Table 2. The frequency of GJB2 mutations and diagnostic age

	GJB2 mutations	Homozygote	Compound heterozygote	Heterozygote
Total (n = 1343)	191 (14.2%)	38 (2.8%)	63 (4.7%)	90 (6.7%)
0–3 y.o. (n = 420)	108 (25.7%)	32 (7.6%)	47 (11.2%)	29 (6.9%)
4–5 y.o. (n = 101)	15 (14.9%)	1 (0.99%)	6 (5.9%)	8 (7.9%)
≥6 y.o. (n = 627)	49 (7.8%)	3 (0.48%)	4 (0.64%)	42 (6.7%)
Unknown (n = 195)	19	2	6	11

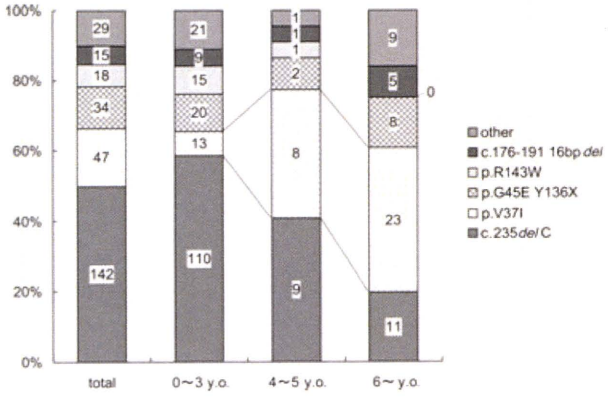

Fig. 1. Frequency of mutant GJB2 alleles in different diagnostic age groups. c.235*del*C was mainly found in the group diagnosed at up to 3 years, where it was significantly higher than in age 6 and over ($p < 0.01$; χ^2 test). On the contrary, p.V37I was mainly found in the diagnostic age groups of 4–5, and 6 and over, at a rate significantly higher than in up to age 3 ($p < 0.01$).

significantly lower rate (7/75: 9.3%) than in all bilateral hearing loss probands (520/1022: 50.9%) ($p < 0.001$; χ^2 test). Concerning episodes of vestibular dysfunction, only 4% (3/75) of those with biallelic *GJB2* mutations had vertigo, dizziness, or faintness, while 25.1% of all hearing loss probands (258/1029) had vertigo ($p < 0.001$; χ^2 test). Inner ear abnormalities were significantly lower in patients with biallelic *GJB2* mutations (5/62: 8.1%) than in all bilateral hearing loss probands (126/599: 21%) ($p = 0.014$; χ^2 test). In the five patients with biallelic *GJB2* mutations who had inner ear abnormalities, enlarged vestibular aqueduct (EVA) was found in three and the other two had hypoplasty of the cochlea and semicircular canals.

Discussion

GJB2 mutations were found in 14.2% of our bilateral hearing loss probands and 25.2% of those diagnosed at age 0–3 (for practicality categorized as congenital hearing loss). In previous studies in East Asia (1–6), frequency of *GJB2* mutations ranged from 10% to 38% in smaller cohorts. In the present large study using Japanese hearing loss patients collected from multiple centers, we could more accurately estimate the frequency of *GJB2* mutations in Japan and the mutation spectrum. We also found two novel mutation candidates, p.N54S and p.M195V, which cause non-conservative amino acid changes.

In Asian populations, c.235*del*C is the most common *GJB2* mutation, and its allele frequency in patients ranges from about 5% to 22% (1–7). The present study reconfirmed this mutation's high frequency in the Japanese hearing loss population. c.235*del*C accounted for 5.3% of the deafness alleles in all patients and 13.1% of those in patients diagnosed at age 0–3.

The p.V37I mutation was originally reported as a polymorphism (8); however, recent reports tend to consider it pathogenic with a milder phenotype (9–12) and this was supported by our results.

Only four out of twenty-six probands showed progressive hearing loss, and bilateral progression was found in only two of those, with a deterioration of less than 20 dB. Therefore, our study supports the previously reported notion that hearing loss due to *GJB2* mutations is typically non-progressive (13–15). With regard to the milder phenotype of p.V37I, none of the five patients with this mutation showed progression. We conclude that this mutation causes milder congenital hearing loss which may not be noticed until age 4 or older.

However, even though it was the second most frequent allele in the hearing loss patients, the p.V37I allele was the most frequent in the control subjects. This may be due to the milder phenotype and non-progression of patients with p.V37I mutation, who therefore either do not visit ENT clinics or do not receive a recommendation for genetic testing from clinicians. Therefore, ENT clinicians should bear in mind the existence of the milder phenotype caused by the p.V37I mutation.

We found that patients with c.235*del*C/p.R143W were significantly more severely affected than those with other c.235*del*C-containing phenotypes. A recent study also reported that the hearing level of c.35*del*G/p.R143W is significantly worse than that of homozygous c.35*del*G (9).

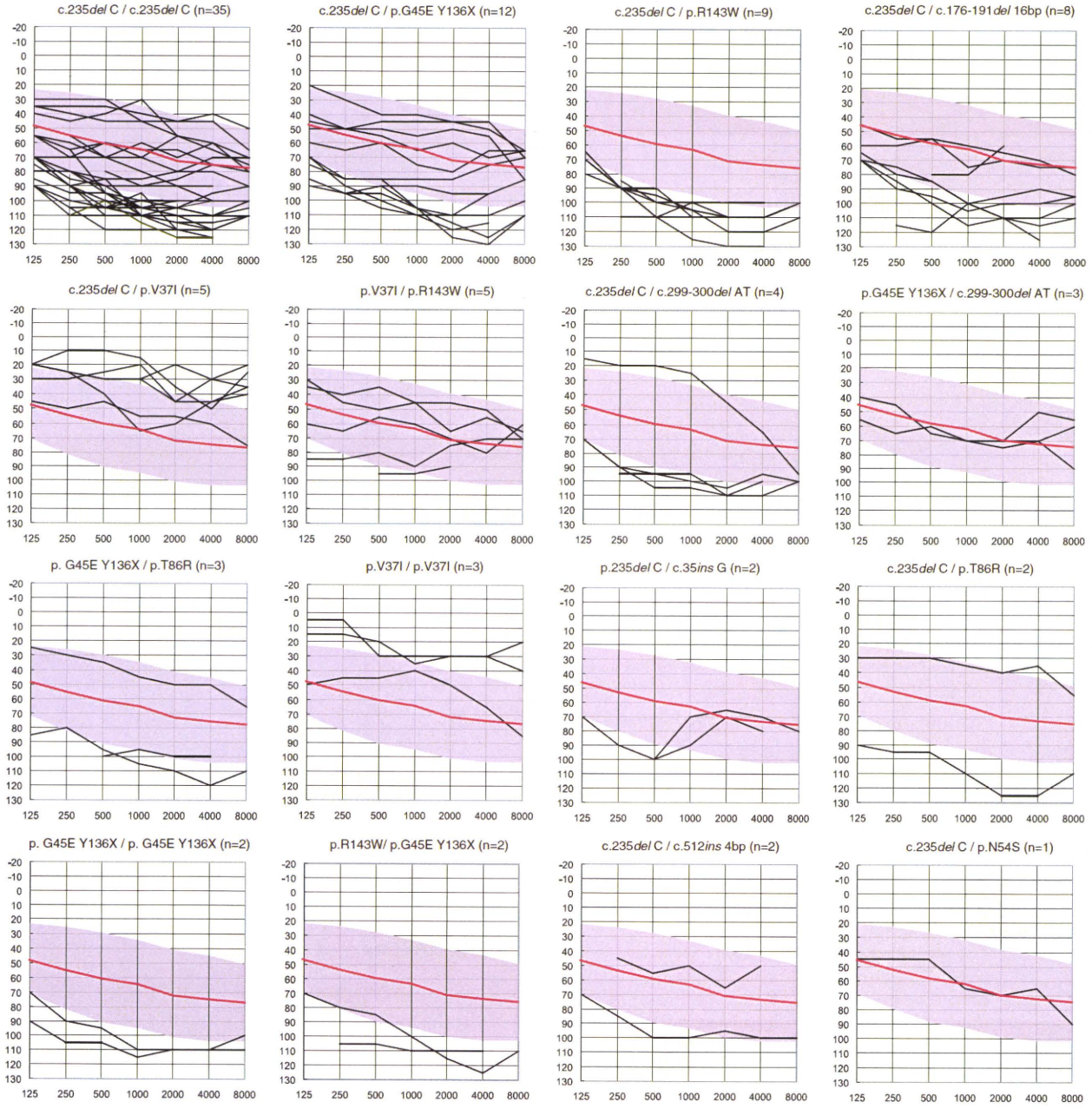

Fig. 2. Overlapping audiograms from the better ear for each genotype. The average audiogram from all subjects (1343 with bilateral sensorineural hearing loss) is indicated by a red line with standard deviation (shadow).

We compared homozygous for c.235*del*C with compound heterozygous with p.R143W (except for the p.V37I allele, which is thought to be a milder phenotype), finding the hearing level of the latter to be significantly worse. Also, comparing only the milder p.V37I allele, the hearing level of p.V37I/p.R143W was worse than that of p.V37I/p.V37I and p.V37I/c.235*del*C. These results suggest that p.R143W leads to a worse phenotype than other *GJB2* mutations.

The majority of our probands did not have tinnitus or vestibular dysfunction. Only 8% (5/65) of the patients with biallelic *GJB2* mutations had inner ear malformation, significantly lower than in the overall population with bilateral hearing loss, and in accordance with previous reports (14, 16, 17). Hearing loss patients with *GJB2* mutations also had a near absence of tinnitus, vestibular dysfunction and inner ear malformations.

