Clinical testing

Pure tone audiometry (PTA) was performed before and after the experiment. The average of 0.5, 1, 2, and 4 Hz is shown in Table I. For vestibular testing, caloric testing and vestibular-evoked myogenic potential (VEMP) testing were performed. In caloric testing, maximum slow eye velocity was measured by cold water irrigation (20°C, 5 ml, 20 s). 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 band-pass filter intensity was 20–2000 Hz, and analysis time was 50 ms. The responses to 200 stimuli were averaged twice.

The Ethics Review Committee of Shinshu University School of Medicine approved the protocol of the study and all patients gave their informed consent to participation.

Results

In this study, 3D-FLAIR MRI clearly showed that the gadodiamide successfully penetrated the round window membrane, entered the perilymphatic space, and delineated the gadodiamide-enhanced perilymphatic and gadodiamide-negative endolymphatic spaces of the inner ear. The endolymphatic space is comparatively small and difficult to identify as a vacant area in the normal side. In contrast, the endolymphatic space in an ear with endolymphatic hydrops is partially or entirely expanded, making identification of the endolymphatic space easier (Figures 2 and 3).

Gadodiamide distribution patterns within the inner ear were variable and differed individually. In patient no. 3, who had definite MD, after 24 h the intratympanic gadodiamide moved toward the perilymphatic space, and the endolymphatic hydrops could be detected as a black area surrounded by the perilymphatic space filled with the gadodiamide in the basal turn of the left cochlea (Figure 2). In the unaffected side, the endolymphatic space (which was significantly small) may have been masked by the strong enhancement of perilymphatic space. In patient no. 6, who also had definite MD, the endolymphatic space in the vestibule on the affected side was significantly larger than that on the normal side (Figure 3). In this patient, in association with the imaging, VEMP was absent, but the caloric test showed normal response.

Table I summarizes imaging results and clinical data obtained for each patient. In the cases such as no. 3 or 6 mentioned above, endolymphatic hydrops could be easily identified qualitatively. However, in some cases, it was difficult to obtain supportive imaging for endolymphatic hydrops. Therefore, the present study tried to perform semi-quantitative analysis by using the MPR image, created from 3D-FLAIR images. Based on the semi-quantitative analysis, the gadodiamide-enhanced area representing the perilymphatic space ratio was 0.14 to 3.86 (Table II). In 9 of 10 patients with definite MD the ratio was reduced, and the quantitative ratio was 0.15 to 0.85 (Table II). In the exception, patient no. 4, gadodiamide was not introduced in the perilymphatic space even on the normal side, probably due to technical failure.

Table I. Summary of bilateral intratympanic gadolinium administration.

Patient no.	Age/sex	Diagnosis	Side	Caloric test CP%	VEMP	PTA-pre (dB) Affected side	PTA-pre (dB) Unaffected side	PTA-post (dB) Affected side	PTA-post (dB) Unaffected side
1	51/M	MD	R	7.2	Depressed	38.8	15.0	38.8	15.0
2	41/F	MD	R	51.2	–	37.5	11.3	32.5	11.3
3	42/M	MD	L	41.3	Depressed	50.0	12.5	53.8	12.5
4	42/F	MD	L	19.9	ND	33.8	12.5	32.5	10.0
5	76/F	MD	L	39	ND	46.5	30.0	40.0	27.5
6	51/F	MD	R	11.9	Absent	22.5	13.8	28.8	12.5
7	53/M	ATMD	R	–	–	58.8	13.8	47.5	13.8
8	38/M	MD	R	22.6	Depressed	20.0	6.3	28.8	5.0
9	76/M	ALSNHL	R	–	–	17.5	46.3	13.8	43.8
10	67/F	MD	L	6.9	ND	55.0	28.8	52.5	26.3
11	52/F	MD	L	6.8	ND	65.0	12.5	62.5	13.8
12	53/F	MD	L	42.3	Depressed	53.8	22.5	47.5	20.0
13	33/M	pMD	R	50.6	Normal	12.5	6.3	6.3	6.3

ALSNHL, acute low tone sensorineural hearing loss; ATMD, atypical Meniere's disease; F, female; L, left; M, male; MD, 'definite' Meniere's disease; ND, not detectable; pMD, 'possible' Meniere's disease; PTA, pure-tone audiometry; R, right.

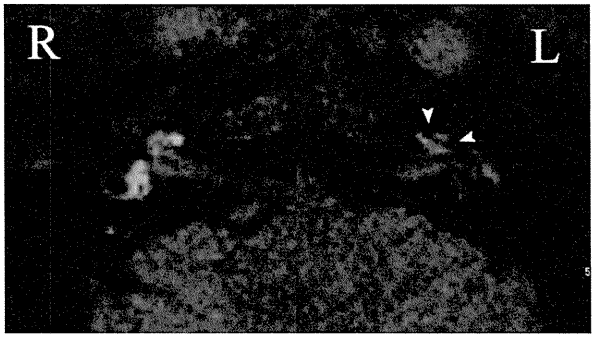

Figure 2. MRI imaging in patient no. 3 (definite Meniere's disease). The endolymphatic hydrops is detectable as a black area (arrowheads) inside the perilymphatic space filled with the gadodiamide in the basal turn of the left cochlea. In the normal side, the endolymphatic space (a significantly small area) is not detectable, probably due to strong signal intensity in the perilymphatic space.

We measured the saccular endolymphatic space by bilateral comparison. Eleven of 13 patients, including 8 with definite MD, 1 with possible MD, 1 with atypical MD, and 1 with ALSNHL, showed differences in endolymphatic space in the saccules. Significant differences (Student's *t* test) in patient nos 6, 8, 10, and 11 were noted (Figure 4)

Concerning vestibular functional testing, caloric testing was performed in all but two patients (nos 7 and 9), and showed decreased response in five cases. VEMP testing was performed in all patients, except nos 2, 7, and 9. In 6 of the 10 patients who underwent the testing, VEMP was either absent on the affected side or depressed compared with the healthy side. VEMP amplitude could not be obtained because of low muscle contraction in patient nos 4, 5, and 10.

No adverse effects, such as vertigo, hearing deterioration, or tinnitus due to the intratympanic injection of gadodiamide were observed and there were no changes in hearing level (Table I).

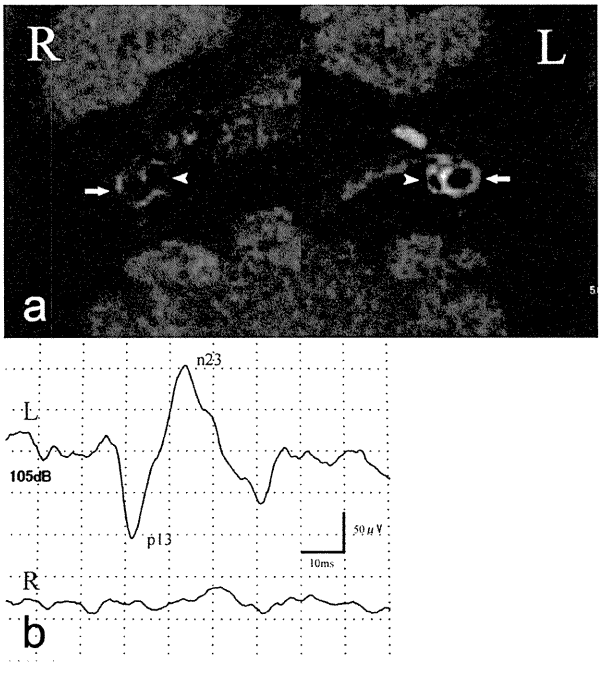

Figure 3. MRI imaging in patient no. 6 (definite Meniere's disease). The endolymphatic space in the saccules is detectable as a black area inside the perilymphatic space filled with gadodiamide in the vestibule (arrowheads). In the normal side (L), the endolymphatic space in the saccules is also detectable in the unaffected side, but is smaller than in the affected area. Arrows indicate lateral semicircular canals. In the affected side (R), enhancement by gadodiamide was weaker than in the unaffected side, indicating that endolymphatic hydrops may be present in the canal. VEMP testing showed no response in the affected side.

