Meanwhile, several etiological studies suggest that at least 60% of congenital hearing loss has genetic causes. Recent advances in molecular genetics have made genetic diagnosis possible [8]. The identification of the mutation responsible for hearing loss may provide some information as to cochlear damage, and help predict the time course and manifestations of hearing loss. Genetic testing can therefore be useful in decision-making regarding cochlear implantation and other necessary treatment.

Evaluation of brain function and diagnosing accurate etiology of hearing loss may be the keys to personalizing post-cochlear implantation habilitation programs and predicting the outcomes thereof.

In this study, we used 18 F-fluorodeoxyglucose (FDG) positron emission tomography (PET) to measure cortical glucose metabolism with a visual language task before cochlear implantation in profoundly deaf patients whose etiologies were identified by genetic testing.

Material and methods

Genetic diagnosis

Genetic screening was performed in two cases using an Invader assay to screen for 41 known hearing loss-related mutations [9] and direct sequencing for *GJB2* and *SLC26A4* mutations [10,11].

FDG-PET scanning and image analysis

FDG-PET scanning and image analysis were performed using the method described by Fujiwara et al. [12]. During the time period between the intravenous injection of 370 MBq 18 F-FDG (the dose was adjusted according to the body weight of each subject) and the PET scanning of the brain, the patients were instructed to watch a video of the face of a speaking person reading a children's book. The video lasted for 30 min, and several still illustrations taken from the book were inserted (for a few seconds each) to help the subjects to follow the story. The subjects were video-recorded to confirm that they were watching the task video. PET images were acquired with a GE ADVANCE NXi system (General Electric Medical Systems, Milwaukee, WI, USA). Spatial preprocessing and statistical analysis were performed with SPM2 (Institute of Neurology, University College of London, UK) implemented in Matlab (Mathworks, MA, USA). The cortical radioactivity of each deaf patient was compared with that of a control group of normal-hearing adults by a *t* test in the basic model of SPM2. The statistical significance level was set at $p < 0.001$ (uncorrected).

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

Control group

The control group consisted of six normal-hearing right-handed adult subjects. The average (mean ± standard deviation) age of the normal-hearing subjects was 27.5 ± 3.8 years. The pure-tone average hearing levels were within 20 dB HL for all.

Case 1

A right-handed 22-year-old female with a *GJB2* mutation (235 delC homozygous) had hearing impairment that was noticed by her parents when she was 2 years old. She had used hearing aids ever since, but with insufficient hearing amplification. She used lip-reading and some sign language, and her speech was not intelligible to hearing people. Computed tomography (CT) findings of the middle and inner ear were normal. Her average pure-tone hearing levels were 102.5 dB for the right ear and 95 dB for the left ear (Figure 1A).

Case 2

A right-handed 26-year-old male with an *SLC26A4* mutation (H723R homozygous) had hearing impairment that was noticed by his parents when he was 2 years old, from which time he had used hearing aids bilaterally. He did not use lip-reading or sign language during the acquisition age for language. He obtained spoken language with hearing aids but had progressive hearing loss, and sometimes suffered vertigo attacks. His pronunciation was clear, and his speech was almost completely intelligible. CT findings exhibited an enlarged vestibular aqueduct on each side. His average pure-tone hearing levels were 106.2 dB for the right ear and 100 dB for left ear (Figure 1B).

Results

Figure 2 shows transaxial PET images of each participant's brain. The visual stimuli resulted in bilateral activation of the superior temporal gyrus, including Heschl's gyrus in case 1 with *GJB2* mutation (Figure 2A, white arrowhead). In contrast, in case 2 with *SLC26A4* mutation, the activation of the superior temporal gyrus was much lower than in case 1 (Figure 2B, white arrowhead).

Figure 3 shows supra-threshold clusters in each case. In case 1, activation higher than normal controls

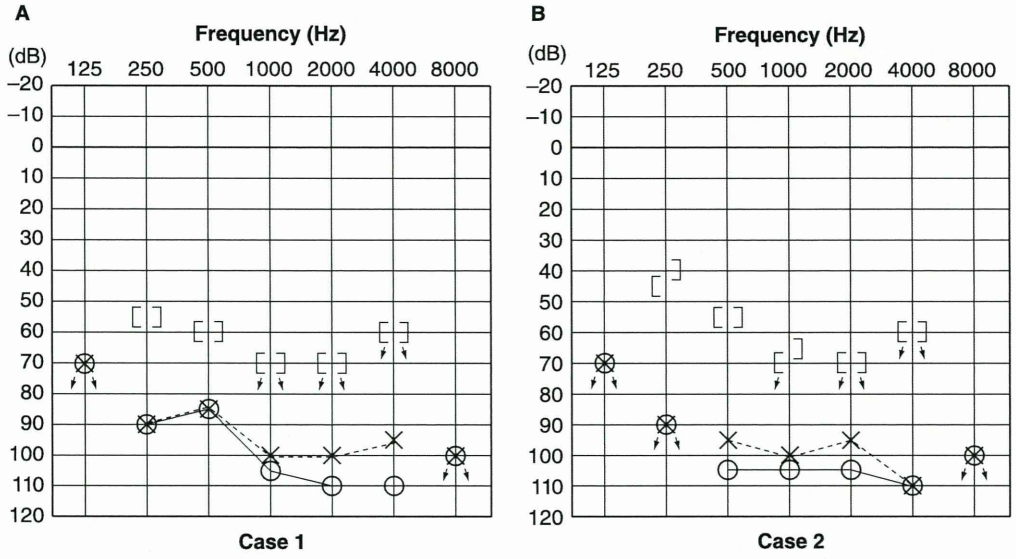

Figure 1. Pure-tone audiograms: (A) a 22-year-old female with a *GJB2* mutation; (B) a 26-year-old male with an *SLC26A4* mutation. There were no clear differences in hearing thresholds in these two cases.

was observed in the right auditory association area [BA21, BA22], and the left auditory association area [BA42] ($p < 0.001$). In case 2, the right superior frontal gyrus [BA9], and the middle temporal gyrus [BA20], showed higher activation than normal controls ($p < 0.001$).

