Original Article

Association between plasma membrane Ca^{2+}-ATPase gene polymorphisms and noise-induced hearing loss in a Chinese population

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Background: Noise-induced hearing loss (NIHL) has become a world-wide high occupational health risk. The current research is aimed at investigating the association of plasma membrane Ca^{2+}-ATPase isoform 2 (*PMCA2*) gene polymorphisms with the susceptibility to NIHL in a Chinese population.

Methods: A total of 2,344 workers exposed to occupational noise were examined with hearing tests. According to the results of audiometry, we selected 613 cases with high frequency hearing threshold worse than 25 dB and 615 controls with high frequency hearing threshold better than or equal to 25 dB and the controls were frequency matched with cases in terms of age, gender and noise exposure level and exposure time. The individual genotypes for *PMCA2* single nucleotide polymorphisms (SNPs) were determined using a TaqMan MGB probe assay performed on an ABI PRISM® 7900 HT Fast Real-Time PCR System (Applied Biosystems). Information about these subjects was collected by questionnaires which were conducted through face-to-face interviews by trained interviewers.

Results: We found that individuals with the rs3209637 CC genotype had a statistically significantly increased risk of NIHL compared with those who carried the rs3209637 TT genotype (adjusted OR =1.26, 95% CI =1.07–1.48) and this increased risk was more pronounced among the workers in the 15- to 25-year noise exposure time, >92 dB(A) noise exposure level. Similar effects were also observed in a recessive model.

Conclusions: Our data suggested that the PMCA2 polymorphisms may be a genetic susceptibility marker for NIHL in the Chinese Han population.

Keywords: Plasma membrane Ca^{2+}-ATPase isoform 2 (*PMCA2*); noise-induced hearing loss (NIHL); polymorphisms; susceptibility

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Introduction

Noise-induced hearing loss (NIHL) is a sensorineural impairment that results from the repeat exposure to detrimental levels of noise. Since the industrial revolution, NIHL has become one of the most important problems in countries with rapidly increasing industrial activities. In Europe, 35 million people are at the risk of developing NIHL (1). In the United States, approximately 10% of the total population (30 million workers) is exposed daily to hazardous noise levels in their work environment (2). Relevant data shows, there are approximately 10 million workers who are working under the environment with excessive levels of noise in our country at present, and about one million people among them suffer from varying degrees of occupational deafness. Consequently, NIHL has become a world-wide high occupational health risk and the second most frequent form of sensorineural hearing loss, after age-related hearing impairment (ARHI) (3). Therefore, NIHL is a disorder with a high priority for occupational health research.

NIHL is a complex disease that is caused by the interaction of genetic and environmental factors (4). Noise is the most common environmental factor causing hearing loss. Beside noise, there are many other responsible environmental factors involved in NIHL. These include chemicals, like organic solvents and heavy metals, ototoxic substances (such as aminoglycosides and carbon monoxide), heat, vibration, smoking, and medical factors such as increased blood pressure, cholesterol, and pigmentation, all of these factors have their effect on the development of NIHL (3). However, individuals show different susceptibility to noise damage even when exposed to identical harmful levels of noise and other similar environmental factors (5). There are two characteristics of NIHL that have been thoroughly established through numerous studies (6). One is that the amount of hearing loss increased with noise intensity and duration of exposure. That is to say, more intense and longer duration of noise exposure may lead to more serious hearing loss. The other one is large individual variability in vulnerability to noise damage as described previous. Not all individuals exposed to a given noise level develop the same degree of hearing loss. Some individuals may develop a severe hearing loss while others exhibit no effect on the ability of hearing in spite of identical noise exposure and other similar environmental factors. This individual variability is an indication that genetic factors may play a part in the predisposition to NIHL. If we can identify the susceptible genes of NIHL, we can make it possible that the individuals who are predisposed to NIHL can be screened out before exposing to prolonged occupational noise. Thereby, the incidence of NIHL may be reduced effectively.

NIHL is a disorder that can be prevented, but once developed, it is permanent and irreversible. There is no effective treatment for NIHL at present. Therefore, we should put our efforts on the prevention of NIHL. It is of critical importance for workers who are surrounded by excessive levels of noise to use hearing protectors for reduction the intensity of noise exposure. As we all know, hearing loss in general has a significant effect on the quality of social, familial, and professional life (7). It brings a heavy economic burden and serious health damage for individuals and society as well. Thus, there is an urgent need for hearing conservation. Nowadays, most countries have enacted legislation to limit the work-related time of noise exposure and oblige the factory to offer hearing protection devices like earplugs to their workers and staff members. Unfortunately, numerous management and plants usually do not execute the rules to the letter. And sometimes workers are not willing to wear earplugs because of the uncomfortable they bring. In addition, wearing earplugs may cover the alarm signal produced by machine fault so as to cause industrial accident (8). As a consequence, NIHL is the most commonly occurring occupational disease in many countries, especially in industrial countries such as India and China.

