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A Comprehensive Study on the Etiology of Patients Receiving Cochlear Implantation With Special Emphasis on Genetic Epidemiology

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Objective: Cochlear implantation is the most important treatment currently available for profound sensorineural hearing loss. The aim of this study was to investigate the etiology of hearing loss in patients with cochlear implantation, and to compare outcomes.

Methods: Japanese hearing loss patients who received cochlear implants (CIs) or electric acoustic stimulation (EAS) in Shinshu University hospital (n=173, prelingual onset: 92, postlingual onset: 81) participated in this study. Invader assay followed by the targeted exon-sequencing of 63 deafness genes using Massively parallel DNA sequencing (MPS) was applied. For prelingual patients, additional imaging examination, cCMV screening, and pediatric examination were performed for precise diagnosis.

Results: Genetic screening successfully identified the causative mutation in 60% of patients with prelingual onset hearing loss and in 36% of those with postlingual hearing loss. Differences in the kinds of genes identified were observed between the two groups. Although there were marked variations in the outcome

of cochlear implantation, patients with specific deafness gene mutations showed relatively good results.

Conclusion: The present study showed genetic etiology is a major cause of hearing loss in CI/EAS patients. Patients possessing mutations in a number of deafness genes known to be expressed within inner ear have achieved satisfactory auditory performance, suggesting that the identification of the genetic background facilitates the prediction of post-CI performance. MPS is a powerful tool for the identification of causative deafness genes in patients receiving cochlear implantation. Therefore, determination of the involved region inside/outside of the cochlea by identification of the responsible gene is essential. **Key Words:** *ACTG1—CDH23—COCH—Cochlear implantation—CRYM—DFNA5—DFNB31—Etiology—GJB2—LOXHD1—MYO7A—MYO6—MYO15A—Next-generation sequencing—OTOF—SLC26A4—TMPRSS3.*

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Genetic factors, the most common etiology in severe-to-profound hearing loss, are one of the key determinants of cochlear implantation (CI) and electric acoustic stimulation (EAS) outcomes. If the genetic background involves an “intracochlear” etiology, there is potential for good outcomes (1). Therefore, it is important to identify the involved region inside/outside the cochlea by identifying the responsible gene. Our recent series of studies on satisfactory auditory performance after receiving CI/EAS in patients with specific deafness gene mutations indicates that genetic

testing would be helpful in predicting CI/EAS outcomes, as well as in deciding treatment choices (2–8). However, because of the extreme genetic heterogeneity of deafness, the clinical application of genetic information continues to entail a number of difficulties. More than 80 genes have already been reported to be associated with nonsyndromic hereditary hearing loss. Recent advances in targeted exon-sequencing of selected genes using massively parallel DNA sequencing (MPS) technology have enabled the successful identification of the causative mutations in relatively rare genes (9). In the present study, to obtain a more complete picture, MPS was applied to a large cohort of CI/EAS patients to 1) clarify their genetic background and 2) compare their etiology and outcomes.

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SUBJECTS AND METHODS

Subjects

A total of 173 consecutive patients who received CI or EAS at Shinshu University Hospital were enrolled in this study.

Among them, hearing loss was prelingual onset in 92 and postlingual onset in 81 patients (male: 92, female: 81).

Of the 92 prelingual patients, hearing loss was congenital onset in 81 (62 were identified during newborn hearing screening), because of meningitis in 3, progressive because of a *CDH23* mutation in 2, and caused by cCMV infection in 3 patients. Age at surgery ranged from 8 months to 58 years (mean = 63.3 mo, median = 31 mo).

For the 81 postlingual patients, the onset age ranged from 7 to 78, and age at surgery ranged from 25 to 89 years.

Written informed consent was obtained from the subjects (or from their next of kin, caretaker, or guardian in the case of minors/children) before enrollment.

This study was approved by the Shinshu University Ethical Committee.

Genetic Screening

Two-step screening (Invader assay followed by MPS analysis) was applied for all patients. In Japan, the cost (approximately \$US320) of genetic testing for deafness using this two-step screening is currently fully covered by social health insurance.

Invader Assay

First, we screened for 46 known mutations in 13 known deafness genes using the Invader assay, which was followed by direct sequencing as necessary (10). At least one deafness gene mutation was found in 29.5% of the subjects (10). This method of simultaneous screening for multiple deafness mutations using the Invader assay, and then direct sequencing where necessary, enables us to detect deafness mutations in an efficient and practical manner. In Japan, genetic testing for deafness using the Invader assay has been covered by social health insurance since 2012.

MPS Analysis

The detailed methodological protocol was described elsewhere (9).