Discussion

The hallmark of MD diagnosis is to prove endolymphatic hydrops, but this has been achieved only in temporal bone histopathology after death. Initial attempts to identify endolymphatic hydrops involved visualization of the Reissner membrane, and it was successfully visualized in animals [7] and human cadavers [8]. The subsequent attempts to identify endolymphatic hydrops used intratympanic GBCA administration with 1.5 T MRI to visualize the

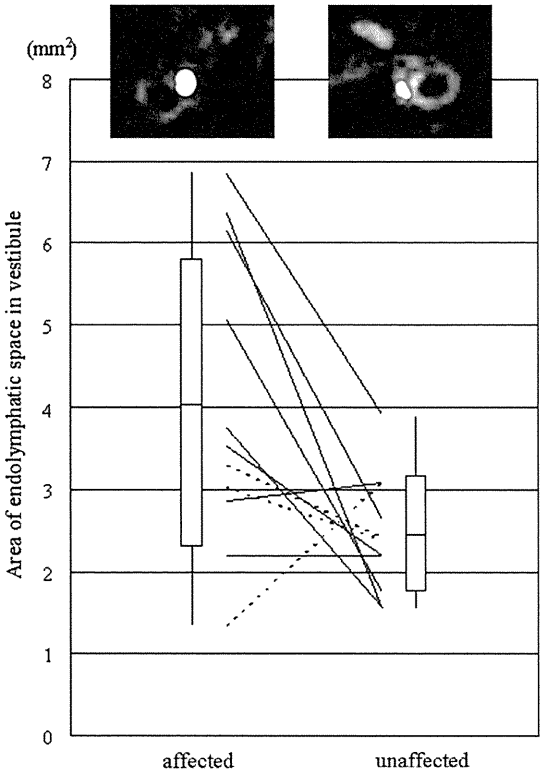

Figure 4. Semi-quantitative analysis of bilateral endolymphatic space in the sacculus.

Table II. Gadolinium distribution in inner ear.

Patient no.	Affected side			Unaffected side			Area			Area (vestibule)	
	Cochlea	Vestibule	Semicircular canals	Cochlea	Vestibule	Semicircular canals	Affected side	Unaffected side	Ratio	Affected side	Unaffected side
1	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole	Whole	17.6	26.8	0.65	2.86	3.08
2	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole	Whole	18.5	21.9	0.85	2.2	2.2
3	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole	Whole	19.6	36.8	0.53	3.74	1.57
4	Faint	Faint	Faint	Faint	Faint	Faint	4.9	1.3	3.86	–	–
5	Basal	Whole	Partial	Basal	Whole	Partial	15.9	19.7	0.81	–	–
6	Faint	Faint	Partial	Basal, second	Whole	Partial	4.6	30.8	0.15	6.84	3.92
7	Basal	Faint	Whole	Basal, second, apical	Whole	Whole	15.0	30.0	0.50	3.02	2.38
8	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole	Whole	20.4	25.6	0.80	6.37	1.54
9	Basal, second	Whole	Whole	Basal, second, apical	Whole	Whole	26.1	27.6	0.95	3.3	2.42
10	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole	Whole	18.1	33.1	0.55	5.05	1.76
11	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole		10.7	20.1	0.53	6.15	2.64
12	Basal, second	Faint	Whole	Basal, second, apical	Whole	Whole	5.6	25.2	0.22	3.52	2.2
13	Basal, second, apical	Whole	Whole	Basal, second, apical	Whole	Whole	21.2	20.9	1.01	1.32	3.08

endolymphatic/perilymphatic space [9]. Recent advances in imaging technology enabled visualization of human endolymphatic hydrops by intratympanic Gd-DTPA administration at 3.0 T MRI [4–6]. The present study adds supportive evidence that endolymphatic hydrops can be diagnosed by the same protocol and expands the findings by semi-quantitative analysis. As in the previous reports [4–6], we could recognize the existence of endolymphatic hydrops as a decreased perilymphatic space, which may indicate an expanding endolymphatic space. Furthermore, semi-quantitative evaluation based on the ratio of the GBCA-enhanced area between affected/control sides represents the degree of endolymphatic hydrops.

In all 10 patients who had definite Meniere's disease, except for 1 (patient no. 4 with failure due to technical error), the ratio was reduced, and the quantitative ratio was 0.14 to 0.85. The present data are the first to indicate that bilateral intratympanic administration of GBCAs is beneficial in the semi-quantitative evaluation of endolymphatic hydrops.

There was inter-individual variation in the pattern of gadodiamide enhancement. For example, in patient no. 3, endolymphatic hydrops was predominantly detected in the cochlea (Figure 2). In contrast, vestibular hydrops was predominantly identified in patient no. 6 (Figure 3), in whom no VEMP response was found, suggesting that imaging findings are well correlated with the functional testing. A series of temporal bone studies also demonstrated that endolymphatic hydrops occurs either locally or entirely [10]. In the cochlea, the endolymphatic space is too small to recognize compared with the perilymphatic space, therefore endolymphatic space is usually undetected in the normal side. Thus, the existence of endolymphatic hydrops, which indicates abnormality, can be qualitatively identified more easily. In contrast, saccular endolymphatic spaces can be identified even in normal ears, making precise diagnosis for endolymphatic hydrops difficult without bilateral comparison. An additional advantage of the present procedure of bilateral intratympanic injection of a GBCA is the enablement of semi-quantitative comparison of endolymphatic space in the vestibule, which is difficult to evaluate by unilateral injection. In this study, four cases with definite MD showed significant differences in endolymphatic space in the saccular region and all of these patients (except patient no. 10 who could not be analyzed due to incomplete myogenic compression) showed decreased response. These correlations between imaging of sacculus associated with saccular functional testing such as VEMP will be of great help in precise diagnosis as well as therapeutic choice in MD.

In the present study, 6 of 11 patients who underwent caloric testing showed unilateral vestibular hypofunction. In comparison with VEMP, in some patients (nos 3, 8, and 12) both were decreased, but the others were shown to be abnormal in VEMP whereas normal in caloric responses (nos 1 and 3). Such discrepancy between the two testing methods, i.e. normal caloric test which is a function of the lateral semicircular canal, and a decrease in or disappearance of VEMP, has recently been reported in MD [11,12]. The present study confirms the saccular hydrops by imaging as well as VEMP, supporting the existence of such pathological conditions. In addition to general quantification, the advantage of bilateral intratympanic injection of GBCAs is semi-quantitative evaluation of saccular endolymphatic hydrops.

