Relationship between CDH23 gene and risk of noise-induced hearing loss

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Abstract

Objective: To investigate the relationship between CDH23 gene and the risk of noise-induced hearing loss (NIHL).

Methods: This was a case-control study. Noise-exposed workers worked in a steel factory in North China was recruited and been divided into two groups: the case group (BHFTA ≥ 40 dB) and the control group (BHFTA < 25 dB). We analyzed the association among 18 single nucleotide polymorphisms (SNPs) in CDH23 and NIHL risk using the generalized multifactor dimensionality reduction (GMDR) method. Logistic regression was performed to analyze the main effects of SNPs and the interactions between CNE and SNPs adjusting cumulative noise exposure (CNE), smoking, drinking, physical exercise and hypertension.

Results: In this study, 776 subjects of period I and 1117 subjects of period I+II were recruited. The results showed that subjects who carried the AA genotype of rs3802711 possessed significantly increased risk of NIHL than those carrying GG (OR: 2.71; 95% CI: 1.15, 6.39) and GA+GG (OR: 2.54; 95% CI: 1.09, 6.00) in period I, respectively. For rs11592462, subjects carrying the GG genotype showed a significantly increased risk of NIHL compared with the subjects. Significant relationships were showed between rs10999947, rs3802711, rs10762480, rs3752751, rs3752752, rs3747867, and rs11592462 for NIHL overall and various CNE strata. There was no significant association between the rs1227049 - rs3752752 - rs10999947 - rs3752751 - rs10762480 - rs3802711 - rs11592462 - rs4747195 - rs4747194 - rs10466026 haplotypes and NIHL risk.

Conclusions: The genetic variation in the CDH23 gene might play an important role in determining individual susceptibility to NIHL.

Introduction

Noise-induced hearing loss (NIHL) is a complex disease resulting from the interaction between environmental and intrinsic factors, studies in environmental factors included noise, organic solvents, heat, vibrations, smoking and drinking.¹⁻³ However, when exposed to similar levels of noise and environmental factors, the morbidity of NIHL varies widely among workers, that is, some workers have had NIHL, others not.⁴ Genetic variation could explain some of the inconsistent results.

CDH23 is one of the most interesting genes to be investigated in humans, which encoded cadherin 23, a component of the stereocilia tip links, which are thought to gate mechanotransduction channel in hair cells.⁵ In animals, CDH23 is the first and the only gene linked with predisposition to noise-induced hearing loss in waltzer mice.⁶ Previous studies revealed that genetic variations in CDH23 is a key determinant of age-related hearing loss and early-onset progressive hearing loss, mice carrying this susceptibility allele was more susceptible to noise.⁶⁻¹⁰ Mutations in CDH23 in mice cause stereocilia disorganization and lead to deafness and vestibular disorders. In humans, mutations in CDH23 lead to both nonsyndromic and syndromic hearing loss. Existing evidence from epidemiological studies suggested an inconsistent association between genetic variations and risk of NIHL.¹¹ One study reported that the associations between 63 polymorphisms in CDH23 and NIHL were all negative in the Polish and Swedish population.¹² Other studies¹³⁻¹⁴ described the positive association of CDH23 polymorphisms with NIHL.

The relationship between genetic variation in CDH23 genes and the risk of NIHL in Chinese population remains unknown. Therefore, we conducted this study to investigate the relationship between CDH23 gene and the risk of NIHL.

1 Materials And Methods

1.1 Subjects

In this study, noise-exposed workers worked in a steel factory in North China was recruited and been divided into two groups: the case group (BHFTA ≥ 40 dB) and the control group (BHFTA < 25 dB). The details had been described in previous trial¹⁵. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of our hospital. All participants had signed the informed consent.

1.2 Inclusion and exclusion criteria

Inclusion criteria: (1) participants who exposed to occupational noise higher than 80 dB and the cumulative time of noise exposure was ≥ 3 years; (2) age was older than 18 years old; (3) patients who have signed informed consent. Exclusion criteria: (1) subjects who served as air force or artillery, have had the history of head trauma, explosion deafness and familial deafness, or had a history of contagious diseases (mumps, measles and rubella) and treatment with an ototoxic drug (aminoglycoside); (2) subjects with tympanitis, Meniere's syndrome, conductive hearing loss, exaggerated hearing loss, feigned deafness, sudden deafness, toxic deafness, deafness caused by contagious diseases, tumors, autoimmune diseases and others; (3) subjects whose pure-tone audiogram showed horizontal lines or near-horizontal lines, and subjects who had impairment of hearing loss in linguistic frequency that was more severe than that of hearing loss in high frequency.