As described earlier, hearing loss was the most common sensory handicap in humans. Owing to the auditory systems of mice and humans are conserved, we can predict the possible human deafness genes through studies conducted on mouse models and identify the new genes involved in hearing (9,10). Street VA *et al*. found that mutations in a plasma membrane Ca^{2+}-ATPase gene caused deafness and vestibular imbalance by impairing the activity of plasma membrane Ca^{2+} pump and reducing its ability to transport Ca^{2+} from the stereociliary cytoplasm in deafwaddler mice (11). Ficarella R *et al*. analyzed an Italian patient affected by severe bilateral sensorineural hearing impairment without vestibular involvement. The G293S mutation of *PMCA2* inherited from the mother with normal hearing and T1999S mutation of *CDH23* inherited from the father who had normal hearing were detected in the affected son (12). Consequently, it is not surprising that *PMCA2* was considered to be the candidate gene involved in hearing loss.

The plasma membrane calcium pump is the system that ejects Ca^{2+} out of eukaryotic cells (13). The *PMCA2* (also known as *ATP2B2*) is located on human chromosome 3p25.3. It is a member of the *ATP2B* gene family, whose products

include four isoforms, PMCA1-PMCA4 with 75–80% amino acid identity among them. The tissue distributions of the four isoforms differ substantially. While PMCA1 and PMCA4 are expressed broadly in different tissues and cell types, PMCA2 and PMCA3 are reported as having a highly restricted expression (14). In sensory hair cells of the inner ear, the major isoform is PMCA2 pump, which is responsible for the extrusion of Ca^{2+} from the outer hair cells (OHCs) stereocilia (15). Fridberger *et al*. [1998] observed that the OHC cytoplasmic Ca^{2+} concentration raised following acoustic overstimulation (16). The PMCA2 pump could protect the inner ear by ejecting the redundant Ca^{2+} from OHCs stereocilia against very large concentration gradients and play a critical role in intracellular calcium homeostasis. Therefore, it was plausible to assume that Ca^{2+} regulation by PMCA2 pump in hair bundles was essential for auditory and vestibular function. In this study, we were aimed to test the hypothesis that polymorphisms of the *PMCA2* might be associated with increased risk of developing NIHL in the Chinese population. For this purpose, we genotyped eight *PMCA2* potentially functional SNPs (single-nucleotide polymorphisms) selected from the data for Chinese in the NCBI SNP database (http://www.ncbi.nlm.nih.gov) to evaluate the association between common genetic variants in *PMCA2* and susceptibility to NIHL in Chinese population.

Methods

Study population

A total of 2,344 volunteers who had normal hearing before joining in the work accompanied with detrimental noise were recruited and 2,140 eligible questionnaires were collected. They were all examined with hearing tests. According to the results of audiometry, we selected 613 cases with high frequency hearing threshold worse than 25 dB and 615 controls with high frequency hearing threshold better than or equal to 25 dB from 2,140 workers who answered the questionnaires. The controls were frequency matched with cases in terms of age, gender and noise exposure level and exposure time. All subjects involved in this study were exposed to occupational noise at least three years and they were recruited from the factories located in the cities of Xuzhou, Nanjing, and Yizheng in Jiangsu province, China, during the period from April 2010 to May 2011. These participants were genetically unrelated individuals of Han Chinese descent. Furthermore, they all meet the following criteria: without the present diseases of high blood pressure, hyperlipidemia and diabetic mellitus *et al*.; no history of middle ear infection and craniocerebral injury; no family history of hereditary deafness and without the history of explosive noise exposure; without auditory system diseases before exposing to occupational noise; no history of using potentially ototoxic drugs (e.g., aspirin, quinolones, and aminoglycosides); not exposed commonly to physical factors (e.g., high temperature and vibration) and chemical factors such as organic solvents and heavy metals; not regularly use individual protective articles (earmuffs and earplugs) *et al*. As a result, there were altogether 613 NIHL cases and 615 controls in this case-control study. The aforementioned information about the study subjects was obtained by the questionnaires that were administered through face-to-face interviews by trained interviewers. Additionally, items concerning individual demographics as well as data on lifestyle habits (smoking and drinking status) and occupational history were involved in the structured questionnaires. According to the previous study (17), those who had smoked ≥100 cigarettes in their lifetimes were defined as chronic smokers and the rest subjects were considered as nonsmokers and those who had consumed alcohol drinks ≥3 times per week for at least one year were defined as chronic drinkers and the others were considered as non-drinkers. Informed consent was obtained from all the subjects who answered the questionnaires and they donated 5 mL venous blood to be used for genomic DNA extraction. The research protocol was approved by the Institutional Review Board of the centers for disease control and prevention of Jiangsu province.

Environmental noise monitoring and audiological assessment

Noise exposure levels at the workplaces were assessed using individual sound pressure noise meters (Noise-Pro, Quest, Oconomowoc, WI). In accordance with the China National Criteria for Noise in the Workplace (GBZ43-2002), representative workers selected in each operating post who were exposed to excessive noise wore the personal audiometers during their work time for three consecutive days and twice per year to measure the noise levels of workplaces. Then, the measuring results were recorded in the form of a nominal 8 hours equivalent continuous A-weighted sound pressure per day (Lex. 8 hours). The measurement was conducted at 10 AM, 3 PM, and 5 PM

during the subjects' normal working time.