No adverse effects, such as vertigo, tinnitus, or hearing deterioration, were noted after intratympanic injection of gadodiamide, indicating that the present protocol can be safely performed in ordinary clinical settings. This is also supported by a recent study using guinea pigs in which diluted GBCAs had no apparent effects on endocochlear potential [13].

EcochG and glycerol testing have been widely performed as useful, but indirect, tests for the detection of endolymphatic hydrops in MD. Because these are indirect tests, EcochG or glycerol testing cannot play a decisive role in determining the presence or absence of endolymphatic hydrops. Unfortunately, in this study, systemic comparison between these tests and imaging results could not be performed. Therefore, a final conclusion concerning the relationship between these previous findings and the current MRI findings await future systemic comparative study.

The number of cases other than MD in this study was limited, and the etiology of each category of disease was not conclusive. In previous studies, 8.5% of patients with ALSNHL progressed to 'definite' MD [14], indicating that some MD was previously diagnosed as ALSNHL. In this study, the patient with ALSNHL was not associated with endolymphatic hydrops. Future study using many cases will subclassify ALSNHL whether it is associated with endolymphatic hydrops or not.

In this study, endolymphatic hydrops was also demonstrated in the patient with atypical MD who had fluctuated low frequency sensorineural hearing loss without vertigo, indicating the possibility that some atypical MD is a continuum clinical entity of MD. Therefore, in the future, the diagnostic criteria for MD may be expanded and reclassified according to image-based diagnosis.

In conclusion, bilateral intratympanic administration of a GBCA was successfully performed and

proved to be beneficial in the semi-quantitative evaluation of endolymphatic hydrops.

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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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Short Report

A large cohort study of *GJB2* mutations in Japanese hearing loss patients

Tsukada K, Nishio S, Usami S, and the Deafness Gene Study Consortium. A large cohort study of *GJB2* mutations in Japanese hearing loss patients. Clin Genet 2010: 78: 464–470. © John Wiley & Sons A/S, 2010

GJB2 is the gene most frequently associated with hereditary hearing loss, and the *GJB2* mutation spectrums vary among different ethnic groups. In this study, the mutation spectrum as well as clinical features of patients with *GJB2* mutations as found in more than 1000 Japanese hearing loss families are summarized. The present results show that the frequency of *GJB2* mutations in the Japanese population with hearing loss is 14.2% overall and 25.2% in patients with congenital hearing loss. c.235*del*C was the most frequent allele (49.8%), was associated with a more severe phenotype, and was mainly found in patients who were diagnosed by the age of 3. In contrast, the second most frequent was p.V37I (16.5%), which has a milder phenotype and was mainly found in patients diagnosed at a higher age. Additional clinical features in hearing loss patients with *GJB2* mutations in this study were the near absence of tinnitus, vestibular dysfunction and inner ear malformations.

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Key words: clinical features – genotype – phenotype correlations – *GJB2* – hearing loss – mutation

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Mutations in the *GJB2* gene have recently been of particular interest because *GJB2* is the commonest causative gene for hereditary hearing loss in all populations. To date, more than 100 variations have been reported worldwide (see the Connexin-deafness homepage: http://www.davinc.crg.es/deafness) and the mutation spectrums vary among different ethnic groups. There have been many papers describing the frequency of *GJB2* mutations among hearing loss populations, but most studies have been based on small numbers of patients from a single center. A large cohort study may prevent bias and provide a more precise estimate of mutation frequencies. Therefore, with the goal of establishing a database of the mutations found in the East Asian populations, we estimated the *GJB2* mutation frequency and spectrum as well as associated clinical features using more than 1500 Japanese hearing loss families collected from multiple centers.

Subjects and methods

Subjects

Data on 3056 Japanese subjects of 1511 independent families were collected from 33 ENT departments nationwide in Japan. All subjects gave prior informed consent for participation in the project, which was approved by the ethical committee of each hospital. Of the 1511 probands, 1343 had bilateral sensorineural hearing loss and 168 had unilateral sensorineural hearing loss. The control group consisted of 252 unrelated Japanese individuals without any noticeable hearing loss evaluated by auditory testing.

Mutation analysis

To identify *GJB2* mutations, a DNA fragment containing the entire coding region was sequenced as described elsewhere (1). Screening for the known

large DFNB1 deletions was performed in the patients with a single heterozygous allele without the presence of a second pathogenic mutant allele, but none were detected (data not shown).

Computational analysis

To evaluate the importance of each amino acid affected by novel missense mutations found in this study, we used a computational analysis program for identification of functionally and structurally important residues in protein sequences: CONSEQ (http://conseq.tau.ac.il/index.html).

Clinical evaluations

Hearing levels were determined by pure-tone audiometry. For the young patients, conditioned orientation response audiometry (COR) or auditory steady-state response (ASSR) were used. Clinical data, including hearing loss progression, episodes of tinnitus and vestibular dysfunction (vertigo, dizziness, faintness), were collected by anamnestic evaluation. Thin section temporal bone computed tomography (CT) was used to investigate inner ear malformations.

Results

GJB2 mutation spectrum in hearing loss probands

There were a total of 26 *GJB2* variants observed in the ascertained probands with bilateral hearing loss (Table 1). Fourteen of those were missense mutations. To evaluate the evolutionary conservation of the amino acids affected by these missense mutations, we used a computational alignment program CONSEQ (not shown). On the basis of this alignment program, all missense mutations had changed evolutionary conserved amino acids, except for p.T123N and p.Y68C. Because p.N54S and p.M195V were found in the compound heterozygous state, they are likely to be pathogenic. Eight of the mutations were found in the control group (Table 1). p.V27I, p.E114G, p.I203T (1, 2), and p.T123N (3), frequently found in both probands and controls, were thought to be non-pathological polymorphisms. The c.235*del*C and p.V37I mutations found in the control group most likely represent the detection of carriers.

Frequency of *GJB2* mutations in hearing loss probands

With regard to the frequency of *GJB2* mutations in the 1343 independently ascertained probands with bilateral hearing loss, 191 (14.2%) had at least one pathogenic *GJB2* mutant allele (Table 2). The most prevalent mutation was c.235*del*C (49.8% of all pathogenic mutant alleles) and the second most frequent was p.V37I (16.5%) (Fig. 1).

The frequency of *GJB2* mutations was significantly higher in probands who were diagnosed at an earlier age: 25.7% (108/420) in those diagnosed at age 0–3, 14.9% (15/101) in those diagnosed at age 4–5, and 7.8% (49/627) in age 6 or over (Table 2). c.235*del*C was also significantly higher in probands diagnosed at an earlier age (58.5%) compared to those who were diagnosed at the age of 6 and over (19.6%) ($p < 0.001$; χ^2 test). In contrast, p.V37I was significantly more frequent in probands who were diagnosed at the ages of 4–5 (36.4%) or 6 and over (41.1%) than in prelingual hearing loss probands (6.9%) ($p < 0.001$; χ^2 test) (Fig. 1).

