | 482 (78.5) | 132 (21.5) | 0.623 | 0.933 (0.709–1.229) | 0.212 | 0.204 | 0.249 |
| | | Control | 198 (64.5) | 93 (30.3) | 16 (5.2) | 489 (79.6) | 125 (20.4) | | | | | |
| | | | AA | AG | GG | A | G | | | | | |
| rs769214 | *CAT* | Case | 31 (10.1) | 120 (39.1) | 156 (50.8) | 182 (29.6) | 432 (70.4) | 0.852 | 0.977 (0.765–1.247) | 0.473 | 0.301 | 0.972 |
| | | Control | 28 (9.1) | 129 (42.0) | 150 (48.9) | 185 (30.1) | 429 (69.9) | | | | | |
| | | | CC | CT | TT | C | T | | | | | |
| rs769217 | *CAT* | Case | 83 (27.0) | 148 (48.2) | 76 (24.8) | 314 (51.1) | 300 (48.9) | 0.864 | 1.020 (0.815–1.275) | 0.263 | 0.493 | 0.333 |
| | | Control | 83 (27.0) | 145 (47.2) | 79 (25.7) | 311 (50.7) | 303 (49.3) | | | | | |
| | | | AA | AT | TT | A | T | | | | | |
| rs7943316 | *CAT* | Case | 160 (52.1) | 112 (36.5) | 35 (11.4) | 432 (70.4) | 182 (29.6) | 0.664 | 1.056 (0.827–1.347) | 0.488 | 0.308 | 0.609 |
| | | Control | 149 (48.5) | 127 (41.4) | 31 (10.1) | 425 (69.2) | 189 (30.8) | | | | | |

***Note.*** MAF: Minor allele frequency; HWE: Hardy–Weinberg equilibrium; [a]1000 genomes; [b]Data form this study; *OR*: odds ratio; *CI*: confidence interval. *P*-values of deviation from HWE between the NIHL group and control group.

**Table 4.** Association of multidimensional gene-gene interactions of 18 SNPs with NIHL risk

| No. of loci | Model | TRBA | TEBA | *P* value | CVC |
|---|---|---|---|---|---|
| 1 | X1 | 0.5480 | 0.5112 | 7 (0.1719) | 8/10 |
| 2 | X3 X18 | 0.5921 | 0.5771 | **9 (0.0107)** | 10/10 |
| 3 | X3 X10 X16 | 0.6186 | 0.5116 | 6 (0.3770) | 3/10 |
| 4 | X3 X8 X10 X18 | 0.6662 | 0.4591 | 2 (0.9893) | 2/10 |
| 5 | X3 X6 X10 X13 X17 | 0.7392 | 0.4650 | 4 (0.8281) | 3/10 |
| 6 | X3 X5 X10 X12 X13 X17 | 0.8290 | 0.5218 | 7 (0.1719) | 8/10 |
| 7 | X3 X5 X7 X10 X11 X12 X17 | 0.9060 | 0.5538 | 6 (0.3770) | 9/10 |
| 8 | X3 X5 X7 X10 X11 X12 X13 X17 | 0.9527 | 0.4351 | 2 (0.9893) | 4/10 |
| 9 | X3 X4 X5 X7 X8 X10 X12 X13 X17 | 0.9927 | NaN | 4 (0.8281) | 4/10 |
| 10 | X3 X4 X5 X7 X8 X10 X11 X12 X13 X17 | 0.9926 | NaN | 4 (0.8281) | 3/10 |
| 11 | X2 X3 X4 X5 X6 X7 X8 X11 X12 X14 X17 | 0.9981 | NaN | 3 (0.9453) | 1/10 |
| 12 | X1 X3 X4 X5 X6 X7 X8 X10 X12 X13 X14 X17 | 1.0000 | NaN | 2 (0.9893) | 1/10 |
| 13 | X1 X2 X3 X4 X5 X7 X8 X9 X11 X12 X14 X16 X17 | 1.0000 | NaN | 3 (0.9453) | 2/10 |
| 14 | X1 X2 X3 X4 X5 X6 X7 X8 X9 X11 X12 X14 X16 X17 | 1.0000 | NaN | 4 (0.8281) | 4/10 |
| 15 | X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X18 | 1.0000 | NaN | 2 (0.9893) | 8/10 |
| 16 | X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15 X18 | 1.0000 | NaN | 0 (1.0000) | 8/10 |
| 17 | X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15 X16 X17 | 1.0000 | N/A | 0 (1.0000) | 10/10 |
| 18 | X1 X2 X3 X4 X5 X6 X7 X8 X9 X10 X11 X12 X13 X14 X15 X16 X17 X18 | 1.0000 | N/A | 0 (1.0000) | 10/10 |

