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Genetic variations in protocadherin 15 and their interactions with noise exposure associated with noise-induced hearing loss in Chinese population

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ABSTRACT

Objectives: The purpose of this study was to examine the associations between genetic variations in the Protocadherin 15 gene (*PCDH15*) and the risk to noise induced hearing loss (NIHL) in a Chinese population.

Methods: A case-control study was conducted with 476 noise-sensitive workers (NIHL) and 475 noise-resistant workers (normal) matched for gender, years of noise exposure, and intensity of noise exposure. 13 tag single-nucleotide polymorphisms in *PCDH15* were genotyped using nanofluidic dynamic arrays on the Fluidigm platform. Multiple logistic regression was used to analyze the associations of genetic variations of *PCDH15* with NIHL adjusted by age, smoking/drinking status, and cumulative noise exposure and their interactions with noise exposure.

Results: The allele frequency and genotypes of rs1104085 were significantly associated with the risk of NIHL($P=0.009$ and 0.005 respectively). The subjects carrying variant alleles (CT or CC) of rs11004085 had a decreased the risk for NIHL (adjusted odds ratio=0.587, 95% confidence interval 0.409–0.842) compared with subjects who had the wild-type (TT) homozygotes. The interactions were found between the SNPs of rs1100085, rs10825122, rs1930146, rs2384437, rs4540756, and rs2384375 and noise exposure.

Conclusions: Genetic variations of *PCDH15* and their interactions with occupational noise exposure are associated with genetic susceptibility to NIHL and modify the risk of noise induced hearing loss.

1. Introduction

Noise is the most common occupational risk factor and induces various harmful effects on health (Nelson et al., 2005). Millions of workers are exposed to harmful levels of noise in the workplace. In Europe, about 20% workers are exposed to noise during $>50\%$ of their working time. In the United States, about nine million workers are exposed to time-weighted average sound levels of ≥ 85 dB (Dobie, 2008). It was estimated that >500 million individuals worldwide may be at risk of developing noise-induced hearing loss (NIHL), which is an irreversible damage to the auditory system and also is a common occupation-associated disease. Worldwide, about 16% cases of disabling hearing loss in adults are attributed to occupational noise (Nelson et al., 2005).

NIHL is a complex disease caused by both environmental and genetic factors (Konings et al., 2009b; Sliwinska-Kowalska and Pawelczyk, 2013). The workers under similar noise exposure may have different outcomes of hearing damage (Śliwińska-Kowalska et al., 2006). These differences indicate that genetic susceptibility plays an important role in NIHL incidence. With the mouse models of Castaneous (CAST/Ei) (resistant inbred strain to NIHL) and C57BL/6 J strain (susceptible strain), White et al. (Davis et al., 2001; Kozel et al., 2002; White et al., 2009) identified a number of genetic loci, which was responded to damaging noise. Candidate-based association studies in humans have identified GSTM1 (Lin et al., 2009; Shen et al., 2012), CAT (Konings et al., 2007), CDH23 (Kazmierczak et al., 2007; Kowalski et al., 2014; Sliwinska-

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Kowalska et al., 2008), KCNE1 (Pawelczyk et al., 2009), and heat shock protein 70 (Konings et al., 2009a) as putative protein products implicated in NIHL incidence. Recently, Shen et al. found one SNP (Ser326Cys /rs1052133) in human 8-oxoG DNA glycosylase1 associated with NIHL (Shen et al., 2014). However, evidence regarding the roles of genetic factors in NIHL pathogenesis remains inadequate. The genetic mechanism of NIHL remains to be further elucidated, and NIHL-associated genes need to be discovered (Konings et al., 2009b; Sliwinska-Kowalska and Pawelczyk, 2013).

The protocadherin 15 gene (PCDH15) is a member of the cadherin superfamily (Kazmierczak et al., 2007). It encodes an integral membrane protein and mediates calcium-dependent cell–cell adhesion. *PCDH15* helps cells to stick together and plays an essential role in maintaining normal retinal and cochlear function (Sotomayor et al., 2010). *PCDH15* is expressed in the inner ear cells, specialized cells in the eye, and retinal photoreceptor cells. The exact function of *PCDH15* in the retina has not been fully determined. *PCDH15* interacts with the proteins Cadherin 23, USH2A, and VLGR1, anchored by harmonin, SANS, or whirlin and makes the synaptic membranes closely appose with homotypic or heterotypic interactions of their large extracellular regions (Kremer et al., 2006; van Wijk et al., 2006). Mutations in this gene are associated with hearing loss and Usher Syndrome Type IF (USH1F) (Kremer et al., 2006; van Wijk et al., 2006). One single-nucleotide polymorphism (SNP) in *PCDH15*, rs7095441, has been reported to be associated with NIHL risk in certain European populations [odds ratio (OR)=2.076, 95% confidence interval 1.344 to 3.206] (Konings et al., 2009c). Although this variation is relatively common [minor allele frequency (MAF) 0.482] in European Ashkenazi Jewish populations, it is rare (MAF 0.006- 0.012) in Chinese and Japanese populations (http://hapmap.ncbi.nlm.nih.gov/). There may be racial differences in the genetic variation of *PCDH15* and the development of NIHL. No findings regarding the effects of *PCDH15* on NIHL have been reported for Asian populations.

