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Association of *Cadherin23* Single Nucleotide Polymorphism with Age-Related Hearing Impairment in Han Chinese

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. Genetic variation of *cadheri23* (*cdh23*; 753G>A in exon 7) has been implicated with age-related hearing impairment (ARHI) in mice. This study aimed to test the association of the *CDH23* tag single nucleotide polymorphism (SNP) in intron 7 with ARHI in Han Chinese.

Study Design. Individual cohort study.

Setting. Tertiary medical center.

Subjects and Methods. A total of 1175 Han Chinese subjects were divided into the case group (n = 310, 26% with poorest hearing) and the control group (n = 308, the 26% with best hearing) according to the Z_{high} score converted from the original frequency-specific hearing thresholds. The *CDH23* SNP locus (rs7087735: C/T) in intron 7 (coordinate: 72996763) shown in the HapMap was genotyped with correlation to the hearing phenotype.

Results. The genotype distributions of *CDH23* (CC/CT/TT) were not significantly different between the case and control group (P = .489). Compared with genotype CC, the odds ratios of the genotypes CT and TT for ARHI were not significantly different after adjustment for other environmental factors (P = .299 for CT; P = .610 for TT).

Conclusions. Despite that the *Ahl* allele of *Cdh23* had been implicated with ARHI in mice, we found no positive association of the *CDH23* tag SNP in intron 7 with ARHI in Han Chinese.

Keywords

cadherin23, single nucleotide polymorphism, age-related hearing impairment, humans, Han Chinese

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Age-related hearing impairment (ARHI) is the most common sensory dysfunction in adults. Environmental factors, including systemic diseases, central obesity, obstructive sleep apnea, noise, chemical exposure, tobacco, ototoxic medication, hormonal replacement therapy, and socioeconomic status, have been reported to be associated with peripheral hearing function.[1-4] In contrast, less is known about the genetic component of ARHI.

In mice, ARHI is linked to 3 loci: *Ahl1*,[5] *Ahl2*,[6] and *Ahl3*.[7] Mitochondrial 4834 deletion in Wistar rats[8] also leads to ARHI. A number of susceptibility genes, including *NAT2*,[9] *KCNQ4*,[10] *APOE*,[11] *GRHL2*,[12] and *GRM7*[13] were associated with ARHI in humans. ARHI was also associated with some mitochondrial DNA (mtDNA) background.[14] However, there was no association between *GRHL2* single nucleotide polymorphism (SNP) in intron 1 and ARHI in Han Chinese.[15]

CDH23, also known as otocadherin, is part of the cadherin superfamily of cell surface adhesion proteins. The hypomorphic *Cdh*23 (753A) allele causes in-frame skipping of exon 7. Altered adhesion or reduced stability of *Cdh23* may confer susceptibility to ARHI and noise-induced hearing loss in mice.[16] In humans, some genetic variations of *CDH23* were also linked to Usher syndrome type 1D (Usher 1D) or DFNB12 patients.[16,17] However, the contribution of

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Table 1. Characteristics of All Subjects (Age ≥ 40 years)

Variable	All (N = 1175)	Control (n = 308)	Case (n = 310)
Age, y, mean ± SD	56.5 ± 8.88	57.4 ± 8.31	56.6 ± 8.95
Sex, female/male	601/574	163/145	137/173
Z_{high}	0.41 ± 0.861	−0.5 ± 0.27	1.5 ± 0.74

CDH23 variations around exon 7 on ARHI was still unclear in humans.

Meanwhile, according to the database of HapMap, there was no tag SNP in exon 7, but 1 in intron 7 beside exon 7 for Han Chinese in Beijing, China. So, this study investigates the association of CDH23 tag SNP in intron 7 (rs7087735: C>T) and ARHI for Han Chinese in Taiwan.