Amplicon Library Preparation

Amplicon libraries were prepared according to the manufacturer's instructions using an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies, Carlsbad, CA) for 63 genes that reportedly cause nonsyndromic hearing loss. The detailed protocol is described elsewhere (2). The amplicon libraries were diluted to 20 pM and equal amounts in six libraries for six patients were pooled for one sequence reaction.

Emulsion PCR and Sequencing

Emulsion PCR and sequencing were performed according to the manufacturer's instructions. The detailed protocol is described elsewhere (2). MPS was performed with an Ion Torrent Personal Genome Machine (PGM) using an Ion PGM 200 Sequencing Kit and an Ion 318 Chip (Life Technologies).

Base Call and Data Analysis

Sequence data were mapped against the human genome sequence (build GRCh37/hg19) with the Torrent Mapping Alignment Program. After sequence mapping, variant regions were piled up with Torrent Variant Caller plug-in software. After variant detection, effects were analyzed using ANNOVAR software (11,12). Missense, nonsense, insertion/deletion, and splicing variants were then selected from the identified

variants. Variants were further selected if they were less than 1% of 1) the 1,000 Genome database (<http://www.1000genomes.org/>) (13), 2) the 6,500 exome variants in the Exome Variant Server (<http://evs.gs.washington.edu/EVS/>) (14), 3) the dataset of 1,208 Japanese exome variants in the Human Genetic Variation Database (<http://www.genome.med.kyoto-u.ac.jp/SnpDB/index.html>) (15), and 4) 269 in-house Japanese normal hearing controls.

To predict the pathogenicity of missense variants, the following functional prediction software was used: PhyloP (16), Sorting Intolerant from Tolerant (SIFT) (17), Polymorphism Phenotyping (PolyPhen2) (18), LRT (19), MutationTaster (20), and GERP++ (21).

Candidate mutations were confirmed by Sanger sequencing and the responsible mutations were identified by segregation analysis using samples from the patients' family members.

CT Imaging and Pediatric Consultation

All patients underwent examination by computed tomography at a slice thickness of 1 mm through the temporal bone to check for the presence of cochlear, vestibular, or inner ear canal malformation. Children with associated symptoms who were suspected of a syndromic disease underwent pediatric consultation and a diagnosis of the coexisting syndrome was made.

Diagnostic Testing for Congenital Cytomegalovirus Infection

For prelingual patients in whom no genetic mutation was detected, examination for congenital cytomegalovirus (cCMV) infection was performed using CMV-DNA quantitative PCR (qPCR) analysis. Before qPCR analysis, total DNA including genomic DNA and CMV DNA was extracted from preserved dried umbilical cords. As a positive control, we used preserved umbilical cords from patients with symptomatic congenital CMV infection. As a negative control, preserved umbilical cords from five healthy children without sensorineural hearing loss were used. Detailed methods are described elsewhere (22).

Outcome of CI

The implant was stimulated for the first time at least 3 to 4 weeks after the operation, and the evaluation of auditory and speech perception skills included the measurement of the aided free-field thresholds for adult patients.

In the prelingual patients, a LittlEARS auditory questionnaire (an assessment of early auditory development in young children) (23,24) was completed by the parents and audiologists. LittlEARS consists of 35 questions, each scored as 1 = yes, or 0 = no. For the postlingual patients, the Japanese monosyllable perception test (67-S test) and word perception test were applied. Assessment was performed at preoperation, and at 3, 6, and 12 months after implantation. We then compared the differences in outcomes for cochlear implantation between the various etiologies, and the distribution patterns of the LittlEARS auditory questionnaire scores were analyzed.

For postlingual patients, the distribution patterns of the Japanese monosyllable and word perception test results were examined and the statistical differences between the group with specific gene mutations and the other etiology group analyzed using Student's *t* test. We further divided all patients into two groups (the good outcome group and the moderate-poor outcome group) with the borderline set at 40% for the Japanese monosyllable perception test and at 60% for Japanese word perception test. Various factors, including age, sex, hearing loss threshold, and etiology, were compared.

RESULTS

Causes of Hearing Loss in Prelingual CI Patients

Of the 92 patients, causative mutations in deafness genes were identified for 55 patients in 49 families (59.8%). Five patients (5%) were diagnosed with cCMV infection on the basis of viral DNA diagnostic testing using dried umbilical cord samples. One patient was diagnosed with congenital rubella syndrome. CT imaging identified anomalies in five patients, including three patients with inner ear malformations (IP-2) and two patients with stenosis of the internal auditory canal. Nine patients (9.8%) were diagnosed with syndromic hearing loss through pediatric consultation, including three with Waardenburg syndrome, two with Usher syndrome (one with *CDH23* biallelic mutations, and one with *PCDH15* biallelic mutations), one with Down syndrome, one with Noonan syndrome, one with CHARGE syndrome, and one with Jervell and Lange-Nielsen syndrome (with *KCNQ1* biallelic mutations). The etiologies of the prelingual CI patients in this study are summarized in Fig. 1A.

