

SOD2 V16A SNP in the mitochondrial targeting sequence is associated with noise induced hearing loss in Chinese workers

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Abstract. *Objective:* To investigate whether single nucleotide polymorphisms (SNPs) in the Mn-superoxide dismutase gene (SOD2) underlie the susceptibility to noise-induced hearing loss (NIHL).

Methods: Audiometric data from 2400 Chinese Han workers who exposed to occupational noise were analyzed. DNA samples were collected from the 10% most susceptible and the 10% most resistant individuals, and five SNPs (SOD2 rs2842980, rs5746136, rs2758331, rs4880 and rs5746092) were genotyped by Taqman SNP Genotyping Kits. The SNP main effects and interactions between noise exposure and SNP were analyzed using logistic regression. Haplotypes were analyzed by using Haploview software.

Results: The CT genotype of rs4880 (SOD2 V16A SNP) was associated with a higher risk of NIHL (covariates-adjusted OR, 2.18; 95% CI, 1.34–3.54, $P = 0.002$). Haplotype analysis revealed that the frequency of AGCCG at the five SNP loci was significantly higher in the susceptible group ($P = 0.020$). With AGCTG as the reference, the OR (95% CI) was 2.63 (1.14, 6.06). The rs4880 polymorphisms imposed larger effects when the carriers were exposed to higher levels of noise, indicating the interaction between SNP and noise exposure.

Conclusions: Our results suggest that SOD2 V16A SNP in the mitochondrial targeting sequence is associated with noise induced hearing loss in Chinese workers, and this effect was enhanced by higher levels of noise exposure.

Keywords: Noise induced hearing loss, association study, SNP, Mn superoxide dismutase

1. Introduction

Noise induced hearing loss (NIHL), one of the most prevalent occupational hazards in countries with rapidly growing activity like China, is considered a complex

disease caused by gene-environment interactions [1]. Noise is the best-known and one of the most studied environmental factors causing hearing loss. Several other environmental factors, such as organic solvents, heavy metals, and heat can augment the effect of noise [2–7]. In addition, individual factors such as smoking, drinking, high blood pressure, and cholesterol levels also can influence the susceptibility to noise [8–12].

There are still limited data about genetic polymorphisms (variations in the genetic code between individuals) that may be involved in the susceptibility to NIHL. Some knockout mice study suggested that the

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gene coding for otocadherin (cadherin 23, CDH23), plasma membrane Ca^{2+} -ATPase isoform 2 gene (PMCA2), Cu/Zn-superoxide dismutase (SOD1, an antioxidant enzyme that protects cells from toxic, reactive oxygen species) and glutathione peroxidase 1 (GPX1) might be involved in the susceptibility of NIHL [13–16]. So far, only a few association studies trying to identify genes involved in human subjects have been performed. Some studies have led to possible identification of NIHL susceptibility genes, i.e. KCNE1, GSTM1, and CAT. Rabinowitz et al. [17] demonstrated a putative association between the absence of GSTM1 and NIHL in a study with limited sample size, but Carlsson et al. [1] could not confirm the observations in a study with higher power. Another study [18], using a Polish sample set, concluded that the role of the GJB2 c.35delG mutation as a determining factor in noise susceptibility is negligible. However, both studies were performed in different sample sets – Van Eyken et al. [18] analyzed Caucasians samples with an age range of 53–67 years, originating from 7 countries and Polish workers exposed to occupational noise; while Carlsson et al. [1] performed this analysis in the Swedish population. Van Laer et al. [19] detected that the variation in genes involved in coupling of cells and potassium recycling in the inner ear such as KCNE1, KCNQ1 and KCNQ4 might partly explain the variability in susceptibility to noise. Konings et al. [20] identified significant associations between CAT SNPs/haplotypes and susceptibility to development of NIHL, but that the effect of CAT polymorphisms can only be detected when noise exposure levels are taken into account. Another study of this group suggested that PCDH15 and MYH14 may be related to NIHL susceptibility in Swedish and Polish sample sets [21], however, further replication in independent sample sets was mandatory. In Chinese automobile workers, Yang et al. [22] suggested that some haplotypes of the 70-kDa heat shock proteins (HSP70) genes may be associated with a higher susceptibility to NIHL, and it was replicated in Swedish and Polish populations by Konings [23], adding to the evidence that HSP70 may be a NIHL susceptibility gene.

