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International Journal of Pediatric Otorhinolaryngology 76 (2012) 433–438

Contents lists available at SciVerse ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Language development in Japanese children who receive cochlear implant and/or hearing aid

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ARTICLE INFO

Article history:
Received 18 October 2011
Received in revised form 27 December 2011
Accepted 27 December 2011
Available online 26 January 2012

Keywords:
Congenital hearing loss
Language development
Cochlear implant
Communication

ABSTRACT

Objectives: This study aimed to investigate a wide variety of factors that influence auditory, speech, and language development following pediatric cochlear implantation (CI).
Study design: Prospective collection of language tested data in profound hearing-impaired children.
Hypothesis: Pediatric CI can potentially be effective to development of practical communication skills and early implantation is more effective.
Methods: We proposed a set of language tests (assessment package of the language development for Japanese hearing-impaired children; ALADJIN) consisting of communication skills testing (test for question–answer interaction development; TQAID), comprehensive (Peabody Picture Vocabulary Test-Revised; PVT-R and Standardized Comprehension Test for Abstract Words; SCTAW) and productive vocabulary (Word Fluency Test; WFT), and comprehensive and productive syntax (Syntactic processing Test for Aphasia; STA). Of 638 hearing-impaired children recruited for this study, 282 (44.2%) with >70 dB hearing impairment had undergone CI. After excluding children with low birth weight (<1800 g), those with >11 points on the Pervasive Developmental Disorder ASJ Rating Scale for the test of autistic tendency, and those <2 SD on Raven's Colored Progressive Matrices for the test of non-verbal intelligence, 190 children were subjected to this set of language tests.
Results: Sixty children (31.6%) were unilateral CI-only users, 128 (67.4%) were CI–hearing aid (HA) users, and 2 (1.1%) were bilateral CI users. Hearing loss level of CI users was significantly ($p < 0.01$) worse than that of HA-only users. However, the threshold level, maximum speech discrimination score, and speech intelligibility rating in CI users were significantly ($p < 0.01$) better than those in HA-only users. The scores for PVT-R ($p < 0.01$), SCTAW, and WFT in CI users were better than those in HA-only users. STA and TQAID scores in CI–HA users were significantly ($p < 0.05$) better than those in unilateral CI-only users. The high correlation ($r = 0.52$) has been found between the age of CI and maximum speech discrimination score. The scores of speech and language tests in the implanted children before 24 months of age have been better than those in the implanted children after 24 months of age.
Conclusions: We could indicate that CI was effective for language development in Japanese hearing-impaired children and early CI was more effective for productive vocabulary and syntax.

1. Introduction

Management of CI in infants and children is one of the most striking advances for congenital severe to profound hearing loss. Several studies have shown that early implantation can be beneficial not only for speech perception, but also for the development of speech and language [1–3]. Moreover, early intervention for children with hearing loss facilitates successful educational integration at the earliest possible age [4].

More than 20 years have passed since the first pediatric CI surgery was performed in Japan. Many hearing-impaired children are now benefiting from this device. However, the long-term benefits for Japanese CI users have rarely been reported. In particular, language development after CI among Japanese children has not often been investigated. Language development outcomes among children with prelingual hearing impairment have been

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doi:10.1016/j.ijporl.2011.12.027

studied in Indo-European languages, but language differences may have an effect on language development in children with CI. In addition, differences in national and local education systems may make a difference to language development. To determine the effect of CI, we examined language development in different language and/or social systems.

Language differences may add other difficulties; for example, interpretation of Japanese language test results may not be comparable with that of English or other European language tests. To reduce these difficulties, we have established the assessment package of the language development for Japanese hearing-impaired children (ALADJIN) as a language performance evaluation tool for hearing-impaired children. ALADJIN includes several Japanese language tests that are directly comparable with previously reported English tests, including the Peabody Picture Vocabulary Test-Revised (PVT-R) and Test for Reception of Grammar (TROG)-like syntax tests (e.g., the Syntactic processing Test for Aphasia; STA). These tests all have their own distinctive emphasis and evaluate different aspects or domains of language.

In 2010, we assessed the current status of hearing-impaired children in Japan through a project called Research on Sensory and Communicative Disorders (RSCD). ALADJIN was used in this nationwide research project. The RSCD was originally intended to assess the effectiveness of interventional methods for hearing-impaired children. As part of the RSCD survey, we evaluated the domain-specific language status of Japanese hearing-impaired children with CI, not only in selected institutes and schools that potentially yield biases, but in a wide variety of institutes in Japan.

Thus, the objective of this study was to evaluate the development of interpersonal communication skills (IPCS) in hearing-impaired children with CI using the ALADJIN data set from the RSCD nationwide research project.

2. Materials and methods

All ALADJIN tests were conducted by trained audiologists, speech pathologists, or deaf school teachers in a noise-minimized compartment. Audiometry for evaluation of hearing level, pure-tone threshold, speech discrimination test, and speech intelligibility rating [5] were measured in a sound-attenuated room of the relevant hospital. The study design was approved by the ethics review board of the Association of Technical Aids.

2.1. Subjects

In 2009, 124 institutes were participated in the RSCD project and 638 hearing-impaired children were registered; written informed consent was obtained from their parents. Open recruitment was conducted not only in institutes for hearing-impaired children, i.e., deaf schools and hard of hearing schools, but also in mainstream schools, day-care nurseries, and hospital/clinic training programs.

Most children included in this project were within the age range from 4 years (2 years before elementary school entrance; −2 grade) to 12 years (6th grade of elementary school; +6 grade) and confirmed to have congenital hearing impairment (average hearing level >70 dB at 4 years of age). Children who were discernibly unable to complete the ALADJIN tests due to additional handicaps were excluded. 282 (44.2%) participating children were CI users, and about 45% of the hearing-impaired children of each age group were CI users (Fig. 1). Subjects were classified into four groups as follows: (1) "unil CI-only" group with unilateral CI users, (2) "CI–HA" group with CI plus conventional HA users (also called the bimodal stimulation group), (3) "bil-CI" group with bilateral CI users, and (4) "HA-only" group with HA users. The number of CI children in each age group is given in Fig. 2. 84 children (35 males and 49 females, 29.8%) in the unil CI-only group were diagnosed as

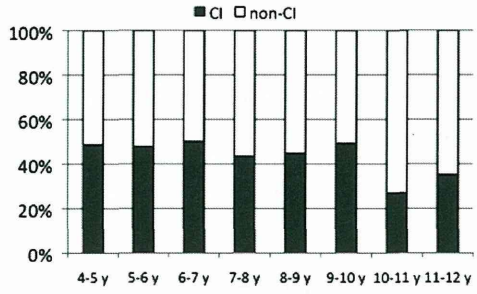

Fig. 1. The rate of CI-only users among the participating hearing-impaired children of each age group. About 45% of hearing-impaired children (>70 dB hearing level) in this study were CI-only users. CI: cochlear implant.

hearing-impaired at 12.5 months on average. In the CI–HA group, 196 children (99 males and 97 females, 69.5%) were diagnosed as hearing-impaired at 10.9 months on average. Two children (1 male and 1 female, 0.7%) were bilateral CI users (bil-CI group). In the HA-only group, 356 children were diagnosed as hearing-impaired at 13.3 months and fitted at first hearing aids at 17.2 months (0–74 months) on average. Age at first fitting hearing aids in the children with CI was 15 months (2–47 months).

In order to reduce the influence of developmental disabilities in our evaluation of the language tests (ALADJIN), participating children with birth weights <1800 g, PARS scores >11 points, and RCPM scores <2 SD of the average were excluded. The numbers of subjects in each group were evaluated in the language tests as follows: 60 unil CI-only users, 128 CI–HA users, and 203 HA-only users. No significant differences in the scores of PARS and RCPM among the unil CI-only, CI–HA, and HA-only users were found (Fig. 3).

2.2. Test battery

We used the test for question–answer interaction development (TQAID) as a tool to measure IPCS function objectively. To let children understand a content of task, their favorite mode of communication (aural, sign language, total communication) were used to perform the language tests. 80% of subjects used aural communication as major mode in the domestic life. The following tests were also used to evaluate IPCS the day after administration of the TQAID.

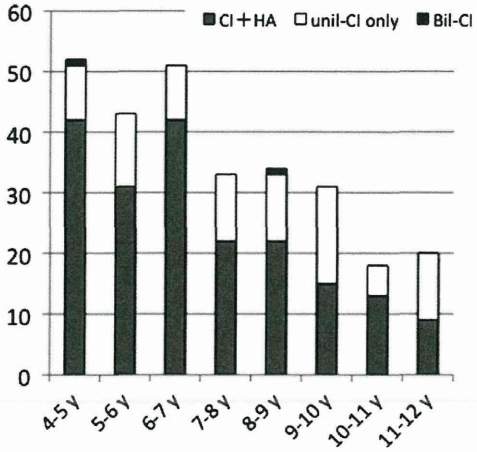

Fig. 2. Number of children in the CI–HA, unil CI-only, and bil-CI groups in each age range. CI plus HA users (bimodal stimulation) make up the majority of CI users. CI: cochlear implant; unil CI: unil CI-only users; bil-CI: bilateral CI users; CI–HA: HA and CI users.