All participants were separated from noise exposure environment 12 to 48 hours before they took the pure-tone audiometry for both ears at 0.5, 1.0, 2.0, 3.0, 4.0, and 6.0 kHz by a trained technician. The pure-tone audiometry was performed in a sound-attenuating chamber in which the level of background noise was lower than 25 dB(A). Furthermore, their ears were examined by an otolaryngologist to determine whether they suffer from tympanitis or other diseases that could affect the normal hearing. In this case, these subjects with tympanitis or other diseases were not included in our research. Threshold value was defined as the lowest signal intensity that was detected in the subject at least half of the time, with a minimum of three tries. NIHL preferentially affected the higher frequencies, with hearing loss beginning typically around 3.0 to 6.0 kHz, creating a V-shape dip or notch because these two audiometric frequencies were most affected by noise (18). Hence, the audiometric profile with a V-shape notch was a principle feature of NIHL. There were two types of hearing loss: low-frequency range (0.5 to 2.0 kHz) and high-frequency range (3.0 to 6.0 kHz). We took the mean threshold of 0.5, 1.0, and 2.0 kHz as low-frequency hearing status and the mean threshold of 3.0, 4.0, and 6.0 kHz as high-frequency hearing status. In our study, those hearing threshold worse than 25 dB in either high frequency or low frequency were defined as NIHL cases. According to the requirement of Diagnostic Criteria of Occupational Noise-induced Hearing Loss, the workers with low-frequency hearing loss should be removed from their initial noise-exposed operating environment immediately. Unfortunately, subjects we collected were all high-frequency hearing loss. In brief, a total of 615 NIHL cases and 615 controls were included in this study. The controls were frequency matched with cases by age, sex, smoking status, drinking status, exposure level, and exposure time.

Single nucleotide polymorphism (SNP) selection and genotyping

Recently, many genome-wide association studies (GWASs) have found that there might be some other functional polymorphisms located downstream or upstream of the genes, even in intergenic regions known as gene deserts. From a gene perspective, the 5' near gene (as promoter region), 5' UTR, 3' UTR, or coding regions with amino acid changes are the most promising potentially functional regions in current association studies. Thus we selected eight SNPs of *PMCA2* from the NCBI database (http://www.ncbi.nlm.nih.gov/) using the SNP selection strategy. These eight SNPs, with a minor allele frequency (MAF) ≥0.05 in Han Chinese, were all potentially functional polymorphisms (meet the following criteria: located in the 5' near gene, 5' UTR, 3' UTR, or coding regions with amino acid changes). Detailed information about the selected SNPs was showed in *Table 1*. Genomic DNA was isolated from the donated venous blood samples by using a Tiangen DNA extraction kit (Tiangen Biotech Company, Beijing, China). We did the experiment strictly according to manufacturer's instructions. The individual genotypes for *PMCA2* SNPs were determined using a TaqMan MGB probe assay performed on a 96-well ABI PRISM® 7900 HT Fast Real-Time polymerase chain reaction (PCR) System from Applied Biosystems Inc. (Foster City, CA, USA). Each 96-well PCR plate included one negative control with no DNA sample to pledge the validity of the experiment. The PCR thermal cycling protocol consisted of three stages: 2 min of 50.0 ℃ for stage 1, 10 min of 95 ℃ initial denaturation for stage 2, then samples pass through 40 cycles of 15 s of 95 ℃ melting, 1 min of 60.0 ℃ annealing and extension for stage 3. PCR amplifications were performed on 1 μL of DNA (0.01 μg/μL) working solution using 2× HoTaq QPCR Reaction Mix, double distilled water, Taqman-MGB probe and primer in a total volume of 10 μL per reaction. Detailed information about the constitution of reaction system is available on request. These reagents involved in the experiment were obtained from an Applied Biosystems-related company. The allelic discrimination was executed with SDS 2.4 software. The genotype analysis was done independently by two persons in a blind fashion. Genotyping was randomly repeated in 10% of samples to check for the typing reliability, and the results were 100% concordant. The call rates were >98% for all variants.

Statistical analysis

Chi-square (χ^2) test was used to evaluate the distribution differences of qualitative data and Student's t-test was used to analyze quantitative data. Unconditional univariate and multivariate logistic regression analyses were done to obtain crude and adjusted odds ratios (ORs) for risk of NIHL and their 95% confidence intervals (CIs). Terms in the models included age, sex, smoking status and drinking status. Hardy-Weinberg equilibrium of the genotype distribution among the controls was tested by a goodness-of-fit χ^2 test. A median value of 20

Table 1 Primary information of genotyped SNPs of *PMCA2*

SNP identifier[a]	Base change	Genomic position[a]	Function	Minor allele frequency			P[b]	P for HWE[c]	Call rate (%)
				Database[a]	Cases	Controls			
rs41147	A>G	Chr3:10368102	3' UTR	0.451	0.395	0.380	0.431	0.647	99.7
rs14154	C>G	Chr3:10368113	3' UTR	0.383	0.392	0.380	0.561	0.805	99.8
rs3209637	T >C	Chr3:10368948	3' UTR	0.500	0.467	0.415	0.011	0.658	99.8
rs1719571	A>G	Chr3:10369180	3' UTR	0.439	0.370	0.364	0.769	0.865	99.5
rs4327369	C>G	Chr3:10370486	3' UTR	0.367	0.393	0.388	0.789	0.453	99.2
rs11916329	C>G	Chr3:10548708	5' near gene	0.275	0.339	0.344	0.794	0.500	99.8
rs34624565	T>C	Chr3:10548799	5' near gene	0.333	0.329	0.308	0.250	0.906	99.8
rs4684700	C>T	Chr3:10548950	5' near gene	0.133	0.141	0.136	0.715	0.975	99.9