In the mid 1990s, a number of studies observed the appearance of increased reactive oxygen species (ROS) and toxic free radicals during and after noise exposure [24]. Cochlear hair cell damage by increased ROS following noise exposure is a potential mechanism for NIHL. Consequently, genes involved in the regulation of ROS, such as Manganese SOD genes (SOD2) may affect the vulnerability of the cochlea to NIHL [1]. Manganese SOD is a homotetramer, and each of its

subunits is encoded by the SOD2 gene on chromosome 6q25. The gene spans five exons and produces a 222-amino acid protein whose first 24 amino acids represent the mitochondrial targeting sequence. In the rat's cochlear labyrinth, the SOD2 enzyme protects against damage caused by free radicals [25]. Furthermore, SOD2-knockout mice have enhanced susceptibility to alterations caused by other mitochondrial enzymes and to diseases resulting from increased concentrations of mitochondrial ROS [26]. However, few studies have addressed the association between the SOD2 gene and NIHL in humans. Only Fortunato et al. showed that the SOD2 polymorphisms IVS3-23T/G and IVS3-60 T/G were clearly associated with NIHL in Italian workers of an aircraft factory (OR=5.09; 95% CI, 1.27–20.47) [27]. The aim of this study was thus to determine whether susceptibility to NIHL is related to SOD2 polymorphisms using five SNPs including one functional SNP (rs4880, i.e. V16A) in Chinese Han industrial workers. Furthermore, we performed a stratified analysis per the levels of noise exposure in order to identify the potential gene-environmental interactions between noise and the SOD2 gene.

2. Materials and methods

2.1. Subjects

Two thousands and four hundreds workers were ascertained from an air-conditioning factory in Southern China where they were all exposed to continuous and steady-state noise. Based on the international reference standard ISO1999:1990 and the noise measurement data, these noise-exposed workers were divided into three categories (≤ 85 dB(A), 700 workers; 85 dB(A) ~, 1100 workers; 92 dB(A) ~, 350 workers; all leq (the time-weighted average exposed sound pressure level), 8 h, 5 days a week) [28]. In each category, the 10% most-susceptible and the 10% most-resistant subjects were identified using the hearing threshold level (HTL) of the left ear at 3 kHz as a measure of noise susceptibility. Then the 10% most-susceptible subjects in the three categories were used as 'case' group and the 10% most-resistant subjects in the three categories 'control' group. The blood sample for each subject in the two groups was collected. As these subjects were very young, almost all with age < 40 years, further sub-grouping per age was not performed. The inclusion criteria were as follows: Chinese Han person, at least 1 year of exposure to the industrial noise, no

history of middle ear disease, no history of causes of hearing impairment other than the noise exposure, no conductive hearing loss or skull trauma and family history of hearing loss, no history of fever or common infections such as influenza, diarrhea, pneumonia or hepatitis within 1 month before medical examination. Furthermore, the subjects did not use any hearing protectors, such as earmuffs and disposable earplugs. A questionnaire was designed to collect the following information from all participants: age, sex, employment years, lifestyle (smoking and drinking), history of diseases, leisure time noise exposure, noise exposure at previous experienced work places and so on. The interview for each subject was performed by an industrial hygienist before the medical examination. Informed consent was obtained from all study participants.