In conclusion, our results describe the frequency of *GJB2* mutations and associated clinical features in a large Japanese cohort. Recently, based on our database of mutation spectrums found in Japanese, we have developed a genetic test for use in

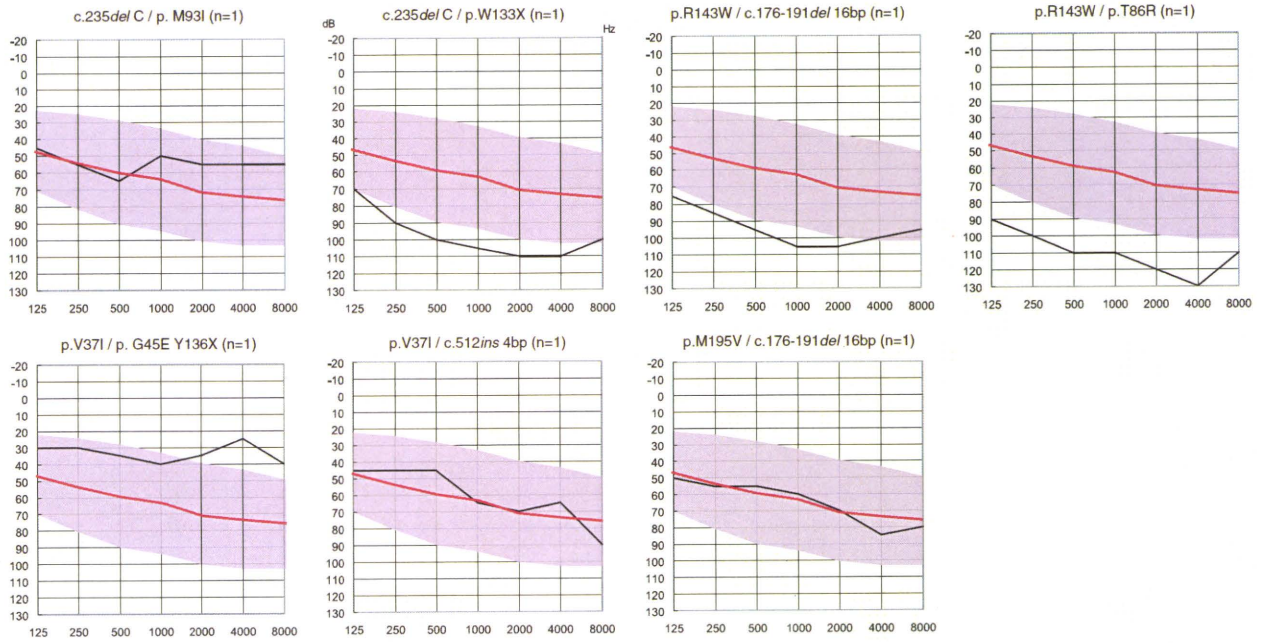

Fig. 2. Continued

diagnostic screening for hearing loss based on the invader assay (18). This database will also facilitate clinical application, and we intend to expand it to cover all Asian populations.

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Conflict of interest

We, the authors, declare that there were no conflicts of interest in conjunction with this paper.

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Genetic background of candidates for EAS (Electric-Acoustic Stimulation)

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Abstract

Objective: There is a certain number of patients with so-called ski-slope hearing loss, in which there is good hearing for lower frequencies in spite of little/no hearing in high frequencies. EAS (electric-acoustic stimulation) has recently been introduced for such patients with residual hearing at lower frequencies. Ski-slope hearing loss can have either a progressive nature or can be rather stable; therefore, decisions regarding timing of surgery are sometimes hampered. One advantage of genetic testing is that the possible prognosis for hearing, i.e. progressive or not, can be predicted for individual patients. The present study was performed to estimate the frequency of ski-slope hearing loss and investigate the genetic background of candidates for EAS. *Study Design:* Using a 2587 subject DNA database of sensorineural hearing loss patients, 1) frequency of patients with ski-slope hearing loss, 2) their clinical features including inheritance mode, onset ages, and progression, and 3) involvement of four common genes with mutations in Japanese hearing loss patients, were evaluated. *Results:* One hundred and fifty-one out of 2587 subjects fulfilled the audiological criteria for EAS. The frequency of patients possibly meeting the criteria for EAS was estimated to be 9.1% by restriction to probands only (139/1520). Various inheritance modes and onset ages were noted, with earlier onset in the patients with sporadic/recessive inheritance mode. Progressiveness was recognized in 56% of the patients. Genetic analysis identified mutations in 26.6% of the patients, including the mitochondrial 1555A>G mutation, and mutations in *SLC26A4, CDH23*, and *GJB2* genes, suggesting that at the least, these four genes may be involved in a certain group of patients, but also leaving possible genetic causes in the majority of the patients undetermined. *Conclusion:* As most of the patients showed a progressive nature in their hearing, genetic testing adds important additional information for candidates for EAS.

Key words: *ski-slope hearing loss, high frequency hearing loss, partial deafness, cochlear implantation*

Introduction

Cochlear implantation is currently the only available device for profound hearing loss patients and therefore has become a standard treatment choice worldwide. Although cochlear implantation has long been applied for patients with severe or profound hearing loss in all frequencies, recent advances in combined electric and acoustic stimulation (EAS) provide a chance of better speech perception for individuals with so-called ski-slope hearing loss. Selection criteria and decision making are sometimes difficult because of individual differences in progression, which is sometimes of a rather rapid progressive nature but other times rather stable. One advantage of genetic testing is that the possible prognosis for hearing, i.e. progressive or not, can be predicted for individual patients. Regarding genes responsible for hearing loss patients, to date, mutations in *GJB2* and *SLC26A4*, and the 1555A>G mutation in the mitochondrial 12S rRNA were found to be the major causes of hearing loss in Japanese patients (1). To date, no study has treated ski-slope hearing loss from an etiological viewpoint. The present study was performed to estimate the frequency of ski-slope hearing loss, audiological characteristics, and genetic background of candidates for EAS.

Subjects and methods

A 2587 subject DNA database of bilateral sensorineural hearing loss patients established by Shinshu University in collaboration with 33 ENT departments (mostly university hospitals) in Japan was used in this study. The database comprises 1520 unrelated Japanese probands (who had made their initial visit to a hospital) and their family members, with various inheritance modes and ages of onset. The composition of the 1520 probands was as follows: 355 subjects

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from autosomal dominant or mitochondrial families (two or more generations affected); 282 subjects from autosomal recessive families (parents with normal hearing and two or more affected siblings); and 738 subjects with sporadic deafness (also compatible with recessive inheritance or non-genetic hearing loss). All subjects gave prior informed consent for participation in the project and the ethics committee of each hospital approved the study.

Audiological selection criteria were based on the pure tone audiogram selection criteria as follows. Pure tone hearing levels were required to be 65dB or under HL for 125 Hz, 250 Hz and 500 Hz; 80dB HL or over for 2000 Hz; 85dB HL or over for 4000 Hz and 8000 Hz. Subjects with one of the above mentioned frequencies being out of the criteria limits by 10dB were included as potential candidates.

Mutation screening for *GJB2*, *SLC26A4*, and the 1555A>G mutation in the mitochondrial 12S rRNA, was performed in all of the patients as follows. Direct sequencing was used for *GJB2* (2), and restriction fragment length polymorphism (RFLP) was used for the 1555A>G mitochondrial mutation, as previously described (3). In patients with enlarged vestibular aqueduct (EVA), direct sequencing was used for *SLC26A4* because mutations in this gene have been restricted to the patients with this particular anomaly (4,5).

For other minor responsible genes, frequencies are relatively small, and therefore one-by-one gene screening was performed in limited numbers of patients (64–319 patients depending on the gene) (see reference (1)). For *CDH23*, 64 probands were analyzed using direct sequencing (6).

Results

One hundred and fifty-one (5.8%) out of the 2587 subjects registered in our database fulfilled the audiological criteria for EAS. The frequency of bilateral sensorineural hearing loss patients in the basic clinical population who may meet the criteria for EAS was estimated to be 9.1% by restriction to probands only (139/1520).

Regarding inheritance mode, 53% (74/139) of these patients had sporadic/recessive inheritance, 28% (39/139) dominant/mitochondrial inheritance, and in 19% (26/139) family history was unavailable (Table I).

Table I. Inheritance mode of candidates for EAS ($n=139$).

Inheritance mode	Number (%)
Sporadic/recessive	74 (53%)
Dominant/mitochondrial	39 (28%)
Data unavailable	26 (19%)

Onset ages are shown in Table II. Onset ages were varied, and earlier onset ages were evident in the patients with sporadic/recessive inheritance mode.

Progressiveness was recognized in 56% (78/139) of the patients, regardless of inheritance mode (54% for sporadic/recessive inheritance, and 56% for dominant/mitochondrial) (Table III).

Genetic analysis identified mutations in approximately 27% of the 145 patients, including the mitochondrial 1555A>G mutation ($n=18$, 12.9%), *SLC26A4* ($n=10$, 7.2%), *CDH23* ($n=6$, 4.3%) and *GJB2* mutations ($n=3$, 2.2%) (Table IV). Among the 2587 subjects, 178 were associated with the 1555>G mitochondrial mutation, 153 subjects harbored biallelic *GJB2* mutations, 61 subjects biallelic *SLC26A4* mutations, and eight biallelic *CDH23* mutations. Overlapped audiograms as well as average audiograms are shown in Figure 1A–D. Candidates rates (number of candidates/total patients with mutations) were high among the patients with the 1555A>G mitochondrial mutation (10.1%, 18/178), *SLC26A4* (16.4%, 10/61) and *CDH23* mutations (75%, 6/8) and low among the patients with *GJB2* mutations (2.0%, 3/153).