With regard to the mode of inheritance, all 49 families were compatible with autosomal recessive inheritance. Segregation analysis as well as prediction software indicated that the identified mutations were compatible with being the responsible gene. The most frequent causative gene was *GJB2*, and c.235delC was found to be the most frequent mutation. The second most frequent genes were *SLC26A4* and *CDH23* (8% respectively). Genetic screening by using MPS identified causative mutations in many rare genes, such as *OTOF*, *MYO15A*, *LOXHD1*, and *PCDH15*.

As *CDH23* heterozygous mutations were identified in two patients, these patients were categorized as unknown etiology.

Clinical Findings and Outcomes for Prelingual CI Patients

Of the 92 prelingual patients, 62 were detected by newborn hearing screening, so that the majority of the patients received CI before 2 years of age. Twenty-one patients, however, received CI passed school age (6 y.o.) because of progressive of hearing loss or a problem with timing, and therefore spent a considerable time with impaired hearing.

Eighty-two out of 92 patients had congenital profound hearing loss, and five patients showed late-onset progressive hearing loss at around the age of 2. Most of the patients with *SLC26A4*, *LOXHD1*, and *MYO15A* mutations showed progressive hearing loss. In contrast, the hearing loss in patients with *GJB2* and *OTOF* mutations was not progressive in nature.

As for the outcomes of CI, in 23 of the 92 pre-lingual patients, early auditory development was assessed using the LittlEARS auditory questionnaire before the operation and at 3, 6, and 12 months after CI. Although scores varied among the patients, the majority of patients with non-syndromic hearing loss with specific deafness gene mutations showed good and rapid development of hearing behavior (Fig. 2). Some of the patients, who had already achieved good behavior using hearing aids, showed high performance preoperation (Fig. 2). In contrast, syndromic hearing loss patients as well as the patients with inner ear anomalies showed comparatively poorer and slower development (Fig. 2). The patients with unknown etiology but without any syndrome or malformation showed relatively good outcomes (Fig. 2). In addition, the cCMV patients, who had already good scores, maintained their high scores (Fig. 2). The distribution patterns of the LittlEARs auditory questionnaire scores are shown in Fig. 3. No statistically significant differences were observed between the group with

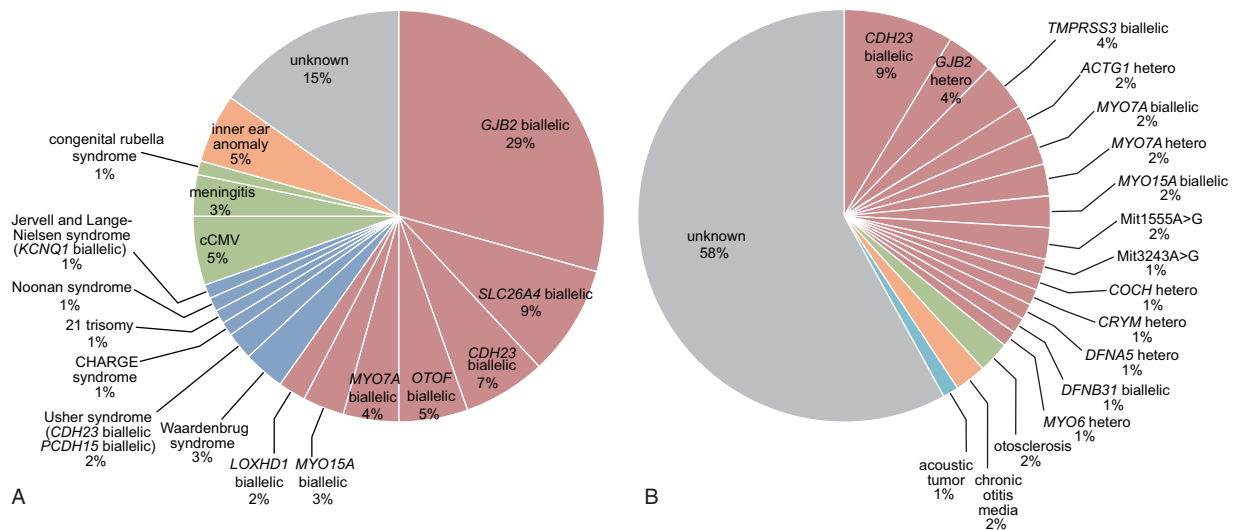


FIG. 1. Etiology of CI/EAS patients. (A) Prelingual CI/EAS patients ($n = 88$). Blue indicates syndromic hearing loss; gray, unknown; green, infection-induced hearing loss; orange, inner ear anomaly; pink, nonsyndromic hearing loss associated with specific gene mutations. (B) Postlingual CI/EAS patients ($n = 77$). Blue indicates acoustic tumor; gray, unknown; green, otosclerosis; orange, otitis media; pink, nonsyndromic hearing loss associated with specific gene mutations.