Discussion

More than half of congenital hearing loss has been estimated to be from genetic causes, and phenotypes are affected by genetic mutations. There have been no reports of the influence of phenotype on brain function associated with hearing. This is the first report on evaluation of cortical processing of language in patients with genetic mutations as a main etiology of hearing loss. The auditory association area was activated bilaterally in case 1 (*GJB2* mutation), but not activated in case 2 (*SLC26A4* mutation). A previous study indicated that the temporal lobe is activated during speech-reading in normal subjects [13] and another study found that the temporal lobe is not activated when reading fluent speech from a talking face [14]. For the present study we used a

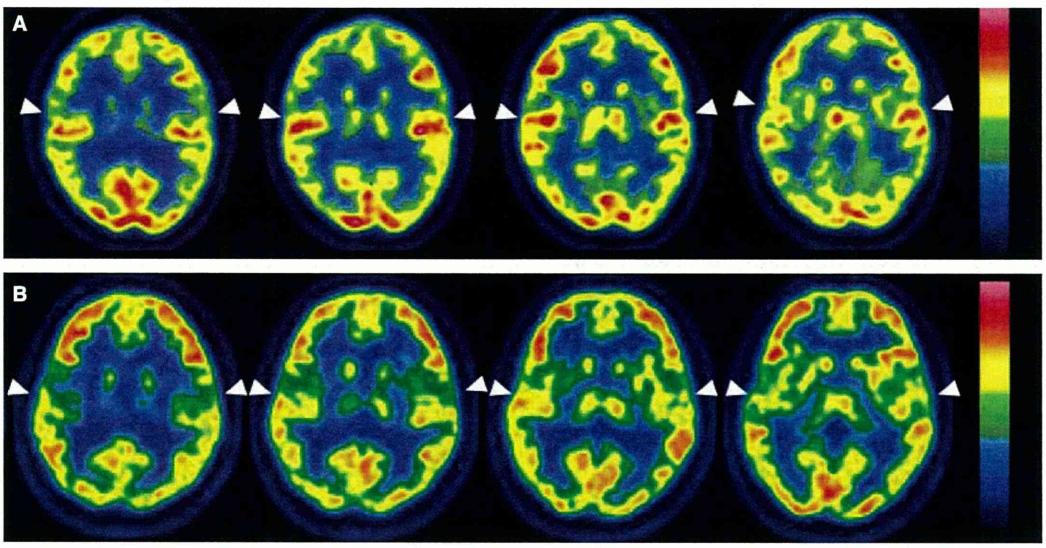

Figure 2. Transaxial PET images of each participant's brain: activation (arrowheads) of the superior temporal gyrus with visual language stimuli in each case. (A) Case 1 (*GJB2* mutation). The superior temporal gyri were strongly activated bilaterally. (B) Case 2 (*SLC26A4* mutation). The superior temporal gyri exhibited less activation than in case 1.

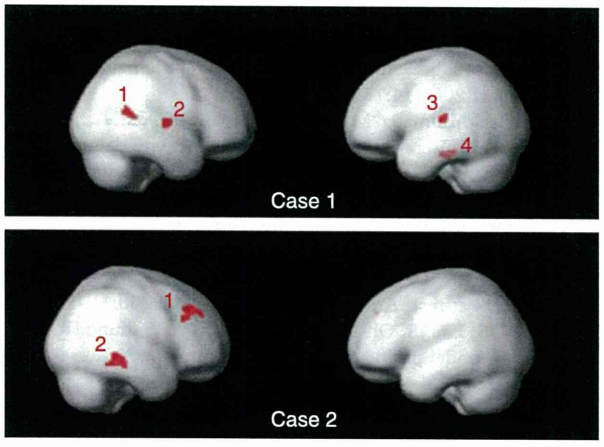

Figure 3. Cortical activation by language-related visual stimuli in the two profoundly deafened cases. Case 1 (*GJB2* mutation) showed significant activation in the right middle temporal gyrus [BA21] (1), superior temporal gyrus [BA22] (2), and left superior temporal gyrus [BA42] (3), and left cerebellum (4), while case 2 (*SLC26A4* mutation) exhibited significant activation in the right superior frontal gyrus [BA9] (1), and middle temporal gyrus [BA20] (2) (SPM2, $p < 0.001$, uncorrected).

fluent speech-reading task, similar to that described by Hall et al. [14]. Fujiwara et al. in a FDG-PET study using the same methods and task as the present study, showed that subjects with better spoken language skills had less temporal lobe activation [12].

To summarize these reports, the patients with hearing aids with better spoken language skills have less temporal lobe activation with a visual language task. Otherwise, Nishimura et al. [15] reported a sign language activation of the bilateral auditory association areas in a congenitally deafened subject. However, detailed clinical data for the subject – including his hearing levels, time course of hearing loss, and the cause of deafness – were not described. The different visual language activation patterns in the auditory cortices revealed in the current two profoundly deafened subjects with different genetic etiologies and hearing loss progressions may, thus, add further knowledge of the cross-modal plasticity brought about in the superior temporal association areas by lack of hearing.

