Ⅱ．　研究成果の刊行に関する一覧表

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Short Report

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

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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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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

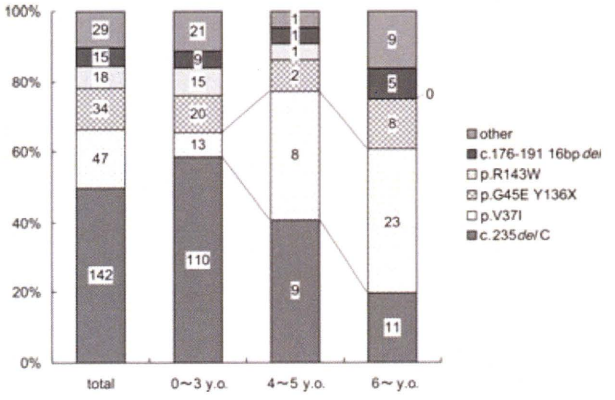

Fig. 1. Frequency of mutant GJB2 alleles in different diagnostic age groups. c.235*del*C was mainly found in the group diagnosed at up to 3 years, where it was significantly higher than in age 6 and over ($p < 0.01$; χ^2 test). On the contrary, p.V37I was mainly found in the diagnostic age groups of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

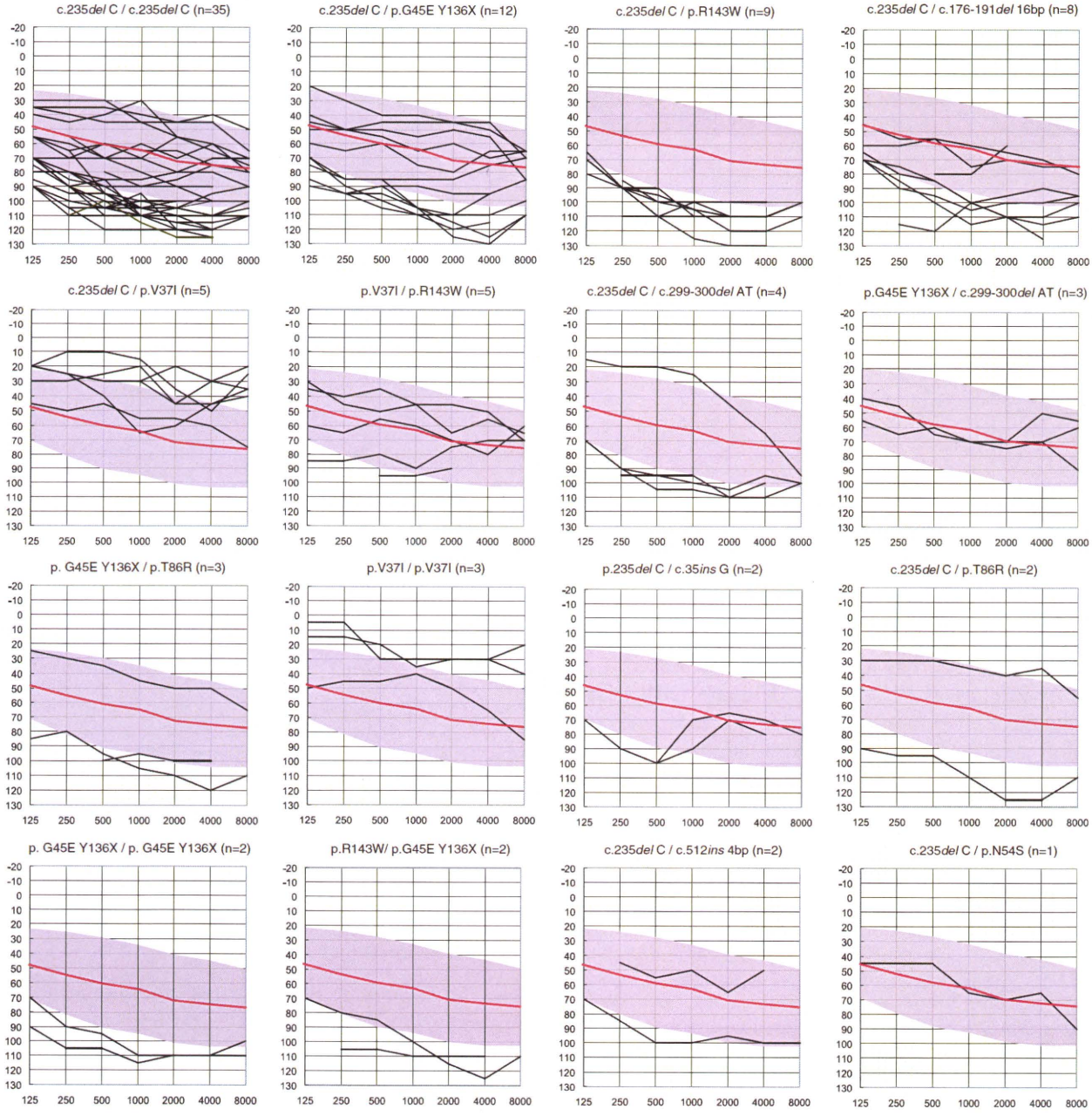

Fig. 2. Overlapping audiograms from the better ear for each genotype. The average audiogram from all subjects (1343 with bilateral sensorineural hearing loss) is indicated by a red line with standard deviation (shadow).

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

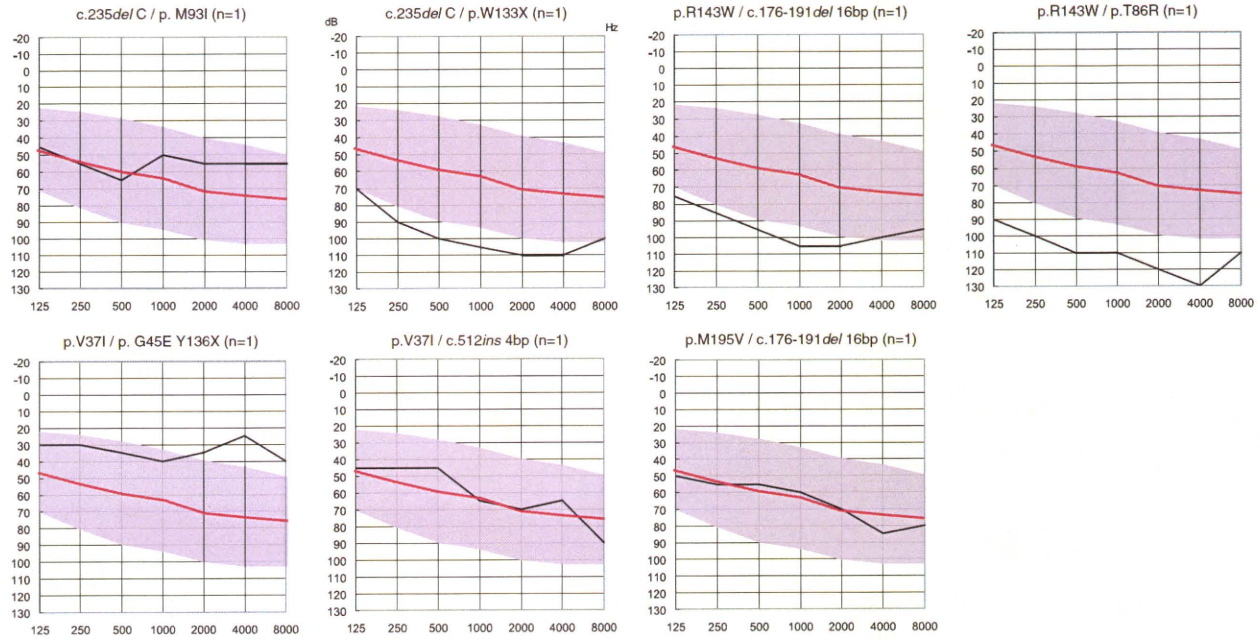

Fig. 2. Continued

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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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).

