NRN1 and CAT Gene Polymorphisms, Complex Noise, and Lifestyles Interactively Affect the Risk of Noise-Induced Hearing Loss

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Research

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1 ***NRN1* and *CAT* gene polymorphisms, complex noise, and lifestyles**

2 **interactively affect the risk of noise-induced hearing loss**

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ABSTRACT

Background: Multiple genetic and environmental factors influence the severity of NIHL. However, few studies have reported interactions among such factors in modulating the risk of NIHL. This study aimed to assess for 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 single nucleotide polymorphisms (SNPs). The generalized multifactor dimensionality reduction (GMDR) method was used to examine the effects of all possible interactions.

Results: The proportion of people with complex noise exposure, high CNE, high adj-CNE, smoking, propensity to watch loud videos, or sedentary lifestyle was significantly greater in the NIHL group than in the healthy group ($P < 0.05$). The GMDR model demonstrated a relevant interaction between *NRN1* rs3805789 and *CAT* rs7943316. Subjects with the SNP pair of *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 ($P < 0.05$). There was an interaction among *NRN1* rs3805789, *CAT* rs7943316, and kurtosis. Subjects exposed to complex noise and carrying both *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 and carrying both *NRN1* rs3805789-CC and *CAT* 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 ($P = 0.0107$).

Conclusions: 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.

Keywords: noise-induced hearing loss; kurtosis; *CAT*; *NRN1*; lifestyle; interaction; generalized multifactor dimensionality reduction

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

74 protection devices [3-6]. Therefore, the single-locus method may not be appropriate to study
75 common complex disorders such as NIHL.

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

92 Increasing evidence has shown the association of susceptibility genes, such as catalase (*CAT*),
93 heat shock protein70 (*HSP70*), cadherin-23 (*CDH23*), caspase (*CASP*), and NADPH oxidase3
94 (*NOX3*), with the development of NIHL [12-18]. Additionally, previous studies have demonstrated
95 that smoking, stressful lifestyle, and physical exercise are associated with hearing loss [19-21].

However, 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 the *Neuritin1* (*NRN1*) gene] and kurtosis. Therefore, in a case-control study with 307 NIHL patients and 307 age- and gender-matched healthy controls, a total of 18 variants 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. 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 (3000, 4000, and 6000 Hz) average hearing threshold > 25 dB. The subjects were divided into two groups—patients with NIHL ($n = 307$) and controls with normal hearing ($n = 307$)—who were matched for gender and age (± 3 years).

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: average daily cigarette use for ≥ 1 year; (2) video volume: high video volume $\geq 40\%$ of the maximum volume; low video volume $< 40\%$ of the maximum volume; (3) regular physical exercise: average physical exercise for once a month or more often for ≥ 1 year. 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**). The measurement time was 8 hours 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.

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

where, x_i is the i^{th} value, $\bar{x}$ is the sample mean, and β 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 impulse 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 [22, 23]. 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]:

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

where, $L_{\text{Aeq},8\text{h}}$ is the equivalent continuous A-weighted noise exposure level normalized to an 8-h working day and T is the time of noise exposure in years. CNE is measured in dB (A) per year.

To incorporate kurtosis (β) 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]:

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

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. Equation (3) shows that when $L_{\text{Aeq},8\text{h}}$ 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 otolaryngologists performed pure-tone audiometry for the left and right ears of

each participant at 500, 1000, 2000, 3000, 4000, 6000, and 8000 Hz in a sound-insulated room with background noise < 25 dB (A) (**Supplementary material 2**). 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. High-frequency NIHL (hNIHL) was diagnosed based on binaural high-frequency hearing threshold levels at 3, 4, and 6 kHz ($HTL_{3, 4, 6}$) using Formula 4:

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

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 **Tables S1 and S2**. 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 [24] 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

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. 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 HTL₃₄₆ 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 ($P > 0.05$).

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, Figure S1**). The CVC of this two-locus model was 10/10, and the TEBA was 0.5768. We then conducted a stratified 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, Table S3**).

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, Figure**

S2). 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, Table S4**). 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**).

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**).

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 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 [25-27].

