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Identifying Children With Poor Cochlear Implantation Outcomes Using Massively Parallel Sequencing

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Abstract: Cochlear implantation is currently the treatment of choice for children with severe to profound hearing impairment. However, the outcomes with cochlear implants (CIs) vary significantly among recipients. The purpose of the present study is to identify the genetic determinants of poor CI outcomes. Twelve children with poor CI outcomes (the ''cases'') and 30 ''matched controls'' with good CI outcomes were subjected to comprehensive genetic analyses using massively parallel sequencing, which targeted 129 known deafness genes. Audiological features, imaging findings, and auditory/speech performance with CIs were then correlated to the genetic diagnoses. We identified genetic variants which are associated with poor CI outcomes in 7 (58%) of the 12 cases; 4 cases had bi-allelic *PCDH15* pathogenic mutations and 3 cases were homozygous for the *DFNB59* p.G292R variant. Mutations in the *WFS1*, *GJB3*, *ESRRB*, *LRTOMT*, *MYO3A*, and *POU3F4* genes were detected in 7 (23%) of the 30 matched controls. The allele frequencies of *PCDH15* and *DFNB59* variants were significantly higher in the cases than in the matched controls (both $P < 0.001$).

In the 7 CI recipients with *PCDH15* or *DFNB59* variants, otoacoustic emissions were absent in both ears, and imaging findings were normal in all 7 implanted ears. *PCDH15* or *DFNB59* variants are associated with poor CI performance, yet children with *PCDH15* or *DFNB59* variants might show clinical features indistinguishable from those of other typical pediatric CI recipients. Accordingly, genetic examination is indicated in all CI candidates before operation.

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Abbreviations: AN = auditory neuropathy, CAP = Categories of Auditory Performance, CI = cochlear implant, HHI = hereditary hearing impairment, MPS = massively parallel sequencing, OAE = otoacoustic emission, SGN = spiral ganglion neurons, SIR = Speech Intelligibility Rating, SNHI = sensorineural hearing impairment.

INTRODUCTION

Approximately 1 in 1000 children suffer from severe to profound sensorineural hearing impairment (SNHI).[1] For these children, cochlear implantation is currently regarded as the best-hearing rehabilitation strategy. Bypassing the deafened cochlea, a cochlear implant (CI) works by directly stimulating auditory nerve fibers, transmitting signals through the central auditory neural pathway, and ultimately yielding speech understanding in the auditory cortex. The benefits of cochlear implantation for spoken language, reading skills, and cognitive development have been well demonstrated.[2] The outcomes after cochlear implantation, however, vary significantly among individuals. Many factors contribute to the outcomes, including age at implantation,[3,4] residual hearing,[5] presence of inner ear malformations,[6] presence of cochlear nerve deficiency,[7] parent−child interactions,[2] and socioeconomic status.[2]

Genetic factors play an important role in pediatric SNHI, with more than 50% of cases having a genetic etiology.[8] To date, more than 100 genes or loci with Mendelian inheritance have been related to deafness, and ~50 genes have been identified to cause nonsyndromic hereditary hearing impairment (HHI) (The Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org/).[9] It has been difficult to address the genetically heterogeneous HHI, as in practice only a limited number of genes could be screened using conventional Sanger sequencing. The advent of massively parallel sequencing (MPS), also known as next-generation sequencing, made comprehensive genetic analysis possible by enabling sequencing of an enormous volume of samples with faster turnaround and relatively low cost. The capability to test all candidate genes simultaneously has made MPS a powerful tool for genetic examination in many diseases with numerous possible causative genes, including mitochondrial diseases,[10] familial hypercholesterolemia,[11] lysosomal storage disorders,[12] neuromuscular diseases,[13] and hearing impairment.[14]

Theoretically, mutations in different deafness genes lead to different pathologies and might result in varied CI outcomes.

Good outcomes have been documented in patients with certain common deafness-associated mutations, including *GJB2* mutations,[15] *SLC26A4* mutations,[16] mitochondrial mutations,[17] and *OTOF* mutations,[18] because the effects of these mutations are confined to the inner ear and the function of the auditory nerve is spared.[19] On the other hand, mutations in genes expressed in spiral ganglion neurons (SGNs), the neurons of auditory nerves, have been hypothesized to portend poor CI performance.[20] It has been estimated that lifetime cost of cochlear implantation might exceed 1 million US dollars,[20,21] but 3% to 7% pediatric recipients might become nonusers of their implants out of various reasons.[22,23] It would be highly beneficial if these poor responders can be identified before the operation. To explore the genetic underpinnings of poor CI outcomes, we conducted comprehensive genetic analyses in children with poor CI outcomes and those with good CI outcomes, using MPS, and then correlated the genetic diagnoses to CI performance.