The differences in cortical processing patterns between cases 1 and 2 – who both had hearing loss of cochlear origin – may have been influenced by the differing clinical courses of hearing loss. *GJB2* is currently known to be the most prevalent gene responsible for congenital hearing loss worldwide. Patients with severe phenotypes who have *GJB2* mutations are good candidates for implantation, because their hearing loss is of cochlear origin and non-progressive [16,17]. *SLC26A4* is known as a commonly found gene and is associated with enlarged vestibular aquaduct [11]. This phenotype includes congenital and progressive hearing loss, usually associated with vertigo [18]. In most cases hearing remains in low frequencies, enabling the understanding of spoken language with hearing aids. Cochlear implantation has resulted in remarkable improvements in auditory skills and speech perception for patients with profound hearing loss associated with *SLC26A4* mutations as well as *GJB2*.

Comparing case 1 (*GJB2* mutation) with case 2 (*SLC26A4* mutation), the crucial importance of the use of hearing aids during childhood up to age 6 years for acquisition of better hearing is evident. In case 1, even though she was able to hear sound with the use of hearing aids, she was unable to recognize enough speech language due to insufficient hearing amplification during the critical periods in her childhood. She therefore used lip-reading and some sign language in addition to hearing aids. Increased metabolism was observed by FDG-PET in the auditory association area, where no significant activation was found in the normal-hearing controls. In contrast, in case 2, a 26-year-old patient with an *SLC26A4* mutation, there was no significant activation in the corresponding area. He obtained rather hearing ability and spoken language by hearing aids with residual hearing at lower frequencies during his childhood. His hearing was supposed to be better than case 1, because 1) he did not use lip-reading or sign language during the acquisition age for language from anamnestic evaluation; 2) his pronunciation was clear, indicating better hearing (at least 40–50 dB) during the acquisition age for language; 3) from an etiological point of view, patients with *SLC26A4* mutation usually have mild to moderate hearing loss during childhood and this shows a progressive nature [18]. He had progressive hearing loss in the natural history as a phenotype of *SLC26A4* mutation. The difference in activation patterns in the cases with *GJB2* and *SLC26A4* mutations was clearly demonstrated by statistical processing with SPM, as well as in the PET scans. These results suggest the importance of hearing during early childhood for the development of a normal cortical language network, and that reorganization had occurred in the auditory cortex of the patient with a *GJB2* mutation; i.e. processing visual aspects of language in the superior temporal gyri. This implies that cross-modal plasticity as a consequence of the lack of hearing during the critical period for spoken language acquisition in early childhood was influenced by the time course of hearing loss characterized by genetic mutations.

Previous studies have suggested that auditory areas presented high accumulation of FDG with deafness of early onset, and plastic changes in auditory cortices

were strongly affected by the duration of auditory deprivation [1,5,6,19,20]. Since low activation of the auditory cortices with visual stimuli suggests the subject's lesser dependence on visual communication methods and substantial residual plasticity in his auditory cortices, case 2 with an *SLC26A4* mutation may be determined to be an appropriate candidate for cochlear implantation.

Accurate diagnosis of hearing loss and early cochlear implantation are important for successful spoken language development. The approach using PET could help those involved in the habilitation and education of prelingually deafened children to decide upon the suitable mode of communication for each individual.

Both of the patients received cochlear implantation after PET examination. Further follow-up of these cases may indicate that efficacy of the combination of genetic diagnosis and functional brain imaging helps to predict long-term outcomes of cochlear implantation. Examination of more cases is necessary to define the relationship of the varying cortical activation patterns with each genetic mutation.

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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

Factors that affect hearing level in individuals with the mitochondrial 1555A>G mutation

Lu SY, Nishio S, Tsukada K, Oguchi T, Kobayashi K, Abe S, Usami S. Factors that affect hearing level in individuals with the mitochondrial 1555A>G mutation.
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The mitochondrial 1555A>G mutation is one of the most common mutations responsible for hearing loss in Asians. Although the association with aminoglycoside exposure is well known, there is great variation in the severity of hearing loss. We analyzed hearing levels in 221 Japanese individuals with this mutation and attempted to identify relevant covariants including (i) age, (ii) aminoglycoside exposure, (iii) heteroplasmy ratio, and (iv) other gene mutations. At every age, average hearing levels were worse than those in normal subjects, suggesting that mitochondrial function itself may affect the severity of hearing loss. Although the hearing loss in individuals with the 1555A>G mutation progressed with age, the rate did not differ from that of the normal subjects. Those who had reported aminoglycoside exposure had moderate-to-severe hearing impairment regardless of age, confirming that such exposure is the most important environmental variable. We also confirmed the presence of heteroplasmy, which is known to modify the expression of other mitochondrial diseases, but found no evidence for a significant correlation with hearing impairment. A high prevalence of *GJB2* heterozygous mutations was noted, indicating that these mutations may exhibit epistatic interaction with the 1555A>G mutation.

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Key words: 12S rRNA – 1555A>G mutation – aminoglycosides – *GJB2* – hearing loss – mitochondria

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The 1555A>G mutation in the mitochondrial 12S rRNA gene (1) is the commonest mitochondrial mutation associated with hearing loss. Generally associated with aminoglycoside exposure (2, 3), there are also well-documented patients without a history of exposure (4–6). Systematic screening of Japanese hearing loss patients revealed that approximately 3–5% of these subjects had the 1555A>G mitochondrial mutation, and in those patients who had reported aminoglycoside exposure, the mutation was found in 33% (1, 7). This mutation has been found not only in patients with late-onset hearing loss but also in those with congenital/early-onset sensorineural hearing loss (8). The mitochondrial 1555A>G mutation has been considered to be transmitted in the homoplasmic state, but there have been recent reports of patients with heteroplasmy (8, 9). In an effort to prevent severe deafness, we distribute a drug use warning card advising avoidance of aminoglycosides to 1555A>G mutation family members who are not yet affected (10). The hearing impairment associated with aminoglycoside exposure is usually a bilateral, progressive, high-frequency sensorineural loss. Although it is clear that the patients who report a history of aminoglycoside exposure have a more severe hearing impairment, the severity of deafness is variable (4, 6), suggesting the contribution of additional factors. Age-related expression/progression of hearing loss is one possible factor (4, 5). The existence of modifier genes has also been postulated (11–14), although no candidate genes have been identified. Finally, it was also recently reported that heteroplasmy ratios of the mitochondrial 1555A>G mutation appear to be associated with phenotype variability (9). In order to clarify the possible involvement of these factors in the severity of hearing loss, we investigated the effect of (i) age, (ii) aminoglycoside exposure, (iii) heteroplasmy ratio, and (iv) other gene mutations in 221 individuals with the 1555A>G mutation.

