Role of CASP7 polymorphisms in noise-induced hearing loss risk in Han Chinese population

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Research

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Abstract

Background: Genetic factors and gene-environment interaction may play an important role in the development of noise induced hearing loss (NIHL). This study explored the relationship between the SNP of CASP genes and the NIHL susceptibility of Chinese workers exposed to occupational noise.

Methods: 191 cases and 191 controls were selected by 1:1 case control study. Among them, case groups were screened from workers exposed to noise at binaural high-frequency hearing thresholds greater than 25 dB (A). The control group selected workers with hearing thresholds≤25 dB (A) in any binaural frequency band based on matching factors such as age, exposure time to noise, and operating position. The blood samples of two groups of workers were subjected to DNA extraction and SNP sequencing of CASP3 and CASP7 genes using the polymerase chain reaction - ligase detection reaction method. Conditional logistic regression correction was used to analyze genetic variation associated with susceptibility to NIHL. Odds Ratios (OR) and 95% confidence intervals (CI) were calculated.

Results: There was an association between rs2227310 and rs4353229 of the CASP7 gene and the risk of NIHL. Compared with the GG genotype, the CC genotype of rs2227310 reduced the risk of NIHL (OR = 0.453, 95% CI = 0.241 ~ 0.852). Compared with CC genotype, the TT genotype of rs4353229 reduced the risk of NIHL (OR = 0.458, 95% CI = 0.243 ~ 0.862). Workers carrying the rs2227310GG and rs4353229CC genotype had an increased risk of NIHL compared to workers without any high-risk genotype (OR = 1.839, 95% CI = 1.033 ~ 3.274). The CASP7 gene interacted with factors such as age, smoking status, dietary taste, and conscious auditory system symptoms on the risk of NIHL (P <0.05).

Conclusions: The single nucleotide polymorphism of CASP7 gene interacts with factors such as age, frequency of smoking, dietary taste, and conscious auditory system symptoms on the risk of NIHL.

Background

Noise refers to sound that can cause physical and mental discomfort to human beings. It exists widely in the manufacturing environment and can cause health damage to workers and reduce production efficiency. With the progress of China's industries, productive noise has become one of the main factors as occupational exposure in working environment. The incidence of occupational noise deafness is the leading occupational disease in China, and it is a serious health hazard to the occupational population. Noise can cause specific hearing damage to the human body and non-specific damage to other organ systems. The specific damage is mainly noise-induced hearing loss (NIHL). Non-specific damage includes impairs on the nervous system, cardiovascular system, Endocrine system and other physical functions [1-2].

Noise-induced hearing loss is related to many factors, including noise exposure levels [3], noise exposure time [3], noise properties [4], interaction with other factors (high temperature, organic solvents, etc.) [5-6], individual health-related behaviors (personal protective measures, smoking, drinking etc.) [7], individual sensitivity [8], individual health status (hypertension, hyperlipemia, etc.) [9] and so on. Genetic factors and gene - environment interactions may also play important roles in the development of NIHL.

Researchers have found in long-term animal experiments and population studies that even under the same noise exposure, the degree of hearing threshold displacement varies among different experimental animals and populations. This shows that individuals with noise-induced hearing loss (NIHL) have different susceptibility [10-11]. We have carried out a lot of researches on the association of NIHL with the genetic variation of genes. We have studied the correlation between SNP of GATA, GPX family genes, TRIOBP genes and NIHL, and have not yet found any meaningful sites.

However, some researchers have reported that cochlear hair cell damage and death can cause NIHL in individuals[12]. Apoptosis is one of the methods that cause cochlear hair cell death, and cochlear hair cell death is attributed to cell-independent and orderly death controlled by specific genes [13-14]. Studies have shown that the cysteine aspartic protease (Caspase) is a cysteine protease with specific aspartic acid, of which the activation is the main step leading to apoptosis. Under the influence of apoptotic signals, caspase is activated by gradual hydrolysis, and the cleavage of cell structure and functional proteins results in apoptosis [15]. Caspase3 and Caspase7 have highly similar functions and substrate specificity. They are the most important effector in the process of apoptosis, and also the converging point of many apoptotic stimulation signals. Their activation marks irreversible apoptosis [16-17]. It has also been shown that the genetic variation of CASP3 gene is related to the risk of NIHL, and the joint effect of working time and CASP3 polymorphism may affect the risk of NIHL[18]. This study assumes that the Caspase7 genes may be associated with the risk of noise-induced hearing loss in the Chinese population. We selected 191 NIHL and 191 noise-exposed workers as the research subjects, and performed genetic analysis of 14 single nucleotide polymorphisms (SNP) in their CASP3 and CASP7 genes, and analyzed their interaction with environmental behavioral factors.

Methods

Participants

In 2019, we selected noise-exposed workers who had undergone occupational health checks from a number of automobile manufacturers in Guangzhou as participants, and the study was conducted from March to October in China. The selected research subjects have relatively fixed job positions and are less mobile in the production process. The study was approved by the Ethics Committee of the 12th People's Hospital of Guangzhou, and all subjects had provided informed consent. The study included 191 NIHL workers and 191 hearing-normal workers, and no workers were exposed to other occupational hazards. We selected 191 cases of noise exposed workers with binaural high-frequency hearing thresholds greater than 25dB (A). This control group was matched according to the following criteria: (1) same enterprises, types of work and operating positions as the case group; (2)

binaural arbitrary frequency bands (including 500, 1000, 2000, 3000, 4000, 6000 Hz) hearing thresholds less than or equal to 25 dB (A); (3) same age (±3 years), same noise exposure time (±1 year).