METHODS

Study Participants and Clinical Evaluations

Participants of the present study were selected from a cohort of ~200 children with CIs.[19] Inclusion criteria were as follows: CIs in use for more than 3 years and no previously detected mutations in common deafness genes. Exclusion criteria were as follows: syndromic hearing loss, acquired hearing loss, cochlear nerve deficiency in the implanted ears, and the presence of additional cognitive or psychological defects. All participants were Han Chinese and came from families whose native language was Mandarin. All children were unilaterally implanted with Nucleus 24 or Nucleus Freedom CIs at National Taiwan University Hospital or Chang Gung Memorial Hospital and received verbal education in mainstream schools or rehabilitation facilities after implantation. This study was approved by the Research Ethics Committees of both hospitals.

For each child, comprehensive family history, history, physical examination, audiological results, and imaging results were ascertained. The preoperative hearing level of the implanted ear was calculated as a 4-tone average (0.5, 1, 2, and 4 kHz).[19] Otoacoustic emissions (OAEs) were recorded preoperatively to assess the physiology of hair cells. Preoperative imaging results were obtained using high-resolution computed tomography and magnetic resonance imaging, and abnormalities of the inner ear and cochlear nerve were determined according to criteria in the literature.[24]

Evaluation of Auditory and Speech Performance

Auditory performance in CI recipients was evaluated using the Categories of Auditory Performance (CAP) scale,[19] which is an ordinal scale of auditory receptive ability composed of 8 categories ranging from "no awareness of environment" (CAP score = 0) to "use of telephone with known users" (CAP score = 7). Speech performance was assessed using the Speech Intelligibility Rating (SIR) scale,[25] which classifies children's spontaneous speech intelligibility into 5 categories ranging from "unintelligible speech" (SIR score = 1) to "speech intelligible to all listeners" (SIR score = 5). The CAP and SIR scales have been confirmed as reliable instruments for measuring CI outcomes.[26,27] Speech perception tests were also conducted to obtain objective recognition scores for 3 parameters: phonetically balanced word, easy sentence, and difficult sentence.[25]

The easy sentence test consisted of 15 sentences varying in length from 2 to 10 words, including 1 to 7 keywords familiar to the CI children in their daily conversations, such as "book" and "car"; whereas the difficult sentence test included 20 sentences varying in length from 2 to 12 words, containing 1 to 10 keywords of lower familiarity to the children, such as "examine" and "dormitory."[25]

In our previous study, median CAP and SIR scores after 3 years of CI use were 6 and 4, respectively.[25] We selected 12 children with CAP scores < 5 (unable to understand conversation without lip-reading) and SIR scores < 3 (speech intelligible only to listeners who concentrate and lip-read, or worse) despite more than 3 years of rehabilitation. We classified these 12 children as "cases" because of their poor CI outcomes. Thirty unrelated children with good CI performance (CAP score = 7 and SIR score = 5) were selected and matched to the cases in terms of implantation age, duration of rehabilitation, and preoperative hearing. These 30 children were the "matched controls" (Figure 1).

Massively Parallel Sequencing and Data Analyses

Genomic DNA was extracted from peripheral blood or saliva samples from the CI patients and their family members. DNA libraries were generated from fragmented genomic DNA and subjected to enrichment with custom probes targeting 129 known human deafness genes.[28] The enriched DNA was sequenced using an Illumina HiSeq 2000 system, and sequence analyses were then performed as previously described.[28] In brief, the paired-end sequence reads were aligned, sorted, and converted by BWA and Picard (http://picard.sourceforge.net) software. Variant calling was performed using GATK, and variants were annotated using ANNOVAR. Integrative Genomics Viewer was used to view the mapping and annotation of sequences. Pindel was used for detecting structural variants such as large deletions/insertions, inversions, and duplications, and ExomeDepth for detecting copy number variants.

Variants with allele frequencies above 5% in both the 1000 Genomes Project[29] and the NHLBI-ESP 6500 exome project (http://evs.gs.washington.edu/EVS/) were excluded because they are unlikely to be the genetic cause of severe single-gene diseases such as SNHI. We retained all highly possible disease-causing variants for further analyses, including frameshift and nonframeshift insertion/deletion (indel) variants, nonsense variants, and splice site variants. PolyPhen-2[30] and SIFT[31] were used to predict the deleterious effects of amino acid substitutions, and we excluded missense variants with PolyPhen-2 scores < 0.95 or SIFT scores > 0.05, except for variants that had been reported in the Deafness Variation Database (http://deafnessvariationdatabase.org/). Sanger sequencing was performed to confirm the filtered variants and examine cosegregation of the genotype and SNHI phenotype among the family members. Allele frequencies of variants segregating with the phenotype were also verified in a panel of 100 normal-hearing Han Chinese.