Materials and methods

Subjects

The subjects in this study were 221 Japanese individuals from 67 families with the 1555A>G mutation, ranging in age from 2 months to 87 years. The number of affected members in individual families ranged from 1 to 24 with an approximate average of 3.3. The control group used to determine *GJB2* allele frequency was composed of 252 independent Japanese subjects with normal hearing.

Methods

Audiological analysis

Hearing level was classified using a pure-tone average over 500, 1000, 2000, and 4000 Hz in the better hearing ear. The hearing tests were performed at ages 4–87 years.

Mutation analysis

We screened for the 1555A>G mitochondrial DNA (mtDNA) mutation as described previously (4). In brief, total DNA including genome DNA and mtDNA was extracted from the blood, and the mitochondrial nucleotides 1252 through 1726 were amplified by polymerase chain reaction (PCR). To identify the *Alw*26I site, digestion was performed with a restriction enzyme (*Alw*26I). An ABI sequencer 3100XL (Perkin Elmer Co., Ltd, Waltham, MA) was used to confirm the 1555A>G mutation by direct sequencing.

To identify *GJB2* mutations, a DNA fragment containing the entire coding region was amplified using the primer pair Cx48U/Cx1040L (15). PCR products were sequenced and analyzed with an ABI sequencer 3100XL (Perkin Elmer Co., Ltd). [See Abe et al. (15) for details of the sequencing analysis methods.]

Heteroplasmy ratio of the 1555A>G mitochondrial mutation

The Hitachi FMBIO II image scanning machine (Hitachi Co., Ltd, Minatoku, Tokyo, Japan), a fluorescence imaging system, was used to quantify the heteroplasmy ratio by detection of fluorescently labeled and digested PCR products as described below. A 459 bp DNA fragment was amplified with Ex *Taq* DNA polymerase (Takara Bio Inc., Ohtsushi, Shiga, Japan) using 200 ng of DNA from the subject as a template. Primer sequences were as follows: upper primer, 5′- GCCTATATACCGCCATCTTC -3′; lower primer, 5′- TCTGGTAGTAAGGTGGAGTG -3′. The upper primer was fluorescently labeled at 5′ with rhodamine. PCR conditions were 95°C for 6 min, followed by 27 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 50 s and 72°C for 7 min. The PCR products were digested with restriction endonuclease *Alw*26I (Fermentas; 2.5 units, 37°C for 8–16 h). The subsequent PCR products were digested at 37°C for 8–16 h with 2.5 units of *Alw*26I (Fermentas). Two fluorescent products, wild type (300 bp) and/or mutant (459 bp), were detected because the 1555A>G mutation destroys the restriction site for *Alw*26I. The fluorescent intensity of the mutant bands in quantification experiments from two independent PCR amplifications was used to estimate the proportion of mutant copies in heteroplasmic subjects. We subcloned the insert including the 1555 position into the pDrive cloning vector using a QIAGEN PCR cloning kit (10) (QIAGEN, Hilden, Germany) as an appropriate standard of mutant heteroplasmy. The standard mixtures containing different amounts of wild-type and mutant synthesized oligonucleotides were used with analytical runs to quantify heteroplasmy of mtDNAs.

Statistical analyses

Student's *t*-test was used to compare average hearing levels of subjects with and without *GJB2* mutations and with and without aminoglycoside exposure.

Results

The hearing loss of individuals with the 1555A>G mutation progressed with age; however, the rate of progression did not differ from that found in the normal population (Fig. 1a). The aminoglycoside exposure group had moderate-to-severe hearing impairment regardless of age (Fig. 1b). The existence of heteroplasmy was confirmed in 10 individuals from eight families; however, no apparent correlation was found between heteroplasmy ratio and hearing loss severity (Fig. 1c). There was a high prevalence of *GJB2* heterozygous mutations in individuals bearing the 1555A>G mitochondrial mutation (Table 1), and their hearing levels tended to be worse (without *GJB2* mutation, 35.4 dB; with *GJB2* mutation, 42.0 dB), but the difference was not statistically significant (Fig. 1d). All the *GJB2* mutations found were in heterozygous state, and no subjects were associated with biallelic mutations. There was no correlation between mutation genotype and hearing level.