1.3 Data collection

Participation was voluntary, and written informed consent was obtained from all participants. Those who signed consent were interviewed by trained study nurses at a convenient location using a standardized and structured questionnaire. Information on name, sex, date of birth, height, weight, noise exposure history (including air force and artillery), tenure, history of past diseases (head trauma, Measles, mumps and rubella, tympanitis, Meniere's syndrome, explosion deafness and familial deafness), smoking history, drinking history, medication history, physical exercise habits and other diseases which can induce the hearing loss were collected through in-person interview, and the data of hypertension and the results of pure-tone audiometry (PTA) were required through professional test. Otology morphological examination and otoscopy were requested, including bilateral auricle malformation, external auditory canal malformation and stenosis, tympanic membrane perforation, adhesion or calcification, etc.

1.4 PTA detection

The audiometry was done using AS216 audiometer (Interacoustics AS Company, Danish). All the audiometric tests were performed among 6297 workers with standard procedures in quiet test rooms with a background noise level of <25 dB (A) by trained occupational health physician. The data of pure-tone air conduction hearing threshold tests were recorded at the frequencies of 500, 1000, 2000, 3000, 4000, and 6000 Hz after subjects stopped noise exposure for at least 48 h. The averages of 3000, 4000, and 6000 Hz were calculated as the threshold levels at high frequency for each ear. The hearing thresholds at speech frequency were calculated by the average of 500, 1000, and 2000 Hz for each ear. The audiometric raw data were polished by the confounding effects of age and gender on the basis of the Diagnostic Criteria of Occupational NIHL [National Health and Family Planning Commission of the People's Republic of China. Diagnosis of occupational noise-induced deafness (GBZ49-2014). Available online: <http://www.moh.gov.cn/zwgkzt/pyl/201410/12e4ec65af8e46248bb45d366a0d5021.shtml> (accessed on 29 October 2014)]. In addition, their ears were inspected according to this standard.

1.5 Cumulative noise exposure calculation

Based on the Occupational Health Standard of the People's Republic of China: Measurement of Noise in the Workplace [National Health and Family Planning Commission of the People's Republic of China. Measurement of noise in the workplace (GBZ/T 189.8-2007). Available online: <http://www.moh.gov.cn/zwgkzt/pyl/201410/1a150c9e20f846b8a651d2fd69c6bdb0.shtml> (accessed on 30 October 2014)], noise exposure levels were assessed from 8 a.m. to 4 p.m. during the subjects' working time at the representational sites of each type of work using Noise Dose Meters (NoisePro series, Quest Technologies, American). Noise exposure was evaluated with equivalent continuous dB(A)-weighted sound pressure levels ($L_{Aeq,8h}$). Besides, the previous recorded data on noise exposure levels of the factory were collected. Cumulative noise exposure (CNE) was calculated to determine the actual noise exposure for each subject based on every period of occupational history, which was defined as: ¹⁶

$$CNE = 10 \log \left[\frac{1}{T_{ref}} \sum_{i=1}^n (T_i \times 10^{L_{Aeq,8h,i}/10}) \right]$$

where $L_{Aeq,8h,i}$ is the equivalent continuous A-weighted noise exposure level in decibels normalized to an 8 h working day, occurring over the time interval T_i in years, with a total of n different noise level exposure periods (i.e., years spent working in different noise tasks/environments), and T_{ref} : 1 year.

1.6 SNP Selection and Genotyping

A total of 18 SNPs in CDH23 genes were selected for genotyping based on the following criteria: minor allele frequencies more than 5%, laboratory evidence of function, or prior association with human disease studies. The detailed information of the 18 SNPs in period I and the 16 SNPs in Period I + II were presented in the Table 1.

Genomic DNA was extracted from blood samples using the DNA Extraction Kit from Biotech Firm. The SNP genotyping work was performed using SNPscan method, which has been described in detail elsewhere,¹⁷ PCR products were sequenced by ABI3730XL DNA analyzer and results were analyzed by GeneMapper 4.1 software (Applied Biosystems, Foster City, CA, USA). The whole analysis process was performed blind. The concordance rates for quality control samples were between 99% and 100% for all assays. All the SNPs in the controls were in Hardy-Weinberg equilibrium (HWE) ($p > 0.05$) except that 2 SNPs (rs1227051 in period I and rs1227049 in period I + II) with minor allele frequency less than 10% were excluded from the final analysis. And that 3 SNPs (rs1227051, rs10823829 and rs1227065) were not significantly associated with NIHL risk in overall or different CNE stata and different noise exposure level were not verified and genotyped in period I + II. A total of 17 SNPs in CDH23 (rs3802720, rs7087735, rs1227049, rs10999947, rs3752752, rs3752751, rs10823829, rs1227065, rs10999978, rs3747867, rs17712523, rs10762480, rs3802711, rs11592462, rs10466026, rs4747194, rs4747195) of period I and 14 SNPs in CDH23 (rs3802720, rs7087735, rs10999947, rs3752752, rs3752751, rs10999978, rs3747867, rs17712523, rs10762480, rs3802711, rs11592462, rs10466026, rs4747194, rs4747195) of period I + II were included in the final analysis.