***Note.*** *P*-values were obtained from the GMDR analysis which adjusted for age, gender, education, years of exposure, kurtosis, CNE, adj-CNE, smoking, video volume, physical exercise, and working pressure. TRBA: Training balanced accuracy; TEBA: Testing Balanced accuracy; CVC: cross-validation consistency; X1: *CASP3* rs1049216; X2: *CASP3* rs6948; X3: *NRN1* rs3805789; X4: *HSPA1L* rs2227956; X5: *HSPA1A*/*HSPA1L* rs1043618; X6: *HSPA1B* rs2763979; X7: *NOX3* rs3749930; X8: *NOX3* rs12665231; X9: *NOX3* rs12195525; X10: *CDH23* rs3752752; X11: *CDH23* rs3802711; X12: *CDH23* rs1227049; X13: *CASP7* rs12415607; X14: *CASP7* rs1127687; X15: *CAT* rs564250; X16: *CAT* rs769214; X17: *CAT* rs769217; X18: *CAT* rs7943316.

analysis for the significant models by using logistic regression. When compared with the subjects carrying *NRN1* rs3805789-CC and *CAT* rs7943316-AA, those with *NRN1* rs3805789-CC and *CAT* rs7943316-AT, *NRN1* rs3805789-CT and *CAT* rs7943316-AA, *NRN1* rs3805789-CT and *CAT* rs7943316-TT, *NRN1* rs3805789-CT/TT and *CAT* rs7943316-AA, or *NRN1* rs3805789-CC and *CAT* rs7943316-AT/TT had higher risks of NIHL (*OR*: 2.276, 95% *CI*: 1.171–4.427; *OR*: 2.213, 95% *CI*: 1.273–3.849; *OR*: 3.169, 95% *CI*: 1.425–7.048; *OR*: 2.005, 95% *CI*: 1.200–3.348; *OR*: 1.892, 95% *CI*: 1.008–3.550; $P < 0.05$) (Figure 1, Supplementary Table S3 available in www.besjournal.com).

### *Associations of the Gene-Noise-Metric Interactions with the Risk of NIHL*

We next asked whether there were any multidimensional interactions between the genes and noise metrics by using the GMDR method. After adjustments were made for age, gender, education, smoking, video volume, physical exercise, and working pressure, the best model for the risk of NIHL was found to be the interaction between *NRN1* rs3805789, *CAT* rs7943316, and kurtosis. This interaction had the score of 10/10 for CVC and 10 for the sign test ($P = 0.0010$; Table 5A, Supplementary Figure S2 available in www.besjournal.com). The joint effects of the individual interactions of *NRN1* rs3805789 and *CAT* rs7943316 with kurtosis on NIHL risk were analyzed via logistic regression analysis. The results showed that, after adjusting age, gender, education level, years of noise exposure, smoking, video volume, physical exercise, and working pressure, the subjects exposed to complex noise who carried *NRN1* rs3805789-CT and *CAT* rs7943316-TT or *NRN1* rs3805789-CT/TT and *CAT* rs7943316-AA had higher risks of NIHL than those exposed to steady noise who carried *NRN1* rs3805789-CC and *CAT* rs7943316-AA (*OR*: 5.961, 95% *CI*: 1.219–29.155; *OR*: 1.607, 95% *CI*: 1.035–2.494; $P < 0.05$) (Figure 2, Supplementary Table S4 available in www.besjournal.com). In the GMDR model, a two-locus model including *NRN1* rs3805789 and *CAT* rs7943316 was found to be significant. This observation is consistent with the results of gene-gene interactions. A four-locus model including *NRN1* rs3805789, *CAT* rs7943316, kurtosis, and adj-CNE was found to be the interaction, in which the CVC was 10/10, and the TEBA was 0.5856 ($P = 0.0107$; Table 5A). In addition, a five-locus model was also identified for the risk of NIHL. In this model, the CVC was 10/10, and TEBA was 0.5856 ($P = 0.0107$; Table 5A).

