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RESEARCH ARTICLE

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The association of genetic polymorphisms in protocadherin 15 with sudden sensorineural hearing loss in a Chinese population

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Abstract

Background: Sudden sensorineural hearing loss (SSNHL) is a multifactorial disease, and its etiology is still unknown. SSNHL may be caused by environmental factors and genetic changes. PCDH15 is associated with susceptibility to hearing loss. The relationship between PCDH15 and SSNHL remains unknown.

Methods: In this study, the potential association between PCDH15 polymorphism and SSNHL in Chinese population was evaluated. Two single nucleotide polymorphisms PCDH15-rs7095441 and rs11004085 in 195 SSNHL patients and 182 healthy controls were determined by TaqMan technology.

Results: In Chinese population, the TT genotype and T allele of rs7095441 are associated with increased susceptibility to SSNHL. The relationships between rs7095441 and the degree of hearing loss were analyzed, and TT genotype increased the risk of hearing loss. Among SSNHL patients, patients with TT genotype of rs7095441 have an increased risk of vertigo.

Conclusion: This study found that the TT genotype of SNP rs7095441 can increase the risk of SSNHL in Chinese population.

KEYWORDS
PCDH15, Rs7095441, single nucleotide polymorphisms, sudden sensorineural hearing loss, vertigo

1 | INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) refers to sensorineural hearing loss with unknown causes within 72 h.[1] Alexander found that the incidence of sudden deafness was 27 per 100,000 people, and the incidence increased with age, with more than 66,000 new cases every year.[2] The onset age of SSNHL is 50–60 years old, and there is no gender difference. Unilateral deafness is frequent, bilateral sudden deafness accounts for about 5% of all SSNHL.[3] The pathogenesis of SSNHL is unclear. The popular theories are virus infection, microcirculation disturbance or thrombosis, immunology, round window membrane rupture, genetics, and so on. Studies have reported a relationship between hearing loss and cognitive impairment. The risk of dementia in SSNHL patients is significantly higher than that in patients without SSNHL. The possible reasons are cognitive decline caused by unwillingness to communicate, lack of physical activity, and self-social isolation after hearing loss.[4] Many neurological and psychological diseases caused by hearing loss seriously reduce the quality of life of patients. Therefore, it is urgent for clinicians to improve the cure rate of SSNHL patients quickly and effectively.

Protocadherin 15 (PCDH15) belongs to a superfamily of calcium-dependent cell adhesion proteins, which is located at

10q21.1 and contains 39 exons. PCDH15 protein encoded by PCDH15 belongs to a subfamily of cadherin, including 11 EC domains, 1 transmembrane domain, and cytoplasmic domain.[5–7] Different from classical cadherin, each EC domain of pro-cadherin is encoded by one or more exons.[8] PCDH15 initially identified the longest transcript with 33 exons encoding a protein containing 1955 amino acids.[6,7] According to different transcription and splicing, PCDH15 protein has three isotypes of cytoplasmic domains. The time and position of these isoforms expressed in hair cells are different, which shows that their roles in hair cells are different. PCDH15 is a cadherin expressed in inner ear hair cells, which can interact with cadherin-23 (CDH23) in inner ear hair cells to form end junction cilia. The end junction destruction caused by the mutation of its coding gene plays an important role in sensorineural hearing loss.

As a multifactorial disease, SSNHL may be caused by environmental factors and genetic changes. Some studies have reported the association between gene polymorphism in SSNHL patients. Chien found that GPX3 polymorphism may affect the risk of SSNHL in southern Taiwan, and GG and AG genotypes of SNP rs3805435 may be the protective genotypes of SSNHL.[9] There is no report on the relationship between PCDH15 gene polymorphism and SSNHL risk. This study investigated whether PCDH15 gene polymorphism is a risk factor for SSNHL. We conducted a case–control study to investigate the role of PCDH15 single nucleotide polymorphisms (SNPs) in the pathogenesis of SSNHL.

2 | METHODS AND MATERIALS

Genomic DNA was extracted from peripheral blood using standard methods. According to previous related studies, two SNPs were selected. These two SNPs include SNP rs7095441 and rs11004085 of PCDH15 gene.[10] The extracted DNA and genotyping assays were added to TaqMan universal PCR master mix (Roche, USA) according to the manufacturer's instructions. The reaction conditions of PCR were initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 minute, with a final extension at 72°C for 10 min. The genotyping procedures were then performed using ABI PRISM–7500 real-time PCR system (Applied Biosystems).