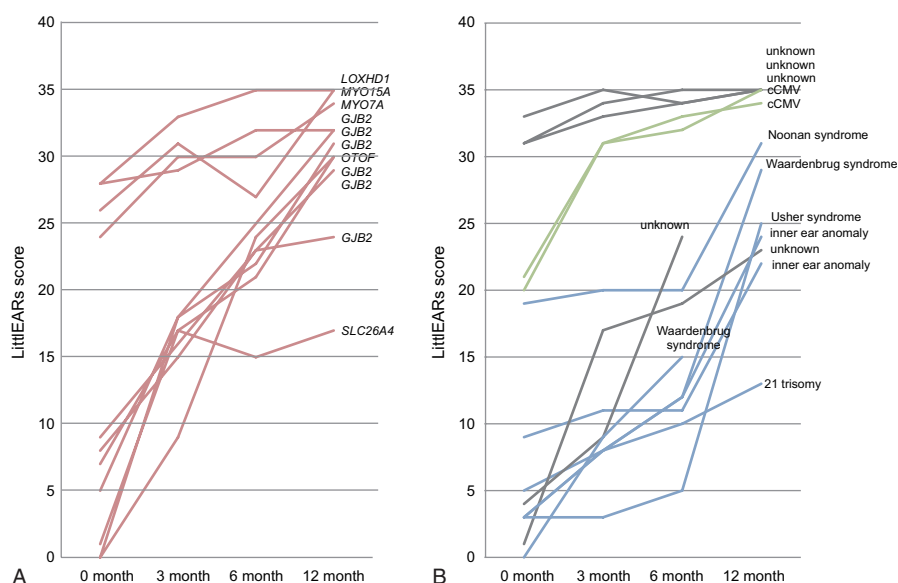


FIG. 2. Early auditory development assessed using the LittlEARs auditory questionnaire. (A) Nonsyndromic hearing loss with specific gene mutations (n = 11). (B) Other etiology (n = 11). Blue indicates syndromic hearing loss; gray, unknown; green, infection-induced hearing loss; pink, nonsyndromic hearing loss associated with specific gene mutations.

specific gene mutations for nonsyndromic hearing loss and the other etiology group (Student’s *t* test).

Causes of Hearing Loss in Postlingual CI/EAS Patients

The cause of hearing loss was identified in 34 out of 81 postlingual patients (42.0%) in this study (Fig. 1B). In 29

patients (35.8%), the hearing loss was caused by mutations in deafness genes, whereas two patients (5%) were diagnosed with otosclerosis, two patients developed hearing loss as a consequence of chronic otitis media, and one patient experienced acoustic neuroma. This patient had some residual hearing and CI was, therefore, indicated for this patient.

The mode of inheritance varied among the 81 patients (22 families were compatible with autosomal dominant inheritance, 7 families were compatible with autosomal recessive inheritance, and 27 patients were sporadic pattern). Mutations were identified on the basis of genetic screening, and segregation analysis and prediction software suggested them to be the responsible mutations. In total, 13 causative genes were identified in this group, although no major genes, such as *GJB2* in the prelingual group, were identified. The most common causative gene was *CDH23* (9%), followed by *MYO7A* (4%), *TMPRSS3* (4%), *MYO15A* (2%), *DFNB31* (1%), *ACTG1* (2%), *DFNA5* (1%), *MYO6* (1%), and *CRYM* (1%). Mitochondrial 3243A >G (3%) and 1555A >G mutation (1%) were also involved in the postlingual CI/EAS patients.

Clinical Findings and Outcomes for Postlingual CI/EAS Patients

Outcomes of CI were shown to vary among the postlingual hearing loss patients (Fig. 4). With regard to the distribution patterns of the Japanese monosyllable and word perception test results, the CI outcomes showed a multi-peaked distribution (Fig. 5). No statistically significant differences were observed between the group with specific gene mutations and the other etiology group. A comparison of the good outcome group and the moderate-poor outcome group (with the borderline set at 40% for the Japanese monosyllable perception test and at 60%