Audiologic studies

Of the total 3056 subjects, 134 with bilateral hearing loss and biallelic *GJB2* mutations were selected for audiologic studies. We excluded 22 subjects who were from a family with another subject who had the same mutation. In the remaining 112 subjects, audiometric results were available for 105 probands, of 23 different genotypes. Figure 2 shows a collection of overlapping audiograms from those 105 subjects. We compared the hearing levels in the six genotypes that were shared by five or more subjects. The subjects with the p.V37I allele had significantly milder hearing loss ($p < 0.027$; Mann–Whitney *U* test).

p.V37I/p.R143W showed a significantly worse hearing level than p.V37I/p.V37I ($p = 0.025$; Mann–Whitney *U* test) and also tended to be worse than p.V37I/c.235*del*C ($p = 0.076$; Mann–Whitney *U* test). Moreover, comparison of c.235*del*C/c.235*del*C ($n = 35$) and c.235*del*C/p.R143W ($n = 13$) revealed that subjects with the p.R143W allele had a significantly worse hearing level than homozygotes ($p = 0.025$; Mann–Whitney *U* test).

Twenty-six subjects with biallelic *GJB2* mutations were followed at least two years by audiometric testing with progression of hearing loss seen in four subjects (15%), two (7%) of those being unilateral progression and two (7%) being bilateral progression.

Clinical findings

Based on the data availability, clinical findings were statistically evaluated. Episodes of tinnitus in patients with *GJB2* mutations were at a

Table 1. *GJB2* variants in deafness patients and controls

		Patients					Controls					
Amino acid change	Nucleotide change	Allele ($n = 2686$)	Allele frequency (%)	Homozygous (n)	Compound heterozygous (n)	Heterozygous (n)	Alleles ($n = 504$)	Allele frequency (%)	Controls ($n = 252$)	Carrier rate (%)	Evolutionary conservation	Reference
—	c.235 *del*C	142	5.29	34	45	28	2	0.40	2	0.80	NA	Fuse et al. (19)
p.V37I	c.109G>A	47	1.75	3	11	30	3	0.60	3	1.20	Yes	Abe et al. (1)
p.G45E[c]	c.134G>A											
p.Y136X[c]	c.408C>A	34	1.27	1	22	10					Yes	Fuse et al. (19)
p.R143W	c.427C>T	18	0.67	0	16	2					Yes	Brobby et al. (20)
—	c.176_191 *del*16bp	15	0.56	0	10	5					NA	Abe et al. (1)
—	c.299-300 *del*AT	11	0.41	0	8	3					NA	Abe et al. (1)
p.T86R	c.257C>A	8	0.30	0	5	3					Yes	Ohtsuka et al. (2)
—	c.512*ins*AACG	3	0.11	0	3	0					NA	Hismi et al. (21)
—	c.35*ins*G	2	0.07	0	2	0					NA	Estivill et al. (22)
p.I71T[b]	c.212T>C	2	0.07	0	0	2					Yes	Ohtsuka et al. (2)
p.T8M	c.23C>T	1	0.04	0	0	1					Yes	Kenna et al. (23)
p. I33N[b]	c.98T>A	1	0.04	0	0	1					Yes	This study
p.A49V[b]	c.146C>T	1	0.04	0	0	1					Yes	Ohtsuka et al. (2)
p.N54S	c.161A>G	1	0.04	0	1	0					Yes	This study
p.Y68C[a]	c.203A>G	1	0.04	0	0	1					No	This study
p.M93I	c.276G>A	1	0.04	0	1	0					Yes	Wu et al. (24)
p.K112M[b]	c.335A>T	1	0.04	0	0	1					Yes	This study
—	c.376-377 *del*AA[e]	1	0.04	0	0	1					NA	This study
p.W133X	c.398G>A	1	0.04	0	1	0					NA	Primignani et al.[f]
p.K168R[b]	c.503A>G	1	0.04	0	0	1					Yes	This study
p.M195V	c.583A>G	1	0.04	0	1	0					Yes	This study
—	c.605*ins*46bp	1	0.04	0	0	1					NA	Yuge et al. (25)
p.F191L	c.571T>C	0	0	0	0	0	1	0.20	1	0.40	yes	Feng et al. (26)
p.R127H	c.380G>A	0	0	0	0	0	1	0.20	1	0.40	yes	Seeman et al.[f]
Polymorphism												
p.V27I	c.79G>A	865	32.20	—	—	—	196	38.90	158	62.70	Yes	Kelley et al. (8)
p.E114G	c.341A>G	259	9.64	—	—	—	64	12.70	62	24.60	No	Fuse et al. (19)
p.T123N[d]	c.368C>A	18	0.67	0	3	15	2	0.40	2	0.80	No	Park et al. (3)
p. I203T	c.608T>C	112	4	—	—	—	21	4.10	21	8.30	No	Abe et al. (1)

[a]Variant probably representing polymorphism because no evolutionary conservation was observed.
[b]Variants with unproven pathogenic nature.
[c]p.G45E and p.Y136X(c.134G>E) mutations are on the same parental allele.
[d]p. T123N was found with equal frequency in the probands and controls, and three out of eight subjects with compound heterozygous state did not have any hearing loss, suggesting the polymorphic nature of p.T123N.
[e]c.376-377 delAA is thought to be a pathogenic mutation, but it was present as a single heterozygous allele without the presence of a second pathogenic mutant allele, therefore it could not clearly be classified as pathogenic in this study.
[f]Ballana E, Ventayol M, Rabionet R et al. Connexins and deafness Homepage. World wide web URL: http://www.crg.es/deafness.

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

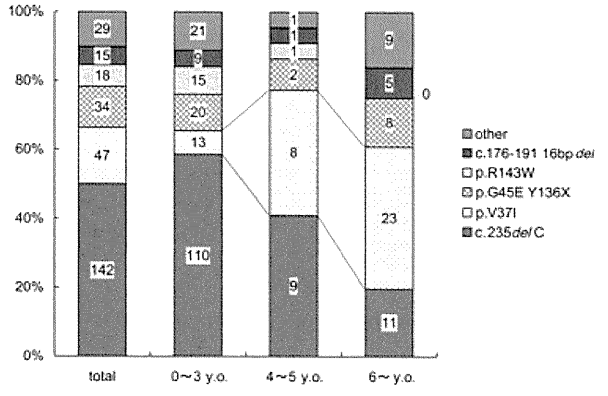

Fig. 1. Frequency of mutant GJB2 alleles in different diagnostic age groups. c.235*del*C was mainly found in the group diagnosed at up to 3 years, where it was significantly higher than in age 6 and over ($p < 0.01$; χ^2 test). On the contrary, p.V37I was mainly found in the diagnostic age groups of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