Discussion

The average hearing level in people with the 1555A>G mutation was worse than that in normal populations at any age (Fig. 1a). This

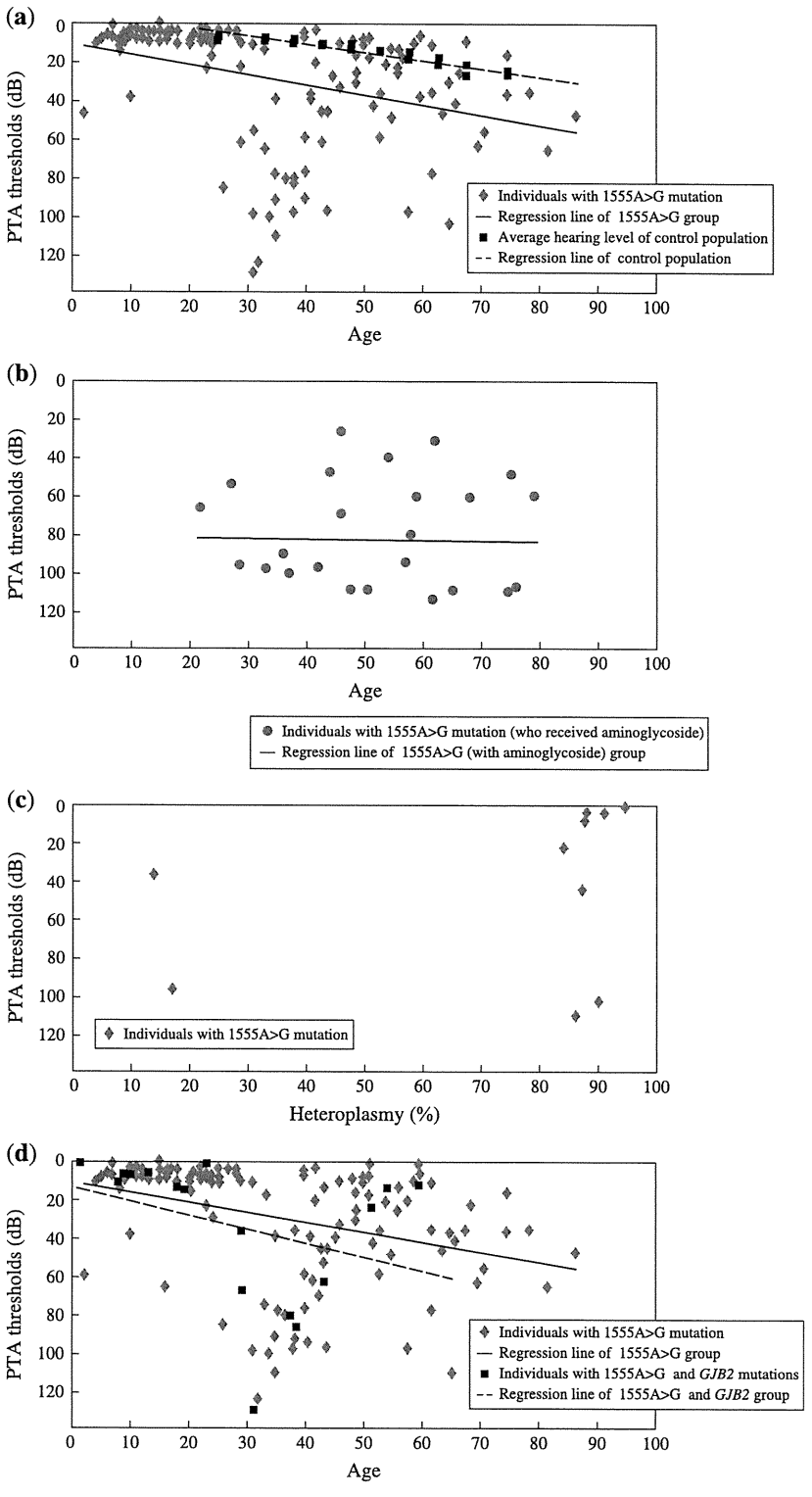

Fig. 1. Hearing levels and various parameters. (**a**) Correlation with age in the 1555A>G mutation group without reported aminoglycoside exposure compared with hearing levels in the normal Japanese population as described by Okamoto et al. (Pure-Tone Hearing Levels According to Age. Audiology Japan 1989: 32:82: 81–86, in Japanese). (**b**) Correlation with age in individuals with the 1555A>G mutation who reported aminoglycoside exposure. (**c**) Correlation with heteroplasmy in individuals with the 1555A>G mutation and no reported aminoglycoside exposure. (**d**) Comparison with age in individuals with the 1555A>G mutation with and without *GJB2* mutations but with no reported aminoglycoside exposure.

suggests that the 1555A>G mitochondria mutation itself or a modifier gene may play a role in aggravating hearing loss. Hearing of the individuals with the 1555A>G mutation also worsened with age; however, the progression speed did not differ from that found in the normal population (Fig. 1a). Interestingly, most of the worst pure-tone audiometry thresholds were clustered in the age range of 30–50 years, indicating possible unreported aminoglycoside exposure as their childhoods coincided with the period in which aminoglycosides were most commonly used in

Table 1. Allele frequency of *GJB2* mutations in 1555A>G and control groups

GJB2 mutations (all hetero genotype)	Mitochondria 1555A>G (n = 26, 14 families)		Control (n = 252)	
	Family number[a]	Allele frequency (%)	Family number	Allele frequency (%)
V37I	2.85	2.13	3	0.60
G45E/Y136X	1.94	1.45	0	0.00
235 del C	1.45	1.08	2	0.40
176-191 del 16bp	0.5	0.37	0	0.00
299-300 del AT	0.19	0.14	0	0.00
Y136H	1	0.75	2	0.40
Total	7.93	5.92	7	1.40

[a]Family numbers in the 1555A>G group were calculated by the following formula: number of family members with the 1555A>G and *GJB2* mutations divided by the total number of family members.

clinical practice including for treatment of childhood infections (1960s to 1980s). Given the above, worsened hearing and mitochondrial function may be related to genetic background (the 1555A>G mitochondrial mutation itself or modifier genes), rather than environmental factors such as noise, because older persons would be expected to have had more exposure to various environmental events and therefore to have a steeper progressive curve.