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 [28], 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 [29-31]. *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 [32] and establishment of functional synapses [33]. Additionally, it is necessary for the survival of neurons [34]. A previous study by our group has shown that a recombinant *NRN1* induced extensive neuritogenesis from PC12 cells [35]. Picard et al. have observed that knocking out *NRN1* impairs the development and plasticity of excitatory visual cortical networks in mice [36]. Taken together, these studies reveal that *NRN1* may play an important role in NIHL by promoting neurodevelopment and neural plasticity.

Complex noise is ubiquitous in industrial environments. Complex noise with impact and pulse damages the auditory system more than steady-state noise at the equivalent level [10, 11, 37]. 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. In this study, we focused on noise kurtosis. This factor was used to describe the characteristics of noise pulse, 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 kurtosis 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. [38] 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.

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 [39, 40]. High-volume noise exposure may lead to hearing loss via a mechanism involving reduced cochlear oxygen tension during and after noise exposure [41]. 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 [42].

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.

Abbreviations

NIHL: Noise-induced hearing loss; CNE: Cumulative noise exposure; adj-CNE: kurtosis-adjusted cumulative noise exposure; EEH: equal-energy hypothesis; ISO: International Standardization Organization; SV: stria vascularis; ROC: receiver operating characteristic; KASP: kompetitive allele specific polymerase chain reaction; Neuritin1: *NRN1*; Catalase: *CAT*; Caspase3: *CASP3*; Caspase7: *CASP7*; NADPH Oxidase3: *NOX3*; Cadherin-23: *CDH23*; Heat shock protein: *HSP*; CV: coefficient of variation; OR: Odds ratio; CI: Confidence interval; SD: Standard deviation.

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Authors' contributions

SYL and JRX are joint first authors. SYL oversaw data analysis and wrote the manuscript. JRX edited the article and 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 and LY was responsible for data collection and final manuscript. All authors approved the final manuscript.

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Availability of data and materials

Please contact author for data requests.

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).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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References

1. WHO. Deafness and hearing loss [online] [DB/OL]. Available at: <http://www.who.int/media centre/fact sheets/fs300/en/>.
2. Chen YL, Hu WJ. Research progress on mechanisms of noise-induced hearingloss. Chinese J Ind Med. 2018;31(6):438-440.
3. Stanbury M, Rafferty AP, Rosenman K. Prevalence of hearing loss and work-related noise-induced hearing loss in Michigan. J Occup Environ Med. 2008;50(1):72-79.
4. Shargorodsky J, Curhan SG, Curhan GC, et al. Change in prevalence of hearing loss in US adolescents. JAMA. 2010; 304(7):772-778.
5. Lao XQ, Yu ITS, Au DKK, et al. Noise Exposure and Hearing Impairment among Chinese Restaurant Workers and Entertainment Employees in Hong Kong. Plos One. 2013; 8(8):e70674..
6. Frederiksen TW, Ramlau-Hansen CH, Stokholm ZA, et al. Noise-Induced Hearing Loss - A Preventable Disease? Results of a 10-Year Longitudinal Study of Workers Exposed to Occupational Noise. Noise Health. 2017; 19(87):103-111.
7. Clark WW, Bohne BA, Boettcher FA. Effect of periodic rest on hearing loss and cochlear damage following exposure to noise. J Acoust Soc Am. 1987; 82(4):1253-1264.
8. Erdreich J. A distribution based definition of impulse noise. J Acoust Soc Am. 1986; 79(4):990-998.
9. Pourbakht A, Yamasoba T. Cochlear damage caused by continuous and intermittent noise exposure. Hear Res. 2003; 178(1-2):70-78.
10. Zhao YM, Qiu W, Zeng L, et al. Application of the kurtosis statistic to the evaluation of the risk of hearing loss in workers exposed to high-level complex noise. Ear Hear. 2010; 31(4):527-532.
11. Davis RI, Qiu W, Heyer NJ, et al. The use of the kurtosis metric in the evaluation of occupational hearing loss in workers in China: Implications for hearing risk assessment. Noise & Health. 2012; 14(61):330-342.
12. Wang SL, Yu LG, Liu RP, et al. Gene-gene interaction of GJB2, SOD2, and CAT on occupational noise-induced hearing loss in Chinese Han population. Biomed Environ Sci. 2014; 27(12):965-968.
13. Yang JH, Zhang JY, Wang XM, et al. Identification of functional tag single nucleotide polymorphisms within the entire CAT gene and their clinical relevance in patients with noise-induced hearing loss. Int J

Clin Exp Pathol. 2015; 8(3):2852-2863.

