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 pathogenic mutations and 3 cases were homozygous for the DFNB59 variants. In the 7 CI recipients 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.

INTRODUCTION

Approximately 1 in 1000 children suffer from severe to profound sensorineural hearing impairment (SNHI). 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. The outcomes after cochlear implantation, however, vary significantly among individuals. Many factors contribute to the outcomes, including age at implantation, residual hearing, presence of inner ear malformations, and socioeconomic status.

Genetic factors play an important role in pediatric SNHI, with more than 50% of cases having a genetic etiology. 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/). 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, familial hypercholesterolemia, lysosomal storage disorders, neuromuscular diseases, and hearing impairment.

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, 13 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, 15 which is an ordinal scale of auditory receptive ability composed of 8 categories ranging from “no awareness of environment” (CAP score ¼0) to “use of telephone with known user” (CAP score ¼7). 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 ¼0) to “speech intelligible to all listeners” (SIR score ¼5). 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 matching 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 20 and the NHBLI-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 20 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.
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 two 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

![Children with cochlear implants

Inclusion criteria:
- CIs in use for > 3 years
- No mutations in common deafness genes

Exclusion criteria:
- Syndromic hearing loss
- Acquired hearing loss
- Cochlear nerve deficiency in the implanted ears
- Additional cognitive or psychological defects

12 cases (poor CI outcome) 30 matched controls (good CI outcome)

Comprehensive genetic analyses with MPS

- Comparison of genotypes
- Phenotype characterization of audiological features, imaging findings and CI performances

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.

| 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                | $P = 0.25$|
| Proband | Function | Gene   | Affected Transcripts (UCSC or RefSeq) | Affected Proteins | Novel Mutations | Genotype | SIFT ¹ | Poly-Phen-2 ² | 6500 NHLBI Exomes | 1000 Genomes ALL |
|---------|----------|--------|--------------------------------------|------------------|-----------------|----------|--------|--------------|-----------------|-----------------|
| Cases   |          |        |                                      |                  |                 |          |        |              |                 |                 |
| DE2885  | Nonsense | PCDH15 | c.145G>T (uc010qht.2)                | p.E49X           | Yes             | Heterozygous | NA     | NA           | 0               | 0               |
|         | Deletion | PCDH15 | c.4744delC (uc010qht.2)              | p.L1582fs        | Yes             | Heterozygous | NA     | NA           | 0               | 0               |
| DE2901  | Insertion| PCDH15 | c.1863_1864dup (NM_033056)           | p.S622fs         | Yes             | Homozygous   | NA     | NA           | 0               | 0               |
| DE3033  | Insertion| PCDH15 | c.4320_4328dup (NM_033056)           | p.P1441_P1443dup | Yes             | Heterozygous | NA     | NA           | 0               | 0               |
|         | Missense | PCDH15 | c.3451G>A (NM_033056)                | p.G1151R         | Yes             | Homozygous   | 0.01   | 1            | 0               | 0.003            |
| DE3321  | Missense | PCDH15 | c.4812G>T (NM_033056)                | p.R1604S         | Yes             | Homozygous   | 0.05   | 0.129        | 0.000077        | 0.005            |
| DE3039  | Missense | DFNB59 | c.874G>A (NM_001042702)              | p.G292R          | No               | Homozygous   | 0.07   | 0.879        | 0.024145        | 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            |
| 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.V841           | No               | Homozygous  | 0      | 0.59         | 0               | 0.002            |
| DE1520  | Missense | LRTOMT | c.154C>T (uc010qww.2)                | p.R52W           | Yes             | Heterozygous | 0.001  | 0.988        | 0               | 0               |
|         | Missense | LRTOMT | c.655C>T (uc010qww.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             |
|         | Missense | MYO3A  | c.4681C>T (uc001isn.2)               | p.R1561X         | Yes             | Heterozygous | NA     | NA           | 0               | 0.000538         |
| 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.