2.2. Environmental noise monitoring and audiological assessment

Noise exposure levels at the workplaces were assessed with a sound pressure audiometer (CEL-500; Casella CEL LTD, England) at 10 AM, 3 PM, and 5 PM for three consecutive days, twice per year, according to the Chinese national criteria for noise in the workplace (GBZ43-2002, <http://www.zybw.net>). To determine the actual noise exposure level that a worker received, cumulative noise exposure (CNE) was calculated, according to monitoring data on A-weighted sound pressure level and employment time, as follows [29]: $CNE (dB) = Leq + 16.61 \times \log_{10}(T/T_0)$, where Leq was the time-weighted average exposed sound pressure level; T , the total adjusted time worked (in years); and T_0 , the figure for year 1. The occupational risk factors which may affect the hearing system, such as organic solvents, heavy metals, heat, and so on were identified. All subjects received 0.5, 1, 2, 3, 4, 6 kHz pure-tone air conduction hearing threshold tests and ear inspections according to the standards set in 'Diagnostic Criteria of Occupational Noise-induced Hearing Loss' (Chinese occupational health standard, GBZ49-2002, <http://www.zybw.net>). The audiometry was done by using Denmark AS-72 Audiometer (International Acoustic, Denmark). However, based on the characteristics of our sample and several previous studies, 3 kHz and left ear were finally chosen to be the criteria to measure the hearing loss in these workers. In reference [20], Konings et al. believed that compared with thresholds of 4 kHz and 6 kHz, 3 kHz was able to more accurately and sensitively reflect the hearing impairment of the individuals who had long-time ex-

posure to strong noise. The study of reference [1] indicated that the left ear of human beings was more sensitive and thus vulnerable to noise. In our study, all the workers were not wearing any hearing protectors, and in fact they had long-time exposure to strong noise. According to the Chinese legislation for occupational disease prevention and control, the enterprises must provide the workers adequate hearing protectors (e.g. earmuff). Most of workers could comply with the law by wearing some hearing protectors. Nevertheless, it has been seen occasionally that the workers in some factory did not wearing any hearing protector such as observed in this study. Furthermore, our choice was to reduce the potential effect of temporary threshold shift on permanent threshold shift. Based on a previous study [30], compared with 3 kHz, post-exposure temporary threshold shift of the left ear at frequencies of 4 kHz and 6 kHz was more salient. Overall, based on the above considerations, we determined to use 3 kHz as the hearing threshold, reflecting permanent hearing impairment due to noise exposure. Finally, otoscopic examination of the external acoustic meatus and tympanic membrane was performed to exclude any ear disease.

2.3. Genotyping

Informative SNPs were selected based on the data in dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>), Genebank (<http://www.ncbi.nlm.nih.gov/Genebank>) and the International HapMap database (<http://www.hapmap.org>). Based on markers' heterozygosity and MAF (minor allele frequency) in Chinese Han population, the SOD2 rs2842980, rs5746136, rs2758331, rs4880 and rs5746092 were selected to reveal the association between SOD2 and the risk of NIHL. The tagSNP selection was made by using htSNP2 that maximizes the minimum value (0.85) of the R^2 which measures the proportion of variance of each remaining SNP "explained" by grouping on full htSNP haplotype (r^2 min option), and the chosen SNPs were rs2842980, rs5746136, rs2758331, rs5746092. Therefore, the four loci should capture most of the information within the gene. In addition, rs4880 (V16A), a missense mutation, was also included in this study.

Genomic DNA was extracted from all blood samples (5 ml) using the standard procedures of Qiagen DNA extraction kit (QIAGEN company, German). Genotypes were determined using commercial Taqman allelic discrimination assay kits that utilize a 5' nuclease assay with fluorogenic allele-specific TaqMan MBG

probes with the ABI 7900HT real time PCR system (Applied Biosystems, Foster City, CA) [31]. A multi-component algorithm was used to calculate distinct allele/marker signal contributions from fluorescence measurements for each sample, reading with the ABI SNP auto-caller and then automatically determining the sample's genotypes. TaqMan assays C_11414443_10, C_29322854_10, C_16288770_10, C_8709053_10 were used for genotyping SOD2 rs2842980 (3' near gene), rs5746136 (3' UTR), rs2758331 (intron), rs4880 (missense), and rs5746092 was genotyped by a self-designed assay (forward primer: 5'-CTCTTCCATCTGGCTGTTCATCT-3', reverse primer: 5'-GATTACAGGTGTGAGCCATTGC-3'; probe: VIC-AGGATATTTCAGAGAATAC-MGB and FAM-AGGATATTTCAAGAATAC-MGB).