[a], data were taken from NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP); [b], two-sided χ^2 test for allele frequencies between the cases and controls; [c], Hardy-Weinberg equilibrium P value in the control group. SNP, single nucleotide polymorphism; *PMCA2*, plasma membrane Ca^{2+}-ATPase isoform 2.

years for noise exposure time in the recruited subjects was considered as a cut-point dividing all subjects into two levels to conduct the stratification analysis. For stratification analysis by noise exposure levels, we divided it artificially into three groups according to the international reference standard ISO1999:1990 and our noise measurement data [<85, 85–92, and >92 dB, (Lex. 8 h)]. All statistical analyses were two sided and performed with Statistics Analysis System software (version 9.1.3; SAS Institute, Cary, NC). A criterion of P<0.05 was chosen to define differences as statistically significant.

Results

Characteristics of the study population and single nucleotide polymorphism (SNP) genotyping

The demographic and occupational characteristics of the study subjects were presented in *Table 2*. There was no significant difference in the distribution of age (P=0.978) and gender (P=0.686). In life habit respects, the distribution of drinking and smoking status between the NIHL cases and controls without hearing loss had no significant difference as well (P=0.594, 0.780, respectively). In addition, we did not observe the statistically significant difference in noise exposure time and exposure level. The cases and controls appeared to be well matched. However, the average threshold value of NIHL cases was more than two times higher than that of controls with normal hearing (37.2±11.8 *vs*. 14.1±4.1, P<0.001). The primary information about SNP identifiers, genomic positions, allele frequencies and call rates were shown in *Table 1*. The allele frequencies and genotype frequencies of these eight polymorphisms among the controls were all in agreement with Hardy–Weinberg equilibrium (P>0.05). And the MAF of all the polymorphisms was consistent with that reported in the HapMap database.

Associations of plasma membrane Ca^{2+}-ATPase isoform 2 (PMCA2) polymorphisms with the susceptibility to noise-induced hearing loss (NIHL)

All of the observed genotype frequencies in the controls were in agreement with the Hardy-Weinberg equilibrium (*Table 1*). The genotype distributions of the eight SNPs and their associations with NIHL risk were presented in *Table 3*. We compared the genotype results of NIHL cases with the controls. As shown in *Table 3*, in the single SNP analysis of all eight SNPs, one SNP had a significant main effect P value (P=0.015 for rs3209637). For the SNP rs41147, rs14154, rs1719571, rs4327369, rs11916329, rs34624565 and rs4684700, the frequency distributions of genotypes in NIHL cases were similar with the controls. Thus, we did not observe the statistically significant difference in these seven SNPs between the cases and controls (P=0.736 for rs41147, P=0.809 for rs14154, P=0.870 for rs1719571, P=0.966 for rs4327369, P=0.980 for rs11916329, P=0.477 for rs34624565, and P=0.825 for rs4684700). Furthermore, in the dominant model of the eight SNPs, the rs41147 AG + GG, rs14154 CG + GG, rs3209637 CT + CC, rs1719571 AG + GG, rs4327369 CG + GG, rs11916329 CG + GG, rs34624565 CT + CC and rs4684700 CT + TT genotypes were not associated with risk of NIHL compared with their

Table 2 Demographic and occupational characteristics of cases and controls

Variables	Cases (n=613), N (%)	Controls (n=615), N (%)	All (n=1,228), N (%)	P[a]
Age (years)	40.4±6.3	40.4±6.2	40.4±6.3	0.978[b]
<35	122 (19.9)	120 (19.5)	242 (19.7)	
35–45	369 (60.2)	369 (60.0)	738 (60.1)	0.962
>45	122 (19.9)	126 (20.5)	248 (20.2)	
Gender				
Male	566 (92.3)	564 (91.7)	1,130 (92.0)	0.686
Female	47 (7.7)	51 (8.3)	98 (8.0)	
Smoking status				
Nonsmokers	246 (40.1)	256 (41.6)	502 (40.9)	0.594
Smokers	367 (59.9)	359 (58.4)	726 (59.1)	
Drinking status				
Non-drinkers	353 (57.6)	359 (58.4)	712 (58.0)	0.780
Drinkers	260 (42.4)	256 (41.6)	516 (42.0)	
Exposure time (years)	18.6±7.6	18.2±7.4	18.3±7.5	0.391[b]
<15	189 (30.8)	194 (31.5)	383 (31.2)	0.653
15–25	309 (50.4)	318 (51.7)	627 (51.1)	
>25	115 (18.8)	103 (16.8)	218 (17.7)	
Exposure level [dB(A)]	87.1±7.7	87.0±7.6	87.1±7.6	0.807[b]
<85	234 (38.2)	246 (40.0)	480 (39.1)	
85–92	162 (26.4)	151 (24.6)	313 (25.5)	0.710
>92	217 (35.4)	218 (35.4)	435 (35.4)	
Threshold (dB)	37.2±11.8	14.1±4.1	26.9±14.7	<0.001[b]

[a], two-sided χ^2 test for the frequency distributions of selected variables between cases and controls; [b], two-sided t test for the frequency distributions of selected variables between cases and controls.