We recruited a total of 377 people in this case–control study, including 195 SSNHL patients and 182 healthy controls from the Third Affiliated Hospital of Guangxi Medical University. Patients with SSNHL were recruited according to the diagnostic criteria in the Clinical Practice Guideline: Sudden Hearing Loss from the American Academy of Otolaryngology–Head and Neck Surgery.[11] Healthy volunteers with no history of hearing loss or any hearing impairment were included in the control group. Auditory test for patients with SSNHL using a pure tone audiometer (Astera, GN Otometrics, Denmark). Pure tone hearing thresholds were assessed according to standard procedures at frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz. Pure tone threshold average (PTA) was ciphered from frequencies of 500, 1000, 2000, and 4000 Hz. The level of the individual subject's hearing loss was graded based on disparity levels against PTA, as normal hearing (<20 dB), mild hearing loss (20–40 dB), moderate hearing loss (40–70 dB), severe hearing loss (70–95 dB), or profound hearing loss (>95 dB). The audiograms were categorized into 4 patterns, which were low tone, high tone, flat, and total hearing loss pattern. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Guangxi Medical University (IRB number: Y2022164). All patients have signed informed consent forms.

SPSS 19.0 (SPSS) was used for statistical analysis. The chi-square test was used to evaluate the frequency of alleles and genotypes between case and control groups. Continuous variables were analyzed using independent *t*-tests, the results of which are presented as mean ± SD. The Hardy–Weinberg equilibrium (HWE) was examined in the controls by using the chi-squared test. Multivariate logistic regression analysis was performed to adjust for the effects of age, gender, vertigo, tinnitus, hypertension, and diabetes while assessing the genetic effects. $P < .05$ was considered significant.

3 | RESULTS

The characteristics of the 195 SSNHL patients and the 182 controls are shown in Table 1. The mean age of the SSNHL group was 71.9 ± 5.9 years, and the mean age of the control group was 50.7 ± 14.6 years. The SSNHL group was older than the control group ($P < .0001$). There was no significant difference between SSNHL cases and controls on the aspect of gender. Eighty-four SSNHL patients had vertigo (43.1%), and 65 SSNHL patients had tinnitus (33.3%). Regarding the degree of hearing loss, 51 (26.2%) patients had mild hearing loss; 96 (49.2%) patients had moderate hearing loss; 33 (16.9%) patients had severe hearing loss; and 15 (7.7%) patients had profound hearing loss. According to the main frequency affected, 47 (24.1%) patients had low-frequency hearing loss; 74 (37.9%) patients had high-frequency hearing loss; 61 (31.3%) patients had flat-type hearing loss; and 13 (6.7%) patients had total hearing loss configuration. 28.2% of SSNHL patients had hypertension. 21.5% of SSNHL patients had diabetes.

The associations of PCDH15 polymorphism at rs7095441 and rs11004085 in 195 patients and 182 controls were analyzed (Table 2). SNPs were genotyped in HWE ($P > .05$). The TT genotype in rs7095441 (OR = 1.841, 95%CI = 1.167–2.904, $P = .009$) and the T allele carrier in rs7095441 frequency ($P = .001$) were both significantly higher in patients than in controls. These results suggest that TT genotype and T allele of PCDH15 at rs7095441 are associated with increased susceptibility to SSNHL in the Chinese population. No significant association of rs11004085 was found with SSNHL incidence.

The TT genotype in rs7095441 (OR = 5.042, 95%CI = 1.401–18.140, $P = .013$) was significantly higher in patients with profound type than in patients with mild-type (Table 3). These results suggest that TT genotype of PCDH15 at rs7095441 is associated

TABLE 1 The characteristics of the SSNHL patients and controls.

Variables	Case group[%] (*n* = 195)	Control group[%] (*n* = 182)	*P*-value[a]
Age(years)			
18–64	77 (39.5)	104 (57.1)	.001
≥65	118 (60.5)	78 (42.9)	
Mean Age(years)	71.9 ± 5.9	50.7 ± 14.6	.001
Gender			
Male	90 (46.2)	89 (48.9)	.593
Female	105 (53.8)	93 (51.1)	
Hearing status			
Normal <20 dB	0		
Mild 20–40 dB	51 (26.2)		
Moderate 40–70 dB	96 (49.2)		
Severe 70–95 dB	33 (16.9)		
Profound>95 dB	15 (7.7)		
Audiometric pattern			
Low	47 (24.1)		
High	74 (37.9)		
Flat	61 (31.3)		
Total	13 (6.7)		
Associated risk factors			
Tinnitus	65 (33.3)		
Vertigo	84 (43.1)		
Comorbidities			
Hypertension	55 (28.2)		
Diabetes	42 (21.5)		

[a]Chi-squared test.