Discussion

There is a certain number of patients with residual hearing (sometimes normal or slightly elevated thresholds) at the lower frequencies, and profound deafness at the higher frequencies (the so-called ski-slope type hearing loss or partial deafness). Most of these patients do not show any abnormal pronunciation of consonants, indicating that they likely acquired progressive hearing loss at the higher frequencies. In spite of being hard of hearing due to the high-frequency involved hearing loss, they usually do not use hearing aids or use only standard hearing aids with limited efficiency. These cases also do not meet criteria for traditional cochlear implantation.

Recent advances in surgical technique, and electrode design, and newly developed devices enable preservation of residual hearing (see reference 7, for review). The concept of EAS has expanded indications for cochlear implantation from profoundly deaf patients in all frequencies to patients with residual hearing at the lower frequencies. According to the present data based on a multicenter collaborative study, 9.1% of the patients who visited the academic referral center were estimated to fulfill the audiological criteria for EAS.

There has been no aetiological study of ski-slope hearing loss, and although symmetrical audiograms strongly indicate the majority of cases are due to genetic causes, there have been few reports

Table II. Onset ages of the candidates for EAS (n=139).

	Number (%)					
Inheritance mode	–2 y.o	3-10	11-30	31-50	51-	Unknown
Sporadic/recessive	24 (32%)	12 (16%)	16 (22%)	7 (9%)	5 (7%)	10 (13%)
Dominant/mitochondrial	7 (18%)	12 (30%)	9 (23%)	6 (16%)	1 (2%)	4 (11%)

discussing the genetic background. According to Liu and Xu (1994) (8), non-syndromic hearing loss can be classified into several types on the basis of audiograms. In the autosomal dominant group there are three types of audiograms – sharply sloping, flat, and gently sloping; and two types in autosomal recessive – residual and sharply sloping. The present study is in agreement with their report where cases with a sharply sloping audiogram (which may correspond with ski-slope type) are either autosomal dominantly or autosomal recessively inherited. Dominant high-frequency sensorineural hearing loss can be classified into four types – steepest, less steep, gently sloping, and horizontal (9). Together with similarity of audiograms within the same family, Higashi hypothesized heterogeneity of dominant high-frequency sensorineural hearing loss, and actually the former two types may correspond with ski-slope hearing loss.

In the present study, to understand the etiology of ski-slope hearing loss, genetic as well as clinical feature analyses were performed in the patients who fulfilled the audiological criteria. With regard to inheritance mode of these patients, 53% had sporadic/recessive inheritance, and 28% dominant/mitochondrial inheritance (Table I), indicating that various genes are involved in this category of hearing loss.

A high rate of patients with progressiveness was noted (56%) compared to overall (48%), and progressive nature was observed regardless of inheritance mode, indicating that progressiveness is one of the characteristic features of ski-slope hearing loss.

Onset ages were of great variation, also suggesting there are many responsible genes for this category of hearing loss. Earlier onset ages were noted in the patients with sporadic/recessive inheritance mode.

Table III. Progressiveness in the candidates for EAS (n=139).

	Number (%)		
Inheritance mode	Progressive	Non-progressive	Unknown
Overall	78 (56%)	44 (32%)	17 (12%)
Sporadic/recessive (n=74)	40 (54%)	24 (32%)	10 (14%)
Dominant/mitochondrial (n=39)	22 (56%)	10 (26%)	7 (18%)

Ski-slope hearing loss may occur at various ages, and can have either a progressive nature or be rather stable; therefore, decisions regarding timing of surgery are sometimes hampered. There may be a great inter-individual variation regarding progressiveness, indicating that many different etiological differences may interact. Screening for commonly found responsible genes, proved at least four genes, including mitochondrial 12SrRNA, *SLC26A4*, *CDH23*, and *GJB2* are involved in this type of hearing loss, although candidate rates were different among the genes.

The 1555A>G mitochondrial mutation, which is known to result in high susceptibility to aminoglycoside antibiotics, has been identified as the most prevalent mitochondrial mutation (10). Hearing loss is usually high-frequency involved and progressive (3). Therefore, the present higher candidacy rate (10.1%) among the patients with this mutation, together with overlapped audiograms as well as average audiograms (Figure 1A), is consistent with the previously reported phenotype and there is a certain number of candidates for EAS in patients with this mutation.

The *SLC26A4* gene was initially identified as the gene responsible for Pendred syndrome, and is known to be involved in transportation of the chloride ion (11). The phenotype associated with the mutations is known to range from Pendred syndrome to non-syndromic hearing loss associated with EVA (enlarged vestibular aqueduct) (12). Hearing is congenital/progressive, and usually high-frequency involved hearing loss (13). Patients acquire language but sometimes have incomplete pronunciation of consonants, indicating they may already have hearing loss at higher frequencies at the earlier (peri-lingual) ages. Overlapping audiograms (Figure 1B) suggested that some patients with this mutation are good candidates for EAS, but generally the slope is rather gentle. However, from the recent concept of preserving residual hearing it is still worth

Table IV. Responsible genes in the candidates for EAS (n=139).

Genes identified	Number (%)
Mitochondrial 1555A>G	18 (12.9%)
SLC26A4	10 (7.2%)
CDH23	6 (4.3%)
GJB2	3 (2.2%)

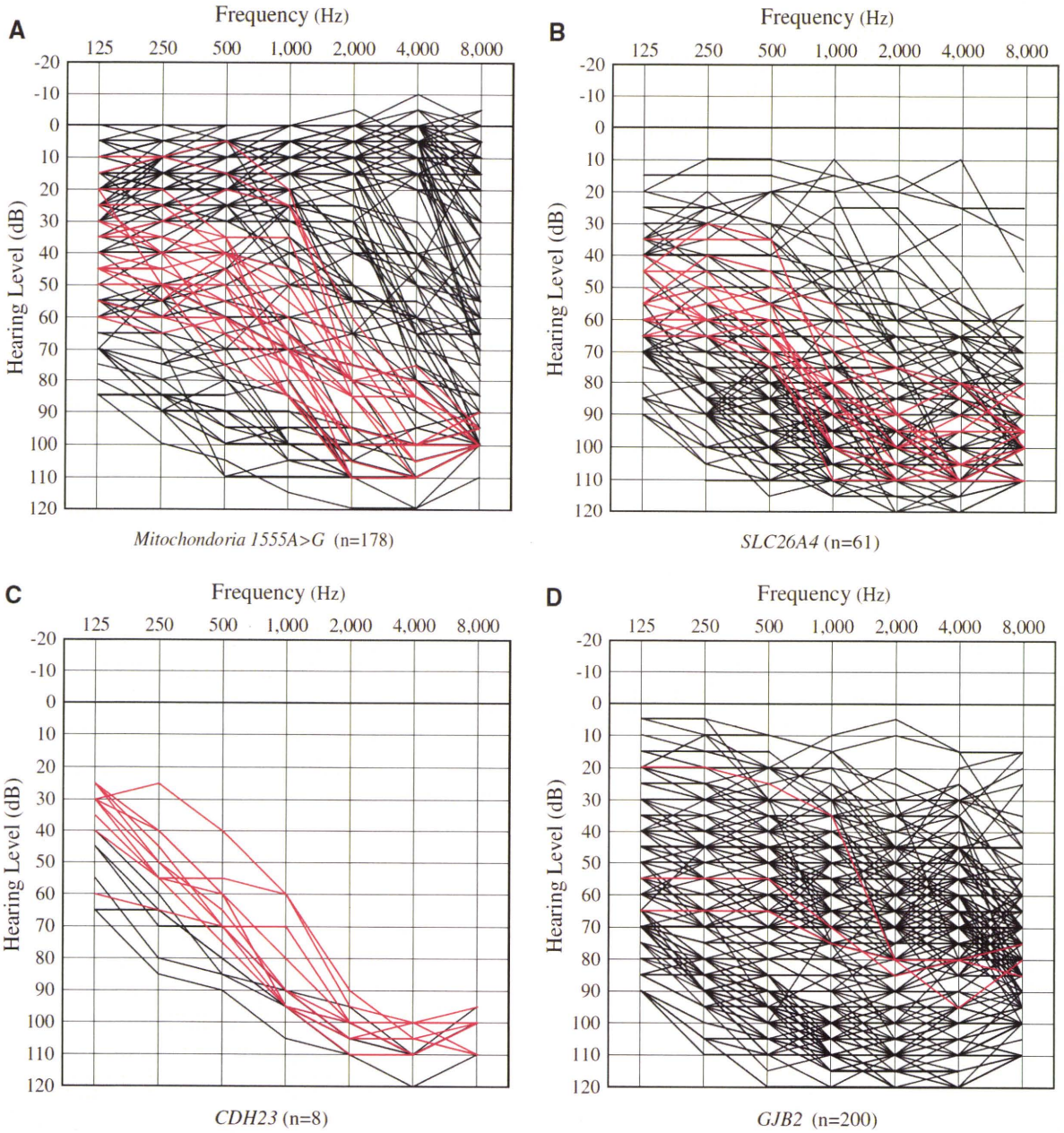

Figure 1. Overlapping audiograms of the patients with mutations. Candidates for EAS are indicated with red lines (A, mitochondrial 1555A>G; B, *SLC26A4;* C, *CDH23;* D, *GJB2*).

trying EAS for such patients with some (but not much) residual hearing at the lower frequencies.

CDH23 is known as the responsible gene for USH1D and DFNB12.

Encoded protein cadherin 23 is important for maintaining tip links (14). Patients with this mutation have high-frequency involved progressive hearing loss (6), suggesting that there is a significant number of EAS candidates. Although only a limited number of patients ($n=64$) with *CDH23* mutations were analyzed in this study, overlapping audiograms also indicated that they are good candidates for EAS (Figure 1C).