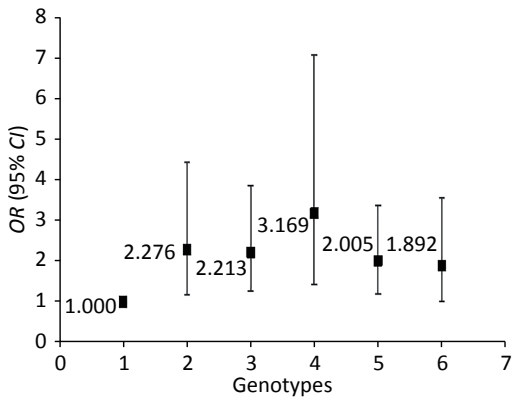


**Figure 1.** Stratified analysis for gene–gene interaction on NIHL risk using logistic regression. The odds ratios (*ORs*) were calculated after adjustment for age, gender, education, years of exposure, kurtosis, CNE, adj-CNE, smoking, video volume, physical exercise, and working pressure. 1: rs3805789-CC and rs7943316-AA; 2: rs3805789-CC and rs7943316-AT; 3: rs3805789-CT and rs7943316-AA; 4: rs3805789-CT and rs7943316-TT; 5: rs 3805789-CT/TT and rs7943316-AA; 6: rs3805 789-CC and rs7943316-AA/TT.

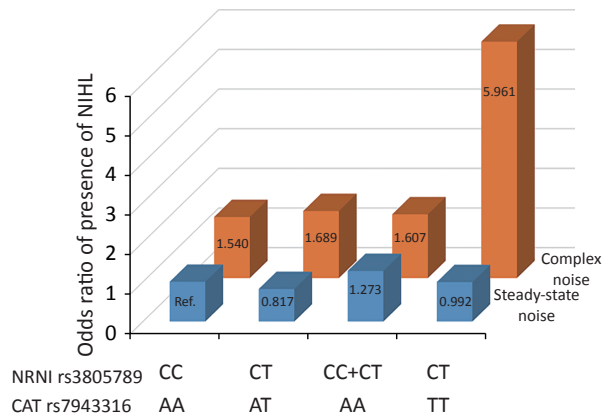


**Figure 2.** Joint effects of the *NRN1* rs3805789 and *CAT* rs7943316 with kurtosis on NIHL risk. The reference group was defined as subjects exposed steady-state noise who carry *NRN1* rs3805789 CC and *CAT* rs7943316 AA. Ref: reference group. The odds ratios (*ORs*) were calculated by the logistic regression analysis after adjustment for age, gender, education, years of exposure, smoking, video volume, physical exercise, and working pressure.

### *Associations of Gene-Lifestyle-Factor Interactions with the Risk of NIHL*

The GMDR model was used to screen for the best gene-lifestyle-factor combinations. After adjusting age, gender, education level, years of noise exposure, kurtosis, CNE, and adj-CNE, the best six-locus model involving *NRN1* rs3805789, *CAT* rs7943316, smoking, video volume, physical exercise, and working pressure for the risk of NIHL was found to be the interaction, which scored 10/10 for CVC and 9 for the sign test ($P$ = 0.0010; Table 5B). A four-locus model involving *NRN1* rs3805789, *CAT* rs7943316, smoking, and physical exercise was found to be the interaction, which scored 9/10 for CVC and 9 for sign test ($P$ = 0.0107; Table 5B). A three-locus model involving *NRN1* rs3805789, *CAT* rs7943316, and working pressure was found to be the interaction, which scored 5/10 for CVC and 9 for the sign test ($P$ = 0.0107; Table 5B). Moreover, a five-locus model was also identified for the risk of NIHL. The corresponding CVC and TEBA were 7/10 and 0.5570, respectively ($P$ = 0.0107; Table 5B).

### *Associations of Noise-Metric-Lifestyle-Factor Interactions with the Risk of NIHL*

We next evaluated the interaction combinations between noise metrics and lifestyle factors via the GMDR model. The results revealed that, after adjusting age, gender, and education level, a five-locus model involving smoking habit, video volume, physical exercise, working pressure, and kurtosis was found to be the interaction, which scored 10/10 for CVC and 9 for the sign test ($P$ = 0.0107; Table 5C). A