14. Li YH, Yu SF, Gu GZ, et al. Polymorphisms of heat shock protein 70 genes (HSPA1A, HSPA1B and HSPA1L) and susceptibility of noise-induced hearing loss in a Chinese population: A case-control study. Plos One. 2017;12(2): e0171722..
15. Zhang XH, Ni YQ, Liu Y, et al. Screening of noise-induced hearing loss (NIHL)-associated SNPs and the assessment of its genetic susceptibility. Environ Health-Glob. 2019; 18(1):30.
16. Wu YY, Ni JT, Qi MJ, et al. Associations of genetic variation in CASP3 gene with noise-induced hearing loss in a Chinese population: a case-control study. Environ Health-Glob. 2017; 16(1):78.
17. Zhao TY, Wang YN, Li Z, et al. Associations of noise kurtosis, genetic variations in NOX3 and lifestyle factors with noise-induced hearing loss. Environ Health-Glob. 2020;19(1):1-13.
18. Henderson D, Bielefeld EC, Harris KC, et al. The role of oxidative stress in noise-induced hearing loss. Ear Hearing. 2006; 27(1):1-19.
19. Rybak LP, Whitworth CA, Mukherjee D, et al. Mechanisms of cisplatin-induced ototoxicity and prevention. Hearing Res. 2007; 226(1-2):157-167.
20. Wang XC, Xu PF, Li P, et al. Alterations in gray matter volume due to unilateral hearing loss. Sci Rep-Uk. 2016; 6:25811..
21. Nakajima K, Kanda E, Hosobuchi A, et al. Subclinical hearing loss, longer sleep duration, and cardiometabolic risk factors in Japanese general population. Int J Otolaryngol. 2014;2014:218218.
22. Qiu W, Davis B, Hamernik RP. Hearing loss from interrupted, intermittent, and time varying Gaussian noise exposures: the applicability of the equal energy hypothesis. J Acoust Soc Am. 2007; 121(3):1613-1620.
23. Qiu W, Hamernik RP, Davis RI. The value of a kurtosis metric in estimating the hazard to hearing of complex industrial noise exposures. J Acoust Soc Am. 2013; 133(5):2856-2866.
24. Lou XY, Chen GB, Yan L, et al. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. Am J Hum Genet. 2007; 80(6):1125-1137.
25. Chen B, Du Z, Dong X, et al. Association of Variant Interactions in RANK, RANKL, OPG, TRAF6, and NFATC1 Genes with the Development of Osteonecrosis of the Femoral Head. DNA Cell Biol. 2019; 38(7):734-746.
26. Feng C, Yang Y, Yang S, et al. Effect of gene-gene and gene-environment interaction on the risk of first-ever stroke and poststroke death. Mol Genet Genomic Med. 2019; 7(8):e846.
27. Zhang LX, Ding RH, Kuang P, et al. Interaction between CONNEXIN37 and PDE4D gene polymorphisms with susceptibility to ischemic stroke in Chinese population. Exp Biol Med. 2019; 244(18):1642-1647.
28. Li J, Wei Z, Hakonarson H. Application of computational methods in genetic study of inflammatory bowel disease. World J Gastroenterol. 2016; 22(3):949-960.
29. Wichmann C, Moser T. Relating structure and function of inner hair cell ribbon synapses. Cell Tissue Res. 2015;361(1):95-114.
30. Moser T, Starr A. Auditory neuropathy - neural and synaptic mechanisms. Nat Rev Neurol. 2016;12(3):135-149.
31. Roux I, Safieddine S, Nouvian R, et al. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. Cell. 2006; 127(2):277-289.
32. Karamoysoyly E, Burnand RC, Tomlinson DR, et al. Neuritin mediates nerve growth factor-induced axonal regeneration and is deficient in experimental diabetic neuropathy. Diabetes. 2008;57(1):181-189.
33. Fujino T, Leslie JH, Eavri R, et al. CPG15 regulates synapse stability in the developing and adult brain.