GJB2 is known to be the most prevalent gene responsible for congenital hearing loss worldwide (see reference 15, for review). Encoded protein, Connexin 26, is known to participate in potassium ion recycling in the inner ear. Currently, more than 100 different *GJB2* mutations are associated with recessive forms of non-syndromic hearing loss (see reference 15, for review). Overlapping audiograms of the 153 patients with bi-allelic *GJB2* mutations showed rather flat or gently sloping audiograms (Figure 1D). As hearing loss is usually reported to be non-progressive, there may be only a small number of the patients with *GJB2* mutations who are indicative

for EAS. Only 2.0% of the patients with *GJB2* mutations in this study fit the criteria for EAS.

The present study clearly revealed some genes responsible for ski-slope hearing loss, and genetic testing is potentially useful for estimating progressiveness and decision making for EAS in the future.

However, at the same time, in the majority of patients the cause is still unknown, and screening for various genes should be continued to understand the aetiology of this type of hearing loss. In the literature, there have been many genes described as being responsible for high-frequency involved hearing loss (16).

In the present study, progression is based on anamnesic information; therefore the actual rate of progression should be determined by future studies.

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ORIGINAL ARTICLE

Achievement of hearing preservation in the presence of an electrode covering the residual hearing region

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Abstract

Conclusions: With full insertion with a long electrode, hearing preservation can be achieved even in the presence of a long electrode covering the residual hearing region. *Objectives:* Advances in developing new atraumatic concepts of electrode design as well as surgical technique have enabled hearing preservation after cochlear implantation surgery, and EAS (electric acoustic stimulation) accompanied with hearing preservation is a new trend for patients with residual hearing at the lower frequencies. However, full insertion with a long/medium electrode and hearing preservation is still a challenging field that calls for discussion. *Method:* In this study, round window insertion, an atraumatic electrode, and dexamethasone administration were used and atraumaticity (hearing preservation and conservation of vestibular function) was evaluated with full insertion of the electrode. *Results:* Postoperative evaluation after full insertion of the electrodes showed that hearing at low frequencies was well preserved in all five cases. Combined postoperative imaging with the referential tonotopic map confirmed achievement of full insertion and indicated the corresponding frequencies and the depth of the electrode. Achievement of atraumaticity of round window insertion in the present cases was confirmed from the viewpoint of the minimal drilling time as well as the preserved vestibular function.

Keywords: *EAS, electric acoustic stimulation, high frequency hearing loss, cochlear implantation, deep insertion, atraumaticity*

Introduction

Advances in developing new atraumatic concepts of electrode design as well as surgical technique have enabled hearing preservation after cochlear implantation surgery, and EAS (electric acoustic stimulation) accompanied with hearing preservation is a new trend for patients with residual hearing at the lower frequencies.

However, a recent review collecting the data obtained by previous studies demonstrated that substantial acoustic hearing loss occurred in 24% of the patients, and among them 13% showed total loss [1]. Various techniques to preserve residual hearing at the lower frequencies have been attempted, including soft surgery technique when performing cochleostomy [2], round window insertion [3], use of atraumatic electrodes [4,5], and postoperative steroid administration.

Partial insertion up to 20 mm (where there is no residual hearing) is currently often performed [1], and full insertion with a long/medium electrode and hearing preservation is still a challenging field that calls for discussion. In this study, the method was based on atraumatic concepts and used round window insertion, an atraumatic electrode (in four of five cases), and dexamethasone administration. Hearing preservation and conservation of vestibular function were evaluated with full insertion of the electrode.

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Material and methods

We performed cochlear implantation with full insertion of the electrode (MEDEL COMBI40+® with a 31.5 mm standard electrode in one case, PULSAR® with a 24 mm FLEXeas® in three cases, and PULSAR® with a 31.3 mm FLEXsoft® in one case). The patients were aged from 38 to 68 years; two male, three female. All cases had post-lingual hearing loss at higher frequencies, starting from 30 to 40 years old and slowly progressive. The round window approach was applied to reduce the insertion damage of the cochlea. All surgeries were performed by a single surgeon (S.U.). Intraoperative infusion of dexamethasone (8 mg) was applied before drilling of the bony edge of the round window niche. Also postoperative dexamethasone treatment was administered for 6 days (8, 8, 4, 4, 2, and 2 mg, respectively). Insertion depth of the electrode and the corresponding frequencies were estimated by using postoperative X-ray (the X-ray digital linear tomosynthesis [6]). For comparison between round window insertion and cochleostomy insertion, drilling time to reach the perilymphatic space was averaged based on the video recording of 21 cases (round window insertion, 12 cases including the present 5 cases; cochleostomy insertion, 9 cases).

In addition to postoperative assessment of audiological testing, vestibular evoked myogenic potential (VEMP) as well as caloric response were analyzed to monitor atraumaticity of the surgery using nine cases (either round window insertion or

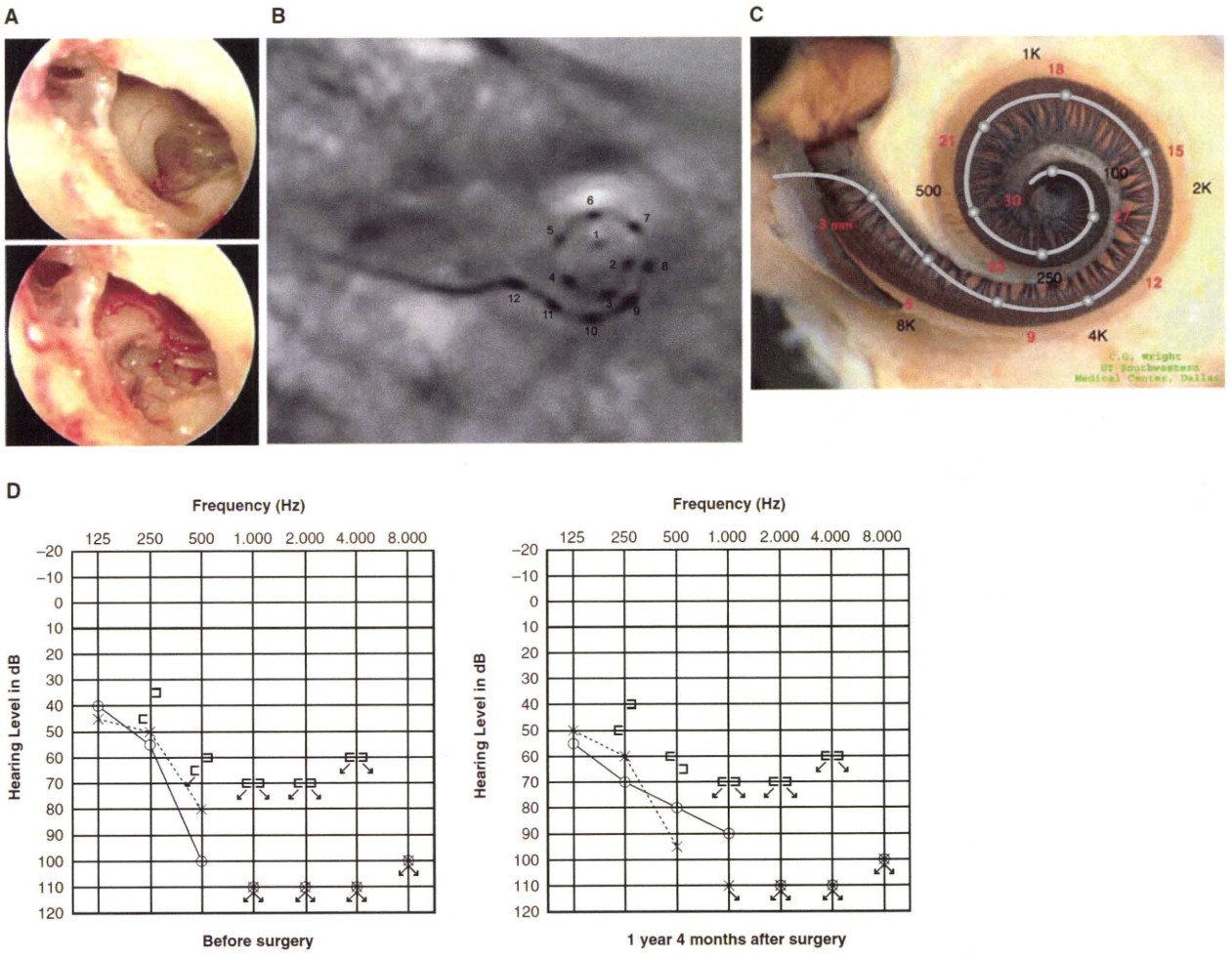

Figure 1. Case 1. A 60-year-old woman presented with slowly progressive bilateral hearing loss from age 40. By age 50 she had only minimal gain from hearing aids and when we first saw her they were nearly useless in her daily life. COMBI40+ with regular electrode was used for this patient on Dec 10, 2008. For insertion, the round window approach was applied, and full insertion was achieved. Complete preservation of residual hearing was obtained. (A) Endoscopic view of round window insertion, (B) postoperative X-ray finding, (C) imaging with putative location of electrode and the referential tonotopic map, (D) preoperative and postoperative audiograms. The image of human cochlea neural tissues stained by osmium tetroxide used in Figures 1–5 was kindly provided by Dr C.G. Wright, USWT, Dallas, USA (red, mm from round window; black, corresponding frequency).

cochleostomy), including the present five cases. In VEMP testing, the electrographic signal from the stimulated side was amplified and averaged using a Neuropack evoked potential recorder (Nihon Kohden Co. Ltd, Tokyo, Japan). Clicks lasting for 0.1 ms at 105 dBnHL were presented through a headphone. The stimulation rate was 5 Hz, the bandpass filter intensity was 20–2000 Hz, and analysis time was 50 ms. The responses to 200 stimuli were averaged twice. In caloric testing, maximum slow eye velocity was measured by cold water irrigation (20°C, 5 ml, 20 s). Postoperative VEMP and caloric responses of the implanted ears and contralateral ears were compared.