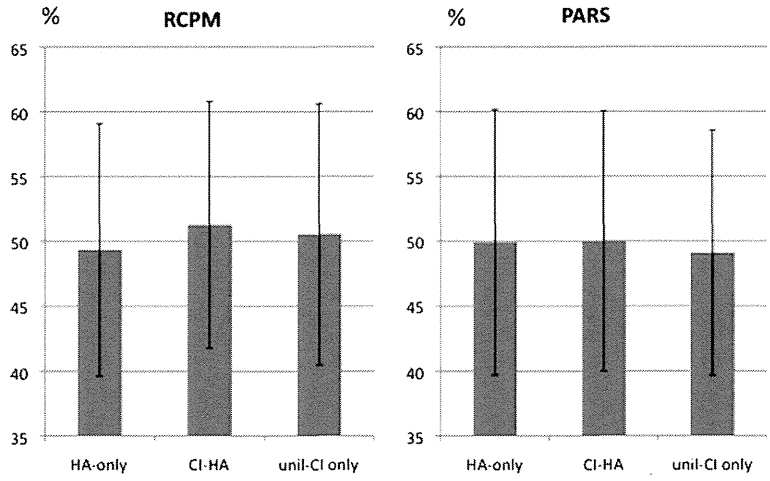

Fig. 3. The scores of PARS and RCPM tests in the HA-only, CI–HA, and unil CI-only groups. There were no significant differences in average scores among the groups. PARS: Pervasive Developmental Disorder ASJ Rating Scales for evaluating autistic tendency; RCPM: Raven's Colored Progressive Matrices test for evaluating non-verbal intelligence; CI: cochlear implant; HA: hearing aid.

The Word Fluency Test (WFT) was conducted as a measure of productive vocabulary [6,7]. Children were asked to produce as many words as possible from a certain category in 60 s. The words, represented either orally or manually, were carefully counted, excluding onomastic words. The Japanese version of the PVT-R [8] and the Standardized Comprehension Test for Abstract Words (SCTAW) [9] were also conducted to evaluate comprehensive vocabulary. An adjusted score was used in this study. The SCTAW consists of 32 or 45 abstract words selected from Japanese school textbooks. The details of how this method has been adapted for hearing-impaired children have been reported in previous studies [9,10]. Only school-aged children were subjected to this test.

The STA evaluates comprehension and production of syntactic structures. The children were asked to choose one of the four pictures appropriate to the tester's presentation (comprehension test) or to express a sentence according to a picture that the tester indicated (production test) [11]. The tests evaluated understanding and expression of irreversible sentences, reversible sentences, Japanese suffixes (Jyo-Shi), and other syntactic structures, including relative pronouns.

To evaluate additional handicaps other than hearing impairment, the Pervasive Developmental Disorder ASJ Rating Scale (PARS) test for autistic tendency [12] and Raven's Colored Progressive Matrices (RCPM) test of non-verbal intelligence [13] were used only in school-aged children.

2.3. Statistical analyses

All statistical values were calculated using IBM SPSS Statistics 18 software (IBM Corp., Armonk, NY, USA). Correlations and standard deviations within each group were examined. The scores of the language tests (PARS, RCPM, PVT-R, SCTAW, WFT, STA, and TQAID) were translated as *Z*-scores from the results of each test in each age group.

3. Results

There were significant ($p < 0.01$) differences in the scores of average hearing loss level, average threshold level with hearing devices, maximum speech discrimination score, and speech intelligibility rating between CI users (unil CI-only or CI–HA users) and HA-only users (Fig. 4). Hearing loss level of CI users was significantly lower than that of HA-only users. However, the threshold level, maximum speech discrimination scores, and speech intelligibility rating of CI users were significantly better than those of HA-only users. The scores of the PVT-R, SCTAW, and WFT tests, which evaluate vocabulary, were higher in CI users than in HA-only users (Fig. 5). There was a significant difference ($p < 0.01$) in the results of the PVT-R test. The scores of the STA (Fig. 6) and TQAID (Fig. 7) in CI–HA users were significantly higher ($p < 0.05$) than those in the unil CI-only group.

The high correlation ($r = 0.52$) has been found between the age of CI and maximum speech discrimination score (Fig. 8). The average scores of speech and language tests in the implanted children before 24 months of age have been better than those in the implanted children after 24 months of age (Table 1). The average scores of WFT (evaluation of productive vocabulary) and comprehension and production tests of STA (evaluation of syntactic structure) were significantly better in the implanted children before age of 24 months compared with the implanted children after age of 24 months.

4. Discussion

To evaluate the language development in the typical hearing-impaired children, we have made exclusionary criteria to standardize the subjects in this study. We excluded the hearing-impaired children with birth weights <1800 g who scored >11 points on the PARS test and <2 SD on the RCPM. Very low birth weight children are at a high risk of neurosensory disability, including developmental delay, behavioral problems, and learning disabilities [14]. Long-term follow-up studies have also emphasized the prevalence of significant neuropsychological and behavioral deficits at school age in children of very low birth weight [15]. Therefore, we excluded children with birth weights <1800 g to reduce the influence of developmental disabilities in our evaluation of communication skills. The PARS and RCPM tests determine the presence of pervasive developmental disorders and non-verbal intelligence, respectively. The scores in these tests were not significantly different among unil CI-only, CI–HA, and HA-only users. Consequently, children with developmental disabilities were excluded from the present study. However, children with ANSD (auditory neuropathy spectrum disorder) could not been excluded, because we did not get the data of ABR and OAE in this study.

Speech development for prelingual deaf children depends on optimal amplification with a CI or HA. Language acquisition is a

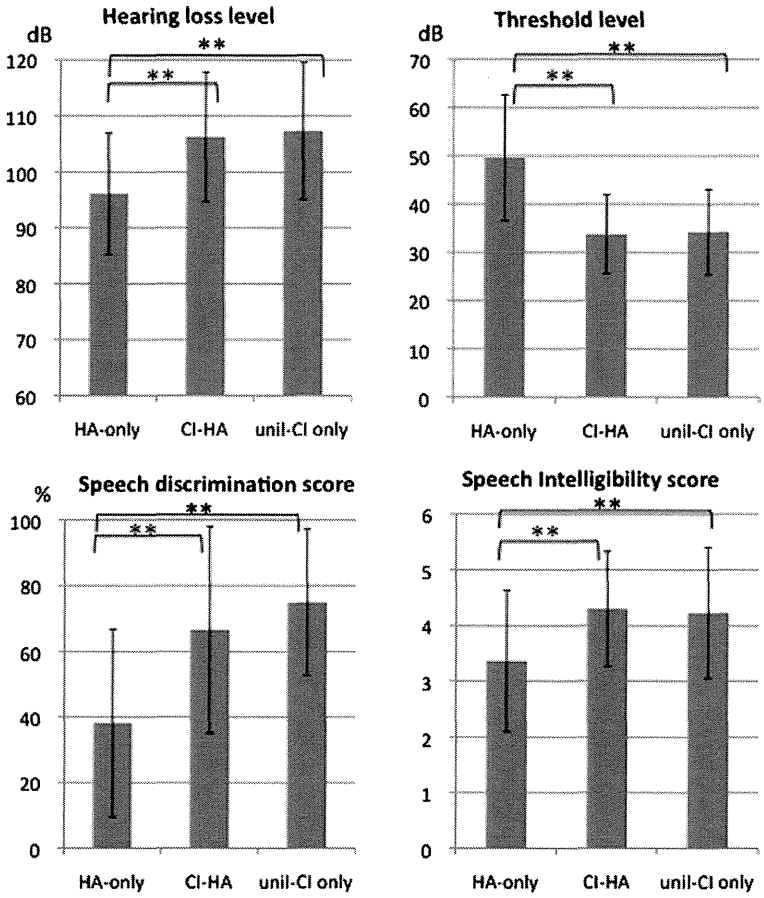

Fig. 4. Hearing loss levels, threshold levels, maximum speech discrimination scores, and speech intelligibility scores in the HA-only, CI–HA, and unil CI-only groups. There are significant differences ($p < 0.01$) in hearing levels, threshold levels, speech discrimination scores, and intelligibility scores between the CI group (CI–HA or unil CI-only groups) and HA-only group. Children with CI achieve better threshold levels, speech discrimination, and intelligibility compared with HA-only users. $^{**}p < 0.01$, CI: cochlear implant; HA: hearing aid.

high priority among deaf children who receive CI. During the 1990s, the following factors were considered to be associated with good speech development: age at implantation, duration of deafness, amount of daily use, mode of communication, and absence of other handicaps. Dettman et al. [16] reported that infants with implantation during the first year of life had significantly faster rates of receptive and expressive language development than those with implantation in the second year of life. On the other hand, another study found no significant differences in the performance in terms of spoken word recognition and expressive language development between children with implantation in the first and second years of life [17]. In our study,

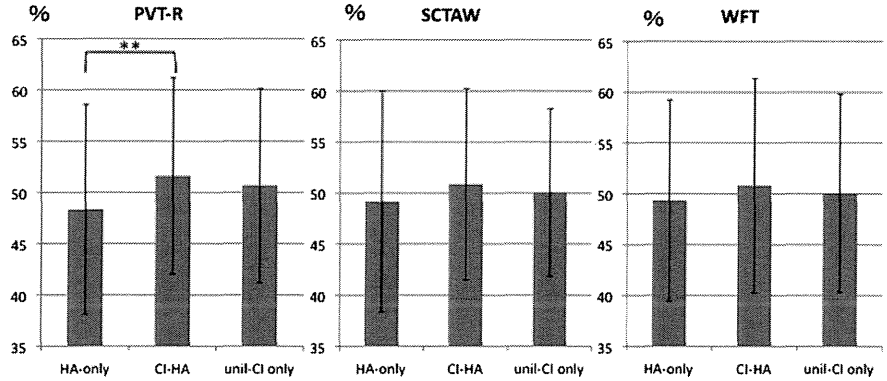

Fig. 5. Scores of the PVT-R, SCTAW, and WFT tests in the HA-only, CI–HA, and unil CI-only groups. Scores of the PVT-R, SCTAW, and WFT tests in the CI–HA and unil CI-only groups were better than those in the HA-only group. A significant difference ($p < 0.01$) was found in the scores of the PVT-R test. $^{**}p < 0.01$, PVT-R: Peabody Picture Vocabulary Test-Revised; SCTAW: Screening Test for Abstract Words; WFT: Word Fluency Test. Values in the longitudinal line indicate *Z*-score.