Genes Dev. 2011; 25(24):2674-2685.

34. Sharma TP, Liu Y, Wordinger RJ, et al. Neuritin 1 promotes retinal ganglion cell survival and axonal regeneration following optic nerve crush. *Cell Death Dis.* 2015; 6:e1661.
35. Zhang P, Luo X, Guo Z, et al. Neuritin Inhibits Notch Signaling through Interacted with Neuralized to Promote the Neurite Growth. *Front Mol Neurosci.* 2017; 10:179.
36. Picard N, Leslie JH, Trowbridge SK, et al. Aberrant development and plasticity of excitatory visual cortical networks in the absence of cp15. *J Neurosci.* 2014; 34(10):3517-3522.
37. Xie HW, Qiu W, Heyer NJ, et al. The Use of the Kurtosis-Adjusted Cumulative Noise Exposure Metric in Evaluating the Hearing Loss Risk for Complex Noise. *Ear Hearing.* 2016; 37(3):312-323.
38. Goley GS, Song WJ, Kim JH. Kurtosis corrected sound pressure level as a noise metric for risk assessment of occupational noises. *Journal of the Acoustical Society of America.* 2011; 129(3):1475-1481.
39. Hwang JH, Chen JC, Hsu CJ, et al. Plasma reactive oxygen species levels are correlated with severity of age-related hearing impairment in humans. *Neurobiol Aging.* 2012; 33(9):1920-1926.
40. Fechter LD, Thorne PR, Nuttall AL. Effects of carbon monoxide on cochlear electrophysiology and blood flow. *Hear Res.* 1987; 27(1):37-45.
41. Su BM, Chan DK. Prevalence of Hearing Loss in US Children and Adolescents: Findings From NHANES 1988-2010. *JAMA Otolaryngol Head Neck Surg.* 2017; 143(9):920-927.
42. Han C, Ding D, Lopez MC, et al. Effects of Long-Term Exercise on Age-Related Hearing Loss in Mice. *J Neurosci.* 2016; 36(44):11308-11319.

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Table 1 Basic Information for 18 SNPs

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

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

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Table 2 General characteristics of subjects between NIHL and control groups

Characteristics		Total (n=614)	NIHL (n=307)	Control (n=307)	χ^2/z	<i>P</i>
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)		
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

Physical exercise, n (%)	High	465 (75.7)	247 (40.2)	218 (35.5)	6.745	0.009
	Never	418 (68.1)	224 (36.5)	194 (31.6)		
Working pressure, n (%)	Regular	196 (31.9)	83 (13.5)	113 (18.4)		
	Low	113 (18.4)	49 (8.0)	64 (10.4)	2.440	0.118
	High	501 (81.6)	258 (42.0)	243 (39.6)		

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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	<i>CASP3</i>	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)					
rs6948	<i>CASP3</i>	Case	GG	GT	TT	G	T	0.064	1.334 (0.982- 1.809)	0.426	0.143	0.431
		Control	10 (3.3)	92 (30.0)	205 (66.8)	112 (18.2)	502 (81.8)					
rs3805789	<i>NRN1</i>	Case	8 (2.6)	72 (23.5)	227 (73.9)	88 (14.3)	526 (85.7)	0.864	1.020 (0.815-1.276)	0.300	0.466	0.216
		Control	CC	CT	TT	C	T					
rs2227956	<i>HSPA1L</i>	Case	84 (27.4)	163 (53.1)	60 (19.5)	331 (53.9)	283 (46.1)	0.942	0.989 (0.744-1.316)	0.123	0.191	0.671
		Control	93 (30.3)	142 (46.3)	72 (23.5)	328 (53.4)	286 (46.6)					
rs1043618	<i>HSPA1A/ HSPA1L</i>	Case	CC	CT	TT	C	T	0.903	0.985 (0.777-1.250)	0.481	0.332	0.587
		Control	15 (4.9)	86 (28.0)	206 (67.1)	116 (18.9)	498 (81.1)					
rs2763979	<i>HSPA1B</i>	Case	CC	CG	GG	C	G	0.453	1.098 (0.860-1.403)	0.448	0.306	0.387
		Control	168 (54.7)	102 (33.2)	37 (12.1)	438 (71.3)	176 (28.7)					
rs3749930	<i>NOX3</i>	Case	GG	GT	TT	G	T	0.123	1.192 (0.953-1.492)	0.197	0.477	0.662
		Control	86 (28.0)	148 (48.2)	73 (23.8)	320 (52.1)	294 (47.9)					
rs12665231	<i>NOX3</i>		68 (22.1)	157 (51.1)	82 (26.7)	293 (47.7)	321 (52.3)					
			CC	CT	TT	C	T					