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).

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%)

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%)

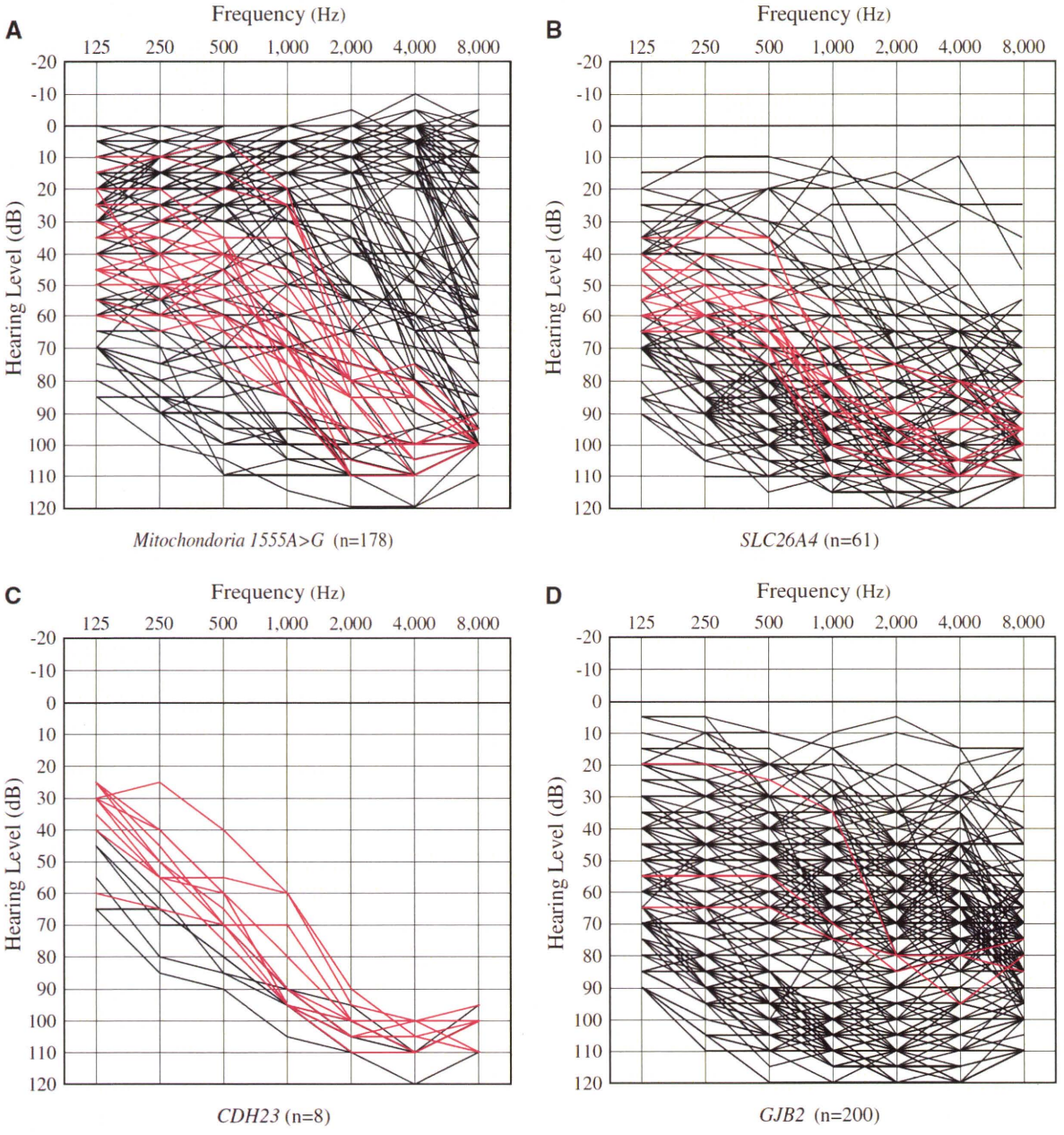

Figure 1. Overlapping audiograms of the patients with mutations. Candidates for EAS are indicated with red lines (A, mitochondrial 1555A>G; B, *SLC26A4;* C, *CDH23;* D, *GJB2*).