Analyses of Genotypes and Phenotypes

To investigate the genetic determinants of CI outcomes, the frequencies of variant alleles of specific genes were compared between the cases and matched controls. Audiological features, imaging findings, and auditory and speech performance with CIs were further analyzed in children with genetic variants associated with poor CI outcomes.
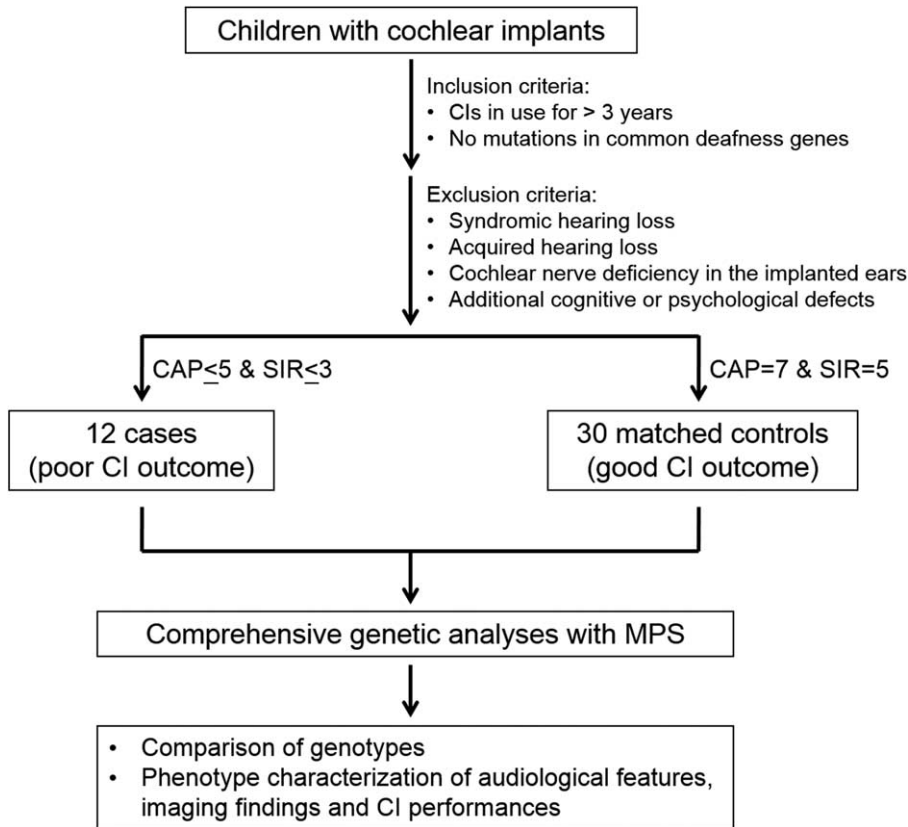
FIGURE 1. Study design. From a cohort of children with cochlear implants (CIs), *12 with poor CI outcomes* were selected as ''cases'' and 30 with good CI outcomes were selected as ''matched controls.'' These 42 children were subjected to comprehensive genetic analyses using massively parallel sequencing, which targeted 129 known deafness genes. Genotypes were then compared between ''cases'' and ''matched controls,'' and phenotypes were correlated to the genetic diagnoses. CAP, Categories of Auditory Performance; MPS, massively parallel sequencing; SIR, Speech Intelligibility Rating.

RESULTS

Demographic Characteristic

The demographic characteristics of the 12 cases and 30 matched controls are summarized in Table 1. There was no significant difference in implantation age (Student t test, $P = 0.84$), residual hearing before operation (Student t test, $P = 0.48$), rehabilitation duration (Student t test, $P = 0.25$), and sex distribution (Fisher exact test, $P = 0.31$) between the 2 groups.

Identification of Genetic Variants

Variants associated with CI outcomes were detected in 7 of the 12 cases (58%) and 7 of the 30 matched controls (23%) (Table 2). In 4 cases, a total of 6 novel *PCDH15* variants were identified (Figure 2A); and each case had 2 variant *PCDH15*

alleles which cosegregated with the SNHI phenotype in the pedigrees (Figure 2B). Of the 2 missense variants (p.G1151R and p.R1604S) which predictably resulted in amino acid changes, the pathogenicity of p.G1151R was supported by its low SIFT (0.01) and high PolyPhen-2 (1) scores, its low frequency (0.00279553) in 1000 Genomes, its absence in the 6500 NHLBI exomes and the 100 normal-hearing Han Chinese (Table 2), and the evolutionary conservation of the p.G1151 amino acid residue (Figure 2C). The pathogenicity of p.R1604S was less confirmable given its low PolyPhen-2 score (0.129) and the lower evolutionary conservation of the p.R1604 amino acid residue (Figure 2C). Nonetheless, the frequency of this variant was very low in the general population (<0.005 in both the 6500 NHLBI exomes and 1000 Genomes), so it was extremely unlikely that the hearing-impaired proband of a nonconsanguineous family segregated 2 variant alleles by