Table 3 Association between *PMCA2* polymorphisms and risk of noise-induced hearing loss

Genotypes	Cases (n=613)	Controls (n=615)	Crude OR (95% CI)	Adjusted OR (95% CI)[b]	P[a]
rs41147	N=612	N=612			
AA	227 (37.1)	238 (38.9)	1.00 (reference)	1.00 (reference)	
AG	286 (46.7)	283 (46.2)	1.06 (0.83–1.35)	1.06 (0.83–1.35)	0.665
GG	99 (16.2)	91 (14.9)	1.07 (0.90–1.26)	1.08 (0.91–1.27)	0.405
AG/GG	385 (62.9)	374 (61.1)	1.08 (0.86–1.36)	1.08 (0.86–1.36)	0.528
AA/AG	513 (83.8)	521 (85.1)	1.00 (reference)	1.00 (reference)	
GG	99 (16.2)	91 (14.9)	1.11 (0.81–1.51)	1.11 (0.81–1.51)	0.527
rs14154	N=613	N=613			
CC	231 (37.7)	237 (38.7)	1.00 (reference)	1.00 (reference)	
CG	284 (46.3)	286 (46.6)	1.02 (0.80–1.30)	1.01 (0.79–1.30)	0.910
GG	98 (16.0)	90 (14.7)	1.06 (0.89–1.25)	1.06 (0.90–1.26)	0.489
CG/GG	382 (62.3)	376 (61.3)	1.04 (0.83–1.31)	1.04 (0.83–1.31)	0.741
CC/CG	515 (84.0)	523 (85.3)	1.00 (reference)	1.00 (reference)	
GG	98 (16.0)	90 (14.7)	1.11 (0.81–1.51)	1.11 (0.81–1.51)	0.524

Table 3 *(continued)*

Table 3 *(continued)*

Genotypes	Cases (n=613)	Controls (n=615)	Crude OR (95% CI)	Adjusted OR (95% CI)[b]	P[a]
rs3209637	N=612	N=613			
TT	184 (30.0)	207 (33.8)	1.00 (reference)	1.00 (reference)	0.015
CT	285 (46.6)	303 (49.4)	1.06 (0.82–1.37)	1.05 (0.81–1.36)	0.665
CC	143 (23.4)	103 (16.8)	1.25 (1.06–1.47)	1.26 (1.07–1.48)	0.006
CT/CC	428 (69.9)	406 (66.2)	1.19 (0.93–1.51)	1.18 (0.93–1.50)	0.165
TT/CT	469 (76.6)	510 (83.2)	1.00 (reference)	1.00 (reference)	
CC	143 (23.4)	103 (16.8)	1.51 (1.14–2.00)	1.52 (1.14–2.01)	0.004
rs1719571	N=611	N=611			
AA	240 (39.3)	248 (40.6)	1.00 (reference)	1.00 (reference)	
AG	290 (47.5)	281 (46.0)	1.07 (0.84–1.36)	1.06 (0.83–1.35)	0.638
GG	81 (13.2)	82 (13.4)	1.01 (0.85–1.21)	1.02 (0.85–1.22)	0.857
AG/GG	371 (60.7)	363 (59.4)	1.06 (0.84–1.33)	1.05 (0.84–1.33)	0.658
AA/AG	530 (86.8)	529 (86.6)	1.00 (reference)	1.00 (reference)	
GG	81 (13.2)	82 (13.4)	0.99 (0.71–1.37)	0.99 (0.71–1.38)	0.949
rs4327369	N=610	N=608			
CC	229 (37.6)	232 (38.2)	1.00 (reference)	1.00 (reference)	
CG	282 (46.2)	280 (46.0)	1.02 (0.80–1.31)	1.02 (0.79–1.30)	0.907
GG	99 (16.2)	96 (15.8)	1.02 (0.87–1.21)	1.02 (0.87–1.21)	0.787
CG/GG	381 (62.5)	376 (61.8)	1.03 (0.81–1.29)	1.02 (0.81–1.29)	0.842
CC/CG	511 (83.8)	512 (84.2)	1.00 (reference)	1.00 (reference)	
GG	99 (16.2)	96 (15.8)	1.03 (0.76–1.40)	1.04 (0.76–1.41)	0.826
rs11916329	N=612	N=613			
CC	264 (43.1)	261 (42.6)	1.00 (reference)	1.00 (reference)	
CG	281 (45.9)	284 (46.3)	0.98 (0.77–1.24)	0.98 (0.77–1.24)	0.842
GG	67 (11.0)	68 (11.1)	0.99 (0.82–1.19)	0.99 (0.82–1.20)	0.929
CG/GG	348 (56.9)	352 (57.4)	0.98 (0.78–1.23)	0.98 (0.78–1.22)	0.833
CC/CG	545 (89.0)	545 (88.9)	1.00 (reference)	1.00 (reference)	
GG	67 (11.0)	68 (11.1)	0.99 (0.69–1.41)	0.98 (0.69–1.40)	0.916
rs34624565	N=612	N=613			
TT	66 (10.8)	56 (9.1)	1.00 (reference)	1.00 (reference)	
CT	271 (44.3)	264 (43.1)	0.87 (0.59–1.29)	0.88 (0.60–1.32)	0.544
CC	275 (44.9)	293 (47.8)	0.89 (0.73–1.09)	0.89 (0.73–1.08)	0.231
CT/CC	546 (89.2)	557 (90.9)	0.83 (0.57–1.21)	0.84 (0.57–1.22)	0.345
TT/CT	337 (55.1)	320 (52.2)	1.00 (reference)	1.00 (reference)	
CC	275 (44.9)	293 (47.8)	0.89 (0.71–1.12)	0.89 (0.71–1.12)	0.317
rs4684700	N=613	N=614			
CC	10 (1.6)	12 (1.9)	1.00 (reference)	1.00 (reference)	
CT	153 (25.0)	146 (23.8)	1.26 (0.53–3.00)	1.28 (0.54–3.07)	0.575
TT	450 (73.4)	456 (74.3)	1.09 (0.71–1.66)	1.09 (0.71–1.66)	0.703
CT/TT	603 (98.4)	602 (98.1)	1.20 (0.52–2.80)	1.20 (0.52–2.81)	0.668
CC/CT	163 (26.6)	158 (25.7)	1.00 (reference)	1.00 (reference)	
TT	450 (73.4)	456 (74.3)	0.96 (0.74–1.23)	0.95 (0.74–1.23)	0.710