Results

Postoperative evaluation after full insertion of the electrodes showed that hearing at low frequencies was well preserved in all 5 cases, and then a speech processor (DUET EAS®) was applied for electric acoustic stimulation (EAS). Combined postoperative imaging with the referential tonotopic map confirmed achievement of full insertion and indicated the corresponding frequencies and the depth of the electrode (Figures 1–5). Audiological testing showed preservation of residual hearing, especially for bone conduction hearing (Figures 1–5).

Drilling time to reach the perilymphatic space based on the video recording was significantly less in the cases with round window insertion compared with cochleostomy cases (Figure 6, $p = 0.00001$, t test). VEMP responses could be recorded in four of five cases and were well preserved postoperatively. VEMP responses were decreased postoperatively in the cases with cochleostomy, in contrast to the round window insertion cases where the responses were maintained (Figure 7A). The ratio of the corrected amplitude value of cochlear implantation side divided by the normal side value was

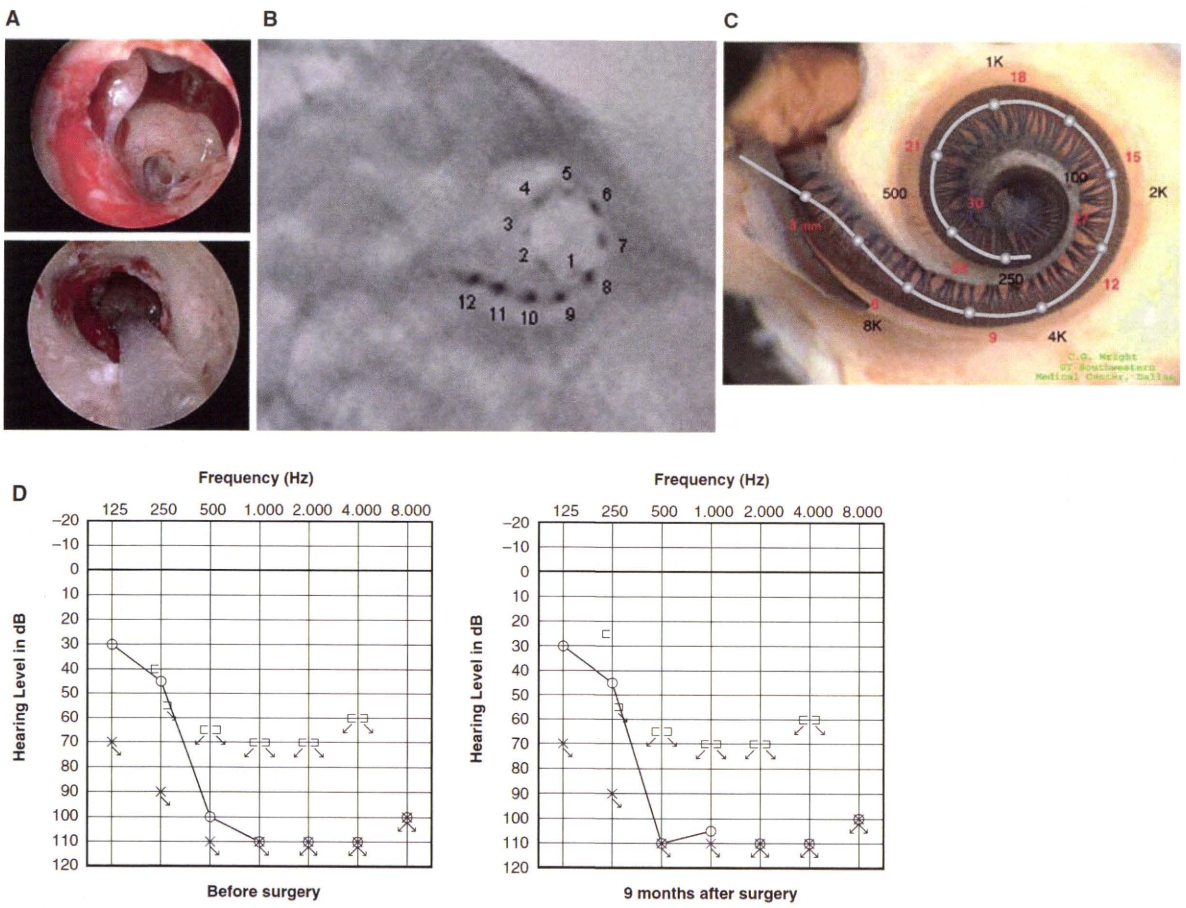

Figure 2. Case 2. This 39-year-old man was congenitally deaf in the left ear. Mild hearing loss in his right ear was noticed in childhood, and he presented with progressive hearing loss of 10 years duration. FLEXeas/RW approach was applied on Nov 16, 2009. Preservation of residual hearing was obtained. (A) Endoscopic view of round window insertion, (B) postoperative X-ray finding, (C) imaging with putative location of electrode and the referential tonotopic map, (D) preoperative and postoperative audiograms.

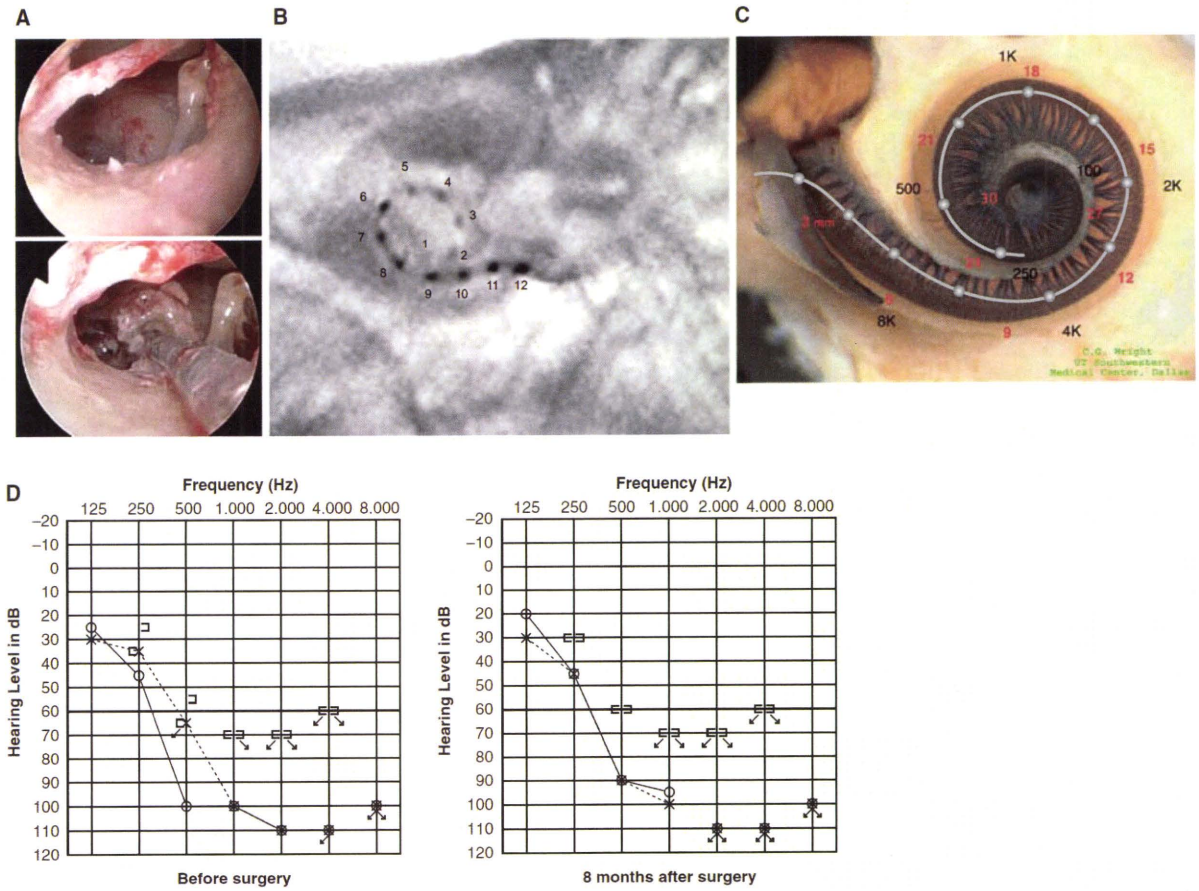

Figure 3. Case 3. This 45-year-old woman became aware of bilateral hearing loss and tinnitus around age 25. When she presented to us it had been slowly progressing for 10 years. PULSAR FLEXeas/RW approach was applied on Nov 18, 2009. Preservation of residual hearing was obtained. (A) Endoscopic view of round window insertion, (B) postoperative X-ray finding, (C) imaging with putative location of electrode and the referential tonotopic map, (D) preoperative and postoperative audiograms.

significantly lower in the cochleostomy cases than in the round window insertion cases ($p = 0.0001$, t test). Caloric response was well preserved and no difference was found between the two groups (Figure 7B, $p = 0.51$, t test).

Discussion

Hearing loss in the majority of these patients is more or less progressive, although the speed of progression, i.e. rapid or rather stable, may be dependent on their etiology. An unresolved issue is the prediction of progressiveness based on the etiology of individual hearing loss, but we have recently reported at least four genes that are responsible for the candidates for EAS, and therefore there is not a single etiology but rather a great genetic heterogeneity involved in this particular type of hearing loss [7]. Since shallow insertion of short electrodes cannot recruit neurons in the apical region, deep insertion would be the best solution to prevent future hearing deterioration at the lower frequencies. Full insertion with a long/medium electrode for the patients with residual hearing at the low frequencies is still a controversial field because of possible loss of their residual hearing due to mechanical trauma of the corresponding area.