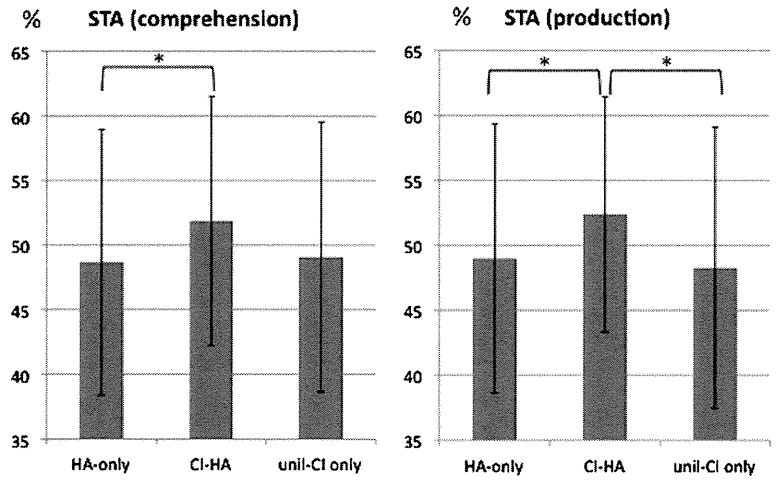

Fig. 6. Scores of the STA test (comprehension and production) in the HA-only, CI–HA, and unil CI-only groups. STA test scores (comprehension) in the CI–HA group were significantly higher ($p < 0.05$) than in the HA-only group. STA test scores (production) in the CI–HA group were significantly higher ($p < 0.05$) than those in the unil CI-only and HA-only groups. *$p < 0.05$. STA: Syntactic processing Test for Aphasia test. Values in the longitudinal line indicate *Z*-score.

early CI was more effective for better speech discrimination and children with CI before the second year of life had significantly better scores of productive vocabulary and comprehensive and productive syntax.

Early intervention has a strong influence on language outcomes in most, but not all, hearing-impaired children. The degree of hearing loss is an important factor in the modeling of speech production and spoken language outcomes. Several studies have demonstrated a clear relationship between the degree of hearing loss and language outcome [18]. In our study, the average age at diagnosis of hearing loss in children with CI was 11.4 months. Age at diagnosis in CI–HA users (10.9 months) was earlier than in unil CI-only users (12.5 months) and HA-only users (13.3 months).

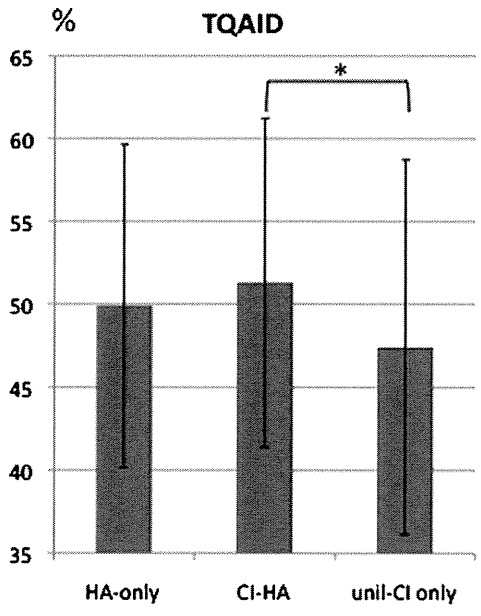

Fig. 7. The scores of TQAID test in the HA-only, CI–HA, and unil CI-only groups. The score of TQAID test in the CI–HA group is significantly ($p < 0.05$) better than that in the unil CI-only group. *$p < 0.05$, TQAID: test for question-answer interaction development is for evaluating the IPCS (interpersonal communication skills) function. Values in the longitudinal line indicate *Z*-score.

Better speech and language development was found in CI–HA users compared with unil CI-only users.

The degree of hearing loss in CI users was higher than in HA-only users. Speech discrimination score and intelligibility rating were higher in CI users than in HA-only users. The degree of hearing loss was significantly negatively correlated with speech discrimination and intelligibility. However, no clear relationship between the degree of threshold with the amplification devices and speech discrimination and intelligibility was found. The degree of threshold with amplification is thus a predictive factor of speech discrimination and intelligibility. It is beneficial for the CI to establish the better threshold level because fitting method is completely different. This study confirmed that CI has a positive influence on speech discrimination and intelligibility in severely hearing-impaired children. However, 124 institutions were participated in this study as nationwide research project, so there might be a confounding variable for selection of amplification devices (CI/HA vs CI/CI vs unil CI).

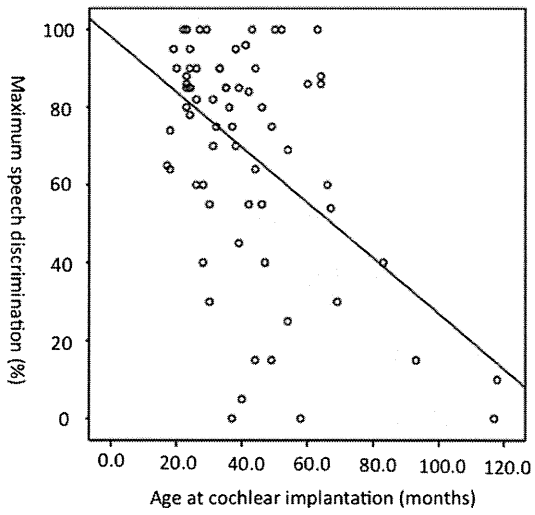

Fig. 8. The correlation between the age of cochlear implantation and maximum speech discrimination score. The high correlation ($r = 0.52$) has been found between the age of CI and maximum speech discrimination score.

Table 1
Average scores of language tests (ALADJIN) in children with CI before and after age of 24 months.

	PVT-R	WFT	SCTAW	STA (Com)	STA (Pro)	TQAID	RCPM	PARS
CI after 24 mo (N=29)	29.5	12.9	13.7	23.1	34.1	209.4	27.5	5.3
CI before 24 mo (N=161)	32.2	15.6	13.8	26.2	39.0	229.7	28.1	4.5
t-Value	0.19	0.02	0.99	0.04	0.04	0.06	0.77	0.30

PVT-R: Peabody Picture Vocabulary Test-Revised; WFT: Word Fluency Test; SCTAW: Standardized Comprehension Test for Abstract Words; STA (Com): Syntactic processing Test for Aphasia (Comprehension); STA (Pro): Syntactic processing Test for Aphasia (Production). TQAID: test for question–answer interaction development; RCPM: Raven's Colored Progressive Matrices; PARS: Pervasive Developmental Disorder ASJ Rating Scale; CI: cochlear implantation; mo: months; N: number.

In evaluating auditory performance, formal speech perception tests, such as open-set and closed-set tests, are often used in children with CI. Communication skills, including auditory, speech, and language development for congenital and prelingual deaf children with CI, are influenced by a wide variety of factors. Several studies have reported that factors such as gender, nonverbal intelligence, estimated family income, communication mode, performance IQ, working memory capacity, articulation rate, and verbal rehearsal speed may predispose a child to better or poorer outcomes with a CI [3,17].

We developed ALADJIN as a set of language tests to evaluate IPCS ability. Results of this assessment showed that CI was more effective for the development of comprehensive and productive vocabulary compared with HA, and bimodal hearing with CI and HA positively influence the development of vocabulary (comprehensive and productive), syntax (comprehensive and productive), and IPCS compared with unilateral hearing with CI. Consequently, we can conclude that early CI, especially in combination with HA, is useful in the development of communication skills.

Acknowledgments

We thank all participants of this study, including children, parents, and staff of the Research on Sensory and Communicative Disorders (RSCD) project who collaborated with the Association for Technical Aids (Tokyo, Japan). This work was supported by the Division of Hearing Impairment, RSCD of the Ministry of Health, Labour and Welfare, Tokyo, Japan.

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Acta Oto-Laryngologica, 2012; 132: 676–682

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CASE REPORT

Experience with the Vibrant Soundbridge RW-Coupler for round window Vibroplasty with tympanosclerosis

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Abstract

Usage of the Vibrant Soundbridge (VSB) with round window (RW)-Coupler placement at the RW has been shown to successfully treat mixed hearing loss. Coupling between the VSB's floating mass transducer (FMT) and the RW membrane is difficult in the case of sclerosis in the RW and drilling down the bony lip until the RW membrane can be seen completely can possibly induce a perilymphatic fistula. A 68-year-old woman who had bilateral mixed hearing loss with sclerosis in the RW due to tympanosclerosis underwent a RW-Vibroplasty with a RW-Coupler. Speech discrimination scores in quiet and noise and functional gain with the VSB with RW-Coupler were better than those using a conventional hearing aid. The results of the present case have shown the feasibility of implanting a VSB with RW-Coupler in patients with mixed hearing loss due to tympanosclerosis.

Keywords: *Floating mass transducer, sclerosis, round window membrane, perilymphatic fistula*

Introduction

Active middle ear implants are an alternative treatment option for various forms of hearing loss. The Vibrant Soundbridge (VSB) comprises an implanted receiver module connected via a conductor link to a floating mass transducer (FMT) and an externally worn audio processor that contains speech-processing circuitry. The classic indication for the use of VSB is sensorineural hearing loss, in which case the FMT is crimped to the long process of the incus with a titanium clip [1]. In 2006, Coletti et al. [2] were the first to describe the application of the VSB on the round window (RW), termed as RW-Vibroplasty, which is emerging as a versatile solution for various causes of conductive or mixed hearing loss. In these patients, conventional hearing aids and/or surgical restoration are either not possible or have failed because of chronic ear disease, extensive otosclerosis, or malformation.

Some surgeons emphasize that the FMT needs to be in complete contact with the round window (RW) membrane for the RW-Vibroplasty to be effective [2]. However, the anatomic mismatch between the diameter of the RW membrane and the FMT diameter does not accommodate complete FMT–RW membrane contact. In cases of sclerosis in the RW, coupling between the FMT and the RW membrane may be difficult because drilling the lesion of sclerosis in the RW can possibly induce a perilymphatic fistula. We report a case of a patient with sclerosis in the RW due to tympanosclerosis who underwent RW-Vibroplasty with a RW-Coupler (Figure 1) used as an alternative coupling aid.