The inclusion criteria for the subjects were as follows:

Cumulative time of occupational noise exposure [noise exposure time ≥ 8 h/day or 40 h/week, noise intensity ≥ 80 dB (A)] >1 year; (2) male and Han; (3) age: 18 ~ 45 year old.

The exclusion criteria were as follows:

Exposure to explosives or head injuries within 1 month prior to physical examination; (2) family history of hearing loss; (3) otitis or other otological diseases; (4) fever or common infections (flu, diarrhea and hepatitis,etc); (5) history of taking ototoxic drugs; (6) participants with bone conduction audiometry suggestive of conductive deafness.

The physical examination was performed by occupational health examiners in accordance with the standard protocol for each participant. Height, weight, blood pressure, blood lipids, pure tone audiometry were measured and we also inquired about the contact situation of other occupational hazards.We used EDTA anticoagulant negative pressure glass tubes to collect peripheral whole blood of empty-stomach subjects . The blood collection tube has a test tube number that can be one-to-one corresponding to the physical examination number, which ensures the consistency of the blood sample, the physical examination result and the questionnaire. Blood samples were temporarily stored and safely transported in a mobile refrigerator after collected. Questionnaires were collected by professionally trained investigators conducting face-to-face surveys and inquiries. The items of the questionnaire include general information, professional history, personal history, past history, personal conscious symptoms and so on.

On the basis of the "Diagnosis of Occupational Noise Deafness" (GBZ 49-2014) and relevant regulations and standards, specialized occupational health doctors performed at least 3 pure-tone hearing tests (the pure-tone hearing threshold test is performed in accordance with GB / T7583 and GB / T16403). Hearing thresholds for both ears were determined in increments of 5 dB in 500 dB, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, and 6000 Hz frequencies. According to GB / T7582–2004, the results were modified by age and gender. The PTA defines the hearing threshold at high frequencies as the average of each ear at 3000, 4000, and 6000 Hz. The hearing threshold at speech frequencies is defined as the average of 500, 1000, and 2000 Hz per ear. All subjects were required to avoid noise exposure for more than 48 hours before conducting audiometry.

The study conducted noise detection in accordance with "Measurement of physical factors in the workplace-Part 8: Noise" (GBZ / T189.8 - 2007). We measured noise after elaborative observation and investigation on the the working environment of spatial distribution, processing procedure, and noise equipment layout in these factories. We used the EDGE individual noise dosimeter produced by the British company CASELLA to evaluate the noise intensity. In this study, Noise exposure was evaluated with A-weighted energy equivalent continuous sound pressure level (Lex.8 h) according to the National Criteria of Measurement of Noise in the Workplace (GBZ/T189.8-2007) (China, 2007). Cumulative noise exposure (CNE) was calculated as

CNE = Lex.8 h + 10logT (Formula 2), where T means years of noise exposure.

SNP selection and genotyping

1. Screening of candidate gene SNP

Find the gene name in NCBI-SNP (http://www.ncbi.nlm.nih.gov/snp/). Open Gene View, refresh after clicking Clinical Source and in gene region. Select functional SNP sites of Promoter proxy (upstream variant 2KB), 5'UTR, Exon (missense, synonymous), 3'UTR region(Relevant optimization parameters are MAF in CHB> 0.05, based on HapMap or 1000 Genomes database) in this gene.

Mark disease-susceptible results by refering to relevant literature

Predict the function of the screened SNP (http://snpinfo.niehs.nih.gov/).

Perform LD analysis of the above SNP and mark the full linkage site of R2 = 1.

(http://asia.ensembl.org/Homo_sapiens/Tools/LD?db=core)

2. The SNP inclusion criteria are as follows:

Functional SNP sites located in Promoter proxy (upstream variant 2KB), 5'UTR, Exon (missense, synonymous), 3'UTR; (2) MAF in CHB> 0.05; (3) The linkage disequilibrium value of r2 is> 0.80; (4) Genetic balance test (Hardy-Weinberg) P value> 0.05. In this study, 14 SNP were screened for the three candidate genes TRIOBP, CASP3, and CASP7 (Table S1).

Table S1. Basic information, primer and probe sequences of selected SNP.