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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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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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

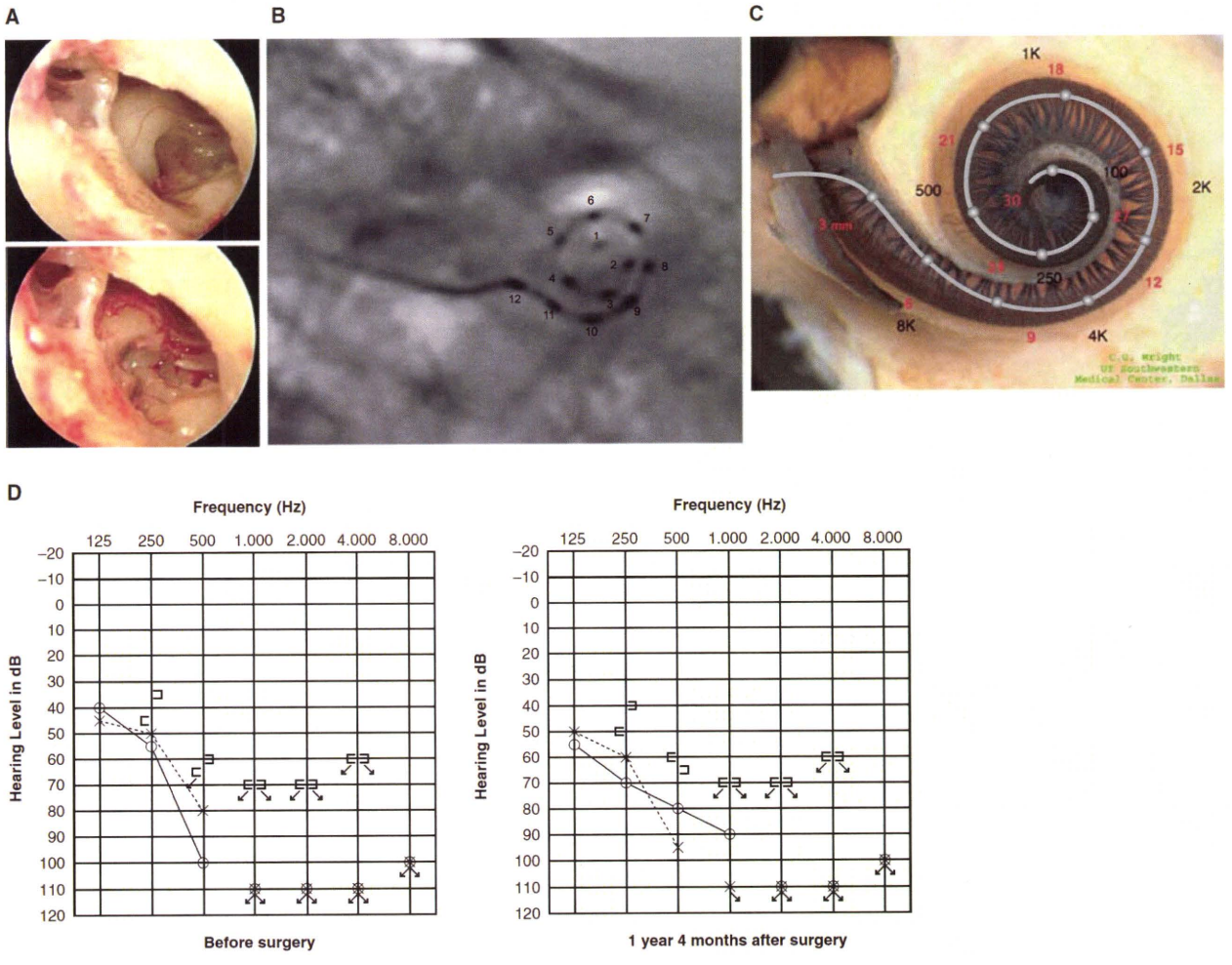

Figure 1. Case 1. A 60-year-old woman presented with slowly progressive bilateral hearing loss from age 40. By age 50 she had only minimal gain from hearing aids and when we first saw her they were nearly useless in her daily life. COMBI40+ with regular electrode was used for this patient on Dec 10,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