One significant factor that determines the expression of mitochondrial disease is heteroplasmy. In this study, we confirmed that heteroplasmy existed in about 5% of the subjects with the 1555A>G mutation. The mitochondrial 1555A>G mutation had been thought to transmit only in a homoplasmic state, but recently, heteroplasmic cases have been found to exist and furthermore to be associated with severe hearing loss (9). Analysis of genotype–phenotype correlation indicated that subjects carrying less than 20% of mutant copies were asymptomatic or had a mild hearing loss (9). However, such correlation was not observed in our sample. It should be noted that it is difficult to determine the correlation of heteroplasmy levels with severity of hearing loss because the mutation load in blood may be different from that occurring in the inner ear.

The group that had reported aminoglycoside exposure had moderate-to-severe hearing impairment regardless of age, confirming that aminoglycoside exposure is the most important environmental factor affecting the phenotypic expression of the 1555A>G mitochondrial mutation.

A series of studies indicated that the nuclear background might be involved in modulating the phenotypic expression of the 1555A>G mitochondrial mutation (11). Genome-wide research has suggested that a region in chromosome 8p23.1 is a candidate region as a modifier gene for phenotypic expression (12). Efforts have been made by genotyping and linkage analysis to find nuclear genes that interact with the 1555A>G mutation to cause hearing loss, but no such single gene has yet been identified. Recently, mutations in TRMU were shown to modify the phenotype of the patients with the 1555A>G mutation (14). According to Guan et al., homozygous mutation in this gene leads to a marked failure in mitochondrial tRNA metabolisms, causing impaired mitochondrial protein synthesis.

We previously reported a high prevalence of *GJB2* heterozygous mutations in patients bearing the 1555A>G mitochondrial mutation and described a family in which potential interaction between *GJB2* and a mitochondrial gene appears to be the cause of hearing impairment (13). In that family, patients who are heterozygotes for the *GJB2* mutant allele showed hearing loss more severe than that seen in siblings lacking a mutant *GJB2* allele, suggesting that heterozygous *GJB2* mutations may synergistically cause hearing loss in the presence of a 1555A>G mutation. This indicates that *GJB2* mutations may sometimes be an aggravating factor in addition to aminoglycosides in the phenotypic expression in the non-syndromic hearing loss associated with the 1555A>G mitochondrial mutation (13). Our updated results in this study revealed that 5.92% of the alleles harbored the *GJB2* mutation, and this frequency is significantly (approximately) fourfold higher than that in the normal population, in line with our previous data. However, on average, in the patients without reported aminoglycoside exposure, the hearing loss severity in the 21 individuals with the *GJB2* mutation tended to be worse but not statistically significant when compared with the 165 individuals without the *GJB2* mutation.

Alternatively, it may merely be due to assortative mating having caused accelerated accumulation of various genes in one family (16).

Further study is needed to elucidate the interaction between the *GJB2* mutations and the 1555A>G mutation.

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CASE REPORT

Endolymphatic hydrops and therapeutic effects are visualized in 'atypical' Meniere's disease

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Abstract
A 53-year-old male with fluctuating low frequency sensorineural hearing loss and tinnitus, but without vertigo, was evaluated by MRI obtained by intratympanic injection of a gadolinium-based contrast agent (GBCA) before and after the administration of isosorbide. The endolymphatic hydrops was semi-quantitatively evaluated by a 3.0-T MR scanner. For quantification, the affected side/contralateral side ratios were calculated. A gadodiamide (a kind of GBCA)-enhanced space surrounding the endolymph in the affected side with a 0.50 ratio (which may have represented endolymphatic hydrops) improved after isosorbide therapy to a 0.98 ratio. Thus, endolymphatic hydrops was demonstrated in a patient with 'atypical' Meniere's disease (MD), suggesting that at least some atypical MD may share similar etiology with, and therefore be a continuum of, MD. Also, therapeutic effects could be visualized by using MRI. Therefore, MRI-based diagnosis of MD-related disease will be a powerful tool not only because of its precision but also its usefulness for therapeutic evaluation.

Keywords: *Atypical Meniere's disease, cochlear Meniere's disease, endolymphatic hydrops, MRI, gadolinium-based contrast agents (GBCAs), osmotic diuretics, isosorbide*

Introduction

Meniere's disease (MD) is an idiopathic disorder of the inner ear characterized by fluctuating sensorineural hearing loss (SNHL), tinnitus and aural fullness, and recurrent spontaneous episodic rotational vertigo (see Sajjahi and Paparella for review [1]).

Clinical diagnosis of MD has sometimes been hampered by the diagnostic criteria, because the full complement of symptoms does not develop simultaneously in some cases. These cases have been reported as so-called 'atypical' MD. 'Vestibular MD' is characterized by recurrent episodic vertigo but without hearing loss. In contrast, 'cochlear MD' is defined as having fluctuating low frequency hearing loss without vertigo [2,3].

Although, in 1995, the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) limited the diagnostic term MD to those patients in whom the full complement of symptoms was present, functional testing results for atypical MD mimic classical MD [1]. For example, cochlear MD shows positive glycerol test and histopathologic findings similar to classical MD [2,3], and increased summating potential to eighth nerve action potential (SP/AP) was reported in vestibular MD [4,5]. On the basis of these findings, atypical MD is thought to be a continuum of MD but no additional supportive evidence has come to light for quite some time.

Recent advances in imaging by three-dimensional, fluid-attenuated inversion recovery (3D-FLAIR) of magnetic resonance imaging (MRI), in association with gadolinium-based contrast agents (GBCAs) enhancement, enables visualization of endolymphatic hydrops in patients with MD [6–8]. Interesting questions were 1) whether atypical MD is associated

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with endolymphatic hydrops or not, and 2) whether endolymphatic hydrops can be visualized by MRI. In the present study, we evaluated endolymphatic hydrops, found in cochlear MD, in a quantitative manner before and after osmotic diuretic therapy.