2.4. Statistical analyses

Hardy-Weinberg equilibrium was checked for all individual SNPs using a χ^2 test. Haplotypes were estimated using Haploview version 3.0 software (<http://www.broad.mit.edu/mpg/haploview>). Data were presented as number (frequency), mean \pm standard deviation, and 95% confidence intervals. χ^2 test was used to evaluate differences in the rates of the categorical variables between the two groups (cases and controls). Logistic regression was used to assess the independent contribution of the SOD2 polymorphisms to the NIHL after adjusting for the potential confounding factors like age, CNE, sex (0, male; 1, female), smoking (1, yes; 0, no), drinking (1, yes; 0, no), organic solvents exposure (1, yes; 0, no), heavy metals exposure (1, yes; 0, no), heat exposure (1, yes; 0, no) and dust exposure (1, yes; 0, no), respectively. For the genetic parameters, the three genotypes of each SNP were coded as 0, 1, and 2, according to the number of the mutant gene (distinct from the ancestral allele defined by dbSNP database), respectively, corresponding to general genetic model. The variables included in the analyses were related to the dependent variables with a logit link. The relationship was measured as odds ratios with 95% confidence intervals. The Statistical Package for the Social Sciences (SPSS for windows, version 11.0.1, 2001, SPSS Inc, Chicago, IL) was used for all the analyses.

3. Results

3.1. Characteristics of subjects

All resistant individuals had normal hearing in both ears (with the hearing threshold $<$ 25 dB in every fre-

quency). The hearing threshold of 3 kHz in left ear was significant higher in the susceptible workers than in the resistant workers (40.23 ± 9.53 dB vs. 16.15 ± 2.58 dB). Balance comparison of the two groups found that age (24.42 ± 4.74 vs. 24.52 ± 5.21 years), employment time (5.20 ± 3.82 vs. 4.89 ± 2.86 years), noise exposure level (86.90 ($83.05, 92.90$) dB vs. 86.90 ($83.73, 92.70$) dB), and CNE (76.58 ($73.74, 79.76$) dB vs. 76.88 ($73.57, 80.50$) dB) had no statistical difference, with all $P > 0.05$. As shown in Table 1, categorical variables like gender (male/female, 190/11 vs. 179/23), heavy metals exposure (27.4% vs. 15.8%), heat exposure (42.2% vs. 26.2%), and dust exposure (46.3% vs. 33.7%) were significantly different between two groups ($P < 0.05$, two-sided χ^2 test). Therefore, in the following multivariate logistic regressions, the covariates of these factors were included for adjustment. All the remaining variables were not significantly different. As all the subjects were living in the same community nearby the factory and had a consistent low leisure time exposure to noise, this factor was therefore not analyzed in this study. In addition, almost all the subjects were recent high-school or college graduates, this job was their first employment, and thus they had no history of exposure to noise at previous workplaces.

3.2. Distributions of SOD2 SNPs

The distributions of the SOD2 SNP genotypes and alleles in the susceptible workers and the resistant workers are shown in Table 2. The minor allele frequency (MAF) for all SNPs in SOD2 were higher than 5% (or percentage of heterozygotes higher than 10%), indicating that those SNPs were frequent in Chinese Han population. All the five polymorphism loci were in Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) between the SNPs were estimated by using Haploview version 3.0 software, showing that most of the LD values were very small, with the largest ($r^2 = 0.77$) between rs5746136 and rs4880, indicating that the five SNPs were in separated LD blocks.