OR, odds ratios; CI, confidence intervals; *PMCA2*, plasma membrane Ca^{2+}-ATPase isoform 2. [a], two-sided χ^2 test for the frequency distributions of genotype between cases and controls; [b], adjusted for age, sex, smoking and drinking status in the unconditional logistic regression model.

Table 4 Stratified analysis of the *PMCA2* rs3209637 polymorphism (CC *vs.* TT genotype) associated with NIHL risk

Variables	Cases, N (%)		Controls, N (%)		Adjusted OR (95% CI)[b]	P[a]
	TT	CC	TT	CC		
Exposure time (years)						
<15	61 (60.4)	40 (39.6)	55 (56.1)	43 (43.9)	0.92 (0.69–1.23)	0.541
15–25	91 (54.8)	75 (45.2)	118 (72.8)	44 (27.2)	1.48 (1.17–1.87)	0.001
>25	32 (53.3)	28 (46.7)	34 (68.0)	16 (32.0)	1.37 (0.91–2.04)	0.118
Exposure level [dB(A)]						
<85	60 (52.6)	54 (47.4)	74 (32.2)	42 (36.2)	1.25 (0.95–1.63)	0.086
85–92	57 (62.6)	34 (37.4)	53 (67.9)	25 (32.1)	1.13 (0.82–1.57)	0.470
>92	67 (54.9)	55 (45.1)	80 (69.0)	36 (31.0)	1.37 (1.05–1.79)	0.026
Smoking status						
Never	77 (57.5)	57 (42.5)	95 (66.9)	47 (33.1)	1.22 (0.95–1.57)	0.106
Ever	107 (55.4)	86 (44.6)	112 (66.7)	56 (33.3)	1.27 (1.02–1.57)	0.029
Drinking status						
Never	71 (53.8)	61 (46.2)	79 (62.7)	47 (37.3)	1.18 (0.92–1.52)	0.147
Ever	113 (57.9)	82 (42.1)	128 (69.6)	56 (30.4)	1.30 (1.05–1.61)	0.019
Age (years)						
<35	33 (51.6)	31 (48.4)	40 (62.5)	24 (37.5)	1.31 (0.91–1.88)	0.211
35–45	114 (60.0)	76 (40.0)	134 (71.3)	54 (28.7)	1.29 (1.04–1.60)	0.021
>45	37 (50.7)	36 (49.3)	33 (56.9)	25 (43.1)	1.15 (0.81–1.63)	0.479

[a], two-sided χ^2 test for the frequency distributions of the selected variables between the cases and controls; [b], adjusted for age, sex, smoking, and drinking. *PMCA2*, plasma membrane Ca^{2+}-ATPase isoform 2; NIHL, noise-induced hearing loss.

wild homozygous types respectively. We did not find the statistically significant difference in the recessive model but rs3209637. The frequency of rs3209637 CC genotype in the normal hearing workers (16.8%) was statistically lower than the frequency in the NIHL workers (23.4%, P=0.006). Unconditional logistic regression was used to calculate the Crude and adjusted ORs for NIHL to eliminate the effects of confounding factors and the results were listed in *Table 3*. Multivariate logistic regression analyses also revealed that individuals with the rs3209637 CC genotype had a 1.26-fold risk of NIHL compared with individuals carrying a TT genotype (adjusted OR =1.26, 95% CI =1.07–1.48). When we combined the TT and CT genotypes as a reference to conduct a recessive model, the number of workers with CC genotype was greater among the NIHL group than in the normal hearing worker group (23.4% *vs.* 16.8%, P=0.004). Workers who carried the CC genotype had a higher risk of NIHL (1.52, 1.14–2.01). We also found that there were more C allele among the NIHL workers than among the normal hearing workers (P=0.011).