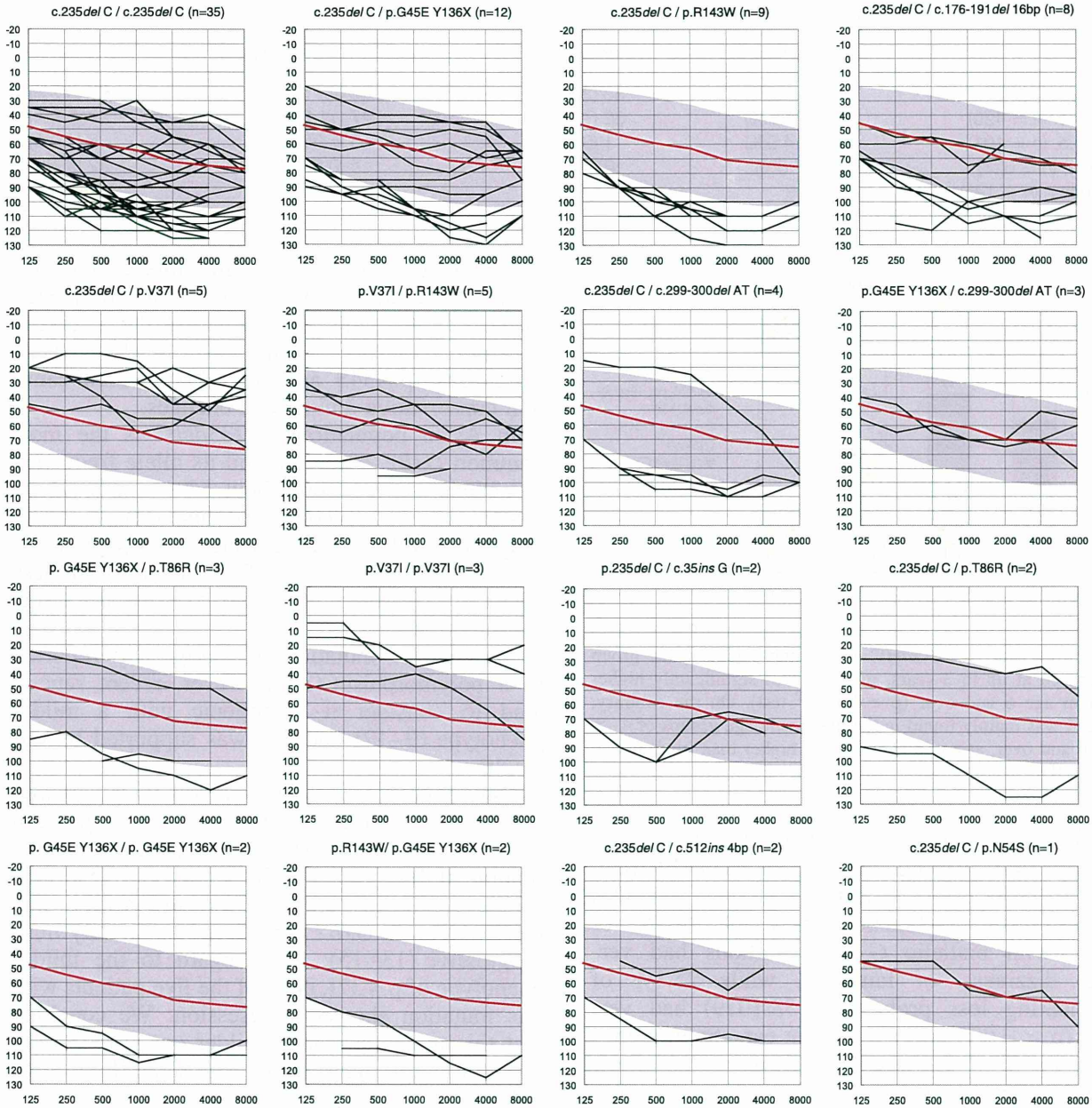

Fig. 2. Overlapping audiograms from the better ear for each genotype. The average audiogram from all subjects (1343 with bilateral sensorineural hearing loss) is indicated by a red line with standard deviation (shadow).

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

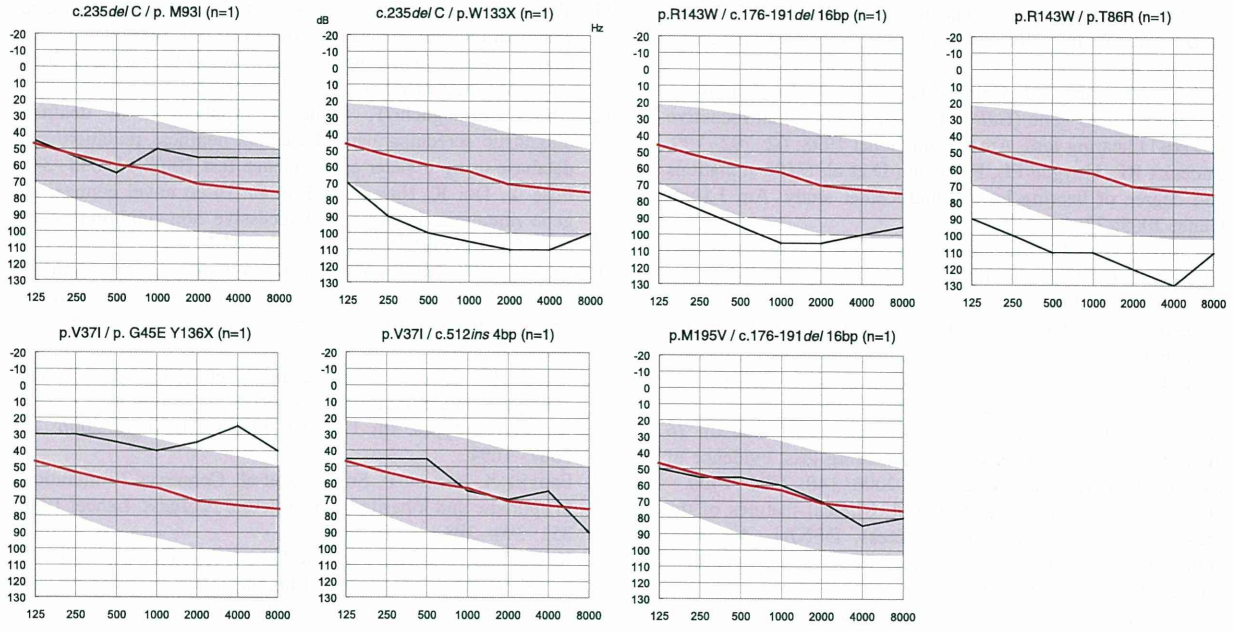

Fig. 2. Continued

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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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