3.3. Association of SNPs with NIHL

Crude and adjusted ORs for NIHL were estimated separately, derived without or with adjustment for confounding factors. As shown in Table 3, the frequencies of CT genotype and C allele of rs4880 in the susceptible workers were significantly higher than in the resistant workers ($P = 0.001$ and $P = 0.006$ respectively). After adjusted for age, sex, smoking, drinking,

Table 1
Difference of risk factors in NIHL sensitive workers and resistant workers

Characteristic	NIHL sensitive workers (<i>n</i> = 201)	NIHL resistant workers (<i>n</i> = 202)	<i>P</i> value
Mean HT of 3000Hz in left ear (SD)*	40.23 (9.53)	16.15 (2.58)	< 0.01
Mean age [years] (SD)*	24.42 (4.74)	24.52 (5.21)	> 0.05
Mean employment time [years] (SD)*	5.20 (3.82)	4.89 (2.86)	> 0.05
Median noise exposure [dB(A)] (range)	86.90 (83.05–92.90)	86.90 (83.73–92.70)	> 0.05
Median CNE [dB(A)] (range)	76.58 (73.74–79.76)	76.88 (73.57–80.50)	> 0.05
Gender, <i>n</i> (%)			< 0.05
male	190 (94.5)	179 (88.6)	
female	11 (5.5)	23 (11.4)	
Smoking <i>n</i> (%)			> 0.05
Yes	95 (47.3)	108 (53.5)	
No	106 (52.7)	94 (46.5)	
Drinking <i>n</i> (%)			> 0.05
Yes	121 (60.2)	106 (52.5)	
No	80 (39.8)	96 (47.5)	
Organic solvents exposure <i>n</i> (%)			> 0.05
Yes	36 (17.9)	23 (11.4)	
No	165 (82.1)	179 (88.6)	
Heavy metals exposure <i>n</i> (%)			< 0.01
Yes	55 (27.4)	32 (15.8)	
No	146 (72.6)	170 (84.2)	
Heat exposure <i>n</i> (%)			< 0.01
Yes	89 (42.2)	53 (26.2)	
No	112 (57.8)	149 (73.8)	
Dust exposure <i>n</i> (%)			< 0.01
Yes	93 (46.3)	68 (33.7)	
No	108 (53.7)	134 (66.3)	

HT: threshold of hearing, CNE: cumulative noise exposure; Median values are given with the range of p₂₅–p₇₅.

*Independent samples Student's *t* test; Rank sum test; Two-sided χ^2 test.

organic solvents exposure, heavy metals exposure, heat exposure and dust exposure using multivariate logistic regression model, genotype CT of rs4880 remained significantly associated with a higher risk of NIHL (adjusted OR, 2.18; 95% CI, 1.34–3.54, *P* = 0.002).

3.4. Interaction between noise exposure level and SNPs

In order to reveal the interactions between SNPs and noise exposure level, we performed stratified analysis per noise exposure level. Inconsistent genotypic distributions between the susceptible and the resistant group were found for different noise exposure levels, suggesting that SNP genotypic effects on the susceptibility to noise induced hearing loss were dependent on the environmental noise exposure level and CNE. In the studied Chinese Han sample, significant gene-environment interactions between rs4880 SNPs and noise exposure were identified (Table 4). In the 85~ and 92~ dB (A) exposure levels, the susceptible workers were more likely to be the carriers of CC or CT genotype, whereas in the resistant workers TT genotype was more frequent, rendering that CC or CT were risk genotypes (respectively with adjusted OR, 2.38 and 95% CI, 1.27–

4.46, *P* = 0.006; adjusted OR, 3.67, 95% CI, 1.10–12.30, *P* = 0.035). Similarly, in the 82 dB(A) ~ CNE level, comparing with TT genotype, we found that the CC and CT were the risk genotypes (*P* = 0.023), with adjusted OR of 2.59 and 95% CI of 1.14–5.90. However, there was no such a trend in the lower noise exposure/CNE levels. These results suggest that hearing loss in these workers were resulted from synergic effects of both genetic and environmental factors.

3.5. Association of haplotypes with NIHL

Haplotypes were estimated by using Haploview software, followed by an association analysis by considering a haplotype as an allele (Table 5). The frequencies of haplotype AGCCG was significantly higher in the susceptible group than in the resistant group (*P* = 0.020), rendering it to be a risk haplotype. With the haplotype AGCTG as the reference, the corresponding OR and 95% CI were 2.63 and 1.14–6.06.