1.7 Statistical Analysis

We used the software program SPSS 22.0 (IBM, Chicago, USA) to conduct the statistical analysis. Before analysis, Hardy-Weinberg equilibrium (HWE) test was checked for each SNP among control subjects using χ^2 -test. Continuous variables were expressed as mean $\pm$ standard deviation (SD) while categorical variables were expressed as frequencies (%). Paired Samples t-test was used for two comparisons when each datum conformed to normal distribution, while the non-normally distributed continuous data were compared using non-parametric tests. The counting data were tested by chi-square test. Four genetic models were used, that were additive-inheritance model, dominant-inheritance model, recessive-inheritance model and co-dominant-inheritance model. Unconditional logistic regression model was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for associations between SNPs, risk of NHL, different CNE strata (CNE<97dB(A)·year and CNE $\geq$ 97dB(A)·year) and different noise level (noise level<85dB and noise level $\geq$ 85dB) in different genotype strata after adjusting for the potential confounding factors such as smoke, drink, [hypertension](#), physical exercise and CNE. And Bonferroni correction was performed to control for multiple testing. Haploview was used to investigate the linkage disequilibrium (LD) between the SNPs (Haploview version 4.2 software, <http://www.broad.mit.edu/mpg/haploview>). Generalized multifactor dimensionality reduction (GMDR) software (version 0.9, <http://sourceforge.net/projects/gmdr/>) was applied for further detecting best multilocus model associated with NIHL after adjustment for covariates including smoke, drink, blood pressure, physical exercise and CNE. Finally, 1000 permutations were performed to get a permuted value of these models.

2 Results

2.1 The general Characteristics

In this study, 776 subjects of period I and 1117 subjects of period I+II were included. The concrete characteristic of the subjects were presented in the Table 2. The results showed that no statistically significant differences were seen between case and control individuals in the distribution of age, tenure exposure to noise, CNE, drinking status, blood pressure in both period I and period I + II ($p > 0.05$), and so physical exercise in period I ($p > 0.05$), but for period I + II, the results of physical exercise was just the opposite ($p \leq 0.05$). However, the BHFTA in the case group was significantly greater than that of the control subjects both period I (51.9 ± 9.1 vs. 19.2 ± 7.5 , $p < 0.05$) and Period I + II (51.3 ± 9.0 vs. 18.6 ± 8.2 , $p < 0.05$). Besides, the risk of NIHL in smokers both above two period group increased significantly than that of non-smokers ($p < 0.05$). The variables (CNE, smoking, drinking, physical exercise and blood pressure) were further adjusted in the unconditional logistic regression.

2.2 Associations of CDH23 Variants and the Risk of NIHL

As shown in Table 3 (presented only for the SNPs with significant results, the whole results of associations of CDH23 variants and the risk of NIHL were detailed in supplementary Table S1), after adjusting for CNE, smoking, drinking, physical exercise and blood pressure, subjects carrying the AA genotype of rs3802711 were significantly associated with a significantly increased risk of NIHL than those carrying GG (adjusted OR: 2.71; 95% CI: 1.15, 6.39) and GA+GG (adjusted OR: 2.54; 95% CI: 1.09, 6.00) in period I, respectively. For rs11592462, subjects carrying the GG genotype showed a significantly increased risk of NIHL compared with the subjects carrying CC (adjusted OR: 2.40; 95% CI: 1.26, 4.56) and CG+CC (adjusted OR: 2.47; 95% CI: 1.31, 4.66) both in period I and period I + II. However, no significant differences were detected in genotype of the other 15 SNPs of CDH23 in period I and the other 13 SNPs in period I + II between case and control subjects ($p > 0.05$). However, none of the associations remained statistically significant after Bonferroni adjustment.

2.3 Stratification Analysis of CDH23 by Noise Exposure Level and CNE

The results of associations between CDH23 SNPs and NIHL risk stratified by noise exposure level and CNE were listed in the Table 4 (presented only for the SNPs with significant results, the whole results of stratification analysis of CDH23 by noise exposure level and CNE were detailed in supplementary Table S1). For rs10999947 in period I + II, when the noise exposure levels > 85 dB (A), compared with the GG genotype, an increased NIHL risk were observed for workers carrying GA (adjusted OR: 1.43; 95% CI: 1.01, 2.01) and GA+AA (adjusted OR: 1.40; 95% CI: 1.01, 1.95) genotype; when CNE<97dB(A)·year in period I, compared with the GA+GG genotype, a decreased NIHL risk was observed for subjects with the AA genotype (adjusted OR: 0.42; 95% CI: 0.18, 0.99).