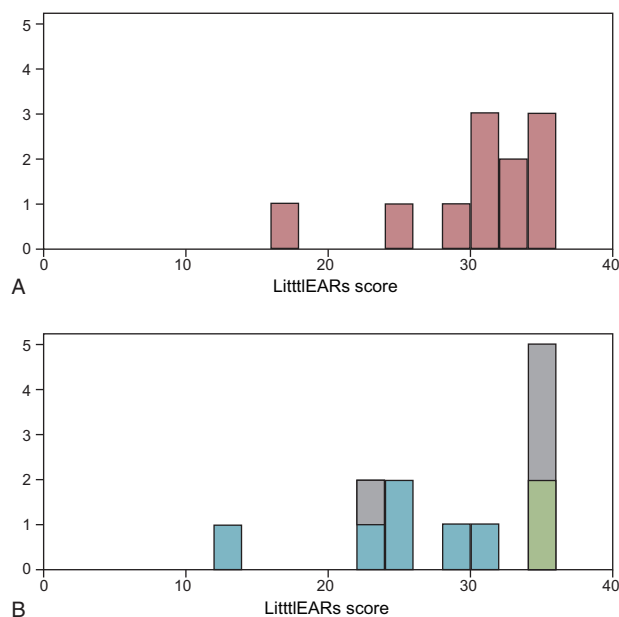


FIG. 3. Distribution of auditory behavior assessment (LittlEARs) scores for prelingual CI patients. (A) Nonsyndromic hearing loss with specific gene mutations (n = 11). (B) Other etiology (n = 11). Blue indicates syndromic hearing loss; gray, unknown; green, infection-induced hearing loss; pink, nonsyndromic hearing loss associated with specific gene mutations.

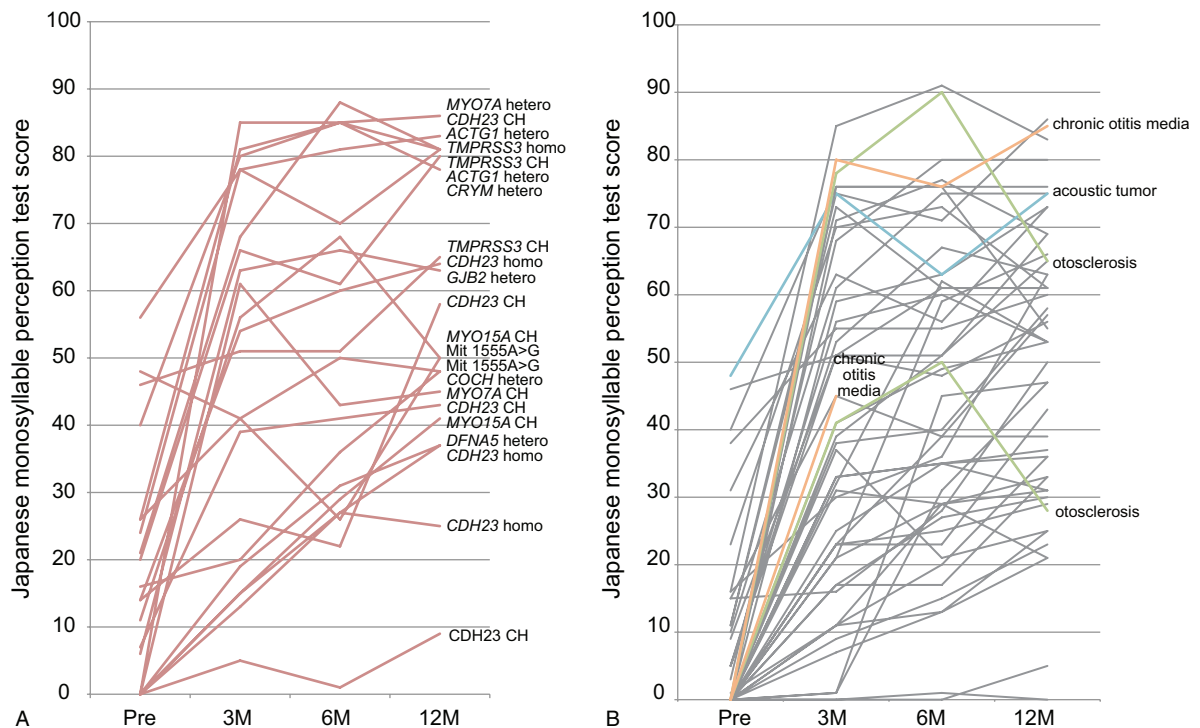


FIG. 4. Japanese word perception test results for postlingual CI patients. (A) Nonsyndromic hearing loss with specific gene mutations ($n = 22$). (B) Unknown or other etiology ($n = 50$). Blue indicates acoustic tumor; gray, unknown; green, otosclerosis; orange, otitis media; pink, nonsyndromic hearing loss associated with specific gene mutations.

for the Japanese word perception test) revealed that 1) the good outcome group was significantly younger (mean age = 52.79 for monosyllable test, 54.23 for word test) than the moderate-poor outcome group (mean age = 66.88 for monosyllable test, 64.85 for word test), and 2) there were significant differences in etiology; i.e., 40% for monosyllable test and 43% for word test of the good outcome patients were found to have specific gene mutations whereas only 27% for monosyllable test and 23% for word test of the poorer outcome group had the same specific gene mutations (Table 1).