**Table 5.** Associations of interactions among genes, noise metrics and lifestyle factors with the risk of NIHL

| No. of loci | Model | TRBA | TEBA | *P* value | CVC |
|---|---|---|---|---|---|
| A. Gene–noise-metric interaction[a] | | | | | |
| 1 | K4 | 0.5363 | 0.4907 | 5 (0.6230) | 4/10 |
| 2 | X3 X18 | 0.5903 | 0.5762 | **9 (0.0107)** | 10/10 |
| 3 | X3 X18 K4 | 0.6003 | 0.5863 | **10 (0.0010)** | 10/10 |
| 4 | X3 X18 adj-CNE K4 | 0.6125 | 0.5856 | **9 (0.0107)** | 10/10 |
| 5 | X3 X18 adj-CNE CNE K4 | 0.6125 | 0.5856 | **9 (0.0107)** | 10/10 |
| B. Gene–lifestyle-factor interaction[b] | | | | | |
| 1 | Y3 | 0.5554 | 0.5356 | 6 (0.3770) | 9/10 |
| 2 | X3 X18 | 0.5927 | 0.5349 | 7 (0.1719) | 7/10 |
| 3 | X3 X18 Y4 | 0.6291 | 0.5377 | **9 (0.0107)** | 5/10 |
| 4 | X3 X18 Y1 Y3 | 0.6830 | 0.5850 | **9 (0.0107)** | 9/10 |
| 5 | X3 X18 Y1 Y3 Y4 | 0.7387 | 0.5570 | **9 (0.0107)** | 7/10 |
| 6 | X3 X18 Y1 Y2 Y3 Y4 | 0.7946 | 0.5866 | **9 (0.0010)** | 10/10 |
| C. Noise-metric–lifestyle-factor interaction[c] | | | | | |
| 1 | Y3 | 0.5543 | 0.5340 | 6 (0.3770) | 9/10 |
| 2 | Y2 Y3 | 0.5816 | 0.5209 | 7 (0.1719) | 5/10 |
| 3 | Y2 Y3 Y4 | 0.6069 | 0.4792 | 3 (0.9453) | 6/10 |
| 4 | Y1 Y2 Y3 Y4 | 0.6494 | 0.5509 | **9 (0.0107)** | 10/10 |
| 5 | Y1 Y2 Y3 Y4 K4 | 0.6778 | 0.5467 | **9 (0.0107)** | 10/10 |
| 6 | Y1 Y2 Y3 Y4 K4 adj-CNE | 0.6970 | 0.5437 | **9 (0.0107)** | 7/10 |
| 7 | Y1 Y2 Y3 Y4 K4 adj-CNE CNE | 0.6920 | 0.5503 | **9 (0.0107)** | 10/10 |

***Note.*** [a]Adjusted for age, gender, education, smoking, video volume, physical exercise, and working pressure; [b]Adjusted for age, gender, education, years of exposure, kurtosis, CNE, adj-CNE; [c]Adjusted for age, gender, education; TRBA: Training balanced accuracy; TEBA: Testing Balanced accuracy; CVC: cross-validation consistency; X3: *NRN1* rs3805789; X18: *CAT* rs7943316; K4: kurtosis; Y1: smoking; Y2: video volume; Y3: physical exercise; Y4: working pressure.

four-locus model involving smoking, video volume, physical exercise, and working pressure was found to be the interaction ($P$ = 0.0107; Table 5C), in which the CVC was 10/10, and the TEBA was 0.5509. A seven-locus model involving smoking, video volume, physical exercise, working pressure, kurtosis, CNE, and adj-CNE was found to be the interaction, which scored 10/10 for CVC and 9 for the sign test ($P$ = 0.0107; Table 5C). Furthermore, a six-locus model was also identified for the risk of NIHL. The corresponding CVC and TEBA were 7/10 and 0.5437, respectively ($P$ = 0.0107; Table 5C).

## DISCUSSION

In the current study, the association between gene polymorphisms, noise metrics, lifestyle factors, and NIHL was preliminarily explored using univariate analysis. The GMDR method was used to detect the association of the interaction among multiple factors with the risk of NIHL. The GMDR method explores interactions by collapsing the high-dimensional interactions of multiple factors into a single dimension. This method not only avoids biases associated with disease risk by adjusting confounding covariates, but also explores complex multi-locus interactions between genetic and environmental factors. Over the past ten years, the GMDR method has been widely applied to analyze the associations of gene–gene and gene–environment interactions with many complex diseases[26-28].