As shown in Table 4, for rs3802711 in period I, not only noise exposure levels ≥ 85 dB (A) but also CNE ≥ 97 dB(A)·year, compared with workers carrying GG genotype, the increased NIHL risk were observed for GA (noise exposure levels ≥ 85 dB (A): adjusted OR: 1.55; 95% CI: 1.02, 2.35; CNE ≥ 97 dB(A)·year: adjusted OR: 1.63; 95% CI: 1.04, 2.55), AA (noise exposure levels ≥ 85 dB (A): adjusted OR: 4.93; 95% CI: 1.26, 19.24; CNE ≥ 97 dB(A)·year: adjusted OR: 5.88; 95% CI: 1.43, 24.13), GA+AA (noise exposure levels ≥ 85 dB (A): adjusted OR: 1.68; 95% CI: 1.12, 2.52; CNE ≥ 97 dB(A)·year: adjusted OR: 1.79; 95% CI: 1.16, 2.76); compared with workers carrying GA+GG genotype, the increased NIHL risk was observed for AA genotype (noise exposure levels ≥ 85 dB (A): adjusted OR: 4.29; 95% CI: 1.11, 16.64; CNE ≥ 97 dB(A)·year: adjusted OR: 5.01; 95% CI: 1.23, 20.41). Similarly, rs3802711 in period I+II, not only noise exposure levels ≥ 85 dB (A) but also CNE ≥ 97 dB(A)·year, compared with workers carrying GG genotype, the increased NIHL risk were observed for GA (noise exposure levels ≥ 85 dB (A): adjusted OR: 1.51; 95% CI: 1.06, 2.15;

CNE $\geq$ 97dB(A)-year: adjusted OR: 1.50; 95% CI: 1.04, 2.15), GA+AA (noise exposure levels $\geq$ 85 dB(A): adjusted OR: 1.53; 95%CI: 1.09, 2.15; CNE $\geq$ 97dB(A)-year: adjusted OR: 1.53; 95% CI: 1.07, 2.18).

For rs10762480, not only in period I but also period I + II, when noise exposure levels $\geq$ 85 dB(A), compared with workers carrying CC genotype, the increased NIHL risk was observed for CT (period I: adjusted OR: 1.74; 95% CI: 1.13, 2.68; period II: adjusted OR: 1.47; 95% CI: 1.03, 2.11), CT+TT (period I: adjusted OR: 1.71; 95% CI: 1.12, 2.60; period II: adjusted OR: 1.45; 95% CI: 1.02, 2.06); but for period I, when CNE $\geq$ 97dB(A)-year, compared with workers carrying CC genotype, the increased NIHL risk was observed for CT+TT (adjusted OR: 1.59; 95% CI: 1.01, 2.51).

For rs3752751 and rs3752752 in period I, when noise exposure levels $\geq$ 85 dB (A), compared with workers carrying CC genotype, the TT genotype significantly decreased the risk of NIHL (rs3752751: adjusted OR: 0.43; 95% CI: 0.21, 0.87; rs3752752: adjusted OR: 0.41; 95% CI: 0.20, 0.86); by contrast, when noise exposure levels $\geq$ 85 dB (A), the TT genotype significantly increased the risk of NIHL (rs3752751: adjusted OR: 2.20; 95% CI: 1.24, 3.88; rs3752752: adjusted OR: 2.06; 95% CI: 1.16, 3.66); As such, when noise exposure levels $\geq$ 85 dB (A), compared with workers carrying CT+CC genotype, the TT genotype significantly decreased the risk of NIHL (rs3752751: adjusted OR: 0.43; 95% CI: 0.23, 0.82; rs3752752: adjusted OR: 0.41; 95% CI: 0.21, 0.79); by contrast, when noise exposure levels $\geq$ 85 dB (A), the TT genotype significantly increased the risk of NIHL (rs3752751: adjusted OR: 1.90; 95% CI: 1.16, 3.10; rs3752752: adjusted OR: 1.85; 95% CI: 1.12, 3.04).

For rs3747867 in period I, when CNE $\geq$ 97 dB(A)-year, compared with workers carrying CC genotype, the increased NIHL risk was observed for CT (adjusted OR: 1.61; 95% CI: 1.03, 2.53), CT+TT (adjusted OR: 1.64; 95% CI: 1.05, 2.57).

For rs11592462 in period I + II, not only noise exposure levels $\geq$ 85 dB (A) but also CNE $\geq$ 97dB(A)-year, compared with workers carrying CC genotype, the increased NIHL risk was observed for GG (noise exposure levels $\geq$ 85 dB (A): adjusted OR: 2.53;95% CI: 1.16, 5.52; CNE $\geq$ 97dB(A)-year: adjusted OR: 2.46; 95% CI: 1.09, 5.55); A similar pattern was also observed for that compared with workers carrying GG, the increased NIHL risk was observed for CG+CC (noise exposure levels $\geq$ 85 dB (A): adjusted OR: 2.53; 95% CI: 1.17, 5.48; CNE $\geq$ 97dB(A)-year: adjusted OR: 2.61;95% CI: 1.17, 5.85) .

However, none of the associations remained statistically significant after Bonferroni adjustment.