		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)					
rs12195525	NOX3	GG		TG	TT	G	T	0.057	1.471 (0.987-2.193)	0.129	0.104	0.103
		Case	267 (87.0)	35 (11.4)	5 (1.6)	569 (92.7)	45 (7.3)					
		Control	249 (81.1)	52 (16.9)	6 (2.0)	550 (89.6)	64 (10.4)					
rs3752752	CDH23	CC		TC	TT	C	T	0.954	0.994 (0.794-1.243)	0.445	0.493	0.154
		Case	84 (27.4)	142 (46.3)	81 (26.4)	310 (50.5)	304 (49.5)					
		Control	85 (27.7)	141 (45.9)	81 (26.4)	311 (50.7)	303 (49.3)					
rs3802711	CDH23	AA		CA	CC	A	C	0.305	1.151 (0.880-1.506)	0.139	0.212	0.345
		Case	19 (6.2)	107 (34.9)	181 (59.0)	145 (23.6)	469 (76.4)					
		Control	11 (3.6)	108 (35.2)	188 (61.2)	130 (21.2)	484 (78.8)					
rs1227049	CDH23	CC		CG	GG	C	G	0.325	1.129 (0.887-1.436)	0.185	0.301	0.612
		Case	37 (12.1)	127 (41.4)	143 (46.6)	201 (32.7)	413 (67.3)					
		Control	26 (8.5)	133 (43.3)	148 (48.2)	185 (30.1)	429 (69.9)					
rs12415607	CASP7	AA		CA	CC	A	C	0.035	1.280 (1.017-1.611)	0.268	0.360	0.237
		Case	51 (16.6)	155 (50.5)	101 (32.9)	257 (41.90)	357 (58.1)					
		Control	35 (11.4)	151 (49.2)	121 (39.4)	221 (36.0)	393 (64.0)					
rs1127687	CASP7	AA		GA	GG	A	G	0.103	0.801 (0.613-1.046)	0.230	0.248	0.955
		Case	17 (5.5)	94 (30.6)	196 (63.8)	128 (20.8)	486 (79.2)					
		Control	19 (5.5)	114 (37.1)	174 (56.7)	152 (24.8)	462 (75.2)					
rs564250	CAT	CC		TC	TT	C	T	0.623	0.933 (0.709-1.229)	0.212	0.204	0.249
		Case	195 (63.5)	92 (30.0)	20 (6.5)	482 (78.5)	132 (21.5)					
		Control	198 (64.5)	93 (30.3)	16 (5.2)	489 (79.6)	125 (20.4)					
rs769214	CAT	AA		AG	GG	A	G	0.852	0.977 (0.765-1.247)	0.473	0.301	0.972
		Case	31 (10.1)	120 (39.1)	156 (50.8)	182 (29.6)	432 (70.4)					
		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)					

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

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.

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

^aAdjusted for age, gender, education, smoking, video volume, physical exercise, and working pressure;

^bAdjusted for age, gender, education, years of exposure, kurtosis, CNE, adj-CNE;

^cAdjusted 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.

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: rs3805789-CT/TT and rs7943316-AA; 6: rs3805789-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.