SNP	allele	MAF IN CHB	Function Prediction	Region	Primers for PCR	Probes for detecting variations	P_{HWE}
CASP3							
rs6948	G/T	0.199	–	3' UTR	F:GGAGGCCTCCCGGGCTGAG	TG:GGAGGCCTCCCGGGCTGAGG TT:GGAGGCCTCCCGGGCTGAGT	0.107
rs1049216	G/A	0.209	miRNA binding site	3' UTR	R:TGAAAAAGTTAAACATTGAAGTAA	TG:TGAAAAAGTTAAACATTGAAGTAAC TA:TGAAAAAGTTAAACATTGAAGTAAT	0.107
rs113420705	C/T	0.32	–	5' UTR	F:AGCCTCCTCATACCTTC	TC:AGCCTCCTCATACCTTCC TT:AGCCTCCTCATACCTTCT	0.400
rs12108497	T/C	0.282	TFBS	promoter	R:GGACTCTGTGACTATAAAAGATG	TT:GGACTCTGTGACTATAAAAGATGA TC:GGACTCTGTGACTATAAAAGATGG	0.607
rs1405937	C/G	0.277	TFBS	promoter	F:CCCCCAGGGACCCCATGGCA	TC:CCCCCAGGGACCCCATGGCAC TG:CCCCCAGGGACCCCATGGCAG	0.848
rs4647602	G/T	0.288	TFBS	promoter	F:CTGCAGGGCCGAAAA	TG:CTGCAGGGCCGAAAAG TT:CTGCAGGGCCGAAAAT	0.914
CASP7							
rs2227310	C/G	0.427	nsSNP	Exon-missense	F:AGGGAGCACGGAAAAGA	TC:AGGGAGCACGGAAAAGAC TG:AGGGAGCACGGAAAAGAG	0.610
rs12415607	C/A	0.417	TFBS	promoter	R:TTGAGTACATGCTTAGTGGTC	TC:TTGAGTACATGCTTAGTGGTCG TA:TTGAGTACATGCTTAGTGGTCT	0.491
rs11196418	G/A	0.117	TFBS	promoter	R:CCCAAACACACAGATTCTAGTT	TG:CCCAAACACACAGATTCTAGTTC TA:CCCAAACACACAGATTCTAGTTT	0.598
rs4353229	C/T	0.427	miRNA binding site	3' UTR	F:ACATGCAACAGAAGTGAC	TC:ACATGCAACAGAAGTGACC TT:ACATGCAACAGAAGTGACT	0.611
rs10787498	G/T	0.194	miRNA binding site	3' UTR	F:CAGTGGTAGAGTCATGT	TG:CAGTGGTAGAGTCATGTG TT:CAGTGGTAGAGTCATGTT	0.251
rs12247479	G/A	0.112	miRNA binding site	3' UTR	R:CCATTGGTGGTCCTAA	TG:CCATTGGTGGTCCTAAC TA:CCATTGGTGGTCCTAAT	0.129
rs1127687	A/G	0.204	miRNA binding site	3' UTR	F:CAGCCATGACAAGAACAAA	TA:CAGCCATGACAAGAACAAAA TG:CAGCCATGACAAGAACAAAG	0.881
rs12263370	A/G	0.141	TFBS	promoter	F:GGAAGTAAGCCACCGCGCCT	TA:GGAAGTAAGCCACCGCGCCTA TG:GGAAGTAAGCCACCGCGCCTG	0.193

Primer design and dilution

Sort the rs numbers of the sites before detection, and input the rs numbers to http://agenacx.com/ for primer design. According to the results of the operation, select and determine the appropriate primer design scheme, and order primers. The PCR primer was diluted to 100 μM, and a PCR primer mixture was prepared according to 1: 200. The extension primers were diluted according to the dilution table. Prepare the EXT primer mix at 1:25. After the extension primer mixture was prepared, 2 μl was diluted 25-fold for mass spectrometry. Adjust the extension primer ratio of individual sites according to the test results.

DNA extraction

Sample DNA was extracted using a ThermoFisher automated magnetic bead extractor. For blood samples, the Magpure Buffy Coat DNA Midi KF Kit kit was used. The NanoDrop8000 instrument was used for OD value detection and 1.25% agarose gel electrophoresis. After passing the DNA quality test, the sample DNA were transferred to a 96-well plate and stored at -20 ° C.

Agena MassArray System Genotyping Steps

A target fragment containing the SNP site to be detected was amplified by a PCR reaction. Shrimp alkaline phosphatase (SAP enzyme) was then used to remove the remaining deoxyribonucleoside triphosphate (dNTP) and primers in the PCR system. Then single base extension primers was added, the 3 'terminal base of which is close to the SNP site and is completely complementary to the base on the target fragment. Four types of ddNTP were used instead of dNTP. The probe extended only one base at the SNP site, and the ddNTP on the connection corresponds to the allele of the SNP site. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to detect the molecular weight difference between the extended product and the unextended primer, and the base at this point was determined.

The PCR master mix was configured and oscillated at low speed. We added 4 μl PCR master mix to each well of the 384-well plate, and mixed them after 1 μl of template DNA (20ng / μl) was added. The PCR reaction plate was placed on the PCR instrument and then the program was started. After the PCR reaction was completed, the PCR products were treated with SAP to remove free dNTPs from the system. Next, we prepared alkaline phosphatase treatment in a new 1.5ml EP tube, following by adding the SAP mix to a 384-well PCR reaction plate. After centrifugation, the SAP reaction program was performed, and then a single base extension reaction was activated after the completion of alkaline phosphatase treatment. Subsequently, we prepared a single base extension reaction solution in a new 1.5ml EP tube, and added the EXTEND Mix to the 384-well reaction plate. Again with centrifugation, an extension reaction procedure was performed. The cation exchange resin was used to remove Na +, Mg2 +, K + and other salt ions after the PCR reaction, so as to avoid excessive salt peaks in the analysis spectrum produced by mass detection, which would affect the result judgment. The PCR product plate was contrifuged for 5min (4000r / min), and 19ul of ultrapure water was added to each reaction well and centrifuged for 1min. Resin was applied on the top, and the PCR product plate was left to dry at room temperature for 15-30 minutes. Afterwards, they were mixed up for 40 minute to 1 hour. The sample was then micro-loaded onto a SpectroCHIP with a Mass Array Nanodispenser to prepare a co-crystallized film of the chip matrix and the sample. The prepared chip was put into a mass spectrometer (MassARRAY Analyzer 4 System) for detection, and Typer 4.0 software was used to obtain the original data and cluster map, and check the integrity and accuracy of the data file.`