2.4 Association of CDH23 Haplotypes with NIHL Risk

Haplotypes were inferred based on observed genotypes by using Haploview software, for period I and period I+II, rs1227049, rs3752752, rs10999947, rs3752751, rs10762480, rs3802711, rs11592462, rs4747195, rs4747194 and rs10466026 ten SNPs constructed the haplotypes in period I, but the results for period I suggested that there was no significant association between the rs1227049-rs3752752-rs10999947-rs3752751-rs10762480-rs3802711-rs11592462-rs4747195-rs4747194-rs10466026 haplotypes and NIHL risk, but the results for period I+II was the opposite, after adjusting for CNE, smoking, drinking, physical exercise and blood pressure, the detailed significant association suggested that the decreased NIHL risk was observed for the haplotype of GGGCCGCAAT (adjusted OR: 0.66; 95% CI: 0.43, 0.99) (See Table S2). After applying the Bonferroni correction, all the associations were no longer statistically significant.

2.5 Evaluation of the Interaction Effect between CDH23 SNPs

GMDR was performed to reveal the interactions between SNPs. None of significant SNP-SNP interactions were found in Period I+II of this study (P>0.05, see details in Table S3).

3 Discussion

Our study was the first comprehensive analysis between genetic polymorphisms in CDH23 genes and risk of NIHL. In this study, SNPs located in the CDH23 genes in period I (17 SNPs) and period I + II (14 SNPs), the SNPs in period I with the significant and borderline significant increased or decreased NIHL risk were chosen to verify the association between gene variation in CDH23 and NIHL risk in period I + II. Significant associations were observed for rs10999947, rs3802711, rs11592462, rs10762480, rs3752751, rs3752752, rs3747867 for NIHL overall and/or various CNE strata and noise exposure level.

For overall, not only period I and but also period I + II, the study suggested that CDH23 polymorphism (rs11592462) may have increased NIHL risk which was consistent with the Polish population study,¹⁸ and CDH23 polymorphism (rs3802711) may have increased NIHL risk in period I which was in accordance with the previous study.¹⁹

For stratified analysis by noise exposure level and/or CNE, when noise exposure levels $\geq$ 85dB(A) or when CNE $\geq$ 97dB(A)-year, the study suggested that CDH23 polymorphism (rs11592462) may have increased NIHL risk in period I + II, so did the CDH23 polymorphism (rs3802711) and the CDH23 polymorphism (rs10762480) in period I and period I + II.

In conclusion, the study suggested that CDH23 polymorphisms (rs11592462, rs3802711 and rs10762480) were significantly associated with NIHL risk both period I and period I + II, which was consistent with previous study,^{19–20} which suggested that CDH23 gene polymorphisms play an important role in the development of NIHL, which was proved by the analysis of the association between the CDH23 haplotypes and NIHL risk.

CDH23 plays a necessary long-term role in the normal marshalling course of the ciliary bundle of the cochlea,^{20–23} CDH23 mutations were firstly found to be associated with susceptibility to noise induced hearing loss in the population and the study recommended that CDH23 could be an early indicator of hearing loss in routine screening.¹¹ Although some studies did not manifest the association between gene variations in CDH23 and NIHL risk,^{12,24} however recent one study suggested that CDH23 gene variations could increase NIHL risk,¹⁴ which is consistent with the present study results.

The study has several strengths. Firstly, the study is a NIHL cohort population-based case-control study, in which it has a comprehensive data of noise exposure than previous studies. Secondly, the "cases" of this study were chose by the diagnosis of occupational noise-induced deafness (GBZ 49-2014) and the "controls" of this study were diagnosed by the same standard with the "cases", so the results of this study were different from the previous studies^{11,14} in which study population was chose whether susceptible to noise or not and, to some degree in hearing loss, the results of this study were more valuable and objective than those studies, in that subjects of susceptibility of those studies might have normal hearing loss. Thirdly, the power size of the study in "case" of hearing loss was bigger than previous study. Lastly, the study divided two periods and the results of period I were verified in period II, and then analyze the results of period I + II with a big power size, thus the results appears convincing.

This study also has some limitations that should be considered. Firstly, when the Bonferroni correction was applied in our study, the significance of the findings was overwhelming; However, it is recognized that replication of findings in independent populations is much more important than obtaining highly significant p-values. Secondly, the workers might be exposed to noise in other places, such as the community, but this was too complicated to take into consideration; but one recent review²⁵ based on different high noise exposure groups suggested that for high intensity noise exposure study, the noise intensity of daily life has little effect on the results of the study. Lastly, this study focused on a specific pathway, additional SNPs should be tested in the future.

Conclusions

The genetic variation in the CDH23 gene might play an important role in determining individual susceptibility to NIHL. The results provide new insight into the pathogenesis and early prevention of noise-induced hearing loss.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Henan Medical College.

Consent to publication:

All authors final approval of the version to be published, and informed consent was obtained from all participants.