TABLE 1. Demographic Characteristics of the 12 Cases (Poor CI Outcomes) and 30 Matched Controls (Good CI Outcomes)

Variables	Cases (n = 12)	Matched Controls (n = 30)	*P*-Value
Male, n (%)	8 (66.7)	13 (43.3)	$P = 0.31$
Age at implantation, y	3.2 ± 1.2	3.3 ± 1.5	$P = 0.84$
Residual hearing, dBHL	106.8 ± 10.5	104.3 ± 9.6	$P = 0.48$
Duration of rehabilitation, y	5.1 ± 2.5	4.2 ± 1.2	$P = 0.25$

TABLE 2. Genetic Variants Identified in the 7 Cases and 7 Matched Controls

Proband	Function	Gene	Affected Transcripts (UCSC or RefSeq)	Affected Proteins	Novel Mutations	Genotype	SIFT*	Poly-Phen-2*	6500 NHLBI Exomes	1000 Genomes ALL
Cases										
DE2885	Nonsense	PCDH15	c.145G > T (uc010qht.2)	p.E49X	Yes	Heterozygous	NA	NA	0	0
DE2901	Deletion	PCDH15	c.4744delC (uc010qht.2)	p.L1582fs	Yes	Heterozygous	NA	NA	0	0
DE3033	Insertion	PCDH15	c.1863_1864dup (NM_033056)	p.S622fs	Yes	Homozygous	NA	NA	0	0
	Insertion	PCDH15	c.4320_4328dup (NM_033056)	p.P1441_P1443dup	Yes	Heterozygous	NA	NA	0	0.003
DE3321	Missense	PCDH15	c.3451G > A (NM_033056)	p.G1151R	Yes	Heterozygous	0.01	1	0	0.005
DE3039	Missense	PCDH15	c.4812G > T (NM_033056)	p.R1604S	Yes	Homozygous	0.05	0.129	0.000077	0.044
DE3133	Missense	DFNB59	c.874G > A (NM_001042702)	p.G292R	No	Homozygous	0.07	0.879	0.024145	0.044
DE3138	Missense	DFNB59	c.874G > A (NM_001042702)	p.G292R	No	Homozygous	0.07	0.879	0.024145	0.044
	Missense	DFNB59	c.874G > A (NM_001042702)	p.G292R	No	Homozygous	0.07	0.879	0.024145	
Matched controls										
DE1333	Nonsense	POU3F4	c.950T > A (uc004eeg.2)	p.L317X	Yes	Hemizygous	NA	NA	0	0
DE1381	Nonsense	WFS1	c.1453C > T (uc003giz.3)	p.Q485X	No	Heterozygous	NA	NA	0	0
DE1478	Missense	GJB3	c.250G > A (uc001bxz.4)	p.V84I	No	Heterozygous	0	0.59	0	0.002
DE1520	Missense	LRTOMT	c.154C > T (uc010rqw.2)	p.R52W	Yes	Heterozygous	0.001	0.988	0	0
	Nonsense	LRTOMT	c.655C > T (uc010rqw.2)	p.R219X	Yes	Heterozygous	NA	NA	0	0
DE2865	Missense	MYO3A	c.4462A > G (uc001isn.2)	p.K1488E	Yes	Heterozygous	0.04	0.101	0.000154	0.01
	Nonsense	MYO3A	c.4681C > T (uc001isn.2)	p.R1561X	Yes	Heterozygous	NA	NA	0.000538	0
DE2896	Missense	ESRRB	c.16A > G (uc001xsq.1)	p.R6G	Yes	Heterozygous	0	0.999	0.000308	0.005
	Missense	ESRRB	c.1144C > T (uc001xsq.1)	p.R382C	Yes	Heterozygous	0.02	0.866	0	0
DE4541	Missense	ESRRB	c.16A > G (uc001xsq.1)	p.R6G	Yes	Homozygous	0	0.999	0.000308	0.005