Table S1 Primer information A

SNP ID	Primer Allele FAM	Primer Allele HEX	Primer Common
rs1049216	AGTAATTGTGAAAAAGTTAAACATTGAAGTAAT	AGTAATTGTGAAAAAGTTAAACATTGAAC	CAGTCTTAAGTGGGGGAATATCATAAAA
rs6948	GGAGGCCAGAGCTGAGCC	CGGAGGCCAGAGCTGAGCA	AGCCTGCCTCCCGGGCTGA
rs3805789	TTCCGCCCTGAGGCCGAC	CTTTCCGCCCTGAGGCCGAT	CACTCAGCTTCCAGCCGGGT
rs2227956	ATGGTATTCTCAATGTCACAGCCAC	AATGGTATTCTCAATGTCACAGCCAT	CTTGTTACCTTGCCGGTGCTCTT
rs1043618	CCTGCTCTCTGTCGGCTCC	CCTGCTCTCTGTCGGCTCG	GTGTTCCGTTTCCAGCCCCCAA
rs2763979	ATTCCTGGTTCACCTCTGGACG	AATTCCTGGTTCACCTCTGGACA	CTCGGGACTGTGAGGTCTACTT
rs3749930	ACACAACCACTGAATTGCTAAGGAC	AACACAACCACTGAATTGCTAAGGAA	CAGACCGGTGACGCCTGCTATT
rs12665231	AGCTAAGGAAGTATGGTATAGTGTACT	GCTAAGGAAGTATGGTATAGTGTACC	CCCAGTTGGGAAGTGGTGAATAATAATTA
rs12195525	GTTCTCTTATGAATGAAATAAGGTTTCA	GTTCTCTTATGAATGAAATAAGGTTTCG	CTGTGCCTGAATTTTAACTGCATGCTAAT
rs3752752	AAACATCACCTCCTGGACATCAAT	CATCACCTCCTGGACATCAAC	GTCCTTCCACGTGGGGTGGTT
rs3802711	GTCCAGCTCCACGTCCTTCC	GGTCCAGCTCCACGTCCTTCT	TCTCCTGTGGAGGGGTGCTAA
rs1227049	AGCTGACTTCCCCAAAGGTGG	AGCTGACTTCCCCAAAGGTGC	GCTGTTGCAGGCAACTGACAATGAT
rs12415607	AATGGAGTACATGCTTAGTGGTCG	GAATGGAGTACATGCTTAGTGGTCT	AGAAGATGGCGTTCTTGCCTGGTAT
rs1127687	CACTCCATCTCAGTCAGTGGC	CTCACTCCATCTCAGTCAGTGGT	TGGAAAATGGAGCCATGACAAGAACAAA
rs564250	AGACCTGGAGTCATTTCTTAACCTTTA	GACCTGGAGTCATTTCTTAACCTTTG	CAAATCCATAGTAACAAAAACAAAGGAAAA
rs769214	TTTCAAAATTCCTGCTTACCTGGGA	TCAAAATTCCTGCTTACCTGGGG	ACTGGAGAAATCTGCTTCCCCAAATTTTA
rs769217	GTGGCCAACCTACCAGCGTGAC	AGTGGCCAACCTACCAGCGTGAT	TACCCTGATTGTCCTGCATGCACAT
rs7943316	AAATCTGCCTGTTGCCCCGAGT	AAATCTGCCTGTTGCCCCGAGA	GGTGCTGATTGGCTGAGCCTGAA