Statistical Analysis

Categorical variables were expressed in frequency (%) and analyzed by Pearson's χ^2 independence test. Quantitative variables obeying normal distribution were expressed as mean ± standard deviation (M ± SD) and analyzed by Student's t test. We performed a χ^2 goodness-of-fit test on the frequency of each genotype tested to verify that it complies with Hardy-Weinberg Equilibrium (HWE). Conditional logistic regression was used to correct the statistically significant confounding factors in the study. The OR (Odds Ratio) value and its 95% CI (Confidence interval) were used to analyze the correlation between the gene SNP and the risk of NIHL. We used stratified and crossover analysis methods to analyze the potential relationship between genes and the environment. Multifactor dimensionality reduction (MDR) method was used to explore the potential interactions between genes. Statistical analysis was performed using SAS version 9.2 (SAS INSTITUTE INC, Cary, NCSU, USA). The MDR method uses MDR version 3.0.2 (Computational Genetics Laboratory of the University of Pennsylvania, Philadelphia, PA, USA). We considered all significant statistical tests with p value<0.05 . Bonferroni correction was applied to multiple hypothesis tests.

Results

There were 191 cases and 191 controls in this study, with a mean age of 32.19 ± 6.41 years and 31.88 ± 5.92 years, respectively. There was no significant differences in age, noise exposure time, noise exposure intensity, cumulative noise exposure [CNE, dB (A) .year], and body mass index (BMI) between the case and the control group (P> 0.05)Table S2.

Table S2. Comparison of matching factors between case group and control group.

Matching factors	Cases（n=191）	Controls（n=191）	*t*	*P*
Age(year)	32.19±6.41	31.88±5.92	0.484	0.629
Noise exposure time (year)	9.36±5.52	9.44±5.54	0.146	0.884
Noise intensity [dB(A)]	83.08±2.74	82.91±2.92	0.569	0.569
CNE [dB(A).year]	91.64±4.73	91.53±4.89	0.220	0.826
BMI (kg/m^2)	22.64±2.89	22.35±2.78	0.990	0.323

The differences in the basic characteristics of the study subjects between the two groups are shown in Table 1. Their demographic characteristics include Age, Educational level, Marital status, Personal monthly income, Noise exposure time, Wear noise protection products, Smoking frequency, Drinking frequency, Wear headphones to listen to music / watch videos, Call time per day, Length Of one's sleep, BMI, Total cholesterol, Triglyceride, and CNE, which were not significantly different (P> 0.05). There were significant differences between the two groups on different Diet taste and Auditory system symptoms (P <0.05). Compared with the control group, more workers in the case group had a salty taste and had conscious symptoms such as hearing loss, tinnitus, or ear pain within the last month of the survey.

Table 1. Distribution of different characteristics in the two groups.

Individual characteristics		Cases （n=191）	Controls （n=191）	χ^2	*P*-value
		n（%）	n（%）		
Age(year)	≤25	27（14.1）	32（16.8）	0.571	0.752
	25～35	106（55.5）	105（55.0）		
	≥35	58（30.4）	54（28.3）		
Educational level	High school/secondary school	138（72.3）	120（62.8）	3.869	0.049
	College and above	53（27.7）	71（37.2）		
Marital status	unmarried	57（29.8）	64（33.5）	0.593	0.441
	married	134（70.2）	127（66.5）		
Personal monthly income（RMB）	≤3000	7（3.7）	9（4.7）	0.525	0.769
	3001~8000	119（62.3）	113（59.2）		
	≥8000	65（34.0）	69（36.1）		
Noise exposure time (year)	1～5	45（23.8）	44（23.0）	0.042	0.979
	6～10	48（25.1）	47（24.6）		
	～10	98（51.3）	100（52.4）		
Wear noise protection products	Occasionally wear	127（66.5）	115（60.2）	1.624	0.203
	Standard wear	64（33.5）	76（39.8）		
Smoking frequency （cigarette/d）	0~	97（50.8）	113（59.2）	2.954	0.228
	1~	66（34.6）	52（27.2）		
	11~	28（14.7）	26（13.6）		
Drinking frequency （times/week）	0~	120（62.8）	131（68.6）	3.391	0.183
	1~	52（27.2）	37（19.4）		
	2~	19（9.9）	23（12.0）		
Diet taste	Light	69（36.1）	89（46.6）	28.422	<0.001
	Salty	90（47.1）	53（27.7）		
	Partial sweet	9（4.7）	11（5.8）		
	Partial oil	21（11.0）	17（8.9）		
	Other	2（1.0）	21（11.0）		
Wear headphones to listen to music / watch videos	Never listen	91（47.6）	78（40.8）	2.647	0.266
	Sometimes listen	90（47.1）	97（50.8）		
	Often listen	10（5.2）	16（8.4）		
Call time per day (minutes)	0	22（11.5）	21（11.0）	1.142	0.767
	0~	126（66.0）	129（67.5）		
	15~	27（14.1）	30（15.7）		
	30~	16（8.4）	11（5.8）		
Length of one's sleep（hours）	～7.0	70（36.6）	71（37.2）	1.310	0.519
	7 ~	116（60.7）	118（61.8）		
	9~	5（2.5）	2（1.0）		
Auditory system symptoms	no	96（50.3）	144（75.4）	25.825	<0.001