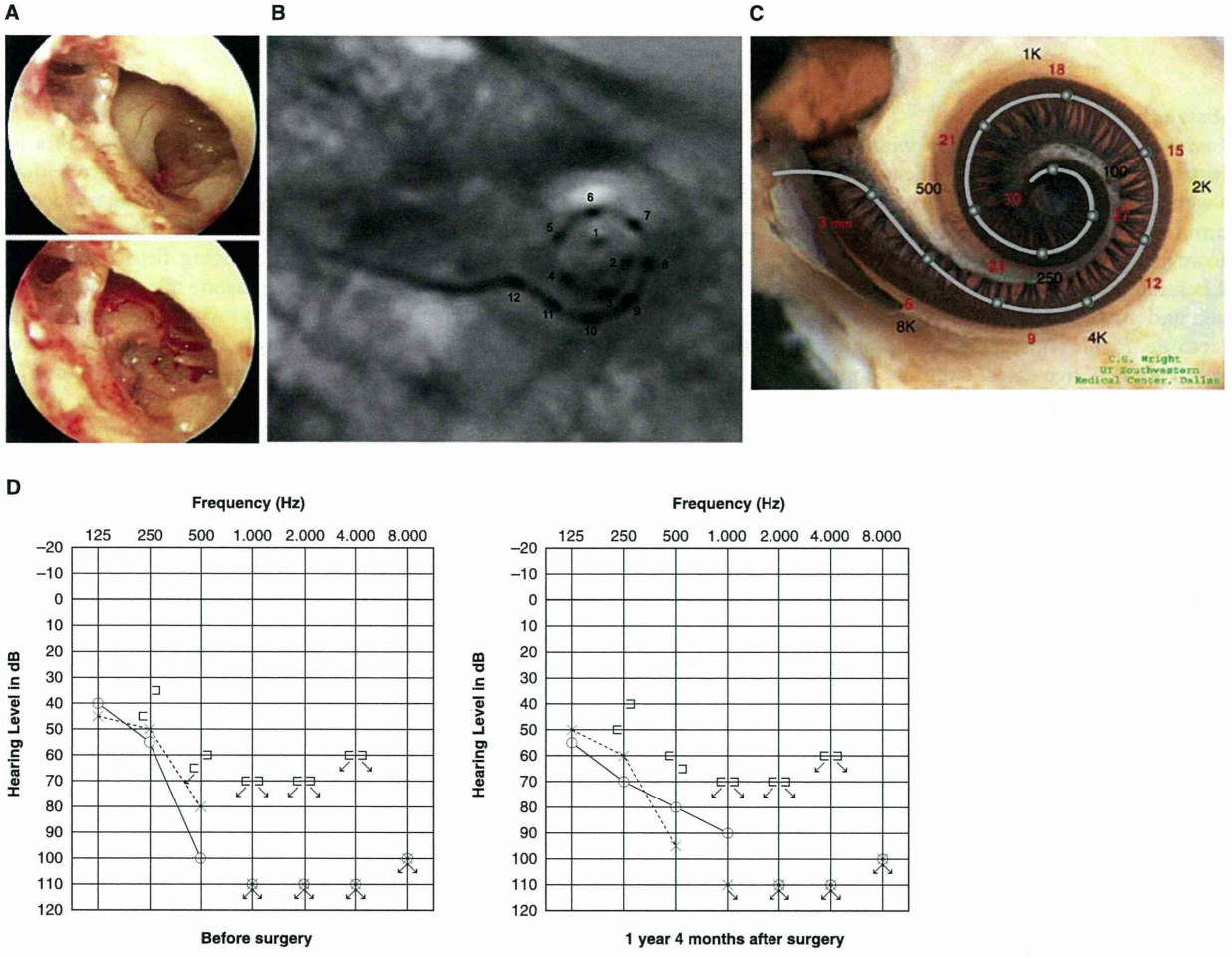

Figure 1. Case 1. A 60-year-old woman presented with slowly progressive bilateral hearing loss from age 40. By age 50 she had only minimal gain from hearing aids and when we first saw her they were nearly useless in her daily life. COMBI40+ with regular electrode was used for this patient on Dec 10, 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

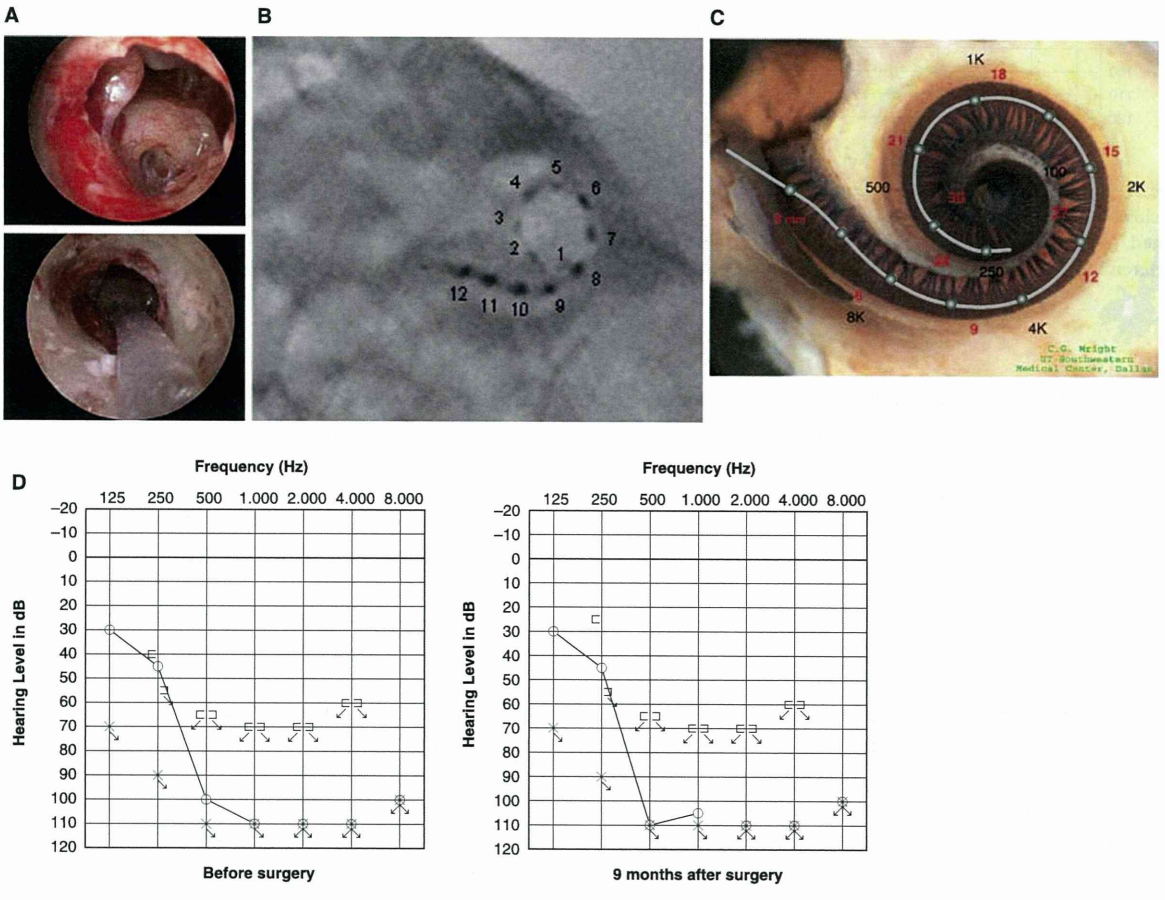

Figure 2. Case 2. This 39-year-old man was congenitally deaf in the left ear. Mild hearing loss in his right ear was noticed in childhood, and he presented with progressive hearing loss of 10 years duration. FLEXeas/RW approach was applied on Nov 16, 2009. Preservation of residual hearing was obtained. (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.