Case report

The patient was a 68-year-old woman who was diagnosed with bilateral mixed severe hearing loss due to

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(Received 8 September 2011; accepted 13 November 2011)

ISSN 0001-6489 print/ISSN 1651-2251 online
DOI: 10.3109/00016489.2011.649492

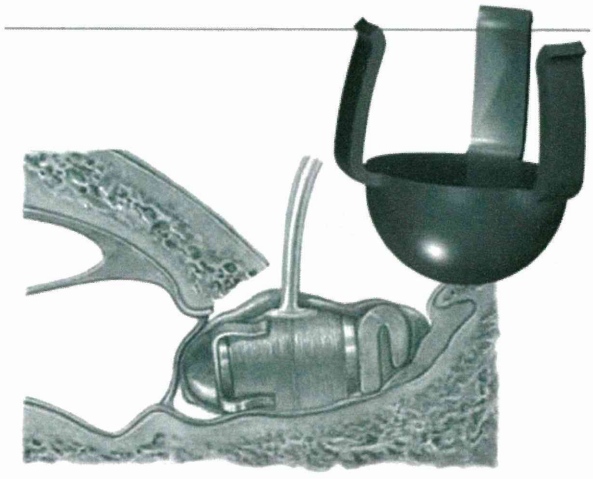

Figure 1. Round window (RW)-Coupler. The RW-Coupler was provided by MED-EL Co.

chronic otitis media (right ear, postoperative cholesteatoma plus tympanosclerosis; left ear, tympanosclerosis). She had undergone a tympanoplasty for the right cholesteatoma at the age of 55 years. She had worn a hearing aid on her left ear since she was 57. Revision surgery for cholesteatoma recurrence was conducted at Shinshu University Hospital when she was 66 years old. No ear ossicles were found except for the footplate, which was fixed, and tympanosclerotic lesions were found in the tympanic cavity. Reconstruction surgery was then conducted at the age of 67 years. The ossicular chain was reconstructed using a cartilage and the mastoid cavity was obliterated, with no resulting hearing improvement. She suffered with bilateral hearing loss and was not satisfied with hearing aid usage.

Otoscopic examination revealed an expansion of the right external auditory canal and a sclerotic lesion on her left tympanic membrane (Figure 2). A pure-tone audiometric examination showed bilateral mixed hearing loss (Figure 3A). Average air conduction hearing loss was 73.8 dBHL in the right ear and 68.8 dBHL in the left ear. The average air–bone gap was 41.3 dB in the right ear and 36.3 dB in the left ear. Her maximum speech discrimination score with monosyllables was 55% (100 dB) in the right ear and 85% (100 dB) in the left ear. We evaluated the effect of her conventional hearing aids as follows. (1) Speech discrimination scores with and without the hearing aids. (2) Questionnaire regarding background noise with the hearing aids. (3) Warble tone threshold level with and without the hearing aids. (4) Speech discrimination scores with the hearing aids in quiet and in noise. (5) Questionnaire with and without the hearing aids. She did not pass items number 2–5, according to the guideline for adaptation tests of conventional hearing aids (2010) in Japan. Computed tomography (CT) of the temporal bone showed poor development of both mastoid cavities, sclerotic lesions in both tympanic cavities, and sclerosis in both RWs (Figure 4A,B).

We judged that it was difficult to improve the hearing loss in the patient's left ear by tympanoplasty, because her left conductive hearing impairment was also caused by tympanosclerosis, as in the right ear. The hearing aid evaluation tests revealed that the hearing aid's effect had been inadequate (Table I and Figure 5A). The maximum speech discrimination score in the right ear was much worse than that in the left ear. We performed a RW-Vibroplasty with VSB in her left ear.

The surgical procedure consisted of a VSB implantation using a retroauricular approach. We found sclerotic lesions in the middle ear cavity and the RW and fixation of the stapes. The bony lip of the RW was drilled down until the RW membrane could be seen. However, drilling down the bony lip until the FMT could be placed completely on the RW membrane meant that a perilymphatic fistula might possibly be induced because of injury to the RW membrane. Therefore, to prevent the occurrence of

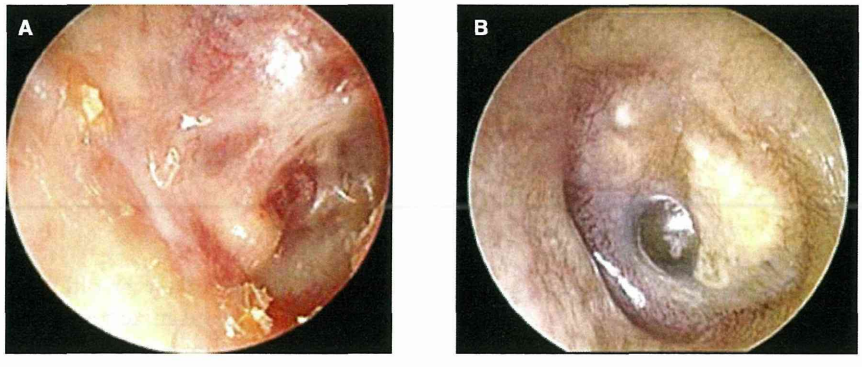

Figure 2. Otoscopic findings of tympanic membrane. The right external auditory canal was expanded (A) and sclerotic lesion was found in the left tympanic membrane (B).

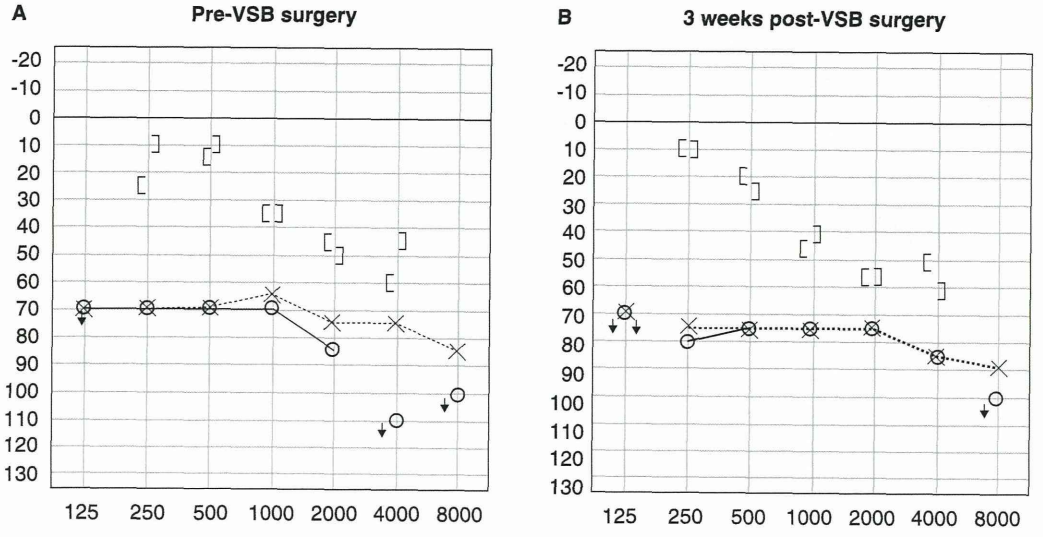

Figure 3. Pure-tone audiogram. (A) Bilateral mixed hearing loss was present. (B) Left hearing level was not changed after the VSB surgery.

a fistula, we decided to use the RW-Coupler, which is designed to fit smaller RW diameters, and were able to confirm good coupling (Figure 6). Perichondrium was placed in contact with the RW membrane, with the FMT interposed and wrapped with the perichondrium.

Fitting of the VSB's audio processor (Amadė®) was performed at 2 months following implantation. Warble tone threshold level at the first fitting according to the postoperative audiogram (Figure 3B) showed that functional gain at low frequencies (0.25–1 kHz) was low (Figure 5B). Amplification was elevated at the low frequencies and the final threshold level was calculated in Figure 5C. The audiologic outcomes 3 month after VSB fitting are shown in Table I. The evaluation tests consisted of the following. (1) Speech discrimination test for monosyllables at 60, 70, and 80 dBSPL. (2) Questionnaire for environmental noise (sentences 65 dBSPL and noise 60 dBSPL in the following conditions: a train station platform, a major intersection, a rub sound with a plastic bag, the sound of dishes being washed). (3) Speech discrimination tests for monosyllables in noise (noise at 60 dBSPL and monosyllables at S/N + 0, 5, 10 dB). Speech and noise signals were delivered by two speakers frontally placed at a distance of 1 m from the center of the subject's head. Speech discrimination scores in quiet and noise and functional gain with the VSB were better than those with the conventional hearing aid. Good placement of the FMT with RW-Coupler at the RW was confirmed 3 months after implantation (Figure 7).

This study was approved by the Ethics Committee of Shinshu University School of Medicine and written consent was obtained from the patient.

Discussion

The rate of tympanosclerosis is reported to be 9–38% in chronic otitis media cases, with bilateral tympanosclerosis seen in about 50% of the patients [3]. Tympanosclerotic plaques are found in the attic and around the stapes, oval window, RW, and promontorium [3]. The second most common site for sclerosis occurrence is the RW region, which is involved in 30% of clinical cases in histologic studies [4]. In radiologic studies, the prevalence of sclerosis in the RW is 3.2–13% [4]. Half of the patients with tympanosclerosis have required second sessions or revision procedures if ossicle fixation was present [5]. The present case involved bilateral mixed hearing loss and sclerotic lesions in both middle ears, which does not seem to be very rare. Figure 8 shows the diameters of the left RW membrane in our case and in 24 patients with sensorineural hearing loss as controls by radiologic evaluation of CT scan, which was measured on the inferior edge of the RW (arrowheads in the diagram in Figure 4) in the transverse slice. The diameter of the left RW in our case (0.9 mm) was remarkably small compared with control cases (1.78 mm on average) because of sclerosis of the inferior edge of the RW. An air–bone gap of 41.3 dB was found, which tympanoplasty in the right ear was unable to improve. We therefore decided that it would also be difficult to improve the hearing loss by tympanoplasty in the left ear. The maximum speech discrimination score in the right ear was much worse than that in the left ear. We decided to perform the RW-Vibroplasty in the left ear.