In the present series, combined postoperative imaging with the referential tonotopic map clearly indicated that hearing preservation is achievable even in the presence of a long electrode covering the residual hearing region. Due to individual variation in the length of the cochlear turn, it is not sufficient to describe the length of the inserted electrode for estimating the corresponding frequencies of the tip of the electrode. In the present study, the X-ray digital linear tomosynthesis, which is known to have less artifacts and provide better understanding of the morphological relationship with the cochlear turn, indicated tonotopic orientation.

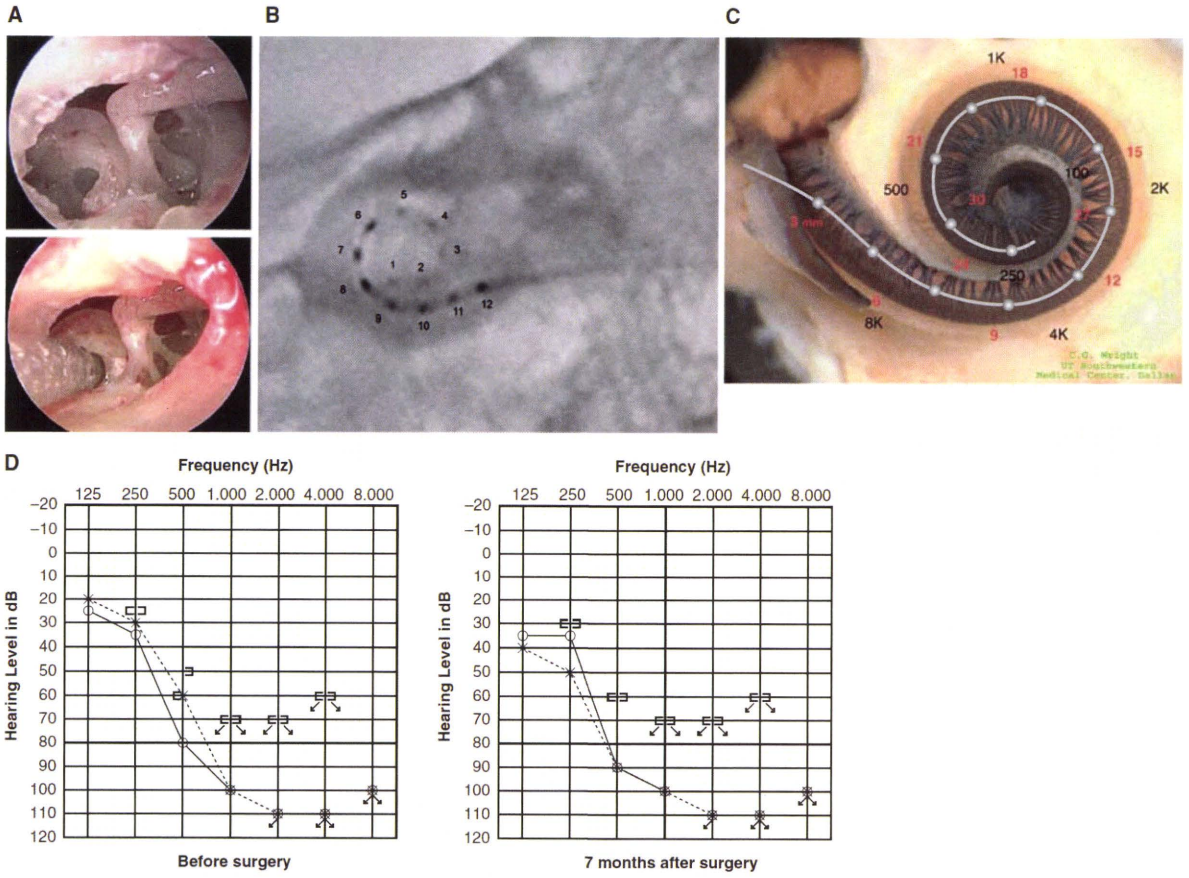

Figure 4. Case 4. This 38-year-old woman had hearing loss detected by mass screening in primary school. It appeared to slowly progress as she grew up, and by age 25 she suffered inconvenience in hearing and communication, mainly using only her left ear. The PULSAR FLEXeas/RW approach was applied on Dec 21, 2009. Preservation of residual hearing was obtained. (A) Endoscopic view of round window insertion, (B) postoperative X-ray finding, (C) imaging with putative location of electrode and the referential tonotopic map, (D) preoperative and postoperative audiograms.

With regard to the vibrations of the basilar membrane in the presence of the electrode, based on histological observations of morphologic changes in temporal bone studies, a close contact or even a slight lifting of the basilar membrane in the ascending basal and middle turns of the cochlea has been described [8]. However, in most cases, in adjacent regions, the basilar membrane was not in direct contact with the electrode, and lower frequencies were not affected by fixation in the basal and middle turn of the cochlea. Kiefer et al. [8] also reported the interesting phenomenon that audiological testing of the patients showed slightly better thresholds of the corresponding frequencies after implantation. Acoustic energy may increase perception in regions adjacent to the fixed regions, and basilar membrane behavior may be altered, i.e. some frequencies are redistributed and more amplified. In this series, some frequencies of the patients represented improvement after cochlear implantation (see Figure 1, air conduction hearing at 500 and 1000 Hz and bone conduction hearing at 500 Hz and Figure 2, bone conduction hearing at 250 Hz), supporting this phenomenon. On the other hand, in some cases, an air–bone gap was slightly recognized postoperatively (air conduction hearing was slightly elevated), perhaps due to a slight lifting of the basilar membrane in the middle turn observed in the temporal bone study [8].

These hearing improvement/deterioration results are not conclusive, because they could also be considered as within the margin of error. Serial testing as well as long follow-up observation period will resolve this issue, and we are currently working on this aspect.

Dexamethasone is known to have protective effects against insertion trauma as well as inflammatory process after implantation [9]. In this series, intraoperative infusion and postoperative dexamethasone treatment was administered systemically.

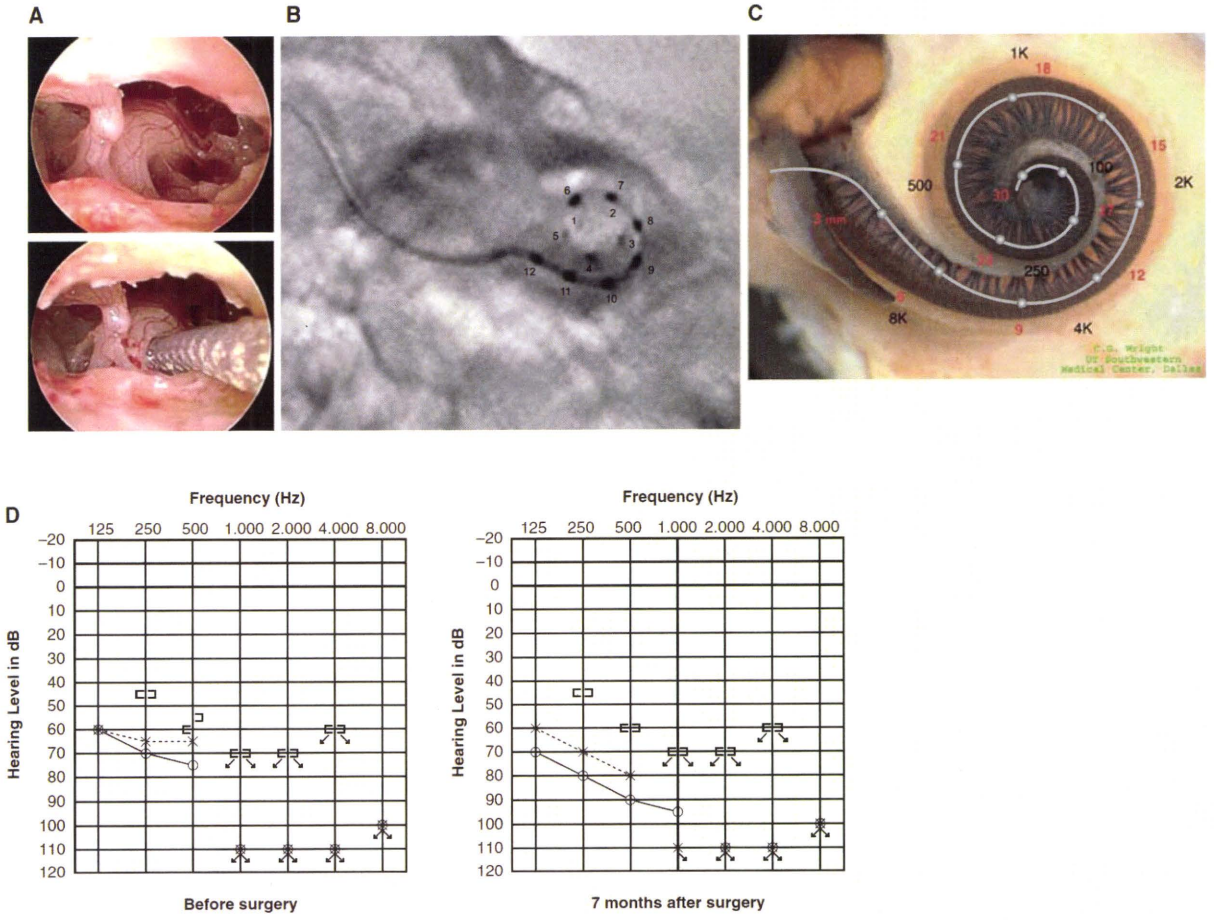

Figure 5. Case 5. This 68-year-old man presented with slowly progressive bilateral hearing loss from around age 40. He had only minimal gain from hearing aids. The PULSAR FLEXsoft/RW approach was applied on May 17, 2010. Preservation of residual hearing was obtained. (A) Endoscopic view of round window insertion, (B) postoperative X-ray finding, (C) imaging with putative location of electrode and the referential tonotopic map, (D) preoperative and postoperative audiograms.