DISCUSSION

Etiology

Because of the extreme genetic heterogeneity of deafness, beyond screening for common genes such as *GJB2*, it has been difficult to identify the responsible gene in individual CI/EAS patients, especially in a clinical setting. However, recent advances in NGS may afford a breakthrough as targeted exon-sequencing of selected deafness genes using MPS technology has enabled the successful identification of causative mutations in relatively rare genes. In fact, the current series using MPS successfully discovered rare causative genes among the enrolled CI/EAS patients. These genes have not usually been screened and, therefore, mutations in these genes have not been clinically diagnosed using a conventional approach. MPS, however, has the potential to identify such rare genes/mutations.

Two previous studies have described the genetic backgrounds of cochlear implant patients, although both studies used selected samples (25,26). A definitive genetic diagnosis was made in 20.6% (37/180) of CI children by the screening of four common deafness-associated genes, *GJB2*, *SLC26A4*, the mitochondrial 12S rRNA gene, and *OTOF* (Wu et al. (25)). More recently, using targeted resequencing of 204 candidate deafness genes and a phenotype-driven candidate gene approach, causative variants were found in 54.8% (51/93) of cochlear implantees (Park et al. (26)). Both studies suggested that genetic causes account for an important proportion of CI patients. The present study is the first to clarify the genetic epidemiology in a more comprehensive way using 1) a consecutive (nonbiased) large cohort of samples, 2) both pre- and postlingual patients, and 3) an updated genetic screening system (Invader assay followed by MPS-based screening).

In this study, two-step genetic screening (Invader assay followed by MPS-based screening) successfully identified causative mutations in 59.8% of congenital hearing loss patients, and 35.8% of postlingual hearing loss patients with cochlear implantation. For the prelingual CI/EAS patient group, in particular, genetic screening together with additional imaging examination, cCMV screening, and pediatric examination was able to detect successfully the etiology of deafness in 85% of the patients. The present high diagnostic rate is expected to have a great impact, with such epidemiological data being essential for decision making with regard to the decision to implement CI/EAS,

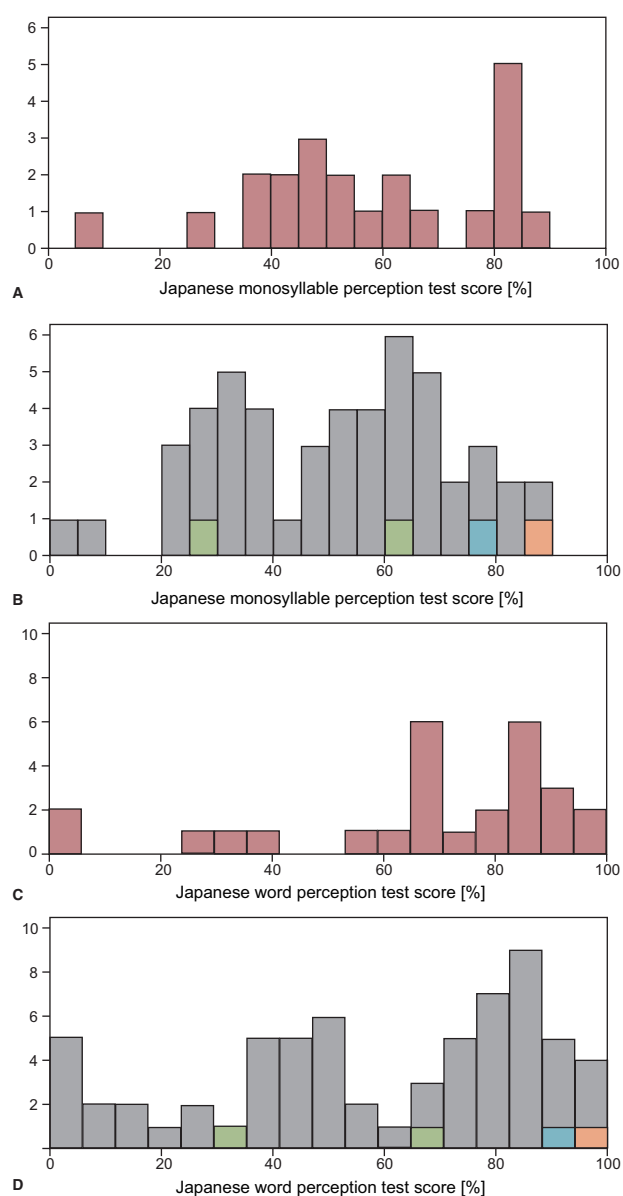


FIG. 5. Distribution of Japanese perception test results for post-lingual CI patients. (A) Monosyllable. (B) Word. Blue indicates acoustic tumor; gray, unknown; green, otosclerosis; orange, otitis media; pink, non-syndromic hearing loss associated with specific gene mutations.

the prediction of outcomes, and the provision of appropriate future intervention.