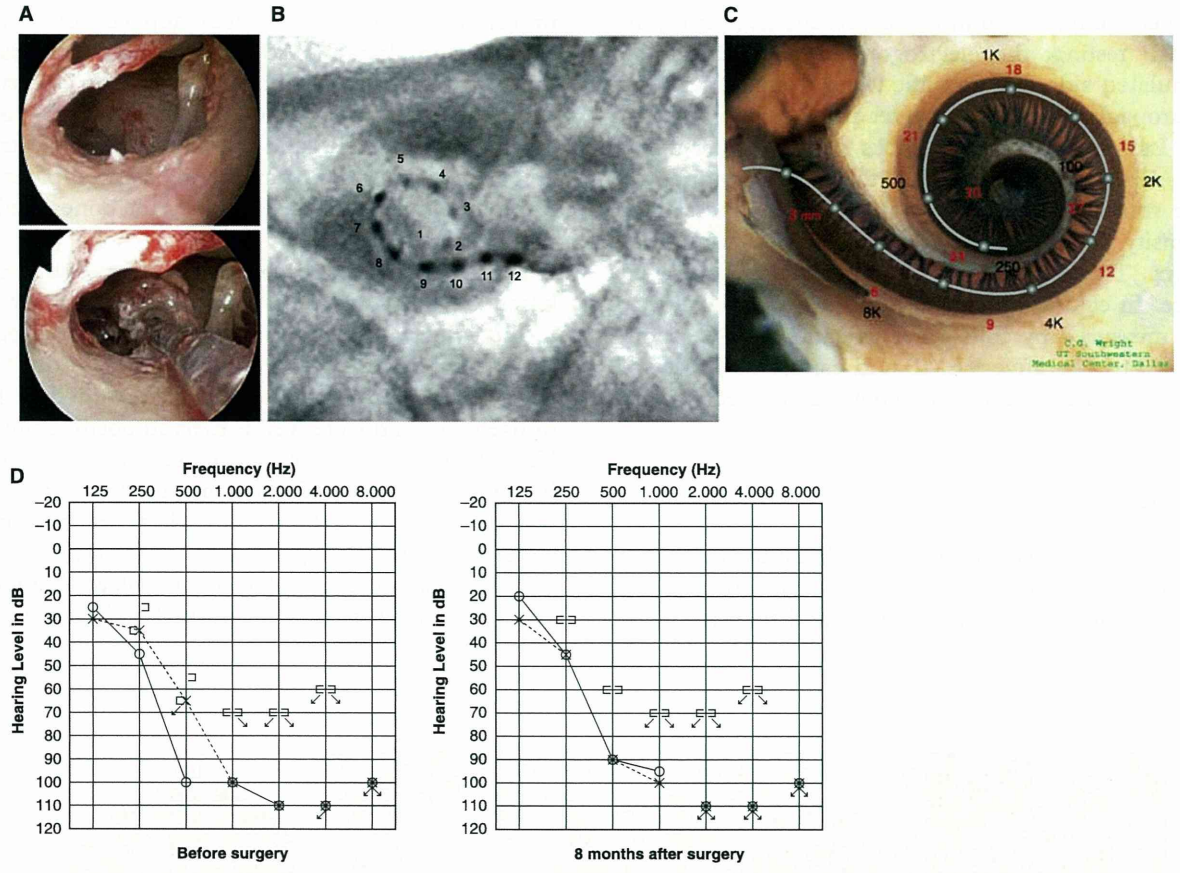

Figure 3. Case 3. This 45-year-old woman became aware of bilateral hearing loss and tinnitus around age 25. When she presented to us it had been slowly progressing for 10 years. PULSAR FLEXeas/RW approach was applied on Nov 18, 2009. Preservation of residual hearing was obtained. (A) Endoscopic view 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.

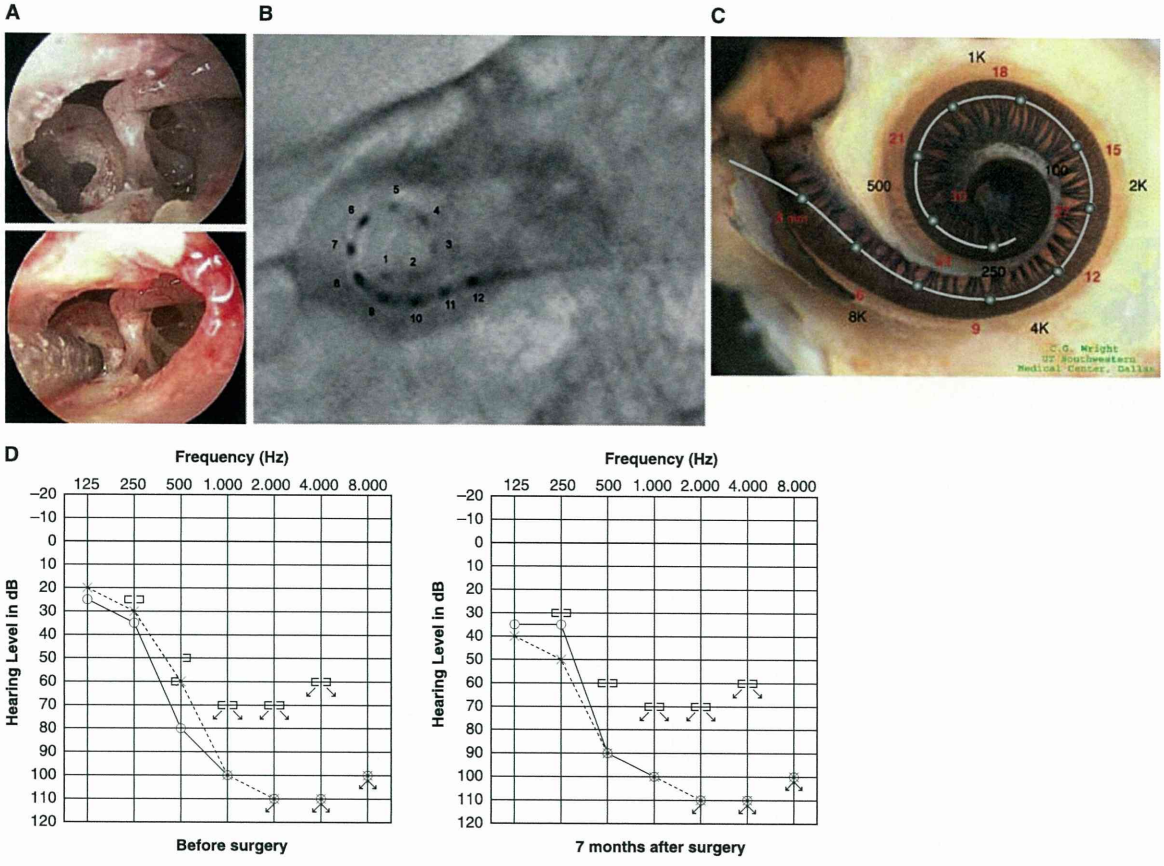

Figure 4. Case 4. This 38-year-old woman had hearing loss detected by mass screening in primary school. It appeared to slowly progress as she grew up, and by age 25 she suffered inconvenience in hearing and communication, mainly using only her left ear. The PULSAR FLEXeas/RW approach was applied on Dec 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.