Stratification analysis between the plasma membrane Ca^{2+}-ATPase isoform 2 (PMCA2) polymorphisms and risk of noise-induced hearing loss (NIHL)

The stratification analysis results of SNP rs3209637 are shown in *Tables 4* and *5*. We found that the individuals with the rs3209637 CC genotype were more susceptible to NIHL than those who carrying the TT genotype, both in the 15- to 25-year noise exposure group (adjusted OR =1.48, 95% CI =1.17–1.87) and in the 35- to 45-year-old group (adjusted OR =1.29, 95% CI =1.04–1.60). Similar effects were also found in >92 dB(A) noise exposure level, smoking and drinking status group. The rs3209637 CC genotype carriers with >92 dB(A) exposure level were at a significantly increased risk of NIHL (1.37, 1.05–1.79). Ever smoking or ever drinking subjects with the rs3209637 CC genotype were more susceptible to NIHL. No effects were observed in <15- and >25-year exposure time groups, <85 and 85 to 92 dB(A)-exposure level groups, nonsmoker group, non-drinker group, and <35 and >45 years old groups. No significant higher risk was found in any genotype of the rest

Table 5 Stratified analysis of the *PMCA2* rs3209637 polymorphism (CC *vs.* TT+CT genotype) associated with NIHL risk

Variables	Cases, N (%)		Controls, N (%)		Adjusted OR (95% CI)[b]	P[a]
	TT/CT	CC	TT/CT	CC		
Exposure time (years)						
<15	149 (78.8)	40 (21.2)	156 (78.4)	43 (21.6)	0.98 (0.59–1.61)	0.915
15–25	233 (75.6)	75 (24.4)	265 (85.8)	44 (14.2)	1.93 (1.28–2.92)	0.001
>25	87 (75.7)	28 (24.3)	89 (84.8)	16 (15.2)	1.84 (0.93–3.65)	0.092
Exposure level [dB(A)]						
<85	180 (76.9)	54 (23.1)	200 (82.6)	42 (17.4)	1.41 (0.89–2.21)	0.120
85–92	127 (78.9)	34 (21.1)	123 (83.1)	25 (16.9)	1.29 (0.72–2.30)	0.345
>92	162 (74.6)	55 (25.4)	187 (83.9)	36 (16.1)	1.79 (1.11–2.87)	0.017
Smoking status						
Never	189 (76.8)	57 (23.2)	216 (82.1)	47 (17.9)	1.42 (0.92–2.20)	0.138
Ever	280 (76.5)	86 (23.5)	294 (84.0)	56 (16.0)	1.62 (1.11–2.35)	0.012
Drinking status						
Never	198 (76.5)	61 (23.5)	208 (81.6)	47 (18.4)	1.40 (0.91–2.15)	0.154
Ever	271 (76.8)	82 (23.2)	302 (84.4)	56 (15.6)	1.64 (1.12–2.39)	0.010
Age (years)						
<35	91 (74.6)	31 (25.4)	99 (80.5)	24 (19.5)	1.44 (0.78–2.65)	0.269
35–45	292 (79.3)	76 (20.7)	310 (85.2)	54 (14.8)	1.50 (1.02–2.20)	0.039
>45	86 (70.5)	36 (29.5)	101 (80.2)	25 (19.8)	1.70 (0.95–3.07)	0.077

[a], two-sided χ^2 test for the frequency distributions of the selected variables between the cases and controls; [b], adjusted for age, sex, smoking, and drinking. *PMCA2*, plasma membrane Ca^{2+}-ATPase isoform 2; NIHL, noise-induced hearing loss.

seven SNPs in the stratification analyses (data not shown).

A stratification analysis between the rs3209637 recessive model and risk of NIHL is shown in *Table 5*. The subjects with the rs3209637 CC genotype had an increased risk of NIHL in the 15- to 25-year exposure group (adjusted OR =1.93, 95% CI =1.28–2.92), the >92 dB(A) noise exposure level group (adjusted OR =1.79, 95% CI =1.11–2.87), the ever smoking group (adjusted OR =1.62, 95% CI =1.11–2.35), the ever drinking group (adjusted OR =1.64, 95% CI =1.12–2.39), and the 35- to 45-year-old group (adjusted OR =1.50, 95% CI =1.02–2.20).

Discussion

It is widely known that NIHL is a complex disease that results from the interaction of genetic and environmental factors. The responsible environmental factors have been clearly demonstrated in previous research (3). However, the genetic basis for susceptibility to NIHL needs further study. Currently, there has been a great increase in association studies attempting to identify the susceptible genes for NIHL in humans. Most studies on NIHL have focused on pathways comprised predominantly the group of oxidative stress genes, inner ear potassium recycling pathway genes and heat shock protein (*HSP70*) genes, as well as other genes. At present, only a limited number of studies have obtained the promising results for *KCNQ4* and *KCNE1* (19), *PON2* (20,21), protocadherin 15 (*PCDH15*) (22), *GSTM1*(17), heat shock protein (*HSP70*) (23,24), myosin 14 (*MYH14*) (22) and catalase (*CAT*) (25). Studies in animal models suggested that genetic factors play a role in the development of NIHL. Several knockout mice including SOD1-/- (26), GPX1-/- (27), *Pmca2*-/- (28) and CDH23+/- (29) were shown to be more sensitive to noise than their wild-type littermates.