There have been a series of trials with the goal of minimizing intracochlear trauma, by both cochleostomy insertion and round window insertion. For cochleostomy insertion, to avoid trauma, much attention has been paid to the cochleostomy site with the aim of avoiding the critical structures of the inner ear [10,11]. According to Lane et al. [12], by using 64-slice multidetector computed tomography (CT), localization of the electrode in the scala vestibuli as well as migration of the electrode array from the scala tympani to the scala vestibuli, which may influence hearing preservation, was observed in the patients with cochleostomy. On that basis, round window insertion was chosen in the present series.

Detailed clinical evaluation has confirmed the atraumaticity of the surgical approach in the present cases from the point of drilling time as well as of vestibular function.

During cochleostomy, noise levels were reported ranging from 114 to 128 dB SPL, indicating that during inner ear surgery they reach levels that can cause noise-induced hearing loss [13].

Our measurements clearly showed that drilling time to reach the perilymphatic space is significantly less for the round window approach compared with cochleostomy insertion, suggesting reduced influence of noise-induced trauma that may cause sensorineural hearing loss.

The importance of conservation of vestibular function is recognized, especially for bilateral cochlear implantation. A recent study suggested that dysfunction of the saccular macula, an integral component of the otolith system, likely resulting from insertion trauma of the cochlear implant electrode, can cause chronic dizziness after cochlear implantation [14]. In the present series, postoperative assessment of VEMPs as well as caloric response also supported

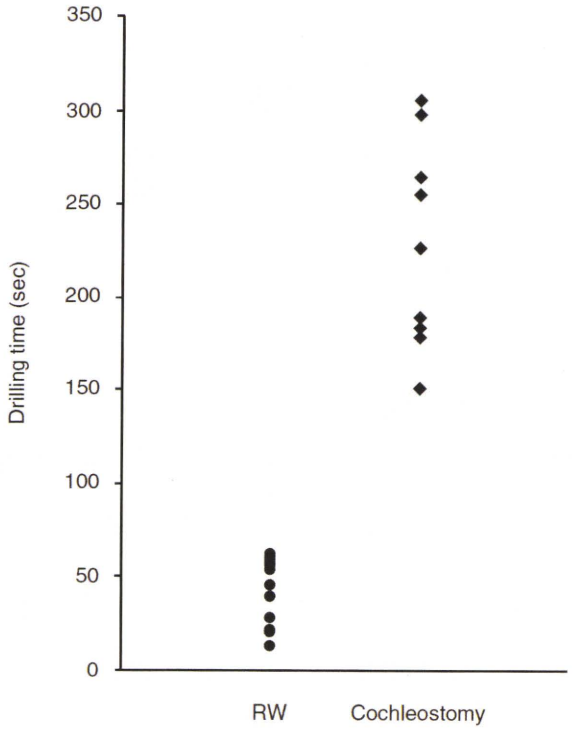

Figure 6. Video recording showing that drilling time to reach the perilymphatic space is significantly shorter for the round window approach compared with cochleostomy insertion.

achievement of atraumatic surgery from the vestibular functional point of view. Comparison with the cochleostomy insertion cases showed symmetrical VEMP scores in round window cases. The cochleostomy cases showed poorer response postoperatively, indicating that saccular function may be affected by the cochleostomy. These data support the recent report that for the sacculus, which is known to be the most vulnerable vestibular organ, the round window approach is preferable from the viewpoint of vestibular function [15].

Conclusion

In our series of experiences with full insertion with a long electrode we were able to preserve residual hearing at low frequencies as well as the vestibular function. Combined postoperative imaging with the referential tonotopic map clearly indicated that hearing preservation can be achieved even in the presence of a long electrode covering the residual hearing region and indicated that development of atraumatic procedures, including fine flexible electrodes, surgical technique (round window insertion), and postoperative steroid application enabled successful hearing preservation.

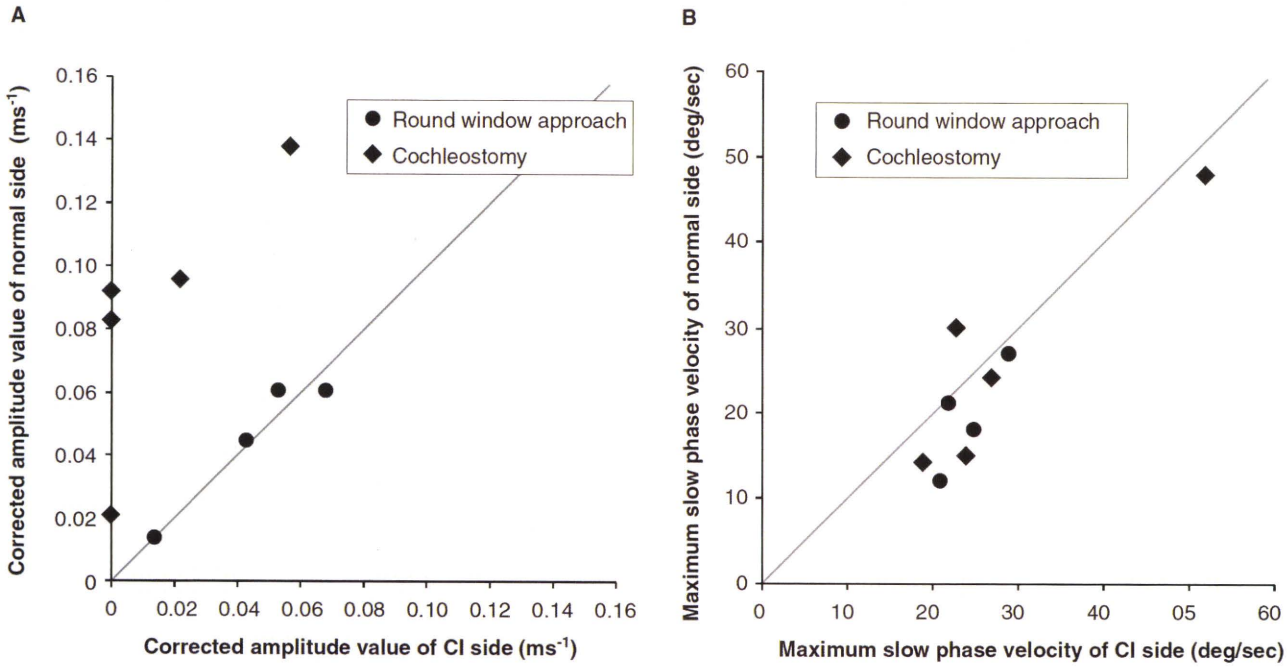

Figure 7. (A) Vestibular evoked myogenic potential (VEMP) responses were recorded in four of five cases and were well preserved postoperatively. VEMP responses decreased postoperatively in the cochleostomy cases while they were maintained in the round window insertion cases. Corrected amplitude value Cp13-n23 (ms^{-1}) = amplitude Cp13-n23 (micro V)/background electromyographic activities (micro V ms). (B) Caloric response was well preserved and there were no differences between the two groups. MSV, maximum slow eye velocity.

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ORIGINAL ARTICLE

Clinical profile of hearing loss in children with congenital cytomegalovirus (CMV) infection: CMV DNA diagnosis using preserved umbilical cord

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Abstract

Conclusions: Congenital cytomegalovirus (CMV) infection is a major cause of bilateral and unilateral sensorineural hearing loss (SNHL) in children, accounting for 9.0% of SNHL cases. The diagnostic rate using combined genetic deafness test and CMV DNA detection test was determined to be 46.4% in bilateral profound SNHL. *Objectives.* The present study investigated the prevalence of congenital CMV infection diagnosed retrospectively by detection of CMV DNA in dried umbilical cord specimens from children with unilateral or bilateral SNHL up to the age of 12 years. *Methods:* Preserved dried umbilical cords were collected from 134 children with bilateral (46 children) or unilateral (88 children) SNHL. DNA was extracted from the dried umbilical cords and CMV DNA was detected by quantitative PCR. Genetic deafness tests based on the invader assay were performed in children with bilateral SNHL. *Results:* CMV DNA from the dried umbilical cords was detected in 8.7% of the bilateral SNHL and 9.1% of unilateral SNHL. Deafness gene mutations were identified in 21.7% (10/46) of children with bilateral SNHL.

Keywords: *Sensorineural hearing loss, GJB2, SLC26A4*

Introduction

Sensorineural hearing loss (SNHL) is one of the most common birth defects. Genetic causes of SNHL can be found in half of prelingual cases and the remaining half are ascribed to environmental or unidentified genetic factors. The most common environmental cause of SNHL is congenital cytomegalovirus (CMV) infection, with an estimated overall birth prevalence of approximately 0.3–2.4% [1]. The vast majority (approximately 90%) of these infants exhibit no signs of congenital infection, which is asymptomatic at birth. Approximately 10% of infected infants are born with clinical symptoms of congenital CMV infection. SNHL reportedly occurs in 22–65% of children with symptomatic congenital CMV infections and 6–23% of children with asymptomatic infections [2].