NA = not available.
* Missense variants with PolyPhen-2 scores close to 1 and SIFT scores close to 0 are more likely to have deleterious effects; whereas nonsense variants and insertions/deletions do not have scores.
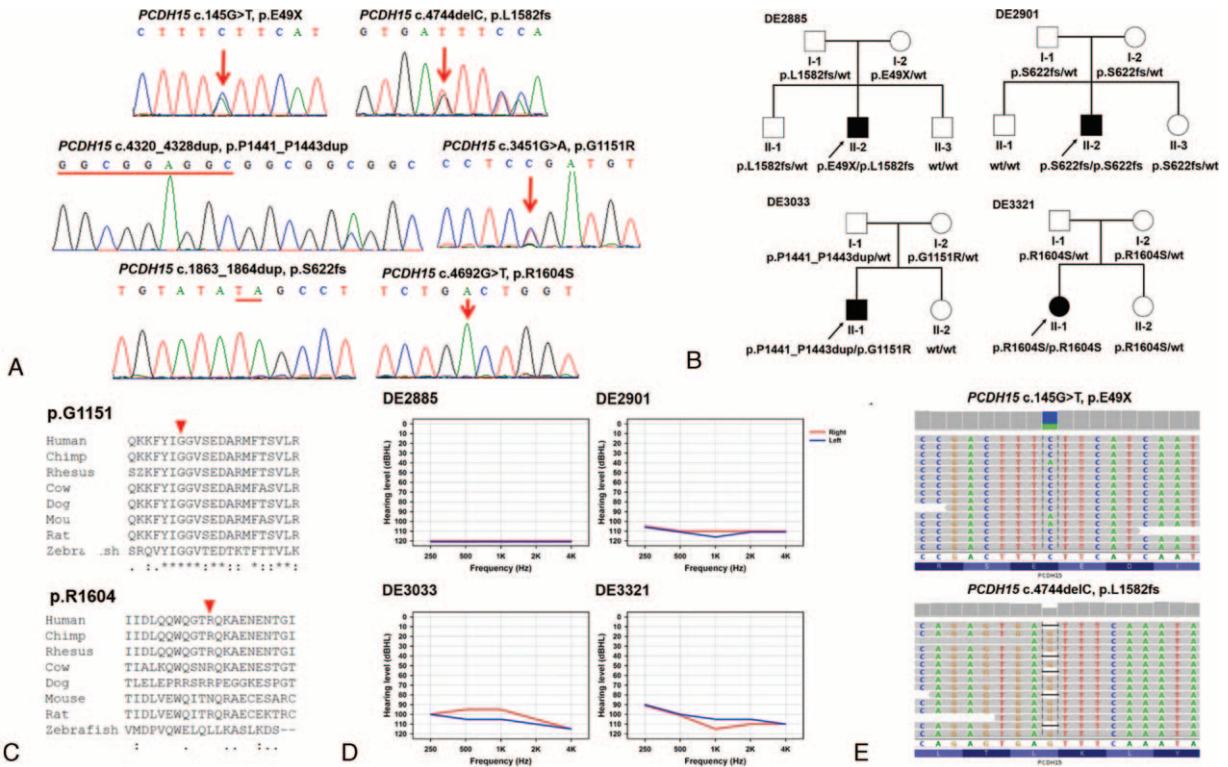
FIGURE 2. *PCDH15* mutations in 4 families with poor CI outcomes. (A) The 6 *PCDH15* mutations identified in the present study, including p.E49X, p.L1582fs, p.P1441_P1443dup, p.G1151R, p.S622fs, and p.R1604S. Sequencing data are shown on either the forward or the reverse strand, while *PCDH15* is a reverse-stranded gene. (B) Pedigrees of the 4 families, showing that bi-allelic *PCDH15* mutations cosegregated with the phenotype of hearing impairment in the family members. (C) Evolutionary conservation of the *PCDH15* p.G1151 and p.R1604 amino acid residues. Arrowhead: variant site. (D) Audiograms of the 4 CI recipients with *PCDH15* mutations. All 4 recipients had bilateral symmetric flat-type audiograms of profound severity. Hearing levels of the right ear and left ear are marked with red and blue lines, respectively. (E) Representative plots of the massively parallel sequencing results. Integrative Genomics Viewer showed a single nucleotide substitution (c.145G > T) and a single nucleotide deletion (c.4744delC) in the same patient (DE2885). Sequencing data are shown on the forward strand, while *PCDH15* is a reverse-stranded gene.

chance. Therefore, we inferred that p.R1604S was causally related to SNHI in family DE3321. The 4 CI recipients with *PCDH15* mutations revealed similar audiographic features, having bilateral symmetric flat-type audiograms of profound severity (Figure 2D). The MPS results visualized by Integrative Genomics Viewer are shown in Figure 2E.

Three cases were homozygous for the *DFNB59* p.G292R (c.874G > A) variant. This variant was previously reported as a rare nonpathogenic polymorphism.[32,33] However, the allele frequency of *DFNB59* p.G292R in the 12 cases, which were recruited from 12 unrelated families, was significantly higher than that in the 1000 Genomes (0.25 vs. 0.044, Fisher exact test, $P = 0.0005$). In addition, except the 3 cases with poor CI outcomes in the present study, homozygosity for *DFNB59* p.G292R has never been found in any of the >70 hearing-impaired Taiwanese families we performed MPS previously.[28,34] Accordingly, from a statistical perspective, *DFNB59* p.G292R might be associated with poor CI performance in the Taiwanese population.