As shown in Fig. 1, the most common etiology was genetic (59.8%), followed by cCMV infection (5%), inner ear malformation (5%), meningitis (3%), and congenital rubella (1%). Among the genetic causes, the most frequent causative gene was *GJB2* (29%), followed by *SLC26A4* (9%), *CDH23* (7%), *MYO7A* (4%), *OTOF* (5%), *MYO15A* (3%), *LOXHD1* (2%). The present results indicated that these deafness genes are typical deafness genes indicative for CI in the prelingual group. A further

TABLE 1. Differences between patients with a good CI outcome and those with a moderate-poor CI outcome

Monosyllable Test (12 mo)	n	Age at Surgery (mean)	Genetic Mutation
> = 40	48	52.79	40%
<40	26	66.88	27%
Word test (12 mo)	n	Age at surgery (mean)	Genetic mutation
> = 60	47	54.23	43%
<60	37	64.85	23%

9% of the patients were diagnosed with syndromic deafness on the basis of associated symptoms.

All of the identified genes are known to be localized and function in the inner ear (27). *GJB2*, the most common cause of congenital deafness worldwide, encodes the gap junction protein connexin 26, which is essential for potassium recirculation and other metabolite transport. *SLC26A4* is a common cause of deafness associated with an enlarged vestibular aqueduct. Pendrin protein, which is encoded by *SLC26A4*, acts as a transporter of chloride, bicarbonate, and iodide ions in the spiral prominence. Pendrin also contributes to pH homeostasis and the mineralization process in the organ of Corti and vestibule. Cadherin 23, which is encoded by *CDH23*, is a component of the tip link and transient lateral links of the stereocilia. *MYO7A*, which encodes unconventional myosin VIIA, acts as a component of the USH complex (including *CDH23*, *SANS*, *USH1C*, and *MYO7A*) in the tip link of the stereocilia. *MYO15A* directly binds to *WHRN* to form the *MYO15A-WHRN-EPS8* complex in the stereocilia, which is essential for stereocilia elongation. *LOXHD1* is also involved in the regulation of stereocilia elongation and mutations in *LOXHD1* cause “fused stereocilia” and “membrane ruffling” at the apical surface of hair cells. *CDH23*, *MYO7A*, *MYO15A*, and *LOXHD1* are all important for the development and maintenance of the stereocilia and have important roles in mechano-electro-transduction. *OTOF* is the most common cause of auditory neuropathy spectrum disorder and encodes the protein otoferin, which is involved in the late step of synaptic vesicle exocytosis as the major Ca^{2+} sensor for the ribbon synapse of inner hair cells.

Among the postlingual CI/EAS patients, the etiology was detectable in approximately 40% of patients. The most frequent etiology was genetic (35.8%), followed by otosclerosis (2%), otitis media (2%), and acoustic neuroma (1%). Interestingly, although genetic causes were the most common, a number of different kinds of causative genes, including various rare genes, were found to be involved in postlingual deafness. Only a small number of patients could be diagnosed by Invader assay, with the majority of the rare genes identified by MPS.

The most common causative gene was *CDH23* (9%), followed by *MYO7A* (4%), *TMPRSS3* (4%), *MYO15A*

(2%), *DFNB31* (1%), *ACTG1* (2%), *DFNA5* (1%), *MYO6* (1%), and *CRYM* (1%). In the postlingual CI/EAS patients, mitochondrial 3243A>G (1%) and 1555A>G mutation (2%) were also found to be involved. Compared with the prelingual group, many dominant genes, such as *MYO7A*, *ACTG1*, *DFNA5*, *MYO6*, and *CRYM*, as well as mitochondrial genes reported to cause progressive hearing loss, were found to be involved.

These genes are also localized and play important roles in the inner ear (27). *TMPRSS3* encodes transmembrane protease serine 3, which is involved in the maturation of the epithelial amiloride-sensitive sodium channel (ENaC) and K⁺ channel (KCNMA1). *DFNB31* (*WHRN*) encodes the scaffolding protein whirlin, which directly binds to *SANS*, *EPS8*, and *MYO15A*, and is colocalized in the tip link of the stereocilia. *ACTG1* encodes cytoskeletal nonmuscle actin protein gamma. This protein is localized in the F-actin gap region of the stereocilia. *MYO6*, which is expressed in the cuticular plate region of IHC and OHCs, is involved in stereocilia formation, and may have an important role as a stereocilia anchor. Mu-crystallin is encoded by *CRYM* and directly binds to thyroid hormone (T3) with high affinity in the presence of NADPH. *CRYM* in complex with *NADPH* transports T3 into the nucleus and activates T3-dependent transcription.