	Yes	95（49.7）	47（24.6）		
BMI(kg/m^2)	＜18.5	7（3.7）	7（3.7）	4.079	0.130
	18.5~	117（61.3）	135（70.7）		
	24.0~	67（35.1）	49（25.7）		
Total cholesterol	normal	163（85.3）	166（86.9）	0.197	0.657
	abnormal	28（14.7）	25（13.1）		
Triglyceride	normal	171（89.5）	174（91.1）	0.269	0.604
	abnormal	20（10.5）	17（8.9）		
CNE[dB(A).year]	<90	58（30.9）	59（30.9）	0.334	0.954
	90~	88（46.1）	90（47.1）		
	95~	42（22.0）	39（20.4）		
	100~	2（1.0）	3（1.6）		

We performed logistic regression analysis on 14 genotypes of the CAPS3 and CAPS7 genes, and found that there was an association between rs2227310 and rs4353229 of the CASP7 gene and the risk of NIHL (Table 2). Compared with the GG genotype, the risk of NIHL in the rs2227310 CC genotype was reduced (OR = 0.453, 95% CI = 0.241 ~ 0.852). Similarly, It was also found that in the recessive genetic model, compared with the GG genotype, the risk of NIHL carrying the (CC + CG) genotype was reduced (OR = 0.528, 95% CI = 0.298 ~ 0.938). The risk of NIHL in rs4353229 TT genotype was reduced (OR = 0.458, 95% CI = 0.243 ~ 0.862) comparing with CC genotype. It was also found in the recessive genetic model that comparing with the CC genotype, the risk of NIHL in the rs4353229 (TT + CT) genotype was reduced (OR = 0.547, 95% CI = 0.308 ~ 0.974). We classified the rs2227310GG and rs4353229CC genotype as high-risk genotype and then calculated the number of high-risk genotype in the combined genotype. We found that workers carrying the rs2227310GG and rs4353229CC genotype had an increased risk of NIHL compared with workers who did not carry any high-risk genes (OR = 1.839, 95% CI = 1.033-3.274). However, after Bonferroni correction, all associations were meaningless.

Table 2. Association between SNP and risk of NIHL.

SNP	Genetic model	Genotype	Cases （n=191）	Controls（n=191）	P#	OR（95%CI）#
rs2227310	Additive	GG	42（22.0）	27（14.1）		1.000
		CC	62（32.5）	70（36.6）	0.014	0.453（0.241~0.852）
		CG	87（45.5）	94（49.2）	0.040	0.538（0.298~0.972）
	Dominant	GG+CG				1.000
		CC	129（67.5）	121（63.4）	0.577	0.878（0.555~1.388）
	Recessive	GG				1.000
		CC+CG	149（78.0）	164（85.9）	0.029	0.528（0.298~0.938）
rs4353229	Additive	CC	41（21.5）	27（14.1）		1.000
		TT	62（32.5）	70（36.6）	0.016	0.458（0.243~0.862）
		CT	88（46.1）	94（49.2）	0.046	0.548（0.303~0.990）
	Dominant	CC+CT				1.000
		TT	129（67.5）	121（63.4）	0.577	0.878（0.555~1.388）
	Recessive	CC				1.000
		TT+CT	150（78.5）	164（85.9）	0.041	0.547（0.308~0.974）
Risk genotype *		0	149（78.0）	164（85.9）		1.00
		1	1（0.5）	0（0.0）	-	-
		2	41（21.5）	27（14.1）	0.038	1.839（1.033-3.274）

#adjusted for age、educational level、marital status、personal monthly income、noise exposure time、wear noise protection products、smoking frequency、drinking frequency、wear headphones to listen to music / watch videos、call time per day、length of one's sleep、BMI、total cholesterol、triglyceride、CNE、diet taste and auditory system symptoms.

* rs2227310GG and rs4353229 CC genotype were classified as high-risk genotype; the number represents the numbers of the two genotype within the combined genotype.

In order to further explore the impact of different exposure levels on the relationship between SNP and NIHL, we performed a stratified analysis of CNE (CNE <90 and CNE≥90). When CNE ≥ 90, the rs2227310 GG and rs4353229 CC genotype can be classified as high-risk genotype. The rs2227310 (CC + CG) genotype had a lower risk of NIHL than the GG genotype (OR = 0.183, 95% CI = 0.035 ~ 0.946). Compared with the CC genotype, the rs4353229 (TT + CT) genotype reduced the risk of NIHL (OR = 0.183, 95% CI = 0.035 ~ 0.946). We found that workers carrying the rs2227310GG and rs4353229CC genotype had an increased risk of NIHL compared with workers who did not carry any of the risk genes (OR = 5.468, 95% CI = 1.057 to 28.295). But after Bonferroni correction, none of it makes sense(Table 3).

Table 3. Association of SNP and NIHL after stratification of different exposure levels.