Genetic causes of SNHI were identified in 7 matched controls. The mutations were scattered in 6 genes completely different from the 2 genes in the cases (Table 2). Two known autosomal dominant mutations, *WFS1* p.Q485X[35] and *GJB3* p.V84I,[36,37] were detected in 2 matched controls. Novel mutations in 4 other genes were detected in the remaining 5

matched controls: 2 had bi-allelic mutations in *ESRRB*, 1 had bi-allelic mutations in *LRTOMT*, 1 had bi-allelic mutations in *MYO3A*, and 1 had a hemizygous mutation in *POU3F4*.

Comparison of Variant Frequencies Between Cases and Matched Controls

The allele frequencies of the detected variants in the 12 cases and 30 matched controls are shown in Table 3. The allele frequency of *PCDH15* mutations was 33% and 1.7% in cases and matched controls, respectively, and the difference between the 2 groups was significant (Fisher exact test, $P = 0.0001$). Similarly, the allele frequency of the *DFNB59* p.G292R variant was significantly higher in cases than in matched controls (25% vs. 0%, Fisher exact test, $P = 0.0003$). In other words, variants in *PCDH15* and *DFNB59* were significantly associated with poor CI outcomes. In contrast, although mutations in certain genes occurred more frequently in matched controls than in cases, the difference in mutation frequency between the 2 groups was not statistically significant.

Clinical Features and CI Outcomes in Patients With *PCDH15* or *DFNB59* Variants

The phenotypes of the 7 CI recipients with *PCDH15* or *DFNB59* variants are summarized in Table 4. None of the 7

TABLE 3. Comparison of Variant Frequencies Between Cases and Matched Controls

Genes/Alleles	Variant Allele no. (%) in Cases[*]	Variant Allele no. (%) in Matched Controls[†]	P-Value[‡]
PCDH15			
p.E49X	1 (4.2)	0 (0)	
p.L1582fs	1 (4.2)	0 (0)	
p.S622fs	2 (8.3)	0 (0)	
p.P1441_P1443dup	1 (4.2)	0 (0)	
p.G1151R	1 (4.2)	0 (0)	
p.R1604S	2 (8.3)	1 (1.7)	
Total	8 (33)	1 (1.7)	$P = 0.0001$
DFNB59			
p.G292R	6 (25)	0 (0)	$P = 0.0003$
POU3F4			
p.L317X	0 (0)	1 (1.7)	$P = 1$
WFS1			
p.Q485X	0 (0)	1 (1.7)	$P = 1$
GJB3			
p.V84I	0 (0)	1 (1.7)	$P = 1$
LRTOMT			
p.R52W	0 (0)	1 (1.7)	
p.R219X	0 (0)	1 (1.7)	
Total	0 (0)	2 (3.3)	$P = 1$
MYO3A			
p.K1488E	0 (0)	1 (1.7)	
p.R1561X	0 (0)	1 (1.7)	
Total	0 (0)	2 (3.3)	$P = 1$
ESRRB			
p.R6G	0 (0)	3 (5.0)	
p.R382C	0 (0)	1 (1.7)	
Total	0 (0)	4 (6.7)	$P = 0.32$

[*] Total 24 alleles in the 12 cases.
[†] Total 60 alleles in the 30 matched controls.
[‡] Fisher exact test.

recipients was diagnosed as having auditory neuropathy (AN) before cochlear implantation, as OAEs were absent in both ears of these patients. Cochlear nerve hypoplasia was identified in the nonimplanted ear of 1 patient (DE3039) homozygous for the DFNB59 p.G292R variant, whereas imaging findings were normal in the other 6 patients. Speech perception scores were documented in 6 patients. One patient (DE3138) homozygous for DFNB59 p.G292R showed moderate speech perception scores, while the other 5 patients revealed poor scores in all 3 parameters despite more than 3 years of rehabilitation.

DISCUSSION

In the present study, we identified genetic determinants in 7 of the 12 cases with poor CI outcomes: 4 cases had bi-allelic PCDH15 mutations and 3 were homozygous for the DFNB59 p.G292R variant. The allele frequencies of PCDH15 and DFNB59 variants were significantly higher in the 12 cases than in the 30 matched controls with good CI outcomes, indicating that variants in these 2 genes were specifically associated with poor CI performance. Moreover, children with PCDH15 or DFNB59 variants revealed audiological and imaging results indistinguishable from those of other typical CI recipients with cochlear SNHI. These findings have important clinical implications. Genetic testing to identify SNHI patients likely to have poor CI outcomes could help in counseling patients

preoperatively. For these patients, the genetic information is critical for determining appropriate rehabilitation programs and setting the expectations of physicians, audiologists, schools, and families, as these patients might need more aggressive aural rehabilitation postimplantation.