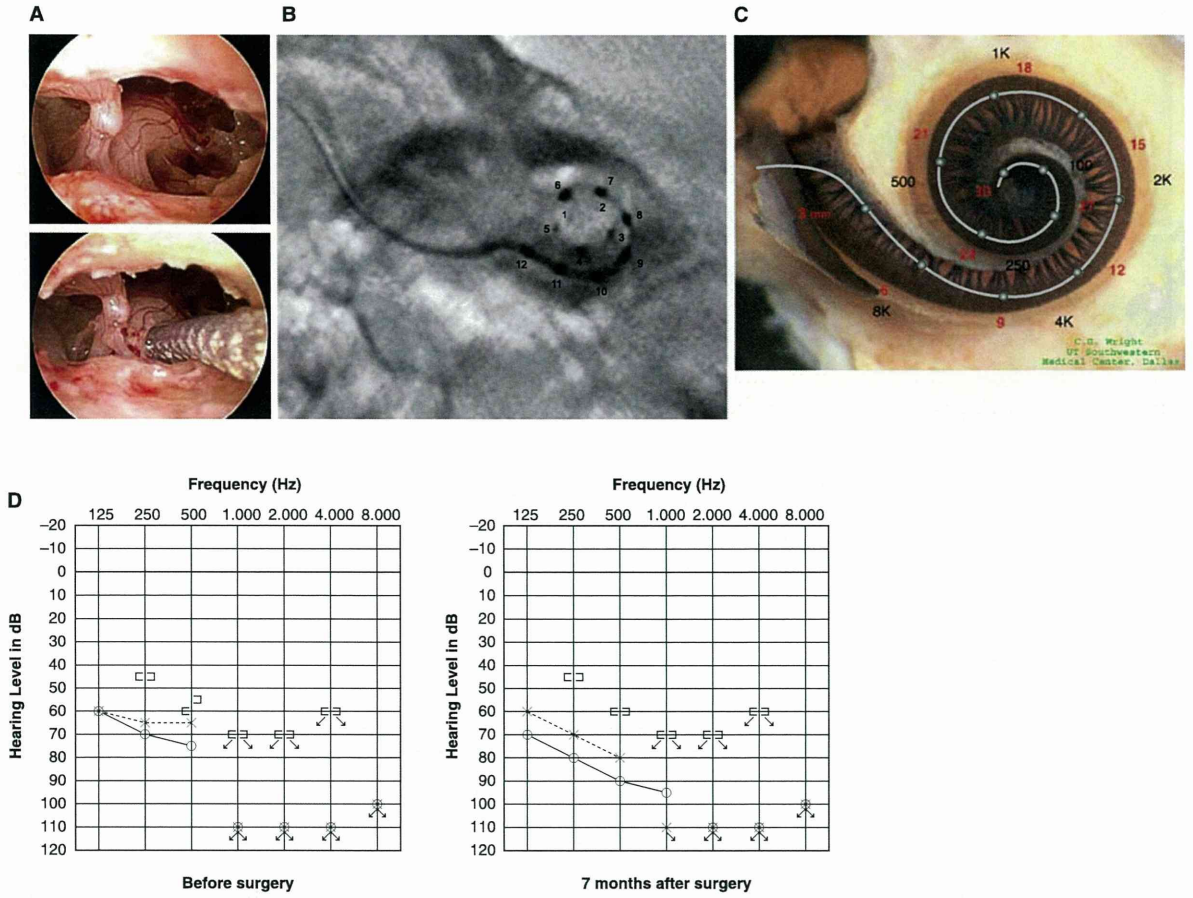

Figure 5. Case 5. This 68-year-old man presented with slowly progressive bilateral hearing loss from around age 40. He had only minimal gain from hearing aids. The PULSAR FLEXsoft/RW approach was applied on May 17, 2010. Preservation of residual hearing was obtained. (A) Endoscopic view of round window 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

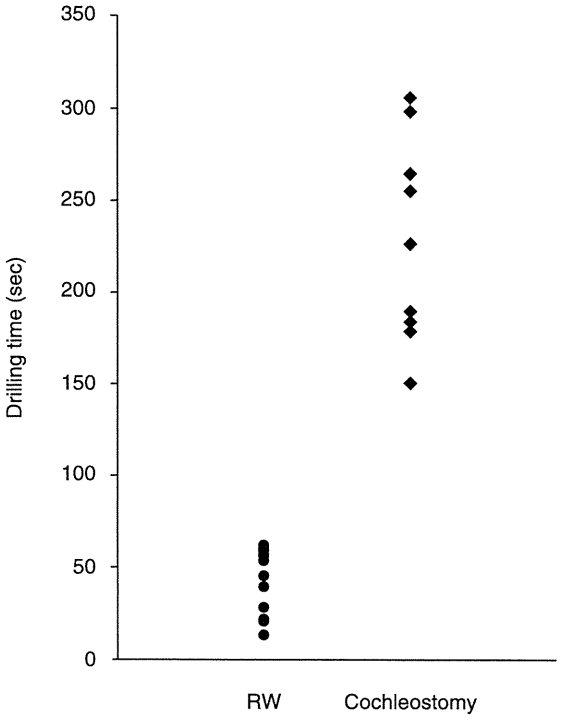

Figure 6. Video recording showing that drilling time to reach the perilymphatic space is significantly shorter for the round window approach compared with cochleostomy insertion.

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.

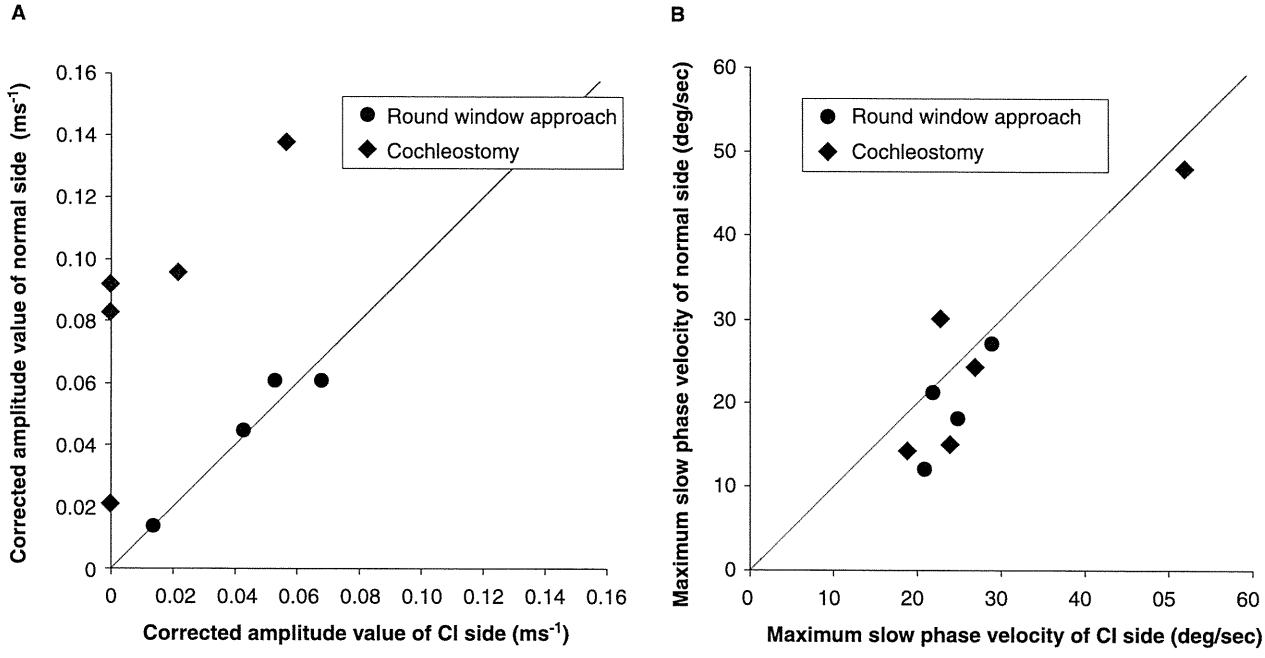

Figure 7. (A) Vestibular evoked myogenic potential (VEMP) responses were recorded in four of five cases and were well preserved postoperatively. VEMP responses decreased postoperatively in the cochleostomy cases while they were maintained in the round window insertion cases. Corrected amplitude value 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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