Table S2 Primer information B

SNP ID	Sequence	CG%_FAM	CG%_HEX	CG% Common
rs1049216	GGGGAATATCATAAAAATTC[A/G] TTACTTCAATGTTTAACTTT	21.2	24.2	37.9
rs6948	AGCCTGCCTCCCGGGCTGAG[G/T] GCTCAGCTCTGGCCTCCGGC	72.2	68.4	73.7
rs3805789	TCTTTCGCCCCTGAGGCCGA[C/T] GAACCCGGCTGGAAGCTGAG	70	61.9	61.9
rs2227956	TATTCTCAATGTCACAGCCA[C/T] GGACAAGAGCACCGGCAAGG	44	38.5	54.2
rs1043618	CCAGCCCCCAATCTCAGAGC[C/G] GAGCCGACAGAGAGCAGGGA	68.4	68.4	59.1
rs2763979	GAGGTCTACTTCTACACAC[C/T] GTCCAGGAGTGAACCAGGAA	54.5	47.8	56.5
rs3749930	GACCGGTGACGCCTGCTATT[G/T] TCCTTAGCAATTCAGTGGTT	44	38.5	59.1
rs12665231	GGAAGTATGGTATAGTGTAC[T/C] GTTCACAATAGTTAATTATT	37	42.3	37.9
rs12195525	TATGAATGAAATAAGGTTTC[A/G] ACTGACAGGTATTAGAAATTA	30	32.3	37.9
rs3752752	ATCACCTCCTGGACATCAA[T/C] GACAACCACCCACGTGGAA	42.3	54.5	61.9
rs3802711	GGAGGGGGTGCTAAGGGTCC[G/A] GAAGGACGTGGAGCTGGACC	65	61.9	59.1
rs1227049	GGCAACTGACAATGATGCAG[C/G] CACCTTTGGGGAAGTCAGCT	54.5	54.5	48
rs12415607	TGGCGTTCTTGCTGGTATC[C/A] GACCACTAAGCATGTACTCC	45.8	42.3	48
rs1127687	GGAGCCATGACAAGAACAAA[G/A] CCACTGACTGAGATGGAGTG	58.3	52.2	39.3
rs564250	GCTTTTTTTTATATAATGAA[C/T] AAAGGTTAAGAAATGACTCC	37	42.3	26.7
rs769214	AAAATTCCTGCTTACCTGGG[A/G] GTAAAATTTGGGGAAGCAGA	49.2	44	37.9
rs769217	GTGGCCAACTACCAGCGTGA[C/T] GGCCCGATGTGCATGCAGGA	61.9	54.5	48
rs7943316	GAGCCTGAAGTCGCCACGGA[A/T] CTCGGGGCAACAGGCAGATT	54.5	54.5	56.5

Table S3 Jointed effects of NRN1 and CAT on NIHL by using the logistic regression models

NRN1 rs3805789	CAT rs7943316	Risk of NIHL			
		Controls/Cases, n (%)	ORs (95% CI) *	P	FDR
CC	AA	55 (17.9)/39 (12.7)	1.00		
CC	AT	29 (9.4)/41 (13.4)	2.276 (1.171-4.427)	0.015	0.041
CC	TT	9 (2.9)/4 (1.3)	0.650 (0.173-2.442)	0.524	0.64
CT	AA	61 (19.9)/87 (28.3)	2.213 (1.273-3.849)	0.005	0.028
CT	AT	67 (21.8)/48 (15.6)	1.036 (0.578-1.857)	0.905	0.905
CT	TT	14(4.6)/28 (9.1)	3.169 (1.425-7.048)	0.005	0.028
TT	AA	33 (10.7)/34 (11.1)	1.620 (0.419-6.205)	0.154	0.282
TT	AT	31(10.1)/23 (7.5)	1.048 (0.514-2.137)	0.898	0.905
TT	TT	8 (2.6)/3 (1.0)	0.558 (0.131-2.377)	0.430	0.591
CT+TT	AA	94 (30.6)/121 (39.4)	2.005 (1.200-3.348)	0.008	0.029
CC	AT+TT	38 (12.4)/45 (14.7)	1.892 (1.008-3.550)	0.047	0.103
CT+TT	AT+TT	120 (39.1)/102 (33.2)	1.255 (0.752-2.094)	0.385	0.591

NRN1: neuritin 1; CAT: catalase; ORs: Odds ratio; CI: confidential interval.

*ORs (95%CI) and P value were obtained with the use of multivariate logistic regression analysis after adjustment for age, gender, education, years of exposure, kurtosis, CNE, adj-CNE, smoking, video volume, physical exercise, and working pressure.

FDR (false discovery rate) was obtained with the use of Benjamin-Hochberg procedure for controlling the false positive rate in multiple comparison.

Table S4 Jointed effects on NIHL between high-risk genotypes of *NRN1* and *CAT* and kurtosis