Prior to the present study, CI outcomes had never been reported in patients with bi-allelic PCDH15 or DFNB59 mutations. PCDH15 encodes protocadherin-15, and PCDH15 mutations have been related to Usher syndrome type 1F and nonsyndromic deafness DFNB23.[38] Besides being a structural protein at the tip links of stereocilia,[39] protocadherin-15 is also expressed in hair cell synapses and SGNs, suggesting its role in synaptic maturation.[40] Mice with defective Pcdh15 gene showed a reduction in the number of SGNs in addition to disordered arrangement of stereocilia in hair cells.[41] It has been documented that CI performance is satisfactory in most patients with type 1 Usher syndrome,[42] but in a patient with digenic CDH23 and PCDH15 mutations, there was no development of open-set word recognition at 2 years after implantation.[43] Therefore, pathogenetic mechanisms of PCDH15 mutations might differ from other Usher syndrome-related genetic mutations in involving auditory nerves additionally, thus contributing to poor CI performance.

The DFNB59 gene encodes pejvakin, which is expressed in hair cells, SGNs, and brainstem auditory nuclei in mammals.[44,45] Corresponding to the histological distributions,

TABLE 4. Clinical Features and Outcomes in the CI Recipients With *PCDH15* or *DFNB59* Variants

Patient No.	Sex	Genotype	Age at Implantation, y	Preoperative Hearing, dBHL*	OAEs Results†	Preoperative Imaging Findings	Duration of Rehabilitation, y	CAP Score	SIR Score	PB Word	Easy Sentence	Difficult Sentence
											Speech Perception Scores	
DE2885	M	*PCDH15* p.E49X/p.L1582fs	2.2	118.3	Bil (−)	Normal	4	4	2	NA	NA	NA
DE2901	M	*PCDH15* p.S622fs/p.S622fs	1.9	110	Bil (−)	Normal	3	5	2	40	32	10
DE3033	M	*PCDH15* p.P1441_P1443dup/ p.G1151R	4.7	103.3	Bil (−)	Normal	4	4	2	32	0	0
DE3321	F	*PCDH15* p.R1604S/p.R1604S	2.6	120	Bil (−)	Normal	10	5	3	20	0	0
DE3039	F	*DFNB59* p.G292R/p.G292R	2.7	120	Bil (−)	Left CN hypoplasia with mild NIAC	4	5	2	12	0	2
DE3133	M	*DFNB59* p.G292R/p.G292R	5.1	118.3	Bil (−)	Normal	4	5	3	32	28	16
DE3138	M	*DFNB59* p.G292R/p.G292R	2.6	98.3	Bil (−)	Normal	6	5	3	76	78	60

CAP = Categories of Auditory Performance, CN = cochlear nerve, NA = not available, NIAC = narrow internal auditory canal, OAEs = otoacoustic emissions, PB = phonetically balanced, SIR = Speech Intelligibility Rating.
* Last measurement in the implanted ears before cochlear implantation.
† Last measurement in bilateral ears before cochlear implantation.

mutations in *DFNB59* have been described in families with either AN or cochlear SNHI.[32,33,44–46] Because SGNs and brainstem auditory nuclei are affected, it is conceivable that patients with *DFNB59* variants may demonstrate poor performance with CIs. The *DNFB59* p.G292R variant is of particular interest. Although this variant was reported in deafness individuals of several pedigrees, it was also found in control individuals with a relatively high allele frequency in 1000 genomes (0.044). Therefore, it was considered to be a benign polymorphism rather than a pathogenic mutation.[32,33] However, to exclude the pathogenecity/susceptibility of an autosomal recessive locus simply by allele frequency might be too arbitrary. Current genetic data from the literature could be consistent with the hypothesis that *DNFB59* p.G292R is a pathogenic allele with incomplete penetrance. Another possible explanation is that the *DFNB59* p.G292R variant is not a causative mutation for deafness, but it is in linkage disequilibrium with an unknown pathogenic mutation which is associated with poor CI outcomes in the Taiwanese population. Under this assumption, although we did not identify the true causative mutation in these 3 patients, the *DFNB59* p.G292R variant still might serve as a predictor for CI performance. Further studies are warranted to confirm the association between *DFNB59* variants and poor CI outcomes.