To investigate whether genetic variations in *PCDH15* are associated with genetic susceptibility to NIHL in Chinese populations, we genotyped 13 tag SNPs (tSNPs) in *PCDH15* in 476 NIHL workers and 475 normal hearing workers, and examined the associations of 13 candidate SNPs and haplotypes with NIHL. We also explored the interaction effects among these SNPs and noise exposure.

2. Methods

2.1. Study subjects and data sources

Subjects in this study included 476 NIHL workers and 475 normal hearing workers. All the subjects were recruited from a cross-sectional survey of 4419 occupational noise- exposed workers conducted between March 1, 2011 and December 31, 2012. In this survey, the workers were employed in the noise-exposed factories of mechanical equipment and household appliance manufacturing, steel construction, and cigarette production/packaging in Hangzhou city, Zhejiang province, China. Intensity of noise in the workplace was determined by a noise statistical analyzer (AWA6218; Westernization Instrument Technology Co., Ltd., Beijing, China). Noise exposure was evaluated with equivalent continuous dB(A)-weighted sound pressure levels ($L_{EX,8\ h}$) (Pawlaczyk-Luszczynska et al., 2011) according to Occupational Health Standard of the People's Republic of China: Measurement of Noise in the Workplace (GBZ/T 189.8-2007) (China, 2007). All the subjects received annual health examinations, including routine physical examination, pure tone audiometry (PTA), epidemiological investigation, and whole-blood collection. The inclusion criteria of subjects in this cross-sectional survey were as follows: (1) Working at noised exposed workplace and $L_{EX,8\ h}$ was >85 dB (A); (2) Cumulative time of noise exposure of >1 year. Cumulative time of noise exposure of each worker was recorded according to the files of occupational health surveillance and verified with epidemiological interview; (3) Han ethnicity. The subjects were excluded if they had a family history of hearing loss or histories of the diseases such as otitis, other otological diseases, head injury, exposure to explosives, or ototoxic drug administration. The study protocol was approved by the Research Ethics Committees of Hangzhou Center for Disease Prevention and Control, Zhejiang, China.

2.2. PTA and NIHL assessment

After participants stopped noise exposure for >12 h, audiometry was carried out for each subject using a Madsen Voyager 522 audiometer (Madsen, Taastrup, Denmark) in a soundproof room with a background noise level of <25 dB (A). Hearing thresholds of both ears were determined with the ascending method in 5-dB steps at frequencies of 500, 1000, 2000, 3000, 4000, and 6000 Hz. All the evaluations were performed by trained physicians using standard procedures. In order to exclude the confounding effects by age and gender, the audiometric raw data were calibrated for the effects of age and gender on the basis of the Diagnostic Criteria of Occupational NIHL (Chinese National Criteria GBZ49-2007, http://www.zybw.net). The hearing threshold at high frequency (HTHF) by PTA was defined as the average at 3000, 4000, and 6000 Hz for each ear, and the hearing threshold at speech frequency (HTSF) was defined as the average at 500, 1000, and 2000 Hz for each ear.

2.3. Physical examination and epidemiological investigation

A physical examination was performed for each subject. Parameters such as height, weight, systolic and diastolic blood pressure levels were measured by trained physicians following a standard protocol. Face-to-face interview was used to collect epidemiological data using a structured questionnaire administered by trained professional physicians. The information in the questionnaire included demographic characteristics; smoking/drinking status; history of medical conditions and drug use; history of exposure to noise, vibration, and toxic chemicals in the workplace; health habits; use of ear protection for noise.

2.4. Definitions of NIHL and control subjects

NIHL group included the workers with normal hearing before exposure, >1 year of occupational noise exposure, and an HTHF >40 dB of hearing level (HL). In order to exclude hearing loss induced by non-noise exposure, the workers were excluded from the study if their differences of HTHF between left and right ears were greater than 35 dB (HL). The normal group included the workers with >1 year of occupational noise exposure, and hearing thresholds <25 dB (HL) at each frequency. In order to control the environmental confounders, control subjects were individually matched with NIHL subjects according to the variables of gender, intensity of noise and years of noise exposure. Because the majority of the subjects in the cross-sectional study were males (about 91.7%), the subjects in this study were restricted on males.

2.5. SNP selection and genotyping

Candidate SNPs in *PCDH15* were selected as tSNPs on the basis of the HapMap database and previous findings from the literature.