### *Associations of the Gene-Gene Interactions with the Risk of NIHL*

Increasing evidence has shown that multiple genes are closely associated with susceptibility to NIHL. Given that multiple genetic loci with moderate effects fail to reach genome-wide significance due to the limited power in most genetic studies[29], the present study focused on the associations of multi-locus interactions with NIHL risk by analyzing 18 variants in 7 susceptibility genes via the GMDR method. These genes were *CAT*, *HSP70*, *CDH23*, *CASP3*, *CASP7*, *NOX3*, and *NRN1*. These risk genes play significant roles in apoptosis, cell adhesion, and oxidative stress during the development of NIHL. We identified for the first time that the interaction between *NRN1* rs3805789 and *CAT* rs7943316 increased susceptibility to NIHL. We further validated this genetic interaction *via* stratified analysis. The results illustrated that subjects carrying *NRN1* rs3805789-CC and *CAT* rs7943316-AT, *NRN1* rs3805789-CT and *CAT* rs7943316-AA, *NRN1* rs3805789-CT and *CAT* rs7943316-TT, *NRN1* rs3805789-CT/TT and *CAT* rs7943316-AA, or *NRN1* rs3805789-CC and *CAT* rs7943316-AT/TT had higher risks of NIHL than those with *NRN1* rs3805789-CC and *CAT* rs7943316-AA. Yang et al.[13] found that *CAT* rs208679 and rs769217 were significantly associated with the risk of NIHL. A study by Wang et al.[12] have studied the association of *CAT* rs7943316 with NIHL susceptibility. Their results indicated that carriers of T allele (AT+TT) of rs7943316 have significantly higher risks of NIHL than those with AA genotype ($P$ < 0.05), and observed that a significant interaction model involving *GJB2* rs4880, *SOD2* rs137852540, and *CAT* rs769214 might associated with NIHL. These results are similar to our results presented here. *CAT* is an oxidative-stress gene. Its mutation weakens the anti-oxidant system in the cochlea, thereby hampering the elimination of the reactive oxygen species generated by noise exposure. Consequently, the structure and function of the cochlea are impaired, ultimately causing hearing loss. Furthermore, noise exposure can damage cochlear hair cells and ribbon synapses between hair cells and nerve fibres[30-32]. *NRN1* is a small polypeptide closely related to the plasticity of neurites in the human central nervous system. As a neurotrophic factor, *NRN1* has multiple effects in the nervous system. It can significantly promote the growth and branch formation of neurites[33] and establishment of functional synapses[34]. Additionally, it is necessary for the survival of neurons[35]. A previous study by our group has shown that a recombinant *NRN1* induced extensive neuritogenesis from PC12 cells[36]. Picard et al. have observed that knocking out *NRN1* impairs the development and plasticity of excitatory visual cortical networks in mice[37]. Taken together, these studies reveal that *NRN1* may play an important role in NIHL by promoting neurodevelopment and neural plasticity.

### *Associations of the Gene-Noise-Metric Interactions with the Risk of NIHL*

Complex noise is ubiquitous in industrial environments. Complex noise with impact and impulse damages the auditory system more than steady-state noise at the equivalent level[10,11,38]. Previous studies on noise have considered only the effect of noise energy on the auditory system, ignored the effect of noise temporal structure, and underestimated the degree of hearing loss associated with complex noise[39]. In this study, we focused on noise kurtosis. This factor was used to describe the characteristics of impulsive noise, distinguish between steady-state and complex noises, and assess the effect of complex noise on

hearing loss. We found that the NIHL group had a higher median noise kurtosis and a larger proportion of workers exposed to complex noise than the control group, consistent with our previous report[17]. In a previous study, mean kurtosis was used to describe the temporal structure of noise, and a mean kurtosis of 10 was used as the boundary value between Gaussian and complex noises. In our study presented here, the median kurtosis of 4 was considered as the boundary. Noise damages the auditory system *via* direct mechanical force and by disrupting the metabolism[10]. Considering that complex noise is more harmful to the auditory system than steady-state noise, researchers have begun to adjust the energy parameters or exposure time by using kurtosis. For example, Zhao et al.[10] and Goley et al.[40] have proposed correction methods for exposure time and noise energy, respectively. In this study, the correction method for the exposure time was used to adjust the CNE. We observed an association between CNE, adj-CNE, and NIHL through univariate analysis. The multidimensional interactions between genes and noise metrics were analyzed using the GMDR method. The best model for the risk of NIHL was found to be the interaction among *NRN1* rs3805789, *CAT* rs7943316, and kurtosis. To date, studies have never reported the associations of such interactions with the risk of NIHL. The further stratified analysis revealed that the subjects exposed to complex noise who carried *NRN1* rs3805789-CT and *CAT* rs7943316-TT or *NRN1* rs3805789-CT/TT and *CAT* rs7943316-AA were at a higher risk of NIHL than those exposed to steady noise who carried both NRN1 rs3805789-CC and *CAT* rs7943316-AA. This observation illustrates that complex-noise exposure increases the effect of the interaction between NRN1 rs3805789 and *CAT* rs7943316 on NIHL risk. In addition, interaction among *NRN1* rs3805789, *CAT* rs7943316, kurtosis, CNE, and adj-CNE was also identified as a risk factor for NIHL. This result indicates that kurtosis, CNE, and adj-CNE may affect the development of NIHL not only through direct effects but also through interactions with genes.