Combination of kurtosis and genotypes of <i>NRN1</i> and <i>CAT</i>		Controls/Cases, n (%)	ORs (95% CI) *	<i>P</i> *	FDR
Steady-state noise	rs3805789 CC- rs7943316 AA	9 (2.9)/8 (2.6)	1.000		
	rs3805789 CC- rs7943316 AT	6 (2.0)/9 (2.9)	2.499 (0.561-11.133)	0.230	0.814
	rs3805789 CC- rs7943316 TT	3 (1.0)/0(0.0)	0.000	0.999	1.000
	rs3805789 CT- rs7943316 AA	19 (6.2)/14 (14.6)	1.317 (0.375-4.626)	0.667	1.078
	rs3805789 CT- rs7943316 AT	15 (4.9)/7 (2.3)	0.817 (0.206-3.234)	0.773	0.895
	rs3805789 CT- rs7943316 TT	3 (1.0)/2 (0.7)	0.992 (0.122-8.078)	0.994	1.047
	rs3805789 TT- rs7943316 AA	7 (2.3)/5 (1.6)	1.289 (0.269-6.185)	0.751	0.987
	rs3805789 TT- rs7943316 AT	4 (1.3)/2 (0.7)	0.734 (0.098-5.485)	0.763	0.941
	rs3805789 TT- rs7943316 TT	1 (0.3)/0 (0.0)	0.000	1.000	1.000
	rs3805789 CT/TT- rs7943316 AA	26 (8.5)/19 (6.2)	1.273 (0.388-4.179)	0.691	1.013
	rs3805789 CC- rs7943316 (AT+TT)	9 (2.9)/9 (2.9)	1.723 (0.422-7.040)	0.449	1.041
	rs3805789 (CT+TT)- rs7943316 (AT+TT)	23 (7.5)/11 (3.6)	0.772 (0.220-2.710)	0.686	1.013
Complex noise	rs3805789 CC- rs7943316 AA	46 (15.0)/31 (10.1)	1.540 (0.348-6.809)	0.569	1.043
	rs3805789 CC- rs7943316 AT	23 (7.5)/32 (10.4)	3.347 (0.740-15.139)	0.117	0.644
	rs3805789 CC- rs7943316 TT	6 (2.0)/4 (1.3)	1.336 (0.193-9.262)	0.770	0.895
	rs3805789 CT-rs7943316 AA	42 (13.7)/73 (23.8)	3.875 (0.895-16.774)	0.070	0.051
	rs3805789 CT-rs7943316 AT	52 (16.9)/41 (13.4)	1.689 (0.391-7.288)	0.483	0.966
	rs3805789 CT-rs7943316 TT	11 (3.6)/26 (8.5)	5.961(1.219-29.155)	0.028	0.374
	rs3805789 TT-rs7943316 AA	26 (8.5)/29 (9.4)	2.625 (0.581-11.864)	0.210	0.843
	rs3805789 TT-rs7943316 AT	27 (8.8)/21 (6.8)	1.750 (0.380-8.057)	0.473	0.966
	rs3805789 CT+TT-rs7943316 AA	68 (22.1)/102 (33.2)	1.607 (1.035-2.494)	0.034	0.051
	rs3805789 CC-rs7943316 (AT+TT)	23 (9.4)/36 (11.7)	1.378 (0.761-2.495)	0.290	0.800

	rs3805789(CT+TT)- rs7943316(AT+TT)	97 (31.6)/91(29.6)	0.945 (0.411-1.270)	0.259	0.800
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*ORs (95%CI) and *P* value were obtained with multivariate logistic regression analysis after adjustment for age, gender, education, years of exposure, smoking, video volume, physical exercise, and working pressure. FDR (false discovery rate) was obtained from the Benjamin-Hochberg procedure.

Figure S1 Interactions of *NRN1* rs3805789 and *CAT* rs7943316 associated with NIHL risk identified by GMDR analysis. In each cell, the left bar represents a positive score and the right bar a negative score. High-risk cells were indicated by dark shading, low-risk cells by light shading.

Figure S2 Interactions of *NRN1* rs3805789, *CAT* rs7943316 and kurtosis associated with NIHL risk identified by GMDR. 0: Steady-state noise; 1: Complex noise. In each cell, the left bar represents a positive score and the right bar a negative score. High-risk cells were indicated by dark shading, low-risk cells by light shading.

Figures

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: rs3805789-CT/TT and rs7943316-AA; 6: rs3805789-CC and rs7943316-AA/TT.

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: rs3805789-CT/TT and rs7943316-AA; 6: rs3805789-CC and rs7943316-AA/TT.

Figure 2

Joint effects of the NRNI rs3805789 and CAT rs7943316 with kurtosis on NIHL risk. The reference group was defined as subjects exposed steady-state noise who carry NRNI 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.

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.

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