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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

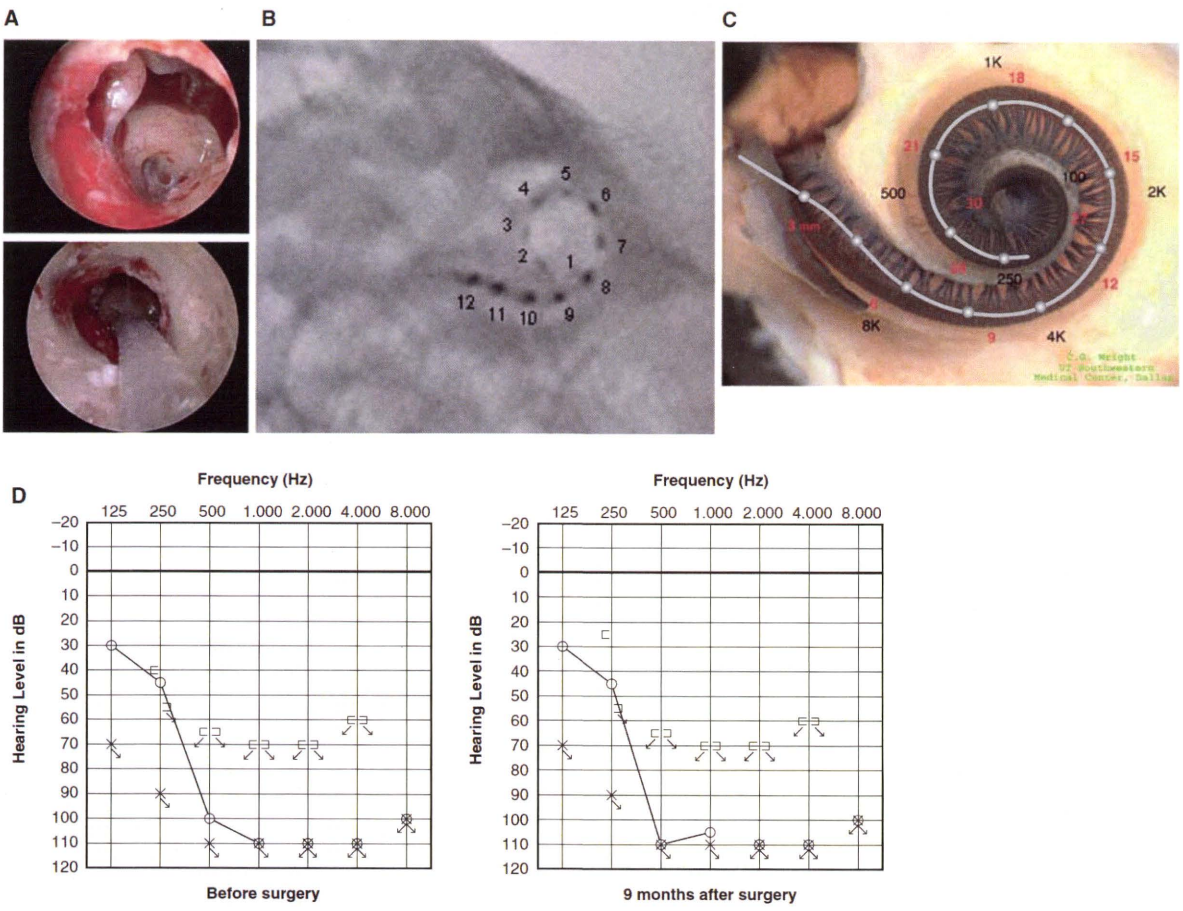

Figure 2. Case 2. This 39-year-old man was congenitally deaf in the left ear. Mild hearing loss in his right ear was noticed in childhood, and he presented with progressive hearing loss of 10 years duration. FLEXeas/RW approach was applied on Nov 16, 2009. Preservation of residual hearing was obtained.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