Competing interests:

All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

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Availability of data and materials

The datesets used or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution

Conception and design of the research: Jiao J, Yu SF. Acquisition of data: Chen GS, Zhang HL. Analysis and interpretation of the data: Jiao J, Gu GZ. Statistical analysis: Zheng YX, Zhang HL. Obtaining financing: None. Writing of the manuscript: Jiao J. Critical revision of the manuscript for intellectual content : Yu SF, Jiao J.

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Tables

Table 1. The Results of Hardy-Weinberg Test and MAF between Period I and Period I + II

SNP	A1 A2	Period I					
		MAF	GENO(A1A1/A1A2/A2A2)	P _{H-W}	MAF	GENO(A1A1/A1A2/A2A2)	P _{H-W}
rs3802720	T C	0.222	16/182/294	0.06	0.214	25/259/441	0.10
rs7087735	C T	0.426	94/241/155	1.00	0.438	136/362/225	0.71
rs1227049	C G	0.303	40/226/226	0.14	0.306	59/337/329	0.04
rs10999947	A G	0.308	45/209/236	1.00	0.310	63/315/345	0.48
rs3752752	T C	0.425	92/229/167	0.40	0.419	139/330/252	0.11
rs3752751	T C	0.429	94/227/167	0.31	0.424	142/328/251	0.07
rs10823829	C T	0.258	25/201/263	0.10	/	/	/
rs1227065	A G	0.122	3/116/360	0.06	/	/	/
rs10999978	T C	0.166	13/130/344	0.87	0.166	20/198/502	0.89
rs1227051	G A	0.218	44/105/303	0.00	/	/	/
rs3747867	T C	0.169	7/141/333	0.09	0.159	13/200/501	0.21
rs17712523	A G	0.152	12/126/354	0.86	0.146	14/183/528	0.77
rs10762480	T C	0.156	10/123/359	1.00	0.151	15/181/529	1.00
rs3802711	A G	0.191	10/151/327	0.16	0.183	20/210/491	0.80
rs11592462	G C	0.191	12/158/322	0.18	0.191	18/230/477	0.14
rs10466026	A G	0.472	101/255/133	0.32	0.466	148/375/199	0.26
rs4747194	A G	0.474	102/257/133	0.32	0.466	148/378/199	0.21
rs4747195	T C	0.472	100/256/133	0.28	0.465	146/377/199	0.20

Note: A1: minor allele; A2: major allele; MAF: minor allele frequency; H-W: Hardy-Weinberg test.

Table 2. The concrete Characteristic of Period I and I+II Subjects

Characteristic	Case	Control	t/ χ^2	P	Case	Control	t/ χ^2	P
	Period I				Period I + II			
	n=284	n=492			n=392	n=725		
Age[$\bar{x} \pm s$, year]	40.5 $\pm$ 8.1	39.4 $\pm$ 8.2	1.71 ^a	0.09	40.9 $\pm$ 8.2	40.3 $\pm$ 8.3	1.21 ^a	0.23
Tenure exposure to noise[$\bar{x} \pm s$, year]	18.9 $\pm$ 9.1	18.0 $\pm$ 8.8	1.32 ^a	0.19	19.6 $\pm$ 9.1	19.3 $\pm$ 9.0	0.65 ^a	0.52
BHFTA[$\bar{x} \pm s$, dB]	51.9 $\pm$ 9.1	19.2 $\pm$ 7.5	54.03 ^a	0.005	51.3 $\pm$ 9.0	18.6 $\pm$ 8.2	61.43 ^a	0.005
CNE[$\bar{x} \pm s$, dB(A)·year]	98.3 $\pm$ 4.7	98.1 $\pm$ 4.7	0.61 ^a	0.54	98.3 $\pm$ 4.5	98.0 $\pm$ 4.5	0.97 ^a	0.33
Sex[n(%)]								
Male	272(95.8)	473(96.1)	0.06 ^b	0.80	375(95.7)	693(95.6)	0.00 ^b	0.95
Female	12(4.2)	19(3.9)			17(4.3)	32(4.4)		
Smoke[n(%)]								
Yes	190(66.9)	273(55.5)	9.86 ^b	0.005	252(64.3)	419(57.8)	4.50 ^b	0.03
No	94(33.1)	219(44.5)			140(35.7)	306(42.2)		
Drink[n(%)]								
Yes	197(69.4)	332(67.5)	0.30 ^b	0.59	268(68.4)	488(67.3)	0.13 ^b	0.72
No	87(30.6)	160(32.5)			124(31.6)	237(32.7)		
Physical Exercise[n(%)]								
Yes	102(35.9)	209(42.5)	3.25 ^b	0.07	162(41.3)	346(47.7)	4.21 ^b	0.04
No	182(64.1)	283(57.5)			230(58.7)	379(52.3)		
BP[n(%)]								
Yes	121(42.6)	206(41.9)	0.04 ^b	0.84	161(41.1)	302(41.7)	0.04 ^b	0.85
No	163(57.4)	286(58.1)			231(58.9)	423(58.3)		

Note: ^at value; ^b χ^2 value; BHFTA: double ear high frequency average hearing threshold; CNE: cumulative noise exposure; BP: blood pressure.