TABLE 2 Distribution of PCDH15 polymorphisms at rs7095441 and rs11004085 in PCDH15 cases and controls.

Allele/Genotype	Case group[%] (*n* = 195)	Control group[%] (*n* = 182)	OR (95%CI)[b]	*P*-value[a]
rs7095441				
C	166 (42.6)	200 (54.9)		
T	224 (57.4)	164 (45.1)		.001
CC + CT	39 + 88 (65.1)	59 + 82 (77.5)	1[c]	
TT	68 (34.9)	41 (22.5)	1.841 (1.167–2.904)	.009
rs11004085				
C	190 (48.7)	181 (49.7)		
T	200 (51.3)	183 (50.3)		.782
CC + CT	49 + 92 (72.3)	47 + 87 (73.6)	1[C]	
TT	54 (27.7)	48 (26.4)	0.935 (0.593–1.474)	.773

Note: Genotype/allele data were shown by *n*(%).

[a]Chi-squared test.

[b]Logistic regression.

[C]Reference group.

with the hearing status of SSNHL. The TT genotype in rs7095441 (OR = 2.966, 95%CI = 1.613–5.455, *P* = .000) was significantly higher in patients with vertigo. The TT genotype of rs7095441 was not associated with tinnitus in SSNHL patients.

4 | DISCUSSION

Although the etiology of SSNHL is unclear, it is considered to be a multifactorial disease, which may be caused by the interaction

TABLE 3 Stratification analysis of PCDH15 polymorphisms in SSNHL patients.

Variables	CC/CT	TT	OR (95%CI)[b]	P[a]
Hearing status				
Normal <20 dB	0	0		
Mild 20–40 dB	33 (26.0)	18 (26.5)	1[c]	
Moderate 40–70 dB	69 (54.3)	27 (39.7)	0.717(0.347–1.483)	.370
Severe 70–95 dB	21 (16.5)	12 (17.6)	1.048(0.421–2.609)	.920
Profound>95 dB	4 (3.1)	11 (16.2)	5.042(1.401–18.140)	.013
Associated risk factors				
Vertigo				
Positive	43 (33.9)	41 (60.3)	2.966 (1.613–5.455)	.000
Negative	84 (66.1)	27 (39.7)	1[c]	
Tinnitus				
Positive	43 (33.9)	22 (32.4)	0.934 (0.499–1.749)	.832
Negative	84 (66.1)	46 (67.6)	1[c]	
Comorbidities Hypertension				
Positive	36 (28.3)	19 (27.9)	0.980 (0.509–1.888)	.952
Negative	91 (71.7)	49 (72.1)	1[c]	
Diabetes				
Positive	27 (21.3)	15 (22.1)	1.048 (0.513–2.140)	.897
Negative	100 (78.7)	53 (77.9)	1[c]	

Note: Genotype/allele data were shown by *n*(%).

[a]Chi-squared test.

[b]Logistic regression.

[C]Reference group.

between genetic and environmental factors. A systematic review in 2019 found an association between polymorphism and SSNHL susceptibility. A total of 47 studies involving 5230 SSNHL patients and 68 genes were included to analyze and discuss the results. Polymorphisms of 26 genes, such as GPX1, SOD-1, IL-1A, ITGB3, PRKCH, TNF-α, and HSP70 were associated with susceptibility to SSNHL.[12,13] This study investigated the relationship between two SNPs of PCDH15 and SSNHL in Chinese population and found that PCDH15 SNP rs7095441 has obvious influence on the risk of SSNHL. We observed that the TT genotype of rs7095441 is associated with an increased risk of SSNHL. There was no significant difference in SNP rs11004085 between SSNHL patients and controls. We further analyzed the relationship between rs7095441 and the degree of hearing loss, and TT genotype increased the risk of hearing loss. Among SSNHL patients, patients with TT genotype of rs7095441 have an increased risk of vertigo.

Protocadherin 15 gene (PCDH15) is a member of cadherin superfamily.[14] It encodes a complete membrane protein and mediates calcium-dependent cell–cell adhesion. PCDH15 helps cells adhere together and plays an important role in maintaining normal retinal and cochlear function.[15] PCDH15 is expressed in inner ear cells, specialized cells in eyes, and photoreceptor cells in retina. The exact function of PCDH15 in retina has not been completely determined. PCDH15 interacts with proteins Cadherin 23, USH2A, and VLGR1, anchored by harmonin, SANS, or whirlin and makes synaptic membranes closely adhere to homologous or heterotypic interactions with their extracellular regions.[16] The mutation of this gene is associated with nonsyndromic hearing loss and Usher syndrome (USH1F). It is reported that a single nucleotide polymorphism rs7095441 in PCDH15 is associated with NIHL risk in the European population.[10]