Case study

A 53-year-old male with fluctuating low frequency SNHL and tinnitus, but without vertigo (i.e. not fulfilling the AAO-HNS diagnostic criteria of MD) was evaluated audiologically and with MRI obtained by a 3.0-T scanner.

The patient had experienced hearing fluctuation without associated episodic vertigo four times from 2006 to 2008, and had been treated with steroids and osmotic diuretics. The audiogram and hearing fluctuation of this patient are summarized in Figure 1. Normal speech discrimination and the recruitment investigation tests including Bekesy audiometry and short increment sensitivity index (SISI) test confirmed that there was cochlear involvement. A positive glycerol test (10% glycerol 500 ml, intravenous administration for 2 h with hearing test performed before/after injection) suggested possible endolymphatic hydrops (Figure 2). Initial treatment was hydrocortisone sodium succinate (300 mg/day for 2 days, 200 mg/day for 2 days, 100 mg/day for 2 days) and isosorbide 90 ml/day for 6 days, and was followed by oral osmotic diuretics (isosorbide 90 ml/day) for 350 days.

For imaging study, the protocol described previously [6–8] was applied bilaterally. In brief, diluted gadodiamide (a kind of GBCA) was administered to the bilateral tympanic cavity by injection through the tympanic membrane. After 24 h, the endolymphatic hydrops was evaluated by MRI using a 3.0-T scanner. The areas enhanced by gadodiamide (a kind of GBCA) were measured by imaging analysis software, and the affected side/contralateral side ratios were calculated.

The perilymphatic space is enhanced by gadodiamide (a kind of GBCA), in contrast to the endolymphatic space, which is not. 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.

A reduced perilymphatic space surrounding the endolymph (which may have represented endolymphatic hydrops) was observed in this patient (Figure 3). The gadolinium-enhanced area representing the perilymphatic space ratio was reduced, and the quantitative ratio was 0.50 (Figure 2).

The patient gradually recovered hearing after 350 days and it was stabilized to symmetricity (Figure 3).

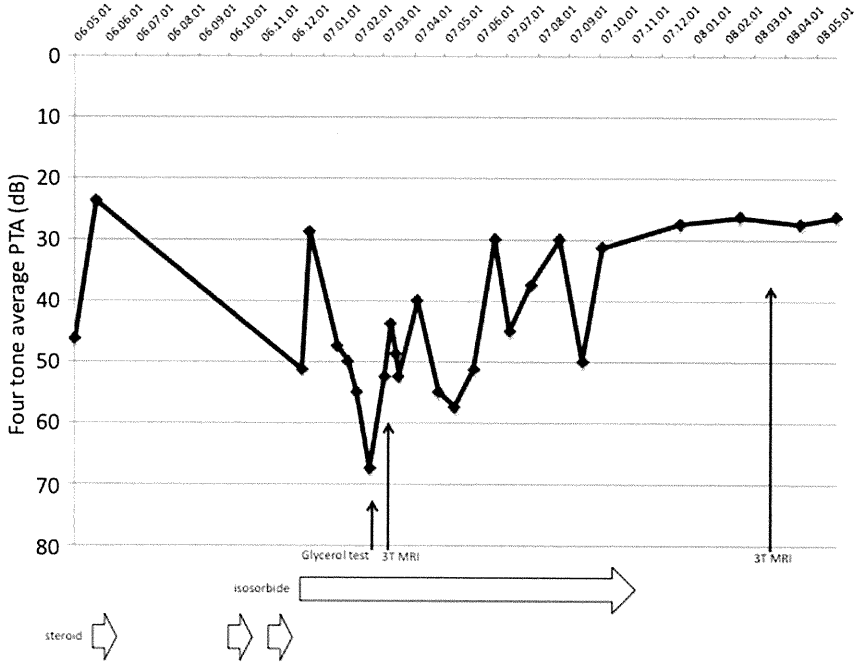

Figure 1. Time course of hearing level (four tone, 500, 1 K, 2 K, 4 K Hz average of pure tone audiometry) and therapeutic agents, indicating that hearing is fluctuated but gradually recovered after long-term osmotic diuretic therapy.