Table 3. The Significant Association between SNPs of CDH23 and NIHL Risk in overall

gene_rs number	genotype	Control(N)	Case(N)	OR(95%CI) #
CDH23_rs3802711 (period I)				
additive model	GG/GA/AA	327/151/10	172/98/13	1.35(1.03,1.76)
co-dominant model	GG	327	172	1
	GA	151	98	1.21(0.88,1.67)
	AA	10	13	2.71(1.15,6.39)
dominant model	GG	327	172	1
	GA+AA	161	111	1.30(0.96,1.77)
recessive model	GA+GG	478	270	1
	AA	10	13	2.54(1.09,5.95)
CDH23_rs11592462 period I + II				
additive model	CC/CG/GG	474/229/18	257/114/23	1.13(0.91,1.41)
co-dominant model	CC	474	257	1
	CG	229	114	0.92(0.70,1.20)
	GG	18	23	2.40(1.26,4.56)
dominant model	CC	474	257	1
	CG+GG	247	137	1.02(0.79,1.33)
recessive model	CG+CC	703	371	1
	GG	18	23	2.47(1.31,4.66)

Note: CNE: cumulative noise exposure; BP: blood pressure; OR: odds ratio; CI: confidence interval; OR(95%CI) #: Adjusted for CNE, smoking, drinking, exercise and BP.

Table 4. The Significant Association between SNPs of CDH23 and NIHL Risk stratified analysis by noise exposure level and CNE

rs	genotype	Noise exposure level<85dB(A)			Noise exposure level≥85dB(A)			CNE<97dB(A)·year			CNE≥97dB(A)·year		
		control	case	OR(95%CI) [#]	control	case	OR(95%CI) [#]	control	case	OR(95%CI) [#]	control	case	OR(95%CI) [#]
l_rs10999947(period I)													
ive	GA+GG	187	107	1	258	155	1	202	125	1	243	137	1
	AA	19	7	0.68(0.27,1.68)	26	15	0.94(0.48,1.85)	27	7	0.42(0.18,0.99)	18	15	1.51(0.72,3.18)
l_rs10999947(period I + III)													
mt	GG	150	83	1	192	90	1	155	79	1	187	94	1
	GA	131	68	0.91(0.61,1.37)	183	119	1.43(1.01,2.01)	141	83	1.17(0.80,1.72)	173	104	1.20(0.85,1.71)
mt	GG	150	83	1	192	90	1	155	79	1	187	94	1
	GA+AA	159	79	0.88(0.59,1.29)	218	140	1.40(1.01,1.95)	175	96	1.09(0.75,1.57)	202	123	1.21(0.87,1.70)
l_rs3802711(period I)													
ive	GG/GA/AA	124/73/7	71/38/5	0.99(0.65,1.51)	203/78/3	101/60/8	1.70(1.18,2.45)	144/78/7	86/40/6	0.99(0.67,1.46)	183/73/3	86/58/7	1.82(1.24,2.69)
	GG	124	71	1	203	101	1	144	86	1	183	86	1
mt	GA	73	38	0.86(0.52,1.42)	78	60	1.55(1.02,2.35)	78	40	0.87(0.54,1.39)	73	58	1.63(1.04,2.55)
	AA	7	5	1.58(0.47,5.34)	3	8	4.93(1.26,19.24)	7	6	1.52(0.49,4.70)	3	7	5.88(1.43,24.13)
mt	GG	124	71	1	203	101	1	144	86	1	183	86	1
	GA+AA	80	43	0.91(0.56,1.48)	81	68	1.68(1.12,2.52)	85	46	0.92(0.59,1.44)	76	65	1.79(1.16,2.76)
ive	GA+GG	197	109	1	281	161	1	222	126	1	256	144	1
	AA	7	5	1.66(0.50,5.55)	3	8	4.29(1.11,16.64)	7	6	1.59(0.52,4.87)	3	7	5.01(1.23,20.41)
l_rs3802711(period I + III)													
ive	GG/GA/AA	200/98/9	108/49/5	0.95(0.66,1.36)	288/111/11	139/81/9	1.44(1.07,1.94)	214/104/12	115/53/7	0.99(0.71,1.38)	274/105/8	132/77/7	1.48(1.08,2.02)
	GG	200	108	1	288	139	1	214	115	1	274	132	1
mt	GA	98	49	0.88(0.58,1.35)	111	81	1.51(1.06,2.15)	104	53	0.94(0.63,1.41)	105	77	1.50(1.04,2.15)
	GG	200	108	1	288	139	1	214	115	1	274	132	1
mt	GA+AA	107	54	0.91(0.60,1.36)	122	90	1.53(1.09,2.15)	116	60	0.96(0.65,1.42)	113	84	1.53(1.07,2.18)
l_rs10762480(period I)													
ive	CC/CT/TT	142/58/6	81/30/4	0.99(0.64,1.54)	218/64/4	111/57/3	1.57(1.07,2.32)	159/63/7	91/39/3	1.02(0.68,1.54)	201/59/3	101/48/4	1.60(1.06,2.41)
	CC	142	81	1	218	111	1	159	91	1	201	101	1
mt	CT	58	30	0.86(0.51,1.47)	64	57	1.74(1.13,2.68)	63	39	1.09(0.68,1.78)	59	48	1.51(0.95,2.40)
	CC	142	81	1	218	111	1	159	91	1	201	101	1
mt	CT+TT	64	34	0.92(0.55,1.53)	68	60	1.71(1.12,2.60)	70	42	1.06(0.67,1.69)	62	52	1.59(1.01,2.51)
l_rs10762480(period I + III)													
mt	CC	220	121	1	307	154	1	233	124	1	294	151	1
	CT	81	38	0.81(0.52,1.27)	98	73	1.47(1.03,2.11)	89	48	1.00(0.66,1.51)	90	63	1.31(0.90,1.92)
mt	CC	220	121	1	307	154	1	233	124	1	294	151	1
	CT+TT	89	42	0.84(0.54,1.29)	105	77	1.45(1.02,2.06)	97	52	1.00(0.67,1.49)	97	67	1.31(0.90,1.90)
l_rs3752751(period I)													
ive	CC/CT/TT	70/87/49	43/57/15	0.70(0.50,0.98)	98/140/44	48/82/41	1.46(1.10,1.93)	79/108/41	40/67/26	1.14(0.84,1.55)	89/119/52	51/72/30	1.04(0.78,1.39)
	CC	70	43	1	98	48	1	79	40	1	89	51	1
mt	TT	49	15	0.43(0.21,0.87)	44	41	2.20(1.24,3.88)	41	26	1.27(0.68,2.37)	52	30	1.08(0.60,1.93)
	CT+CC	157	100	1	238	130	1	187	107	1	208	123	1
ive	TT	49	15	0.43(0.23,0.82)	44	41	1.90(1.16,3.10)	41	26	1.13(0.65,1.96)	52	30	1.04(0.62,1.73)
l_rs3752752(period I)													