4. Discussion

Reactive oxygen species (ROS) play a key role underlying the mechanisms for induction of cochlear

Table 2
The distribution of SOD2 genotypes and alleles in NIHL sensitive workers and resistant workers

SOD2 Alleles	NIHL sensitive workers (n = 201)						NIHL resistant workers (n = 202)													
	Allele			Genotype			Allele			Genotype										
	A*	B	AA*	AB	BB	A	B	AA	AB	BB										
n	%	n	%	n	%	n	%	n	%	n	%									
rs2842980 A/T	210	52.2	192	47.8	59	29.4	92	45.8	50	24.9	192	47.5	212	52.5	41	20.3	110	54.5	51	25.2
rs5746136 A/G	143	35.6	259	64.4	25	12.4	93	46.3	83	41.3	157	38.9	247	61.1	32	15.8	93	46.0	77	38.1
rs2758331 A/C	49	18.2	355	81.8	3	1.5	43	21.4	155	77.1	57	14.6	347	85.4	6	3.0	45	22.3	151	74.8
rs4880 C/T	71	17.7	331	82.3	8	4.0	55	27.3	138	68.7	44	10.9	360	89.1	3	1.5	38	18.8	161	79.7
rs5746092 C/G	147	36.6	255	63.4	22	10.9	103	51.2	76	37.8	161	40.0	241	60.0	26	12.9	109	54.2	66	32.8

* Alleles and Genotypes, two alleles are denoted by A and B, respectively.

Table 3
Association of the genotypes with the risk of NIHL

SOD2	Crude OR value (95% CI)	P value	Adjusted OR* value (95% CI)	P value
rs2842980				
AA/TT	1.47 (0.84, 2.56)	0.177	1.46 (0.82, 2.61)	0.197
AT/TT	0.85 (0.53, 1.38)	0.515	0.82 (0.50, 1.34)	0.430
A/T	1.21 (0.92, 1.59)	0.181		
rs5746136				
AA/GG	0.73 (0.40, 1.33)	0.300	0.73 (0.39, 1.38)	0.337
AG/GG	0.93 (0.61, 1.42)	0.728	0.92 (0.59, 1.43)	0.698
A/G	0.87 (0.65, 1.16)	0.334		
rs2758331				
AA/CC	0.49 (0.12, 1.98)	0.315	0.56 (0.13, 2.39)	0.430
AC/CC	0.93 (0.58, 1.50)	0.767	0.97 (0.59, 1.59)	0.908
A/C	0.84 (0.56, 1.27)	0.404		
rs4880				
CC/TT	3.35 (0.87, 12.90)	0.078	3.68 (0.91, 14.84)	0.068
CT/TT	2.15 (1.36, 3.42)	0.001	2.18 (1.34, 3.54)	0.002
C/T	1.76 (1.17, 2.63)	0.006		
rs5746092				
CC/GG	0.74 (0.38, 1.42)	0.358	0.72 (0.36, 1.42)	0.335
CG/GG	0.82 (0.54, 1.26)	0.363	0.77 (0.49, 1.19)	0.237
C/G	0.86 (0.65, 1.15)	0.310		

*Adjusted for age, CNE, sex, smoking, drinking, organic solvents exposure, heavy metals exposure, heat exposure and dust exposure.