¹ 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.
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. 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. 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 and GJB3 p.V841fs, 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
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, protocadherin-15 is also expressed in hair cell synapses and SGNs, suggesting its role in synaptic maturation. Mice with defective Pcdh15 gene showed a reduction in the number of SGNs in addition to disordered arrangement of stereocilia.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, the genetic distributions were significantly different between the 12 cases and the 30 matched controls.

### 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.S62fs       | 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   |
| WFSI          |                               |                                             |         |
| 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 = 1   |
| 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 = 0.32|
| p.R382C       | 0 (0)                         | 1 (1.7)                                     |         |
| Total         | 0 (0)                         | 4 (6.7)                                     |         |

*Total 24 alleles in the 12 cases.
†Total 60 alleles in the 30 matched controls.
‡Fisher exact test.
Clinical Features and Outcomes in the CI Recipients With DFNB59

Speech Perception Scores

Preoperative Age at Rehabilitation, y

Duration of Preoperative Imaging Findings, y

Preoperative Hearing, dHBHL

Age at Implantation, y

Speech Intelligibility Rating.

Conductive hearing loss has also been reported in children with mutations in a gene, PCDH15, which may lead to good CI outcomes. Good CI performance has been attributed to the hypothesis that DFNB59 deafness is associated with varied CI outcomes, possibly confounded by electrical acoustic stimulation, Miyagawa et al.50 determined that patients with mutations in the DFNB59 gene 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, PCDH15, DFNB59, 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 HI genes, including GJB2,15 SLC26A4,16 OTOF,17 TMC1,18 COCH,19 and LOXHD1.49 By performing MPS of 58 deafness genes in 8 patients with CI or cochlear nerve, NA = not available, NIC = narrow internal auditory canal, OAEs = otocoustic emissions, PB = phonetically balanced.

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

| Patient No. | Sex | Genotype | Age at Implantation, y | Speech Intelligibility Rating | CAP | SIR |
|-------------|-----|-----------|------------------------|-------------------------------|-----|-----|
| DE2885      | M   | PCDH15   | 2.2                    | 118.3                         | 5   | 2   |
| DE2871      | M   | PCDH15   | 1.9                    | 110                           | 4   | 2   |
| DE1033      | M   | PCDH15   | 4.7                    | 103.3                         | 10  | 5   |
| DE3121      | F   | PCDH15   | 2.6                    | 120                           | 4   | 2   |
| DE3036      | F   | GJB3     | 2.0                    | 120                           | 4   | 2   |
| DE3134      | M   | DFNB59   | 3.0                    | 120                           | 4   | 2   |
| DE3133      | M   | DFNB59   | 3.0                    | 120                           | 4   | 2   |
| DE3138      | M   | DFNB59   | 2.4                    | 120                           | 4   | 2   |

CAP = Categories of Auditory Performance; CN = cochlear nerve, NA = not available, NIC = narrow internal auditory canal, OAEs = otocoustic emissions, PB = phonetically balanced.

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.50 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 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 DFNB59 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 pathogenicity/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 DFNB59 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 HI genes, including GJB2,15 SLC26A4,16 OTOF,17 TMC1,18 COCH,19 and LOXHD1.49 By performing MPS of 58 deafness genes in 8 patients with CI or cochlear nerve, NA = not available, NIC = narrow internal auditory canal, OAEs = otocoustic emissions, PB = phonetically balanced.

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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.56 Cochlear implantation in patients with AN requires special consideration because the outcome is more unpredictable than in patients with cochlear SNHL.63–65 Despite the expression of PCDH15 and DFNB59 in SGNs and/or brainstem auditory nuclei, none of the 7 cases with SNHI.63–65 mutations in these genes, particularly those with multiple affected members, SNHI might be attributed to mutations in unknown deafness genes. For these individuals, whole-exome sequencing might be indicated in all 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 1.5 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 mutations 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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