ve	CC/CT/TT	71/85/48	43/57/14	0.70(0.50,0.99)	97/144/43	49/81/39	1.40(1.06,1.87)	80/109/40	42/66/24	1.08(0.80,1.48)	88/120/51	50/72/29	1.04(0.78,1.39)
sl	CC	71	43	1	97	49	1	80	42	1	88	50	1
ant													
sl	TT	48	14	0.41(0.20,0.86)	43	39	2.06(1.16,3.66)	40	24	1.15(0.61,2.17)	51	29	1.08(0.60,1.94)
ive	CT+CC	156	100	1	241	130	1	189	108	1	208	122	1
sl	TT	48	14	0.41(0.21,0.79)	43	39	1.85(1.12,3.04)	40	24	1.06(0.61,1.87)	51	29	1.03(0.61,1.72)
i_rs3747867(period I)													
ve	CC/CT/TT	135/59/5	78/32/3	0.94(0.59,1.48)	200/80/2	103/58/3	1.48(1.00,2.18)	148/72/5	90/36/4	0.93(0.61,1.41)	187/67/2	91/54/2	1.63(1.07,2.48)
sl	CC	135	78	1	200	103	1	148	90	1	187	91	1
ant													
sl	CT	59	32	0.85(0.50,1.45)	80	58	1.46(0.96,2.22)	72	36	0.84(0.52,1.36)	67	54	1.61(1.03,2.53)
ant	CC	135	78	1	200	103	1	148	90	1	187	91	1
sl	CT+TT	64	35	0.89(0.53,1.48)	82	61	1.49(0.98,2.25)	77	40	0.87(0.55,1.39)	69	56	1.64(1.05,2.57)
i_rs1227049(period I + II)													
ant	GG	139	71	1	190	123	1	161	81	1	168	113	1
sl	CC+GC	170	92	1.05(0.71,1.54)	222	108	0.76(0.55,1.05)	169	95	1.12(0.78,1.63)	223	105	0.71(0.51,0.99)
i_rs11592462(period I + II)													
ant	CC	208	114	1	266	143	1	224	116	1	250	141	1
sl													
ant	GG	6	7	2.39(0.77,7.45)	12	16	2.53(1.16,5.52)	7	8	2.26(0.79,6.44)	11	15	2.46(1.09,5.55)
ive	CG+CC	303	156	1	400	215	1	323	168	1	380	203	1
sl													
	GG	6	7	2.55(0.82,7.91)	12	16	2.53(1.17,5.48)	7	8	2.24(0.79,6.34)	11	15	2.61(1.17,5.85)

Note: CNE: cumulative noise exposure; BP: blood pressure; OR: odds ratio; CI: confidence interval; OR(95%CI)[#]:Adjusted for CNE, smoking, drinking, exercise and BP.

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