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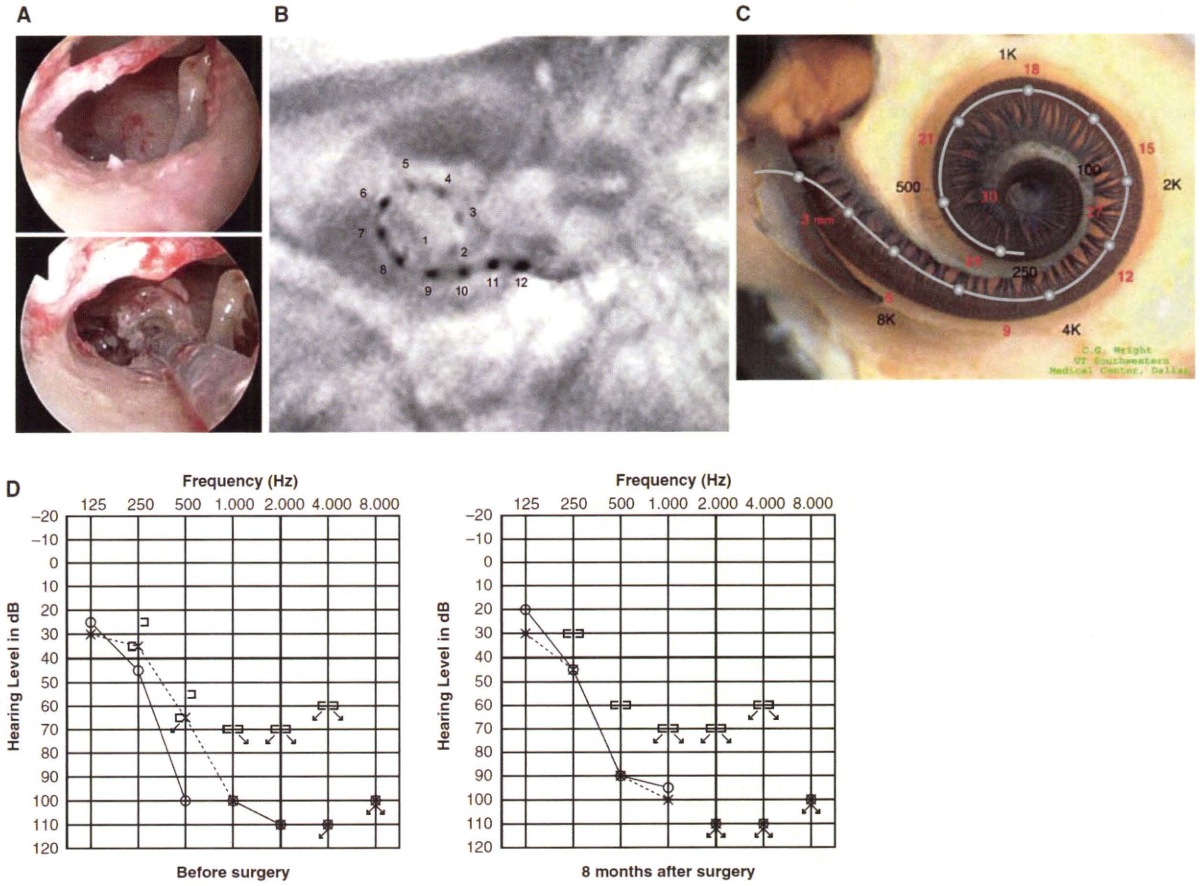