Methods

Subjects

From September 2007 to December 2009, 1175 adult volunteers aged 40 to 86 years old were prospectively recruited and selected randomly from the Health Management Center of National Taiwan University Hospital and from Dalin Tzu-Chi General Hospital. Written informed consent was obtained from all subjects. The study was approved by the institutional review boards of both hospitals.

Clinical Evaluation and Audiological Measurements

The subjects were interviewed by trained assistants with the use of a structured questionnaire detailing demographic data, morphometry, and family history of hearing loss. Audiological results were then assessed with air and bone conduction thresholds of pure tones using an audiometer (GSI 10; Grason-Stadler Inc, Milford, New Hampshire).

Han Chinese with normal or symmetric sensorineural hearing loss and normal cognitive function were included for genotyping and further analysis.

The exclusion criteria included non–Han Chinese, hearing loss developing before 30 years, patients presented with Usher syndrome or nonsyndromic prelingual hearing loss (DFNB12), asymmetric sensorineural hearing loss (defined as 15-dB hearing loss or greater asymmetry in 2 or more frequencies), conductive hearing loss of more than 10 dB, 4-kHz dip on audiogram, high environmental noise exposure, acoustic trauma, exposure to ototoxic drug, major neurological or psychiatric diseases, brain tumor or vestibular schwannoma, pregnancy, vertigo, liver cirrhosis, chronic renal failure (chronic kidney disease) under peritoneal dialysis or hemodialysis, cancer, head and neck radiation exposure, heavy smoking, alcoholism, or substance abuse.

Z-Score Calculation and Phenotype Determination

Based on the ISO 7029 standard, frequency-specific thresholds were converted to sex- and age-independent Z scores.[18] For both ears of each subject, Z-scores at 0.25, 0.5, and 1 kHz were averaged as Z-low, and Z-scores at 2, 4, and 8 kHz were averaged as Z_{high}. Only Z_{high} scores of the better-hearing ear were used for later analysis. Based on the Z_{high} scores, participants were then classified into controls (the 26% of subjects with better hearing) or cases (the 26% of subjects with worse hearing).

Genotyping

Genomic DNA was extracted from peripheral venous blood using standard procedures. A region from exon 7 to intron 7 of *CDH23*, including the tag SNP locus (rs7087735: C/T) in intron 7 (coordinate: 72996763), shown in the HapMap, was amplified with polymerase chain reaction (PCR). The forward and backward primers were TTCATCGT GAATGCCACA GACC and AAG CCT CAG TAA TGC CAG C, respectively. The sequencing was performed by the ABI PRISM 310 Genetic Analyzer.

Statistical Analyses

χ^2 or Fisher exact tests were performed to test the distribution of category variables or *CDH23* genotypes. The difference between the means of the 2 groups was tested using the Student *t* test. Logistic regression analyses were performed using *CDH23* genotypes for the odds ratios (ORs) of ARHI with adjustment for other factors.

Results

Table 1 shows the basic characteristics of all subjects. The mean age was similar in both groups (57.4 ± 8.31 years vs 56.6 ± 8.95 years). As we defined, the mean Z_{high} scores were higher in the case group (1.5 ± 0.74) than in the control group (–0.5 ± 0.27).

Table 2 shows the distribution of *CDH23* genotype and other variables in the control and case groups according to the Z_{high} scores. Genotype distributions of the *CDH23* (CC/CT/TT) were not significantly different between controls and cases classified (χ^2 test, $P = .489$).

Logistic regression models were constructed to control potential confounding of other nongenetic factors on the correlation between *CDH23* genotypes and audiological phenotypes. As revealed in **Table 3**, the OR of the genotypes *CT* and *TT* of *CDH23* for ARHI did not decrease significantly as compared with genotype *CC* of *CDH23*, after adjusting for other environmental risk factors (0.80 ± 0.174, 95% confidence interval [CI] = 0.52~1.22, $P = .299$ for CT; 0.88 ± 0.213, 95% CI = 0.55~1.42, $P = .610$ for TT).