Table 4
Stratified analysis of SOD2 rs4880 per noise exposure level or CNE to identify SNP-environment interactions for risk of NIHL

	NIHL sensitive workers (n = 201)				NIHL resistant workers (n = 202)				χ^2 value	P value	Adjusted OR* value (95% CI), CC+CT vs. TT
	CC+CT		TT		CC+CT		TT				
	n	%	n	%	n	%	n	%			
Noise exposure level [dB(A)]											
< 85	22	33.8	43	66.2	16	23.5	52	76.5	1.733	0.188	1.66 (0.78, 3.56)
85 ~	39	38.5	68	61.5	20	19.4	83	80.6	7.535	0.006	2.38 (1.27, 4.46)
92 ~	12	41.4	17	58.6	5	16.1	26	83.9	4.705	0.035	3.67 (1.10, 12.30)
CNE [dB(A)]											
< 75	22	33.8	43	66.2	17	22.7	58	77.3	2.166	0.141	1.75 (0.83, 3.68)
75 ~	25	36.2	44	63.8	39	27.9	101	72.1	5.684	0.218	1.47 (0.80, 2.72)
82 ~	26	38.8	41	61.2	11	19.6	45	80.4	5.326	0.023	2.59 (1.14, 5.90)

*adjusted for age, sex (0, male; 1, female); smoking (1, yes; 0, no); drinking (1, yes; 0, no); organic solvents exposure (1, yes; 0, no); heavy metals exposure (1, yes; 0, no); heat exposure (1, yes; 0, no); dust exposure (1, yes; 0, no).

Table 5
Assessment of association between SOD2 haplotypes and NIHL

Haplotypes*	NIHL sensitive workers		NIHL resistant workers		χ^2 value	P value	OR value (95% CI)
	n	%	n	%			
AGCTG	177	44.0	169	41.9			1.00
TACTC	124	30.9	133	33.0	0.498	0.480	0.89 (0.65, 1.23)
TGACG	41	10.2	29	7.3	1.284	0.257	1.35 (0.80, 2.27)
AGCCG	22	5.5	8	2.1	5.450	0.020	2.63 (1.14, 6.06)
TGCTG	9	2.2	5	1.4	0.929	0.335	1.72 (0.56, 5.23)

*The allele order is rs2842980, rs5746136, rs2758331, rs4880 and rs5746092 from left to right. Reference group is AGCTG.

damage under various pathological conditions. Superoxide is readily generated in the inner ear following acoustic overstimulation. Superoxide can react in an iron-catalyzed Fenton reaction to form destructive hydroxyl radicals or combine with nitric oxide to form the highly toxic peroxynitrite [32]. Therefore, the regulation of superoxide levels is important for cochlear degeneration caused by ROS. SOD (superoxide dismutase) is an enzyme that converts superoxide to hydrogen peroxide. In humans (as in all other mammals and most chordates), three types of superoxide dismutase are identified. SOD1 is located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular. The first is a dimer (consists of two units), while the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive center. Localization of both SOD1 and SOD2 in the cochlea has been reported, and SOD1 is the most abundant in the cochlea, comprising approximately 74% of total SOD activity. Absence of SOD2 has been shown to lead to an increase in hearing loss related to acoustic trauma. In addition, auditory dysfunction due to noise exposure is attenuated by application of SOD2. Furthermore, transgenic mice over expressing SOD2 were protected against amino glycoside-induced hearing loss, which is also mediated by ROS [33]. However, no protective effects of over expression of SOD2 against acoustic trauma were observed. Therefore, the protective effects of SOD2 against acoustic trauma remain unresolved.

In the present study, we found statistically significant difference between the NIHL susceptible workers and resistant ones in the genotypic and allelic distributions of rs4880 in gene SOD2. As revealed by multivariate logistic regression analysis, the CT genotype of rs4880 was also associated with a higher risk of NIHL (adjusted OR, 2.18; 95% CI, 1.34–3.54, $P = 0.002$). These results suggested that the rs4880 polymorphisms (V16A, or called $-9T > C$) of the SOD2 may confer to the risk of NIHL in the Chinese Hans. A recent study [34] showed that V16A homozygotes may have higher SOD2 activity than V16A heterozygotes. The $-9T > C$ (V16A) polymorphism in the MnSOD mitochondrial targeting sequence significantly reduced MnSOD catalytic activity in cryopreserved hepatocytes [35]. SOD2 contains a signalling peptide of 24 amino acids, and thus $-9T > C$ (or V16A) describes genetic change (T to C) at the ninth position of the upstream of the DNA sequence coding SOD2 signaling peptide. Another study [36] identified a valine/alanine polymorphism (rs4880 or V16A)