The VSB was developed as an active middle ear implant to transmit vibration into the inner ear. It was

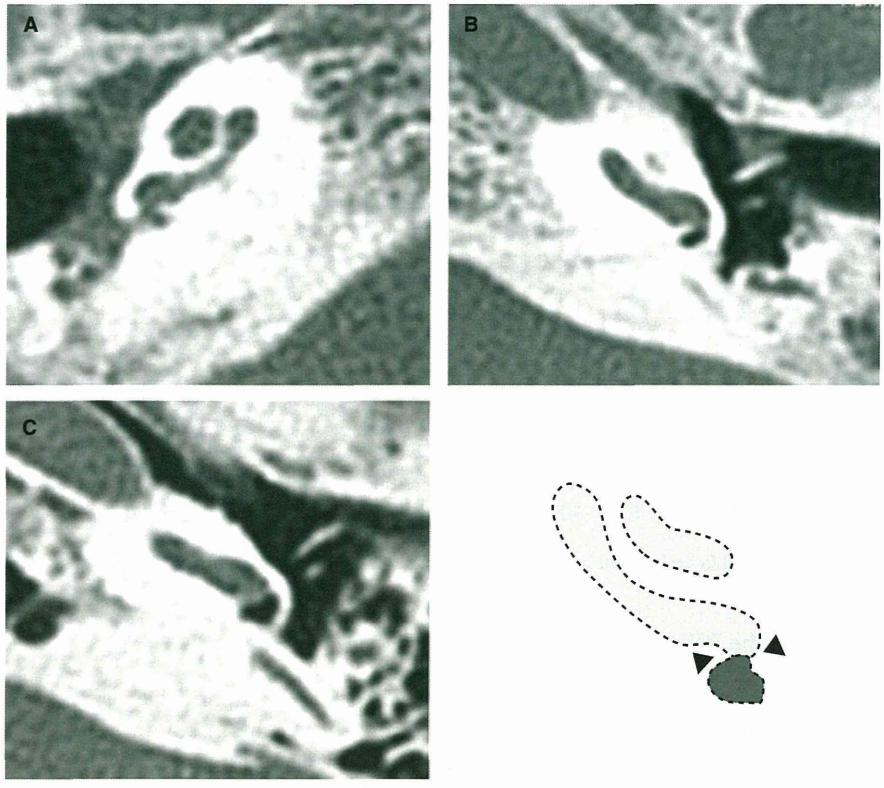

Figure 4. CT scan of region of the round window (RW) in our case. (A) Right ear, (B) left ear, and (C) control case. The RW in our case was remarkably small compared with the control case. The diameter of the RW is shown as arrowheads in the schematic representation.

originally coupled to the long process of the incus in patients with a sensorineural hearing loss and an intact ossicular chain. Recently, application of the middle ear implant has become an efficient procedure to restore mixed hearing loss in selected patients [2]. In these cases, the attachment point of the VSB's FMT has been modified. Different authors have described various modifications, including coupling

Table I. Outcomes of audiological tests in open field.

Parameter	Right ear	Left ear	Hearing aid (left ear)	VSB (left ear)
Max SD score	40%	60%	60%	70%
SD score in quiet				
60 dBSPL	0%	0%	45%	60%
70 dBSPL	0%	0%	55%	80%
80 dBSPL	5%	30%	65%	75%
SD score in noise				
S/N + 0 dB	0%	0%	20%	25%
S/N + 5 dB	0%	0%	30%	35%
S/N + 10 dB	0%	10%	35%	40%
Questionnaire in noise				
Train station platform			Not useful	Useful
Major intersection			Not useful	Useful
Rub sound of plastic bag			Not useful	Useful
Sound of dishes being washed			Not useful	Useful

SD: speech discrimination; VSB: Vibrant Soundbridge.

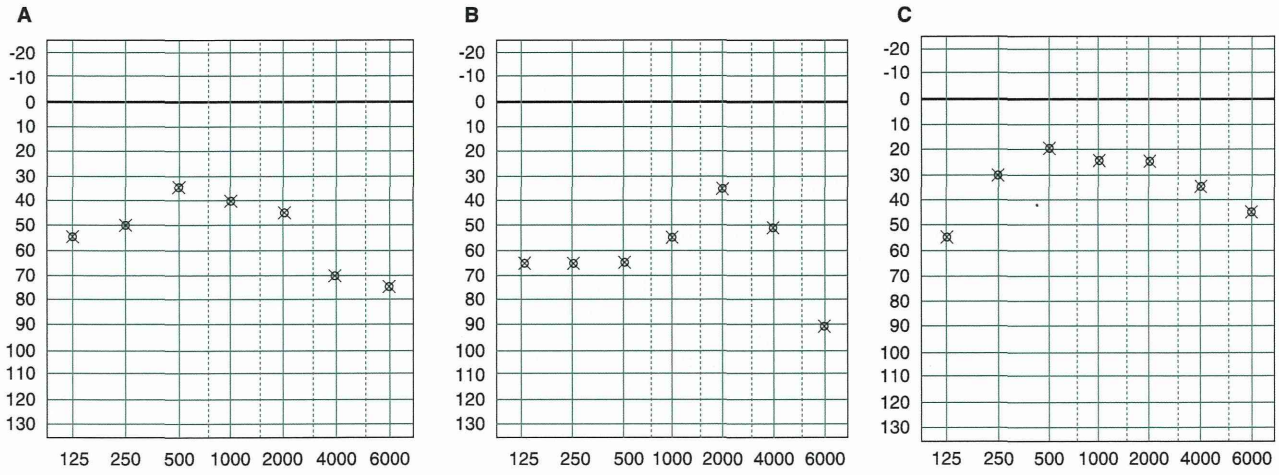

Figure 5. Warble tone threshold level. Compared with the threshold level of the conventional hearing aid (A), the threshold level of the VSB at the first fitting was improved at the high frequencies (B). After refitting, amplification at the low frequencies was elevated (C).

the FMT to the stapes suprastructure, a total ossicular replacement prosthesis (TORP), a partial ossicular replacement prosthesis (PORP), the oval window membrane, and the RW membrane [6-10]. In tympanosclerosis including sclerosis in the oval and round windows with mixed hearing loss, stapes surgery allows one to restore the conductive part of the hearing. However, air–bone gap closure does not systemically improve the auditory threshold enough to avoid use of conventional hearing aids. We considered the present case with mixed hearing loss induced by tympanosclerosis to have an indication for VSB, because the patient was unable to use a conventional hearing aid. We considered that an attachment technique of the FMT to a PORP or the RW membrane could be selected in the present case, because other attachment techniques require a mobile stapes footplate. However, there is no permission to use a PORP in Japan. Therefore, we had concluded that placing the FMT at the RW would improve the mixed hearing loss in the present patient with tympanosclerosis.

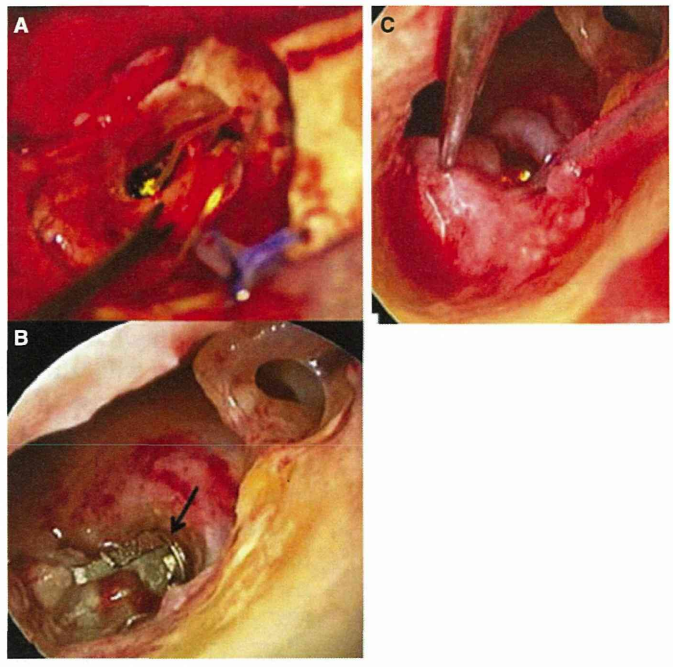

Figure 6. Floating mass transducer (FMT) with round window (RW)-Coupler placement in the RW. The FMT attached with RW-Coupler was placed in the RW (A) after good fitting of the RW-Coupler (arrow) was confirmed (B). The FMT was wrapped in perichondrium (C).

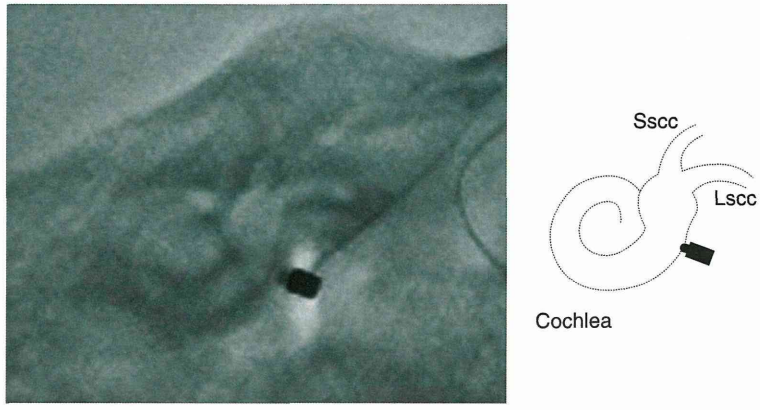

Figure 7. X-P finding of floating mass transducer (FMT) placement after VSB surgery. Good placement of the FMT with the round window (RW)-Coupler was confirmed. Scheme of X-P is shown. Lscc, lateral semicircular canal; Sscc, superior semicircular canal.