Table 2. Distribution of CDH23 Genotype and Other Variables

Variable	Control (n = 308)	Case (n = 310)	*P* Value
CDH23 genotype (CC/CT/TT)	57/163/88	69/153/88	.489
Central obesity (N/Y)	142/166	114/196	.022
CAD (N/Y)	291/17	293/17	1.000
HTN (N/Y)	238/70	252/58	.234
DM (N/Y)	289/19	273/37	.017
Dyslipidemia (N/Y)	283/25	283/27	.885
CKD (N/Y)	308/0	302/8	.007
Smoking (N/Y)	272/36	260/50	.131
Drinking (N/Y)	246/62	237/73	.331

Abbreviations: CAD, cardiovascular disease; CKD, chronic kidney disease; DM, diabetes mellitus; HTN, hypertension.

Table 3. Logistic Regression Model Analyzing the Effects of Both CDH23 Genotypes and Nongenetic Factors in Cases and Controls Classified by the Z_{high} Scores

Variable	Odds Ratio	Standard Error	95% Confidence Interval	*P* Value
CDH23 genotype:				
CC	—	—	—	—
CT	0.80	0.174	0.52∼1.22	.299
TT	0.88	0.213	0.55∼1.42	.610
Central obesity	1.42	0.245	1.01∼1.99	.041
CAD	0.89	0.330	0.43∼1.84	.744
HTN	0.71	0.151	0.47∼1.08	.106
DM	1.77	0.545	0.97∼3.24	.065
Dyslipidemia	1.08	0.335	0.59∼1.98	.807
CKD	Dropped	—	—	—
Smoking	1.52	0.395	0.91∼2.52	.114
Drinking	1.11	0.239	0.72∼1.69	.639

Abbreviations: CAD, cardiovascular disease; CKD, chronic kidney disease; DM, diabetes mellitus; HTN, hypertension; —, no data output.

Discussion

In the present study, we found that the *CDH23* tag SNP in intron 7 did not contribute to ARHI, before and after adjusting for environmental risk factors. These findings might contradict the ones from animal studies: the *ahl1* could be linked to the change of SNP 753G>A in exon 7 of C57BL/6J mice.

The mouse *Cdh23* locus, located on chromosome 10, is composed of two 5′-untranslated exons and 69 coding exons; together they cover a genomic distance of at least 350 kb.[19] A *Cdh*23 transcript with a spliced exon 68 is the predominantly expressed isoform in the organ of Corti. Exon 7 of *Cdh23* encodes 43 amino acids that form sections of the third and fourth EC domains, which is a potential homodimerization site. The *Cdh23Ahl* gene has been characterized as a hypomorphic allele because it may both alter adhesive functions and involve abnormal intracellular targeting due to potential misfolding of the protein product.[19]

The human gene *CDH23* includes exons and introns very similar in size and position to the mouse. Missense mutations in *CDH23* have been associated with DFNB12, whereas null alleles cause the majority of Usher 1D.[16-18] It has been postulated that genes causing single-gene diseases may also be susceptibility genes for multiple traits.[17] Genes of hereditary hearing impairment have been long considered as good candidate genes for ARHI. Except for *KCNQ4*,[10] which is possibly related to one form of autosomal dominant nonsyndromic hearing loss (DFNA2), the susceptibility genes for ARHI documented so far are not associated with monogenic hearing impairment in humans. Now, we showed that the CDH23 SNP in intron 7 is not associated with ARHI in Han Chinese.

In conclusion, this result corroborates the findings of Van Laer et al[12] on Europeans and shows that *CDH23* is not associated with ARHI not only in Europeans but also in Han Chinese and possibly also other human populations.

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Author Contributions

Juen-Haur Hwang, designed study, wrote the article; **Kris Sun Liu**, performed experiment; **Chien-Chi Wu**, consultation; **Tien-Chen Liu**, performed experiment, supervised the study.

Disclosures

Competing interests: None.

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