Late-onset and progressive natures are characteristic of SNHL with congenital CMV infection. The frequency of SNHL in children with asymptomatic congenital CMV infection is also uncertain. The gold standard for diagnosis of congenital CMV infection is the isolation of the virus from urine or saliva in the first 2 weeks of life. However, asymptomatic congenital CMV infection in children who develop late-onset SNHL after 2 weeks of age cannot be diagnosed on the basis of viral isolation from urine or saliva. Detection of CMV DNA in infant blood or umbilical cord using polymerase chain reaction (PCR) assays is a more feasible method to identify children with late-onset of SNHL. Blood stored as dried blood

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spots (DBS) on Guthrie cards and the dried umbilical cord that is generally stored at home as a memento of the birth in the Japanese culture are suitable for retrospective diagnosis of congenital CMV infection.

Congenital CMV infections and genetic defects are the two major causes of SNHL in children. For severe bilateral SNHL children, Ogawa et al. [3] reported that congenital CMV infection, which was diagnosed by detection of CMV DNA in dried umbilical cord, and genetic defects (*GJB2*) were identified in 15% and 30% of the children, respectively. The etiology of SNHL in children including mild to moderate SNHL and unilateral SNHL is still uncertain. The purpose of the present study was to investigate the prevalence of congenital CMV infection diagnosed retrospectively by detection of CMV DNA extracted from dried umbilical cord specimens in children with unilateral or bilateral SNHL defined at an age of months or even years after birth. Genetic testing was also applied to identify the other causes of SNHL.

Material and methods

Subjects

This study evaluated 134 patients (70 males and 64 females) with bilateral (46 patients) or unilateral (88 patients) SNHL who were referred to the Department of Otolaryngology, Shinshu University School of Medicine, from May 2008 to September 2009 (Table I). Informed consent and dried umbilical cord for the preparation of DNA specimens were collected for all of them. The ages of children who were diagnosed with SNHL ranged from 1 month to 138 months (mean age 37.7 ± 36.2 months). Children with deafness syndrome were excluded from this study by an etiologic work-up of their SNHL. Both genetic deafness testing and CMV DNA analysis were performed for children with bilateral SNHL. For children with unilateral SNHL, CMV DNA analysis and genetic test (*GJB2, Mit1555*) were performed.

Audiologic evaluations

Audiometric evaluation was performed for each patient using auditory brainstem response (ABR) and auditory steady-state evoked response (Master 580-Navpro; Nihon Kohden Co. Ltd, Tokyo, Japan) as objective audiologic tests and behavioral audiologic tests and/or pure tone audiometry were also used. Hearing levels (average of 500, 1000, 2000, 4000 Hz) of the patients were classified into two categories on the basis of the severity of the worse ear: severe (71–90 dB) to profound (>90 dB), mild (20–40 dB) to moderate (41–70 dB). The threshold of ABR was determined as a means of hearing level in 5 of 134 children with hearing loss. The follow-up hearing assessments were performed at intervals of 6–12 months. Progressive hearing loss was defined as a decrease in hearing of 10 dB or more at one or more frequencies. Fluctuating hearing loss was defined as a decrease in hearing of >10 dB followed by an improvement of >10 dB at one or more frequencies.

Preparation of DNA samples and real-time PCR analysis and genetic testing

To analyze congenital CMV infection, we used CMV-DNA quantitative PCR (qPCR) analysis. Before qPCR analysis, the total DNA including genomic DNA and CMV DNA was extracted from preserved dried umbilical cords. Preserved dried umbilical cord samples were collected from hearing loss patients and controls. As a positive control, we used preserved umbilical cords from two patients with symptomatic congenital CMV infection, identified by CMV from urine in the first 2 weeks of life at the Department of Pediatrics, Shinshu University Hospital. As a negative

Table I. Summary of children with bilateral and unilateral hearing loss.

				Severe to profound hearing loss		Mild to moderate hearing loss	
Hearing loss	Gender	Hearing level (dB)	Affected side	Hearing level	Age at diagnosis (months)	Hearing level	Age at diagnosis (months)
Total (n = 134)	M = 70, F = 64			101 (75.4%)	34.4 ± 34.7	33 (24.6%)	48.8 ± 38.7
Bilateral (n = 46)	M = 31, F = 15	71.8 (R)		28 (20.9%)	16.6 ± 19.9	18 (13.4%)	11.1 ± 39.1
Unilateral (n = 88)	M = 39, F = 49	89.5 (W)	R = 43, L = 45	73 (54.5%)	41.2 ± 36.6	15 (11.2%)	40.3 ± 36.8
		13.6 (B)		B = 13.0, W = 97.5		B = 14.0, W = 53.5	

M, male; F, female; R, right; L, left; B, better hearing ear; W, worse hearing ear.

control, preserved umbilical cords from five healthy children without SNHL were used. Each 5 mm section of the tissue was incubated in the lysis buffer containing proteinase K and incubated at 56°C overnight. Total DNA was extracted using a DNeasy® Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The total amount of DNA was measured by Qubit® Fluorometer with Quant-iT™ dsDNA BR Assay Kit (Life Technologies-Invitrogen, Carlsbad, CA, USA). Each 10 pg total DNA was analyzed by a Step One Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using a TaqMan® Universal Master Mix II (Applied Biosystems). The qPCR primers and TaqMan® probe used for CMV DNA qPCR analysis are listed below: US14-1F, 5' ACGTCCACGTTAGGATGAGG 3'; US14-1R, 5' GTATGTGGCGCTTCTCTCGT 3'; US14-1 TaqMan probe, 5'-FAM-AACCTGTGCACCACAGCGCC-TAMRA-3'. To quantify the input DNA amount in each sample, qPCR with genome region was also performed, using the primers and TaqMan® probe listed below. GJB2-2F, 5' ACGTCCACGTTAGGATGAGG 3'; GJB2-2, 5' GTATGTGGCGCTTCTCTCGT 3'; GJB2-2 TaqMan probe, 5'-FAM- AACCTGTGCACCACAGCGCC-TAMRA-3'. Initial preheating steps were performed for 2 min at 50°C and 10 min at 95°C. Then qPCR was performed with 43 cycles of 15 s at 95°C and 60 s at 60°C. After qPCR analysis, relative CMV concentrations of each sample were evaluated as ΔCt (delta threshold cycle), which was calculated by threshold cycle of CMV qPCR minus that of GJB2 qPCR. The invader assay described by Abe et al. [4] was used for deafness genetic testing.

Ethics approval

This study was approved by the ethical committee of Shinshu University School of Medicine. Written informed consent was obtained from either the patients or their parents.

Results

Figure 1 shows original amplification curves of real-time PCR of the positive controls, negative controls, blank controls (samples without added DNA), and results of a typical sample (no. 12) for CMV DNA and genomic DNA (*GJB2* gene). CMV DNA was amplified in two of two cases in positive controls, none of five cases in negative controls, and none of two cases in blank controls (data not shown), therefore we considered this method to be appropriate. Comparing ΔCt for each sample and positive control, CMV DNA content was 0.01–0.8 times for positive control. The present study revealed that 9.0% (12/134) of the cases of children with SNHL were attributable to congenital CMV infection. CMV DNA from preserved umbilical cords was detected in 8.7% of bilateral SNHL cases (Table II) and 9.1% of unilateral SNHL cases (Table III) in children with SNHL of unknown causes. Bilateral severe to profound SNHL, bilateral mild to moderate SNHL, unilateral severe to profound SNHL, and unilateral mild to moderate SNHL caused by congenital CMV infection were detected in 14.3% (4/28), 0% (0/18), 9.6% (7/73), and 6.7% (1/15) of the hearing-impaired children, respectively.

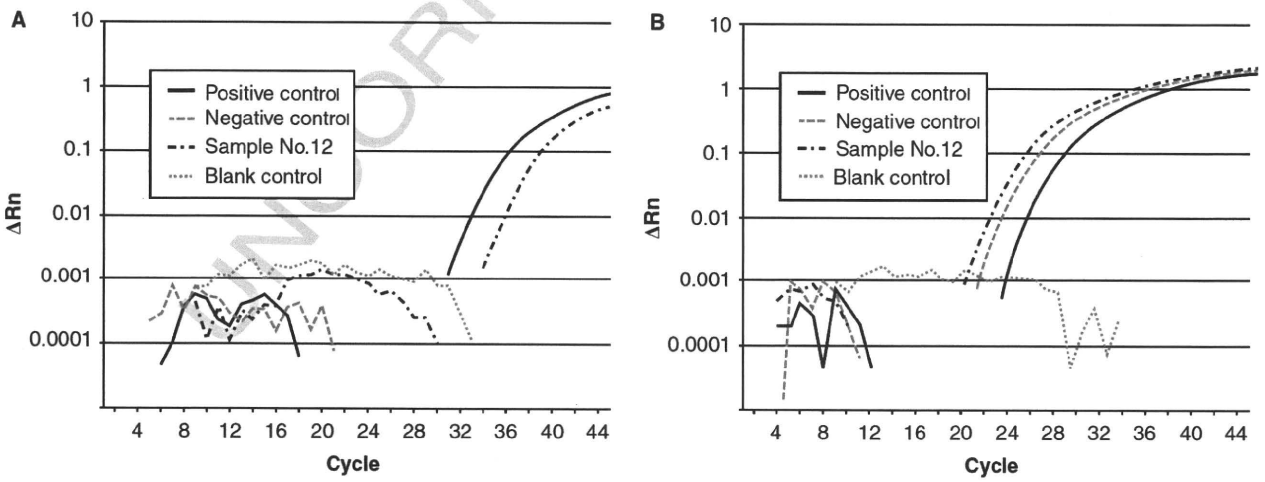

Figure 1. An original amplification plot of real-time PCR in case no. 12 with positive CMV DNA. CMV DNA in positive control and case 11 (A) and genomic DNA (GJB2 gene) in positive control, case no. 12 and negative control (B) are amplified. These results show that our real-time PCR method is precise. Blank, sample without any added DNA.