The attachment of the FMT on the RW was first described by Colletti et al. [2] and is now becoming more frequently used because of its effectiveness and wide range of applications in difficult to treat otologic cases. Clinical data reported by Rajan et al. [11] showed that either partial or complete direct contact of the FMT with the RW membrane increases relative coupling efficiency between the FMT and the RW membrane. The experimental findings in the cadaveric human temporal bone supported the assertion that no acoustic response was measured when the FMT was placed onto the promontory [12]. The postoperative migration of the FMT seems to occur within the first 6 months after surgery [11]. The obliteration of any dead space around the FMT minimizes the chance of FMT migration. Wrapping the FMT with the perichondrium has helped to prevent the migration. It is sometimes not possible to achieve FMT–RW membrane contact because of the depth of the RW niche and the mismatch between the diameter of the FMT and that of the RW membrane. Especially in cases of sclerosis in the RW, the sclerotic lesion should be drilled out to put the FMT in contact with RW membrane, but by so doing, the possibility of inducing a perilymphatic fistula increases. To make sure the FMT contacts the RW membrane while reducing the possibility of perilymphatic fistula, soft tissue such as fascia or perichondrium [7] and cartilage [11] as well as the RW-Coupler can be placed as a coupler between the FMT and RW membrane. However, soft tissue couplers seem to reduce the relative coupling efficiency when compared with direct placement of the FMT onto the RW membrane [11]. Recently, the RW-Coupler has been used as an alternative coupling aid to fit smaller RW diameters and incur less drilling in the RW niche. It is unclear how the RW-Coupler will affect the outcomes, and the present case may be the first report to show these clinical results. Direct coupling with the RW membrane would be expected to achieve complete closure of the air–bone gap, but a large fraction of the gain is required to overcome the soft tissue-related reduction of coupling efficiency [13,14]. In the present case, the perichondrium was placed between the RW membrane and the RW-Coupler attached to the FMT. The functional gain at low frequencies (0.25–1 kHz) after the first fitting according to the postoperative audiogram was less than the predicted ideal acoustic gain. Good functional gain was found at high

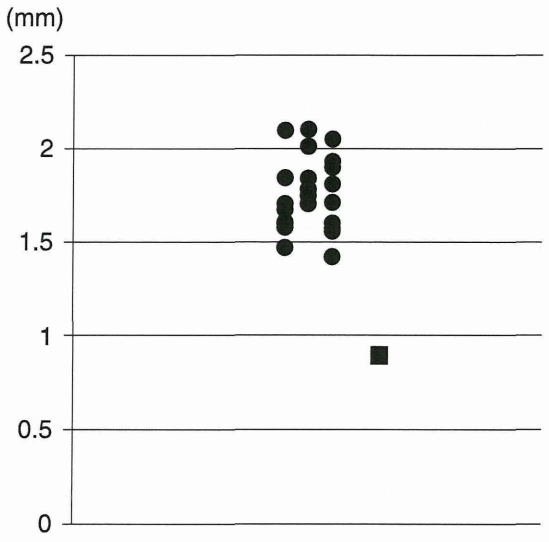

Figure 8. Diameter of the round window (RW) by radiologic evaluation of CT scan. The diameter was measured on the inferior edge of the RW in the transverse slice in the left ear. This case (black square) was remarkably small compared with control cases (black circles). Patients with sensorineural hearing loss were measured as control cases.

frequencies compared with conventional hearing aids. Therefore, we increased the gain offered by the audio processor at low frequencies, resulting in good speech discrimination scores. Beltrame et al. [13] described functional gain in VSB patients with a fixed stapes to be smaller than that in other patients. Consequently, the RW-Coupler has been available for patients with tympanosclerosis involving sclerosis in the RW, and more amplification from the VSB audio processor at low frequencies might be required to provide enough functional gain.

Acknowledgments

We thank Prof. Mueller Joachim for supporting the VSB surgery.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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PLoS one

Simultaneous Screening of Multiple Mutations by Invader Assay Improves Molecular Diagnosis of Hereditary Hearing Loss: A Multicenter Study

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Abstract

Although etiological studies have shown genetic disorders to be a common cause of congenital/early-onset sensorineural hearing loss, there have been no detailed multicenter studies based on genetic testing. In the present report, 264 Japanese patients with bilateral sensorineural hearing loss from 33 ENT departments nationwide participated. For these patients, we first applied the Invader assay for screening 47 known mutations of 13 known deafness genes, followed by direct sequencing as necessary. A total of 78 (29.5%) subjects had at least one deafness gene mutation. Mutations were more frequently found in the patients with congenital or early-onset hearing loss, i.e., in those with an awareness age of 0–6 years, mutations were significantly higher (41.8%) than in patients with an older age of awareness (16.0%). Among the 13 genes, mutations in *GJB2* and *SLC26A4* were mainly found in congenital or early-onset patients, in contrast with mitochondrial mutations (12S rRNA m.1555A>G, tRNA(Leu(UUR)) m.3243A>G), which were predominantly found in older-onset patients. The present method of simultaneous screening of multiple deafness mutations by Invader assay followed by direct sequencing will enable us to detect deafness mutations in an efficient and practical manner for clinical use.

Citation: Usami S-i, Nishio S-y, Nagano M, Abe S, Yamaguchi T, et al. (2012) Simultaneous Screening of Multiple Mutations by Invader Assay Improves Molecular Diagnosis of Hereditary Hearing Loss: A Multicenter Study. PLoS ONE 7(2): e31276. doi:10.1371/journal.pone.0031276

Editor: Francesc Palau, Instituto de Ciencia de Materiales de Madrid - Instituto de Biomedicina de Valencia, Spain

Received July 21, 2011; **Accepted** January 5, 2012; **Published** February 24, 2012

Funding: This work was supported by the (then) Ministry of Health and Welfare, Japan (http://www.mhlw.go.jp/english/) (S.U.) and a Grant-in-Aid for Scientific Research from the (then) Ministry of Education, Science and Culture of Japan (http://www.mext.go.jp/english/) (S.U.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and have the following conflicts. The authors did not receive funding from the Department of Clinical Genomics, Biomedical Laboratories, Inc. They felt that for genetic analysis of patients with hearing impairment in which many gene/gene mutations are involved, Invader Assay is the appropriate choice. However, for patent reasons, the authors cannot develop this method independently. The development of this method was therefore performed in collaboration with Biomedical Laboratories. This relationship had no influence on results and the direct sequencing results were all double checked for accuracy. Although Invader Assay is more efficient, if a method other than Invader Assay had been used, the results would have been identical.

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¶ Membership of the Deafness Gene Study Consortium is provided in the Acknowledgments.

Introduction

From a series of etiological studies, 60–70% of childhood hearing loss has been estimated to be of genetic etiology, with the rest due to environmental causes, including newborn delivery trouble, acoustic trauma, ototoxic drug use, and prenatal/postnatal infection [1]. However, until now, there has been no multicenter study based on genetic testing. Along with early discovery of hearing loss by newborn hearing screening programs and subsequent intervention programs, much attention has been paid to the determination of the hearing loss etiology. Therefore, genetic testing has become more important for highly accurate diagnosis, prediction of severity of hearing loss, estimation of associated abnormalities, selection of appropriate habilitation options, prevention of hearing loss, and better genetic counseling. Although more than one hundred loci have been mapped and 46 genes reported to be responsible for hereditary hearing loss (Hereditary Hearing Homepage; http://webh01.ua.ac.be/hhh/), many may cause similar phenotypes without any abnormality other than hearing loss. This genetic heterogeneity has made clinical application difficult, in spite of the considerable advances in discovery of deafness genes. We have previously established a screening strategy focusing on recurrent mutations and demonstrated its benefits for clinical application [2]. We carried out the current multicenter study to determine 1) whether the simultaneous screening of the multiple deafness mutations by Invader assay is applicable for clinical use, 2) whether the genetic etiology is truly prevalent among hearing loss patients and 3) whether genetic causes differ by ages.

Materials and Methods

Subjects and clinical status

As summarized in Table 1, two hundred sixty-four Japanese patients with bilateral sensorineural hearing loss from 33 ENT departments nationwide participated in the present study. We first applied the Invader assay for screening forty-seven known mutations of 13 known deafness genes, followed by direct sequencing as necessary.

Table 1. Clinical features of subjects in this study.

	Total (n = 264)	*Early onset (n = 141)*	*Late onset (n = 100)*
Severity of HL			
normal – moderate	148	58	78
severe – profound	95	70	21
unknown	21	13	1
Inheritance			
AD or Mitochondrial	38	9	24
AR or Sporadic	119	69	42
unknown	107	63	34
Other clinical features			
inner ear malformations	52	37	10
EVA	30	22	4
goiter	8	4	3
diabetes mellitus	14	3	11

HL: Hearing loss.
AD: Autosomal dominant.
AR: Autosomal recessive.
EVA: Enlarged vestibular aqueduct.
doi:10.1371/journal.pone.0031276.t001

Hearing loss was evaluated using pure-tone audiometry (PTA) classified by a pure-tone average over 500, 1000, 2000 and 4000 Hz in the better hearing ears. For children who were unable to be tested by PTA, we used an average over 500, 1000, 2000 Hz in either auditory steady-stem response (ASSR) or conditioned oriented reflex audiometry (COR), or the response threshold (dB) from auditory brainstem response (ABR). Computed tomography (CT) scans were performed to check for congenital inner ear anomalies.

Status of hearing loss in the 264 patients was: mild (21–40 dB) in 39 patients (14.7%), moderate (41–70 dB) in 84 (31.8%), severe (71–94 dB) in 39 (14.8%) and profound (>95 dB) in 56 patients (21.2%). Twenty-four subjects were classified as having normal hearing due to a specific audiogram with hearing loss only in the high or low frequency portions. With regard to onset age (the age of awareness), 141 patients had early onset deafness (below 6 y.o.), 100 had late onset deafness, and the rest had unknown onset ages.