In the present study, deleterious variants of the *WFS1*, *GJB3*, *ESRRB*, *LRTOMT*, *MYO3A*, and *POU3F4* genes were detected in children with good CI outcomes. Good CI performance has also been reported in children with mutations in a number of nonsyndromic HHI genes, including *GJB2*,[15] *SLC26A4*,[16] *OTOF*,[18] *TMC1*,[47] *COCH*,[48] and *LOXHD1*.[49] By performing MPS of 58 deafness genes in 8 patients with CI or electrical acoustic stimulation, Miyagawa et al[50] determined that patients with mutations in the *MYO15A*, *TECTA*, and *ACTG1* genes also showed relatively good auditory performance after operation. Good CI performance has been attributed to the fact that the expression and pathology of these genes are confined to the cochlea.[19,20,50] Among the 6 genes related to good CI outcomes in the present study, *LRTOMT*,[51] *MYO3A*,[52] and *POU3F4*[53] are expressed only in the cochlea; *GJB3* is expressed in the cochlea and auditory nerves[54]; and *WFS1*[55] and *ESRRB*[56] are expressed in the cochlea and SGNs. Favorable CI outcomes have been documented in patients with *GJB3*[37] and *WFS1*[57] mutations, whereas mutations in *POU3F4* have been associated with varied CI outcomes, possibly confounded by additional cognitive or behavioral issues.[58,59]

Taken together, our results indicate that mutations in genes confined to the cochlea are associated with good CI outcomes in the absence of other cognitive or psychological problems, whereas mutations in genes expressed in SGNs and/or brainstem auditory nuclei might be associated with poor CI outcomes. Eppsteiner et al[20] conducted comprehensive genetic screening of 29 adult CI recipients with idiopathic adult-onset severe-to-profound hearing loss and identified bi-allelic *TMPRSS3* mutations in 2 of 6 patients with poor performance. Because *TMPRSS3* is expressed in SGNs, the authors proposed that mutations in genes expressed in SGNs might portend poor CI performance. Of note, benefits from cochlear implantation were reported in patients with *TMPRSS3* mutations in 3 other studies.[50,60,61] As shown in the present study, although most children with *PCDH15* or *DFNB59* variants had poor speech perception scores, all 7 children achieved CAP scores of 4 to 5 and SIR scores of 2 to 3. Moreover, good CI outcomes were observed in children with mutations in *GJB3*, *WFS1*, and *ESRRB*. In other words, among the genes expressed in SGNs

and/or brainstem auditory nuclei, mutations in certain genes, such as *PCDH15*, *DFNB59*, and *TMPRSS3*, appear to result in more severe pathologies in the auditory neural pathway, thus compromising the utility of CIs. However, the physiology of the auditory neural pathway is not completely abolished, as patients with mutations in *PCDH15*, *DFNB59*, and *TMPRSS3* still gained some benefits from CIs.

Pathologies in SGNs and/or brainstem auditory nuclei clinically might be featured by AN.[62] Cochlear implantation in patients with AN requires special consideration because the outcome is more unpredictable than in patients with cochlear SNHI.[63–65] Despite the expression of *PCDH15* and *DFNB59* in SGNs and/or brainstem auditory nuclei, none of the 7 cases with mutations was diagnosed as having AN before cochlear implantation. In other words, it might be difficult to distinguish patients with *PCDH15* or *DFNB59* mutations from typical pediatric CI candidates based on preoperative audiological evaluations. From this standpoint, genetic diagnosis can be invaluable in elucidating the etiologies and predicting CI outcomes before implantation.

The interpretation and extrapolation of the results in the present study, however, should be done with caution. First, although this study represents the largest series to date of complete MPS analyses in children with long-term CI use, the participants are of a single ethnic background; hence, multicenter studies across populations might be necessary to validate our observations. Second, all 129 genes screened by our MPS panel are known deafness genes; thus, in families without detected mutations, particularly those with multiple affected members, SNHI might be attributed to mutations in unknown deafness genes. For these individuals, whole-exome sequencing might help elucidate the underlying pathophysiology. Third, it has been demonstrated that the optimal time to implant a young deaf child is within age 3.5 years in childhood, and is best by the first 2 years of life.[66] The average implantation age in the 12 cases with poor outcomes was 3.2 years, which is close to the upper limit of the optimal time period and already beyond the best time to implant a child. A possible explanation for the late implantation is that the coverage rate of newborn hearing screening in Taiwan had not increased to 90% until 2012,[67] resulting in delayed diagnosis in certain hearing-impaired children. Although we used a ''matched controls'' study design to control for the confounding effects of the implantation age on the CI outcomes, we could not exclude the possibility that poor performers in the present study might have done better if they were implanted earlier in life.

CONCLUSION

We demonstrated *PCDH15* and *DFNB59* variants were associated with poor CI performance in hearing-impaired children, probably attributable to pathology in SGNs and/or brainstem auditory nuclei. Because children with *PCDH15* or *DFNB59* variants might have clinical features indistinguishable from those of other typical pediatric CI candidates, comprehensive genetic examination might be indicated in all CI candidates before operation.

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