Figure 3. Case 3. This 45-year-old woman became aware of bilateral hearing loss and tinnitus around age 25. When she presented to us it had been slowly progressing for 10 years. PULSAR FLEXeas/RW approach was applied on Nov 18, 2009. Preservation of residual hearing was obtained. (A) Endoscopic view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

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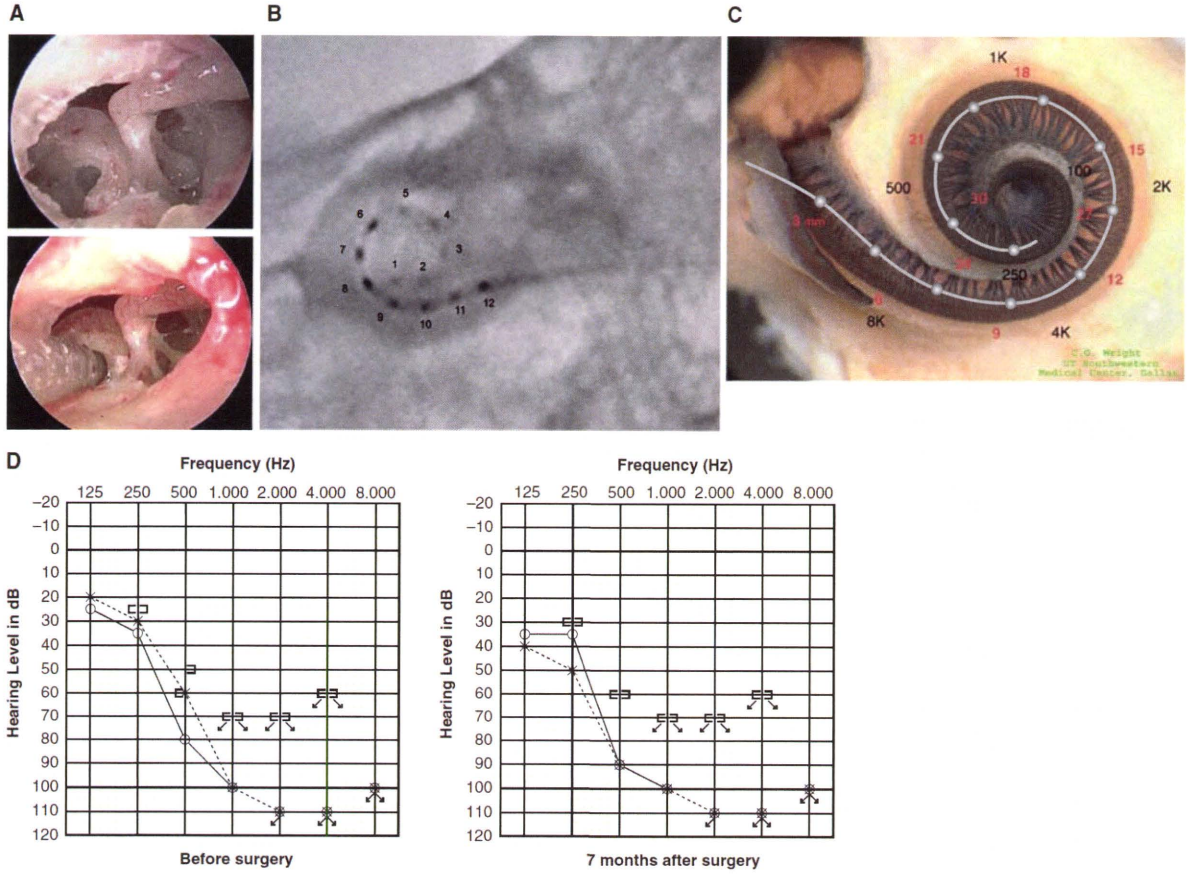

Figure 4. Case 4. This 38-year-old woman had hearing loss detected by mass screening in primary school. It appeared to slowly progress as she grew up, and by age 25 she suffered inconvenience in hearing and communication, mainly using only her left ear. The PULSAR FLEXeas/RW approach was applied on Dec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

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