The inheritance composition of the subjects was as follows: 38 subjects from autosomal dominant or mitochondrial inherited families (two or more generations affected); 119 subjects from autosomal recessive families (parents with normal hearing and two or more affected siblings) or subjects with sporadic deafness (also compatible with recessive inheritance or non-genetic hearing loss). None of the patients had an X-linked pattern of inheritance. The numbers of patients with other manifestations were inner ear malformations (52), enlarged vestibular aqueduct (EVA) (30), goiter (8), and diabetes mellitus (14). None of the patients had typical clinical features of Usher syndrome or BOR syndrome.

All subjects gave prior informed consent for participation in the project and the Ethical Committee of Shinshu University as well as the relevant bodies of the participating institutions of the Deafness Gene Study Consortium approved the study.

Invader assay

Invader technology is convenient for mutation genotyping, offering a simple diagnostic platform to detect single nucleotide changes with high specificity and sensitivity from unamplified genomic DNA.

We applied the Invader assay for screening forty-seven known mutations of 13 known deafness genes [*GJB2*(NM_004004.5), *SLC26A4*(NM_000441.1), *COCH*(NM_001135058.1), *KCNQ4*(NM_172163.2), *MYO7A*(NM_000260.3), *TECTA*(NM_005422.2), *CRYM* (NM_001888.3), *POU3F4*(NM_000307.3), *EYA1*(NM_172060.2), mitochondrial 12 s ribosomal RNA, mitochondrial tRNA(Leu), mitochondrial tRNA(Ser), and mitochondrial tRNA(Lys)] (Table 2). Mutations were selected on the basis of a mutation/gene database established in the Japanese deafness population. The detailed methodological protocol was described elsewhere [2]. In brief, 1.2 ul of primary probe/Invader oligonucleotides mixture (containing 0.5 umol/l wild type primary probes, 0.5 umol/l mutant primary probe, 0.05 umol/l Invader oligonucleotide, and 10 mmol/l MOPS) were poured into each well of 384-well plates. Fluorescent resonance energy transfer (FRET)/Cleavase mixture (Third Wave Technologies, Madison, WI) was added to the probe/Invader oligonucleotide-containing plates. Then, 3 ul of 5–100 fmol/l synthetic target oligonucleotides (positive control), 10 ug/ml yeast tRNA (no target control), and denatured genomic DNA samples (>15 ng/ul) were added. Next, 6 ul of mineral oil (Sigma, St. Louis, MO) were overlayed into all reaction wells and incubated at 63°C for 4 hour. After incubation fluorescence was measured by a Cyto Fluor 4000 fluorescent micro plate reader (Applied Biosystems, Foster CA). The heteroplasmy rate for mitochondrial mutations was quantified by detection of fluorescently labeled and digested PCR products through a fluorescence imaging system [2].

Direct sequencing

Dominant mutations and mitochondrial mutations are themselves diagnostic criteria for molecular diagnosis. But a hallmark of recessive mutations, in *GJB2* and *SLC26A4* for example, is the detection of two mutations in the paternal and maternal alleles. In this study, direct sequencing was further carried out as follows: 1) *GJB2* mutation analysis for all subjects, because the authors wanted to clarify whether the number of mutations on the invader panel are enough (saturated) or not. 2) *SLC26A4* mutation analysis for all the subjects with EVA, 3) *SLC26A4* mutation analysis for heterozygous patients for these genes. DNA fragments containing the entire coding region were sequenced as described elsewhere [3,4].

Results

The mutations found by Invader assay and direct sequencing in this study are summarized in Table 2 and 3.

Invader Assay

A total of 74 (28.0%) hearing-impaired subjects (n = 264) were found to have at least one deafness gene mutation. Among the deafness genes situated on the present diagnostic panel, mutations were most frequently found in the *GJB2* gene. Screening of *GJB2* showed mutations of one or both alleles of the gene in 43 (43/264; 16.2%) samples from the subjects, of which 13 cases had only a single mutation, and 30 cases were compound heterozygotes or homozygotes, confirmed by segregation analysis (Table 4). The most common mutation was c.235delC, accounting for nearly 67% (29/43) of all *GJB2* mutated patients. On the other hand, the *GJB2*: c.35delG mutation, which is known to be the most common mutation in Caucasian or other ethnic populations, was not found in this group. The second most common group of *GJB2* mutations consisted of p.[G45E; Y136X], p.V37I, and c.299_300del. These mutations were detected in more than 5 patients each, and their allele frequencies were relatively high. Three mutations (p.T86R, p.R143W, and c.176_191del) were observed in more than one

Table 2. Mutation list of Invader based genetic screening test.

Gene	*Exon*	*Codon location*	*Nucleotide change*	*Frequency of mutant alleles (n = 528)*	*Number of patients with mutations (n = 264)*
GJB2	exon 2	p.L79fs	c.235delC	43 (8.1%)	29 (10.9%)
GJB2	exon 2	p.V37I	c.109G>A	7 (1.3%)	6 (2.3%)
GJB2	exon 2	p.[G45E; Y136X]	c.[134G>A; 408C>A]	10 (1.9%)	10 (3.8%)
GJB2	exon 2	p.G59fs	c.176_191del	3 (0.6%)	3 (1.1%)
GJB2	exon 2	p.R143W	c.427C>T	4 (0.8%)	4 (1.5%)
GJB2	exon 2	p.H100fs	c.299_300del	5 (0.9%)	5 (1.9%)
GJB2	exon 2	p.T123N	c.368C>A	4 (0.8%)	4 (1.5%)
GJB2	exon 2	p.T86R	c.257C>G	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.F191L	c.570T>C	0	0
GJB2	exon 2	p.I71T	c.212T>C	0	0
GJB2	exon 2	p.A49V	c.146C>T	0	0
GJB2	exon 2	p.G12fs	c.35delG	0	0
SLC26A4	exon 19	p.H723R	c.2168A>G	22 (4.1%)	17 (6.4%)
SLC26A4	int 7/exon 8	splice site	c.919-2A>G	2 (0.4%)	2 (0.8%)
SLC26A4	exon 7	p.T410M	c.1229C>T	4 (0.8%)	3 (1.1%)
SLC26A4	exon 7	p.V306fs	c.917insG	0	0
SLC26A4	exon 19	p.T721M	c.2162C>T	0	0
SLC26A4	exon 8/int 8	splice site	c.1001+1G>A	0	0
SLC26A4	exon 9	p.A372V	c.1115C>T	0	0
SLC26A4	exon 5	p.M147V	c.439A>G	1 (0.2%)	1 (0.4%)
SLC26A4	int 5/exon 6	splice site	c.601-1G>A	0	0
SLC26A4	exon 9	p.K369E	c.1105A>G	1 (0.2%)	1 (0.4%)
SLC26A4	exon 15	p.S551fs	c.1652insT	1 (0.2%)	1 (0.4%)
SLC26A4	exon 15	p.C565Y	c.1693G>A	0	0
SLC26A4	exon 17	p.S666F	c.1997C>T	0	0
SLC26A4	exon 19	p.E704fs	2111ins GCTGG	1 (0.2%)	1 (0.4%)
SLC26A4	exon 4	p.L108fs	c.322delC	0	0
SLC26A4	exon 4	p.P123S	c.367C>T	0	0
SLC26A4	exon 10	p.N392Y	c.1174A>T	0	0
SLC26A4	exon 17	p.S610X	c.1829C>A	0	0
SLC26A4	exon 17	p.S657N	c.1970G>A	0	0
EYA1	exon 12	p.D396G	c.1187A>G	0	0
EYA1	exon 8	p.R264X	c.790C>T	0	0
EYA1	exon 7	p.Y193X	c.579C>G	0	0
COCH	exon 5	p.A119T	c.441G>A	0	0
KCNQ4	exon 5	p.W276S	c.827G>C	0	0
MYO7A	exon22	p.A886fs	c.2656_2664del	0	0
TECTA	exon 16	p.R1773X	c.5318C>T	0	0
TECTA	exon 20	p.R2121H	c.6063G>A	0	0
Mitochondrial 12S rRNA			m.1555A>G	-	5 (1.9%)
Mitochondrial tRNALeu			m.3243A>G	-	6 (2.3%)
Mitochondrial tRNASer			m.7445A>G	-	0
Mitochondrial tRNALys			m.8296 A>G	-	0
CRYM	exon 8	p.K314T	c.941 A>C	0	0
CRYM	exon 8	p.X315Y	c.945 A>T	0	0

doi:10.1371/journal.pone.0031276.t002

Table 3. Mutation list found by direct sequencing analysis.

Gene	*Exon*	*Codon location*	*Nucleotide change*	*Frequency of mutant alleles (n = 528)*	*Number of patients with mutations (n = 264)*
GJB2	exon 2	p.T8M	c.23C>G	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.K12fs	c.35insG	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.F106Y	c.317T>A	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.A171fs	c.511insAACG	2 (0.4%)	2 (0.8%)
GJB2	exon 2	p.C174S	c.522G>C	1 (0.2%)	1 (0.4%)
SLC26A4	exon 14	p.S532I	c.1595G>T	2 (0.4%)	2 (0.8%)
SLC26A4	exon 16	p.R581S	c.1743G>C	1 (0.2%)	1 (0.4%)
SLC26A4	exon 17	p.V659L	c.1975G>C	2 (0.4%)	2 (0.8%)
SLC26A4	exon 10	p.L407fs	c.1219delCT	1 (0.2%)	1 (0.4%)
SLC26A4	exon 15/int 15	splice site	c.1931+5 G>A	5 (0.9%)	4 (1.5%)

doi:10.1371/journal.pone.0031276.t003

patient. p.F191L, p.I71T, p.A49V and c.35delG mutations were not found. One pair of p.[G45E; Y136X] mutations was detected among 10 persons in a heterozygous state. Subsequent parental DNA segregation study through direct sequencing indicated two mutations were in *cis*. The p.T123N mutation was found in 4 subjects but, based on our recent study, is not likely to be a pathologic mutation [5].