### *Associations of Gene-Lifestyle-Factor and Noise-Metric-Lifestyle-Factor Interactions with the Risk of NIHL*

The risk of NIHL was also affected by lifestyle factors. In this study, we found a significant difference in smoking, video volume, and physical exercise between the two groups. We further investigated gene-lifestyle-factor interactions while investigating the effects of noise-metric-lifestyle-factor interactions on NIHL. We observed a cross-reaction involving *NRN1* rs3805789, *CAT* rs7943316, smoking, video volume, physical exercise, and working pressure for the risk of NIHL. Furthermore, we also found a potential five -locus noise-metric–lifestyle-factor interaction model involving smoking, video volume, physical exercise, working pressure, and kurtosis, as well as a seven -locus model including smoking, video volume, physical exercise, working pressure, kurtosis, CNE, and adj-CNE. These results are similar to the previous results of our group[17]. Previous results showed that there were positive interactions between noise kurtosis with smoking, video volume and physical exercise. However, previous studies analyzed only the interactions between two-category variables via crossover analysis and failed to analyze the effects of CNE and adj-CNE on the risk of NIHL. Many studies have shown that smoking-induced hearing loss is likely due to vascular changes, including capillary contraction, increased blood viscosity, and cochlear anoxia[41,42]. High-level noise exposure may lead to hearing loss via a mechanism involving reduced cochlear oxygen tension during and after noise exposure[43]. Moreover, lack of exercise affects blood, oxygen, and nutrient flow to the cochlea, leading to the degradation of the stria vascularis (SV). Blood vessels in the SV are essential for transporting necessary factors, such as oxygen and glucose, to the cochlea[44].

### *Strengths and Limitations of this Study*

This study is superior to previous studies in multiple aspects. First, we firstly focused on the effects of multidimensional interactions on NIHL risk by analyzing 18 variants, three noise metrics, and four lifestyle factors. Second, we identified for the first time that interaction between *NRN1* rs3805789 and *CAT* rs7943316 increases NIHL susceptibility. Third, the associations of the interactions among *NRN1* rs3805789, *CAT* rs7943316, and kurtosis with the risk of NIHL was detected for the first time. However, this study had some limitations as well. First, we could not obtain data regarding other important confounding factors, such as hypertension and diabetes, due to technical reasons. Second, the analyses of lifestyle factors depended on the recollection of the subjects, which can be unreliable. Third, because the sample size is not large enough, the results obtained from this study should be verified by studies involving larger sample sizes. Finally, this study is an association study,

the mechanisms of the gene-gene or gene–environmental-factor interactions should be investigated in future laboratory and clinical studies.

## CONCLUSION

In conclusion, complex noise, high CNE, high adj-CNE, smoking, high video volume, and sedentary lifestyle are environmental risk factors for NIHL. Concurrence of *NRN1* rs3805789 and *CAT* rs7943316 constitutes a genetic risk factor for NIHL. Complex noise exposure significantly increases the risk of NIHL in subjects with a high genetic risk score. Interactions between genes and lifestyle as well as noise metrics and lifestyle affect the risk of NIHL. These results provide a theoretical basis for screening genetic and environmental risk factors to prevent NIHL.

## AUTHORS' CONTRIBUTIONS

SYL and WQS are joint first authors. SYL oversaw data analysis and wrote the manuscript. WQS edited the article and JRX conducted the statistical analysis. ZL, SL, and YQC conducted the study design and revised the manuscript. TYZ, HYW, and LWX carried out the experiment. MBZ, YH, and LY was responsible for data collection and final manuscript. All authors approved the final manuscript.

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the participants signed the informed consent form, and the study was approved by the Science Ethics Committee of Hangzhou Normal University (2017LL107).

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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