in the targeting sequence of SOD2, and an in vitro study [16] showed that valine (Val) instead of alanine (Ala) results in less efficient transport of Mn-SOD into the mitochondrial matrix, which can compromise the ability to neutralize superoxide radicals in the cell. This polymorphism were also associated with diabetic nephropathy in Japanese [37] and Korean patients [38] with type 2 diabetes, breast cancer risk [39], development of Alzheimer's disease, a high degree of carotid atherosclerosis [40], exudative age-related macular degeneration in Japanese individuals, and associated with elevation of prostate cancer risk in Caucasians (Val/Ala versus Val/Val: OR, 1.17; 95% CI, 0.97–1.42; Ala/Ala versus Val/Val: OR, 1.28; 95% CI, 1.03–1.60; $P(\text{trend}) = 0.03$).

However, our positive findings are not in agreement with the results reported by an earlier study using an Italy population of noise-exposed male workers [27] showing that there were no statistically significant difference between the SNPs of rs4880 in SOD2 and the risk of NIHL. However, this study differs from our study in several aspects which may explain the discrepancies. First, there is a large difference in sample size between our study and the previous studies, resulted in different statistical powers achieved. Fortunato et al. analyzed 94 male workers from an aircraft factory [27], while we genotyped > 400 either NIHL sensitive or resistant workers in an air-conditioning factory. Second, this genetic polymorphism may confer a race-specific risk, and our study demonstrated that Chinese Han might be more susceptible to this genetic exposure than the European peoples.

Although sometimes useful, single SNP may not be sufficiently informative for unraveling complex disease [41]. The multipoint haplotype-based association studies by considering linkage disequilibrium pattern and joint effects of several SNPs might overcome this weakness and thus are more powerful for locating the underlying SNP loci for complex disease [42]. Further haplotype analysis using the Haploview version 3.0 software revealed that the frequency of haplotype AGCCG was significantly higher in the sensitive groups than in the resistant groups ($P = 0.020$), compared with haplotype AGCTG, the OR (95% CI) was 2.63 (1.14, 6.06).

In this study, the stratified association analysis between several SNPs in SOD2 and NIHL, according to the noise exposure levels, was also conducted. The strength of the association between gene SOD2 and NIHL is dependent on the noise exposure levels. We observed that the rs4880 SNPs showed more significant

results in the high-level noise exposure group (85 dB (A) ~ or CNE 82 dB (A) ~). These results might be reasonable because there may be an underlying threshold over which these exposed workers were more likely affected. In a previous report, Konings et al. also found that the higher noise exposure may induce a higher risk between the NIHL and the CAT gene [20].

Nevertheless, several weaknesses of this study should be recognized. First, as the fast pace of genomics research continues to identify susceptibility SNP polymorphisms, it becomes apparent that many independent studies are often needed to unequivocally establish a valid genotype-phenotype association across diversities of human populations before their considerations as clinical markers for disease. It is essential that independent replication studies conform to certain standards to ensure proper refutation or acceptance of identified loci. This requirement is particularly salient and time impressive for a small to medium-size genotype-phenotype relationship, such as the association between V16A and NIHL. Second, in this study, no correction for multiple testing was applied. If conservative Bonferroni's correction were applied, the significance of some findings may change although our major findings regarding the association between V16A and NIHL were remained. Finally, it should be cautious to extend the findings to other ethnical populations. Various studies of human hearing loss genes (e.g. SLC26A4 [43]) suggest that there are significant different spectra of mutations between different populations, particularly between populations of European and Asian origins.

In conclusion, SOD2 V16A SNP in the mitochondrial targeting sequence is associated with noise induced hearing loss in Chinese workers. Furthermore, there are significant interactions between these genetic polymorphisms and noise exposure levels, jointly contributing to NIHL. Follow-up functional studies of these genetic variants aiming to understand the underlying molecular mechanism of NIHL are warranted.

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