The second most frequent gene with mutations was the *SLC26A4* gene (23/264; 8.7%). Five cases were homozygotes of p.H723R, one was a homozygote of p.T410M, 3 were compound heterozygotes, and 14 had only one mutation of *SLC26A4* (Table 4). Of the 19 *SLC26A4* mutations, 12 (c.917insG, p.T721M, c.1001+1G>A, p.A372V, c.601-1G>A, p.C565Y, p.S666F, c.322delC, p.P123S, p.N392Y, p.S610X, and p.S657N) were not found in any samples, but the remaining 7 *SLC26A4* mutations were confirmed in more than one subject. Especially, the p.H723R mutation was found to be in high allele frequency (4.1%). All of the patients with *SLC26A4* mutations had EVA, which has been demonstrated to be a result of the mutations of this gene. *SLC26A4* mutations were detected by Invader assay in 63.6% of the patients with EVA.

Mitochondrial m.1555A>G mutations were found in 1.9% (5/264) of the patients and the m.3243A>G mutation was identified in 2.3% (6/264).

Mutations in nine deafness genes (*COCH*, *KCNQ4*, *MYO7A*, *TECTA*, *CRYM*, *POU3F4*, *EYA1*, mitochondrial tRNA(Lys) m.8296A>G, mitochondrial tRNA(Ser) m.7445A>G) were not identified in any patients (Table 2).

Notably, 4 subjects were found to have double gene mutations. Two cases were *SLC26A4* compound heterozygous or homozygous mutations with a *GJB2* heterozygous mutation. One case was a compound heterozygous of *GJB2* with a *SLC26A4* heterozygous mutation and the remaining case was a *GJB2*

Table 4. Diagnostic efficiency of Invader assay alone and Invader assay and direct sequencing.

	Total (n = 264)	*Early onset (n = 141)*	*Late onset (n = 100)*
Invader assay alone			
GJB2 homozygote/compound heterozygote	30 (11.4%)	29 (20.6%)	1 (1.0%)
GJB2 heterozygote	13 (4.9%)	7 (5.0%)	6 (6.0%)
SLC26A4 homozygote/compound heterozygote	9 (3.4%)	9 (6.4%)	0 (0%)
SLC26A4 heterozygote	14 (5.3%)	10 (27.1%)	2 (2.0%)
Mitochondria A1555G	5 (1.9%)	2 (1.4%)	2 (2.0%)
Mitochondria A3243G	6 (2.2%)	1 (0.7%)	5 (5.0%)
Total	74 (28.0%)*	55 (39.0%)*	16 (16.0%)
Invader assay and direct sequencing			
GJB2 homozygote/compound heterozygote	33 (12.5%)	31 (21.9%)	2 (2.0%)
GJB2 heterozygote	13 (4.9%)	7 (5.0%)	5 (5.0%)
SLC26A4 homozygote/compound heterozygote	18 (6.8%)	18 (12.7%)	0 (0%)
SLC26A4 heterozygote	7 (2.7%)	4 (2.8%)	2 (2.0%)
Mitochondria A1555G	5 (1.9%)	2 (1.4%)	2 (2.0%)
Mitochondria A3243G	6 (2.2%)	1 (0.7%)	5 (5.0%)
Total	78 (29.5%)**	59 (41.8%)**	16 (16.0%)

*Three cases carried double mutations (cases 1 to 3 in Table 5).
**Four cases carried double mutations shown in Table 5.
doi:10.1371/journal.pone.0031276.t004

Table 5. Double mutation cases found in simultaneous mutation screening.

Genotype	*Patients Number*
GJB2:p.[V37I];[V37I]; Mitochondria m.1555A>G	1 (0.4%)
GJB2:c.[235delC];p.[R143W]; *SLC26A4*:p.[M147V]	1 (0.4%)
GJB2:p.[V37I]; *SLC26A4*:p.[H723R];[H723R]	1 (0.4%)
GJB2:p.[F106Y]; *SLC26A4*:p.[H723R]; c.[1931+5G>A]	1 (0.4%)
Total	4 (1.5%)

doi:10.1371/journal.pone.0031276.t005

homozygous mutation with a mitochondrial 1555A>G mutation (Table 5).

The detection rate of mutations was 40.4% for the patients with congenital or early-onset hearing loss, i.e. in those with an awareness age of 0~6 years. The rate in congenital hearing loss patients also increased when restricting to the patients with moderate or more severe hearing loss (>50 dB; 40.7%) or severe hearing loss (>70 dB; 44.3%) (Fig. 1). In contrast, the detection rate was only 16.0% in the patients with an older age of onset/awareness (Fig. 1). Among the 13 included genes, mutations is *GJB2* and *SLC26A4* were mainly found in congenital patients or early-onset patients, in contrast with mitochondrial mutations, such as 12S rRNA m.1555A>G or tRNA(Leu(UUR)) m.3243A>G, which were predominantly found in older-onset patients (Table 4). The p.V37I mutation in the *GJB2* gene was also found in older-onset patients (data not shown).

With regard to the relationship between radiographic findings and genetic testing, the mutation detection rate was elevated when restricting to the patients with inner ear anomaly (50.0%) and EVA (63.6%) (Fig. 2).

Direct sequencing

Direct sequencing identified 9 mutations in 15 cases which were not included in the Invader assay panel and improved the mutation detection/diagnostic rate obtained by Invader assay analysis (28.0%/18.6%) to 29.5%/22.7%. (Fig. 1). Combining direct sequencing with invader screening enhanced the diagnostic rate notably but not the mutation detection rate. In detail, direct sequencing identified additional mutations in three cases with single *GJB2* mutations by Invader assay that were finally diagnosed as compound heterozygous mutations of *GJB2* (p.[T86R]; c.[511insAACG], p[T8M];[V37I] and c.[35insG];[235delC]).

In 7 cases only a single *SLC26A4* mutation was found by invader assay, and additional mutations were found by direct sequencing (two cases of p.[H723R];c[1931+5G>A] and one each cases of p.[R581S];[H723R], p.[V659L];[H723R], p.[S532I]; c.[2111insGCTGG], p.[T410M]; c.[1931+5G>A] and p.[K396E];[S532I]). Two cases carried EVA but without any mutations found in Invader assay, c[1931+5G>A]; [1931+5G>A] and p.[V659L];c[1219 delCT] compound heterozygous mutations were found by direct sequencing. With the combination of Invader assay and direct sequencing, and restriction to patients with EVA, the mutation detection rate was elevated to 17/22 cases (77.3%, Fig. 2). Fifteen of them carried homozygous or compound heterozygous *SLC26A4* mutations.

Discussion

We previously reported that simultaneous detection of common deafness gene mutations has excellent sensitivity and accuracy [2]. In this study, using samples from patients at 33 institutions nationwide from northern to southern Japan, we confirmed that the Invader assay based on the Japanese deafness gene mutation database works efficiently in the clinical base to detect the responsible gene mutations from the patients with

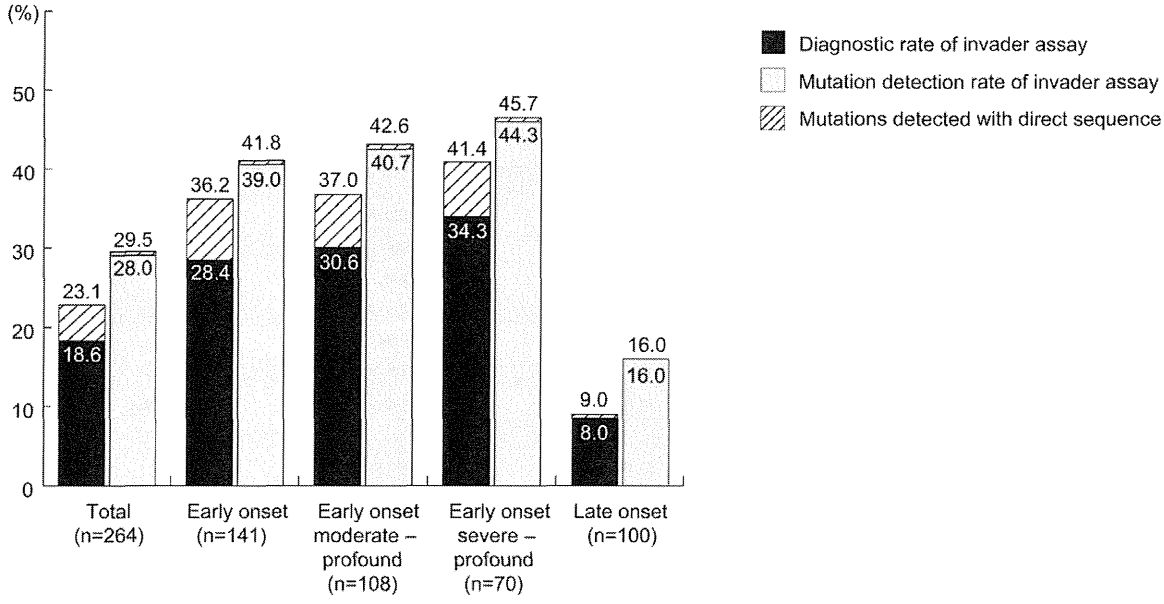

Figure 1. Detection rate by onset/awareness age and severity of hearing loss. Diagnostic rates and detection rates of this simultaneous multiple mutations screening and direct sequencing for biallelic mutations in autosominal recessive genes or mitochondrial mutations increased when restricted to congenital/early-onset hearing loss, and moderate or severe hearing loss. Combined direct sequence and invader screening enhanced the diagnostic rate but not the mutation detection rate.
doi:10.1371/journal.pone.0031276.g001