Interestingly, *CDH23*, *MYO7A*, and *MYO15A* were found in both the pre- and postlingual groups, indicating these genes may express variable phenotypes.

Outcomes

In the prelingual group, the majority of patients with nonsyndromic hearing loss and with specific deafness gene mutations showed good and rapid development of auditory behavior (Fig. 2A). This is in line with the general hypothesis that good outcomes can be expected if the etiology is located within the cochlea (Fig. 2A). According to a previous study, the children with mutations had better auditory nerve responses (CAP scores) than did the children without mutations (25).

In contrast, a number of children in the other etiology group showed moderate-poor CI outcomes. The etiology in these poorer outcome patients involved inner ear/cochlear nerve malformation or syndromic hearing loss (Down syndrome, Noonan syndrome, or Waardenburg syndrome) (Fig. 2B). We compared the distribution patterns of LittleEARs auditory questionnaire scores (Fig. 3), but no statistically significant differences were observed between the two groups, probably because the other etiology group also contained patients showing good outcomes. One example is the patient with unknown etiology but without any malformation or syndrome (Fig. 2B), and another is a cCMV patient who had already recorded good scores before receiving CI because of progressive hearing loss (Fig. 2B).

In the postlingual group, performance after CI varied due to a number of factors; however, the majority of patients showed a good overall outcome after implantation (Fig. 4, A and B).

To identify differences in outcome between patients with specific nonsyndromic deafness gene mutations and those with hearing loss of other etiology, including many unknown patients, we compared the distribution patterns of the Japanese monosyllable perception test results and Japanese word perception test results (Fig. 5). As a result, CI patients with specific nonsyndromic deafness gene mutations tended to show better outcomes than did patients with other etiologies, although this difference was not statistically significant.

Interestingly, with regard to the distribution patterns of the Japanese monosyllable perception test results and Japanese word perception test results (Fig. 5), the CI outcomes showed a multipeaked distribution. These results suggested that patients do not form a homogeneous group. This distribution pattern is commonly observed in both monosyllable and word tests. We divided all patients into two groups (the good outcome group and moderate-poor outcome group) with the borderline set at 40% for the Japanese monosyllable perception test and at 60% for the Japanese word perception test. A comparison of outcomes and various factors, including age, sex, hearing loss threshold, and etiology, revealed that the good outcome group was significantly younger than the moderate-poor outcome group. These results indicated that younger patients can expect a better CI outcome than older patients. In addition to age, significant differences were also observed for etiology; i.e., 40 to 43% of the good outcome patients were found to have specific gene mutations, whereas only 23 to 27% of the poorer outcome group had the same specific gene mutations (Table 1).

With regard to the relationship between etiology and outcome in CI/EAS patients, a large number of articles have focused on the outcomes in patients with *GJB2* (28–39), but not many articles have described outcomes in CI/EAS patients with associated uncommon gene mutations. A series of studies have demonstrated that CI has brought about tremendous improvements in auditory skills as well as in speech production development in patients with profound hearing loss resulting from *GJB2* mutations (28–39). Further, although some literature described comparable results, no articles have reported poorer outcomes for patients with *GJB2* mutations.

There have been fewer reports on the outcomes for CI for other genes, although some reports have described good performances in CI/EAS patients with associated *SLC26A4* (25,39), *OTOF* (25,40,41), *MYO6* (8,42), *MYO15A* (3,4), *TECTA* (3), *CDH23* (2), *COCH* (5,43), *MYH9* (44,45), and *TMPRSS3* mutations (1,3,6,25,46,47). There have also been some reports describing poorer outcomes in patients with *POU3F4* mutations, which are known to cause inner ear anomalies (48–51).

Further, the outcomes of CI for patients with *TMPRSS3* mutations seem controversial. A majority of patients with *TMPRSS3*-associated hearing loss (13 out of 15 based on a literature review) were reported to show good outcomes for CI, whereas two patients reported by Eppsteiner et al. showed a poorer performance (1). We

evaluated the improvement in speech discrimination and perception scores (using the 67S Japanese monosyllable test) preoperatively and at 12 months after initial EAS stimulation in three patients with *TMPRSS3* mutations who underwent EAS and 27 other patients, and confirmed that they showed relatively good outcomes and were good candidates for CI/EAS (6). Our recent gene expression study, in which the *Tmprss3* gene was found to be predominantly expressed within the cochlea (Nishio et al, submitted), supports our clinical data.

CONCLUSION

The present results suggest that a variety of genes may be involved in hearing loss in CI/EAS patients. In the present study, patients with these mutations showed relatively good auditory performance after receiving CI/EAS. Therefore, although many factors may influence outcomes, genetic background can be included as useful in predicting performance after implantation.

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