Exposure level	SNP	Genotype	Cases, n (%)	Controls, n (%)	*P*	OR(95%CI)#
CNE <90 [dB(A).year]	rs2227310	GG	32（21.8）	22（14.8）		1.000
		CC+CG	115（78.2）	127（85.2）	0.125	0.603（0.316~1.152）
	rs4353229	CC	31（21.1）	22（14.8）		1.000
		TT+CT	116（78.9）	127（85.2）	0.167	0.632（0.330~1.211）
	Risk genotype	0	115（78.2）	127（85.2）		1.000
		1	1（0.7）	0（0.0）	-	-
		2	31（21.1）	22（14.8）	0.157	1.600（0.834~3.068）
CNE ≥90 [dB(A).year]	rs2227310	GG	10（22.7）	5（11.9）		1.000
		CC+CG	34（77.3）	37（88.1）	0.043	0.183（0.035~0.946）
	rs4353229	CC	10（22.7）	5（11.9）		1.000
		TT+CT	34（77.3）	37（88.1）	0.043	0.183（0.035~0.946）
	Risk genotype	0	34（77.3）	37（88.1）		1.000
		1	0（0.0）	0（0.0）	-	-
		2	10（22.7）	5（11.9）	0.043	5.468（1.057~28.295）

#adjusted for age、educational level、marital status、personal monthly income、noise exposure time、wear noise protection products、smoking frequency、drinking frequency、wear headphones to listen to music / watch videos、call time per day、length of one's sleep、BMI、total cholesterol、triglyceride、diet taste and auditory system symptoms.

We used crossover analysis to explore the potential interactions between SNP and environmental factors. SNP were classified into protective genotype and dangerous genotype by effect value. Compared with individuals carrying the (CC + CG) genotype and less than 25 years of age, workers with the rs2227310 GG genotype and ≥35 years of age had a significantly higher risk of NIHL (OR = 5.651, 95% CI = 1.215 ~ 26.297). Compared with individuals who carried the (CC + CG) genotype and did not smoke, workers carrying the rs2227310 GG genotype and did not smoke had a significantly higher risk of NIHL (OR = 2.380, 95% CI = 1.103 ~ 5.136), and individuals who smoked and carried rs2227310 (CC + CG) genotype had a significantly increased risk of NIHL (OR = 2.061, 95% CI = 1.156 ~ 3.674). Moreover, individuals with the GG genotype and smoked 11 or more cigarettes a day had a significantly increased risk of NIHL (OR = 5.202, 95% CI = 1.199 ~ 22.578). Compared with individuals with (CC + CG) genotype and light diet taste, individuals with rs2227310 GG genotype and salty taste have a significantly increased risk of NIHL (OR = 4.123, 95% CI = 1.486 ~ 11.438). Compared with individuals who carried the (CC + CG) genotype and did not experience conscious auditory symptoms, individuals who carried the rs2227310 (CC + CG) genotype and had conscious auditory symptoms were more likely to develope NIHL (OR = 3.236, 95 % CI = 1.938 ~ 5.401), individuals who carried the rs2227310 GG genotype and had conscious auditory symptoms had a significantly increased risk of developing NIHL (OR = 5.898, 95% CI = 2.180 ~ 15.962) (Table 4). We found similar results in exploring the potential interaction of rs4353229 with environmental factors(Table 5).

Table 4. Crossover analysis of interaction between rs2227310 and environmental factors on NIHL risk.

environmental factors	Genotype	Cases, n (%)	Controls, n (%)	*P*[#]	OR（95%CI）[#]
Age(year)					
≤25	CC+CG	20（10.5）	27（14.1）		1.000
≤25	GG	7（3.7）	5（2.6）	0.459	1.705（0.416~6.989）
25（35	CC+CG	84（44.0）	87（45.5）	0.692	1.186（0.509~2.761）
25（35	GG	22（11.5）	18（9.4）	0.381	1.554（0.579~2.761）
≥35	CC+CG	45（23.6）	50（26.2）	0.804	1.139（0.407~3.191）
≥35	GG	13（6.8）	4（2.1）	0.027	5.651（1.215~26.297）
Smoking frequency （cigarette/d）					
0	CC+CG	74（38.7）	97（50.8）		1.000
0~	GG	23（12.0）	16（8.4）	0.027	2.380（1.103~5.136）
1~	CC+CG	54（28.3）	44（23.0）	0.014	2.061（1.156~3.674）
1~	GG	12（6.3）	8（4.2）	0.294	1.762（0.611~5.082）
11~	CC+CG	21（11.0）	23（12.0）	0.389	1.387（0.659~2.917）
11~	GG	7（3.7）	3（1.6）	0.028	5.202（1.199~22.578）
Diet taste					
Light	CC+CG	55（28.8）	78（40.8）		1.000
Light	GG	14（7.3）	11（5.8）	0.286	1.656（0.655~4.187）
Salty	CC+CG	69（36.1）	47（24.6）	0.051	1.727（0.997~2.990）
Salty	GG	21（11.0）	6（3.1）	0.007[b]	4.123（1.486~11.438）
Partial sweet	CC+CG	8（4.2）	10（5.2）	0.815	1.135（0.392~3.292）
Partial sweet	GG	1（0.5）	1（0.5）	0.528	2.555（0.139~47.078）
Partial oil	CC+CG	15（7.9）	12（6.3）	0.413	1.459（0.591~3.598）
Partial oil	GG	6（3.1）	5（2.6）	0.216	2.305（0.615~8.644）
Other	CC+CG	2（1.0）	17（8.9）	0.019	0.156（0.033~0.737）
Other	GG	4（2.1）	0（0.0）	-	-
Auditory system symptoms					
no	CC+CG	74（38.7）	123（64.4）		1.000
no	GG	22（11.5）	21（11.0）	0.08	1.863（0.928~3.742）
Yes	CC+CG	75（39.3）	41（21.5）	0.000[b]	3.236（1.938~5.401）
Yes	GG	20（10.5）	6（3.1）	0.000[b]	5.898（2.180~15.962）

[#]adjusted for age，educational level，marital status，personal monthly income，noise exposure time，wear noise protection products，smoking frequency，drinking frequency，wear headphones to listen to music / watch videos，call time per day，length of one's sleep，BMI，total cholesterol，triglyceride，CNE，diet taste and auditory system symptoms.