As far as we all know, plasma membrane Ca^{2+}-ATPase isoform 2 (*PMCA2*) plays a key role in the course of maintaining the function integrity for hair cell stereocilia. Ca^{2+} enters the stereocilia of hair cells through mechanoelectrical transduction (MET) channels and is exported back to endolymph by *PMCA2* (30), which is very concentrated in the stereocilia membrane (≈2,000 per squared micrometer) (31). Yamoah *et al.* [1998]

demonstrated that PMCA2 contributed significantly to regulate the hair bundle Ca^{2+} concentration and indicated that it could also elevate the extracellular Ca^{2+} concentration in the endolymph immediately surrounding the hair bundles (32). It was plausible to assume that the normal function of stereocilia demanded for a microenvironment with high calcium concentration. A study on the *Pmca2*-deficient mouse showed that PMCA2 played critical roles in both the vestibular and auditory systems (33). In addition, PMCA2 might also play an important part in the clearance of intracellular Ca^{2+} which might cause toxic effect on stereocilia hair cells. Fridberger *et al.* found that acoustic overstimulation caused sustained increases in the Ca^{2+} concentration of OHCs (16). It is well established that a sustained increase in intracellular Ca^{2+} concentration is toxic to cells (34). The elevated intracellular Ca^{2+} concentration may result in rounding of the hair cells or the formation of cytoplasmic blebs and then lead to the dysfunction of stereocilia (16). PMCA2 could extrude the excessive Ca^{2+} from hair cell stereocilia and prevent the cells from toxic effect induced by intracellular Ca^{2+} load.

The results from research on animal models suggested that *Pmca2* is closely related to hearing loss. A previous study on *Pmca2*-deficient mice has found that $Pmca2^{-/-}$ mice are profoundly deaf and $Pmca2^{+/-}$ mice exhibit a severe hearing loss (33). It was identified that a missense mutation in *PMCA2* was associated with autosomal dominant hearing loss. The mutated allele was a G→A transition, leading to the replacement of a highly conserved glycine with a serine at position 293, and was detected in an Italian patient affected with bilateral sensorineural hearing impairment (12). Mutations of *Pmca2* were reported in deafwaddler (dfw) mouse (11), dfw^{2J} mouse and Wriggle (wri) Mouse Sagami (35). The wri mutant was deaf and displayed abnormal movement and the dfw mutant was also deaf and displayed vestibular/motor imbalance. The phenotypes of dfw^{2J} mouse included deafness, very unbalanced movement and a slight body tremor (36). A G-to-A transition was detected in wri mouse, changing glutamic-to-lysine within a conserved transmembrane domain. The deafwaddler mouse mutant was a G→A nucleotide transition at position 283 which caused a glycine-to-serine substitution. In another allele, dfw^{2J}, a 2-base-pair deletion caused a frameshift mutant and yielded a truncated protein (36). Therefore, we assumed that deficiency in *PMCA2* might increase susceptibility to noise induced hearing loss. Currently, the association study between *PMCA2* polymorphisms and NIHL risk in humans is scarce. As a consequence, we performed this case-control study to investigate whether *PMCA2* polymorphisms were associated with susceptibility to NIHL in Chinese population.

In this case-control study, eight SNPs in *PMCA2* gene were investigated with respect to the association with risk of NIHL in a Chinese population. We found that the *PMCA2* rs3209637 CC genotype was statistically significantly associated with NIHL. Moreover, the synergistic effects of the *PMCA2* rs3209637 polymorphism and noise exposure time, noise exposure level, smoking status, drinking status, and age on NIHL were observed in our study. Observably, when the noise exposure time or age were combined with the *PMCA2* rs3209637 CC genotype, the effects were more significant. NIHL is positively correlated with noise exposure time. However, we failed to find evidence for an association between the rs3209637 polymorphisms and NIHL risk in <15 and >25 years exposure time groups, and <35 and >45 years old groups. Alternatively, the effect of rs3209637 polymorphisms on the development of NIHL was too small to be detectable with these two sample size. When the noise exposure level was combined with the *PMCA2* rs3209637 CC genotype, the effects were more significant. Similar effects were also found in smoking and drinking status group. The study results show that *PMCA2* may be a susceptible gene that predisposed individuals to NIHL. Furthermore, we could analyze the association between *PMCA2* and NIHL risk in a larger population and its possible interactions with other genes involved in NIHL. It was worth mentioning that the cases involved in this study were all high frequency hearing loss. Further studies trying to identify the underlying mechanism of NIHL should be performed in individuals with low frequency hearing loss.

Conclusions

In conclusion, we found that *PMCA2* gene rs3209637 CC genotype might be risk factor for NIHL. *PMCA2* versus noise exposure time, exposure level, smoking and drinking had interaction effects on NIHL susceptibility. Higher exposure time and exposure level might expand the NIHL risk of individuals *PMCA2* susceptibility SNP. In a preliminary conclusion, the genetic variation in *PMCA2* gene may contribute to the susceptibility of NIHL. Further cohort studies with different races and more subjects should be conducted to confirm our findings.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jphe.2016.12.01). BZ serves as an Editor-in-Chief of *Journal of Public Health and Emergency* from Jan 2017 to Dec 2022. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The research protocol was approved by the Institutional Review Board of the centers for disease control and prevention of Jiangsu province. Informed consent was obtained from all the subjects.

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