Compared with mild-type, the TT genotype in rs7095441 (OR = 5.042, 95%CI = 1.401–18.140, *P* = .013) was significantly higher in patients with profound type. PCDH15 gene is a member of cadherin superfamily. A potential biological function of PCDH15 is to mediate calcium-dependent cell–cell adhesion and to maintain normal retinal and cochlear functions.[14] Mutations in this gene can lead to hearing loss and USH1F. In 2009, Koning reported that SNP rs7095441 of PCDH15 gene was related to the increased risk of NIHL in Swedish and Polish populations.[10] The gene span is close to 1 Mb, and the corresponding ORF is 7021 bp. The intron size of PCDH15 is as high as 150 kb, and the first three exons of this gene cover 0.42 Mb. The six breakpoint intervals are located in introns of 22 to 140 kb in size, which are located in the first third of the gene. Because of the large size of PCDH15, especially the coding ratio is very low, mainly at the 5′end of the gene, it is not surprising that large deletions with different breakpoints form a significant proportion of PCDH15 mutations.[17] Missense mutation of PCDH15 conserved sequence may change the spatial morphology of protein EC domain and disturb the interaction between PCDH15 and CDH23,

which destroys the integrity of apical junction, weakens the interaction between static cilia and abnormal mechanical gating channel, resulting in deafness.[14,18] Chien investigated the relationship between six SNPs of the NR3C1 gene and the outcomes of SSNHL in a Taiwanese population and found that NR3C1 has an evident genetic effect on the outcomes of SSNHL. The significant result stemmed from the rs17100289 polymorphisms of the NR3C1 gene, highlighting the role this rare TT genotype of rs17100289 has in the protection against SSNHL in Taiwan.[19]

Sudden deafness can occur at all ages. This study found that patients over 65 years old have a higher probability of SSNHL. With the increase of age, the function of body organs degenerates and the compliance of blood vessel wall decreases. With the increase of chronic diseases and microcirculation disturbance, the risk of poor prognosis of sudden deafness increases.[20] The mean age of the SSNHL group was 71.9 ± 5.9 years. But, the mean age of most SSNHL study was younger than 70 years old. Tian found that the mean age of SSNHL was 50.7 ± 16.7.[21] The age difference between SSNHL group and control group may be due to the selection bias.

Vertigo is a clinical manifestation of vestibular dysfunction. Our study found that the probability of vertigo in patients with sudden deafness was 43.1%. Analysis showed that patients with TT genotype had an increased risk of vertigo (OR = 2.966, 95%CI = 1.613–5.455, $P = .000$). The nutrient vessels of cochlea and vestibule come from labyrinthine artery, and the common cochlear artery supplying cochlea branches off vestibular cochlear artery supplying vestibule. When blood supply disorder occurs in any vessel, besides hearing loss symptoms, vertigo is often accompanied by vestibular involvement. When vestibular function is examined in patients with vertigo, vestibular organs near cochlea are often involved first, and the heavier the hearing loss, the more extensive the vestibular lesions are.[22]

There are some limitations in this study. First of all, we did not determine the expression level of PCDH15 in peripheral blood and did not analyze its function. Therefore, we cannot draw a conclusion about the influence of these polymorphisms on cytokine levels. Secondly, due to the small number of SSNHL patients participating in the study, we cannot draw strong conclusions to explain the different effects among different subgroups. Third, in this study, we did not examine other predisposing factors that may lead to SSNHL, such as hyperlipidemia. Therefore, it is necessary to conduct extensive research on various potential factors of SSNHL in order to clarify its etiology.

In conclusion, we demonstrated an association between PCDH15 gene polymorphism and susceptibility to SSNHL. Our results suggest that PCDH15 is a susceptibility gene for SSNHL in Chinese population. However, resource-intensive studies using genome-wide association, gene–gene, gene–environment, and large-scale association studies are needed to further clarify the molecular mechanism of SSNHL and the possible complex interactions between PCDH15 gene polymorphism and other susceptible factors of SSNHL. The results provide information for further understanding of the pathogenesis of SSNHL.

AUTHOR CONTRIBUTIONS

Shihua Yin designed the experiment. Ying Lan, Tao Hou, Lu Peng, and Yongpeng Li collected samples and conducted the study. Shihua Yin and Ying Lan analyzed the data. Ying Lan wrote the article. All the authors read and approved the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

All of the data used to support the findings of this study are available from the corresponding author upon request.

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