[B] The Bonferroni correction after multiple comparisons also made sense.

Table 5. Crossover analysis of interaction between rs4353229 and environmental factors on NIHL risk.

environmental factors	Genotype	Cases, n (%)	Controls, n (%)	*P*[#]	OR（95%CI）[#]
Age(year)					
≤25	TT+CT	20（10.5）	27（14.1）		1.000
≤25	CC	7（3.7）	5（2.6）	0.454	1.714（0.419~7.023）
25（35	TT+CT	84（44.0）	87（45.5）	0.703	1.179（0.507~2.742）
25（35	CC	22（11.5）	18（9.4）	0.387	1.545（0.576~4.142）
≥35	TT+CT	46（24.1）	50（26.2）	0.767	1.169（0.418~3.269）
≥35	CC	12（6.3）	4（2.1）	0.040	5.070（1.076~23.897）
Smoking frequency （cigarette/d）					
0	TT+CT	75（39.3）	97（50.8）		1.000
0~	CC	22（11.5）	16（8.4）	0.044	2.219（1.022~4.819）
1~	TT+CT	54（28.3）	44（23.0）	0.017	2.021（1.135~3.597）
1~	CC	12（6.3）	8（4.2）	0.308	1.735（0.602~5.003）
11~	TT+CT	21（11.0）	23（12.0）	0.416	1.361（0.648~2.859）
11~	CC	7（3.7）	3（1.6）	0.030	5.092（1.174~22.081）
Diet taste					
Light	TT+CT	56（29.3）	78（40.8）		1.000
Light	CC	13（6.8）	11（5.8）	0.424	1.469（0.573~3.768）
Salty	TT+CT	69（36.1）	47（24.6）	0.059	1.694（0.979~2.931）
Salty	CC	21（11.0）	6（3.1）	0.007[b]	4.044（1.458~11.216）
Partial sweet	TT+CT	8（4.2）	10（5.2）	0.844	1.113（0.384~3.223）
Partial sweet	CC	1（0.5）	1（0.5）	0.538	2.496（0.136~45.917）
Partial oil	TT+CT	15（7.9）	12（6.3）	0.441	1.425（0.578~3.511）
Partial oil	CC	6（3.1）	5（2.6）	0.227	2.256（0.602~8.454）
Other	TT+CT	2（1.0）	17（8.9）	0.018	0.152（0.032.720）
Other	CC	0（0.0）	4（2.1）	-	-
Auditory system symptoms					
no	TT+CT	75（39.3）	123（64.4）		1.000
no	CC	21（11.0）	21（11.0）	0.117	1.757（0.868~3.554）
Yes	TT+CT	75（39.3）	41（21.5）	0.000[b]	3.193（1.914~5.328）
Yes	CC	20（10.5）	6（3.1）	0.001[b]	5.826（2.153~15.767）

[#]adjusted for age、educational level、marital status、personal monthly income、noise exposure time、wear noise protection products、smoking frequency、drinking frequency、wear headphones to listen to music / watch videos、call time per day、length of one's sleep、BMI、total cholesterol、triglyceride、CNE、diet taste and auditory system symptoms.

[b] The Bonferroni correction after multiple comparisons also made sense.

We used Multifactor dimensionality reduction(MDR) method to explore the potential interactions within genes, but no statistical significance was found (Table S3).

Table S3. The best combination models identified by MDR.

No.	Best model*	Training balanced accuracy (%)	Testing balanced accuracy (%)	P	Cross-validation consistency
1	rs2227310	0.5416	0.4921	0.9055	5/10
2	rs1405937, rs10787498	0.5859	0.4764	0.7688	5/10
3	rs4647602, rs12415607, rs10787498	0.6041	0.4817	0.8206	5/10

Discussion

By genotyping and analyzing 14 SNP in CASP3 and CASP7 genes, we found that rs2227310 and rs4353229 of CASP7 genes was associated with the risk of NIHL. Caspase 7 is an important regulatory factor and executive factor in the process of apoptosis, and it plays an important role in the development of tumors. Lee SY and other studies found that CASP7 rs2227310 polymorphic variant alleles increased the risk of lung cancer in recessive and dominant models [19]. Yan S have also studied that rs2227310, rs3124740, and rs12415607 of CASP7 may be an increased risk of cancer [20]. Wang MY and other studies discovered that CASP7 rs4353229TT genotype may be associated with reduced risk of gastric cancer [21]. Studies have reported that CASP7 gene mutations in the Chinese population may modulate overall survival and progression-free survival rate of patients with advanced non-small cell lung cancer platinum chemotherapy [22]. There are few studies exploring the association between CASP7 gene polymorphisms and noise-induced hearing loss, and the main studies are related to tumors. In our study, we found that in the CAPS7 gene, carrying the rs2227310CC genotype compared with carrying the GG genotype reduced the risk of NIHL (OR = 0.453, 95% CI = 0.241 ~ 0.852). It suggested that the mutated C allele may be a protective factor for NIHL risk. Compared with CC genotype, the rs4353229 TT genotype in CAPS7 gene has a lower risk of NIHL (OR= 0.458, 95% CI = 0.243 ~ 0.862), indicating that the mutant T allele may be a protective factor for NIHL. We classified the rs2227310 GG and rs4353229 CC genotype as high-risk genotype, and found that workers carrying the rs2227310 GG and rs4353229 CC genotype had an increased risk of NIHL compared with workers who did not carry any of the high-risk genotype (OR= 1.839, 95% CI = 1.033-3.274).

NIHL is a disease caused by the interaction of genes and the environment. Noise is the main environmental factor. The relationship between genes and susceptibility to NIHL is affected by the intensity of noise exposure [23]. The study found that when CNE ≥ 90 [dB (A) .year], the rs2227310 (CC + CG) genotype had a lower risk of NIHL than the GG genotype (OR = 0.183, 95% CI = 0.035 ~ 0.946). Compared with the CC genotype, the rs4353229 (TT + CT) genotype reduced the risk of NIHL (OR = 0.183, 95% CI = 0.035 ~ 0.946). We found that workers carrying the rs2227310GG and rs4353229CC genotype had an increased risk of developing NIHL compared to workers without them (OR = 5.468, 95% CI = 1.057-28.295). This phenomenon may be due to the fact that noise-exposed persons are more susceptible to NIHL at high noise levels [24].

In order to explore whether there is a potential interaction between genes and environmental behavioral factors, this study conducted a cross-analysis between CASP7 gene polymorphisms and non-genetic factors. We observed that the two SNP and the salty taste were significantly associated with auditory symptoms. Compared with individuals carrying the (CC + CG) genotype and less than 25 years of age, workers with the rs2227310 GG genotype and ≥35 years of age had a significantly higher risk of NIHL. Compared with individuals who carried the (CC + CG) genotype and did not smoke, workers who carried the rs2227310 GG genotype and did not smoke had a significantly higher risk of NIHL, individuals carried rs2227310 (CC + CG) genotype and smoked had a significantly increased risk of NIHL , and individuals with the GG genotype smoked 11 or more cigarettes a day had a significantly increased risk of NIHL. In contrast with individuals with (CC + CG) genotype and light diet taste, individuals with rs2227310 GG genotype and salty taste had a significantly increased risk of NIHL. Compared with individuals who carried the (CC + CG) genotype and did not experience conscious auditory symptoms, individuals who carried the rs2227310 (CC + CG) genotype and had conscious auditory symptoms had a significantly higher risk of developing NIHL, and the risk of developing NIHL significantly increased in individuals who carried the rs2227310 GG genotype and had conscious auditory symptoms. We found similar results in exploring the potential interaction of rs4353229 with environmental factors. The results showed that individuals with rs2227310 and rs4353229 mutant alleles should pay more attention to smoking cessation and light diet. Workers older than 35 years old and (or) who have recently experienced conscious hearing loss, tinnitus, or ear pain shall be relocated from noise-exposed workplaces to achieve early warning control. Rs2227310 of CASP7 is a missense mutation site in the exon region. C → G leads to changes in its encoded amino acid aspartic acid → glutamic acid, which may lead to abnormal structure and function of CASP7α subtype, thereby promoting the process of apoptosis[25]. Rs4353229 of CASP7 is a miRNA binding site located in the 3'UTR region. MicroRNA (miRNA) is a small class of non-coding RNA molecules. MiRNA regulate gene expression by binding to the 3 'untranslated region (UTR) of their target mRNA, leading to mRNA cleavage or translation inhibition [26]. Sequence variations (such as SNPs) located in the 3'-UTR of miRNA target genes may also eliminate or weaken microRNA targets or produce imperfect sequences that match microRNA seeds.This disrupts the microRNA-mRNA interaction and affects the expression of microRNA targets and the expression of CASP7 [27]. There may be some limitations to our research. First of all, the research subjects we selected were all male Han workers (fewer women in the automobile manufacturing industry), and there may be gender and ethnic differences. Second, the sample size in the case groups and control groups we selected was small, and we may have missed some significant outcomes that could only occur with large sample sizes. Third, our selection of subjects may lead to selection bias.

Conclusions

In summary, our study found that the rs2227310 CC and rs4353229 TT genotype of the CAPS7 gene may be less susceptible to the development of NIHL.The single nucleotide polymorphism of CASP7 gene may interact with factors such as age, frequency of smoking, dietary taste, and conscious

auditory system symptoms on the risk of NIHL.

Declarations

Competing Interests

The authors declare no conflict of interest.

Authors' contributions

YMR, ZW, and JWZ conceived and designed the study. YMR and JWZ performed the research. YMR, SQM, CRG, YYM, KPL, LLH and WFZ analyzed and interpreted the data. YMR, SQM, and ZW wrote the report, which was edited by all authors.

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Ethics approval and consent to participate

The study was approved by Guangzhou Twelfth People's Hospital ethics committee. Verbal informed consent was obtained from each subject before study enrollment.

Consent for publication

Not applicable.

Availability of data and materials

Please contact author for data requests.

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