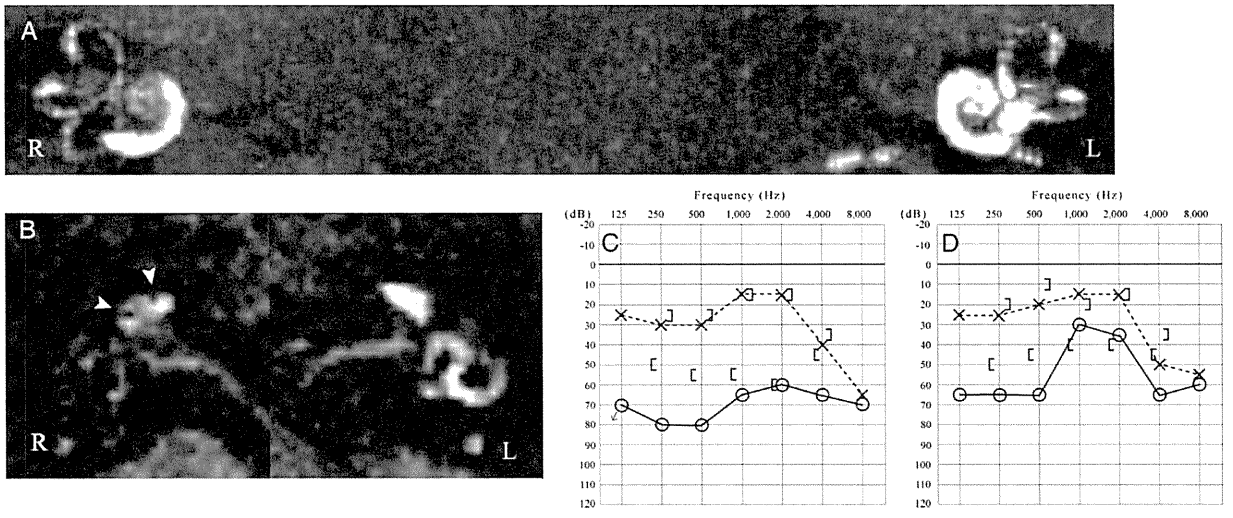

Figure 2. MRI before therapy. (A) The areas enhanced by gadodiamide in the cochlea and vestibule were measured using multi-planar reconstruction (MPR) image by imaging analysis software, and the affected side/unaffected side ratios were calculated. (B) The black area inside the perilymphatic space in the basal turn of the left cochlea that is filled with gadodiamide is the endolymphatic hydrops. On the contralateral side, the endolymphatic space is a significantly small area that is not detectable, likely because of the strong signal intensity in the perilymphatic space. (C, D) Pure tone audiogram: (C) before and (D) after glycerol test.

Discussion

As in definite MD [6–9], in this study, endolymphatic hydrops was demonstrated in an atypical MD patient, who did not fulfill the classical diagnostic criteria for MD, suggesting that at least some atypical MD may share a similar etiology with, and therefore be a continuum of, MD. This concept is supported by the observations that auditory and vestibular symptoms do not always occur simultaneously and the similarity in functional testing results [4]. A series of temporal bone studies also demonstrated that endolymphatic hydrops occurs either locally or entirely [10]. Against this background, recent MRI studies clearly showed inter-individual differences in regional predominance in hydrops; some cases are cochlear predominant whereas some are vestibular predominant. Such differences may lead to a diagnosis of atypical MD, which is a continuum category of disease, and therefore should be treated by the same protocol.

Although MD has been attributed to endolymphatic hydrops, only post-mortem histopathological confirmation has been available. Electrocochleography (EcochG), glycerol test, or other functional

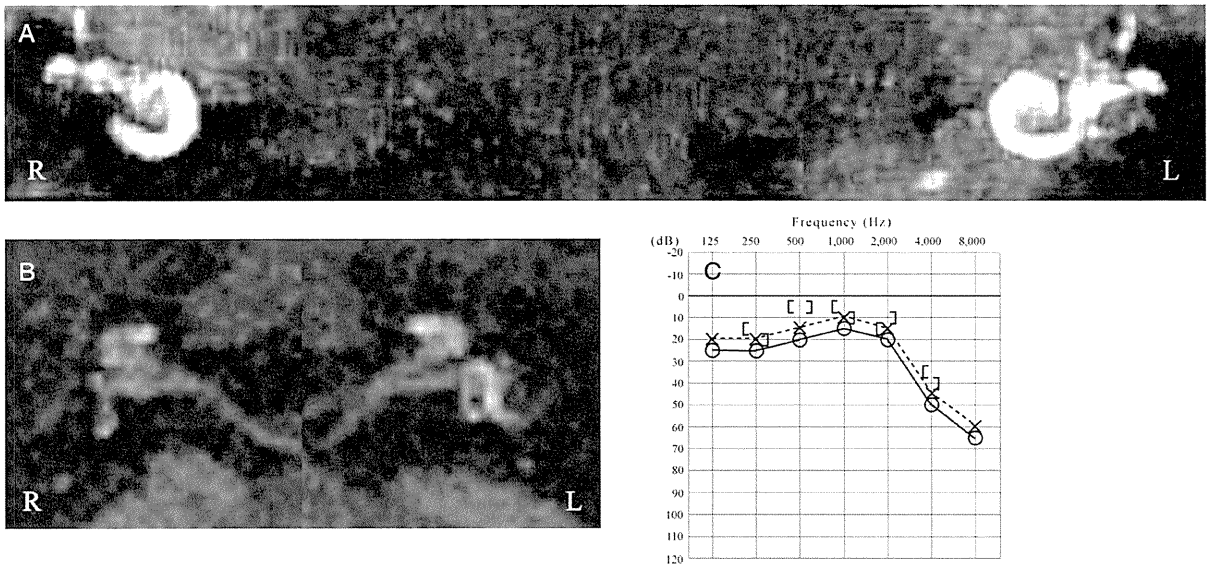

Figure 3. MRI imaging after therapy. (A, B) The area representing the perilymphatic space enhanced by gadodiamide is increased. (C) Hearing is recovered.

testing has been utilized to estimate endolymphatic hydrops, but these do not give direct proof [1]. These limited options of clinical diagnosis and functional testing were all that were available, thus making precise diagnosis of MD difficult.

Accordingly, visualization of endolymphatic hydrops by 3D-FLAIR of MRI, in association with GBCAs enhancement, will be a breakthrough, giving us a powerful tool to confirm endolymphatic hydrops.

The present imaging data demonstrated that cochlear MD is a continuum disease of classical MD; both being characterized by endolymphatic hydrops. Although further study will be necessary to reach any conclusions, in the near future the diagnostic criteria for MD may be reclassified according to image-based diagnosis.

Furthermore, this study is the first to successfully demonstrate the change in the degree of endolymphatic hydrops in the same subject before and after treatment. Quantitative analysis by bilateral administration of GBCAs with 3.0T-MRI is beneficial to such evaluation. Among several treatment choices, the present results demonstrated direct evidence of the change in endolymphatic hydrops, which may be due to the response to therapeutic agents, i.e. osmotic diuretics (isosorbide). Since the possibility remains that these results were a matter of a natural course, a further large cohort study will be necessary. However, this study demonstrated that MRI-based imaging has a great potential to be a powerful tool not only for precise diagnosis of MD and its variants, but also in therapeutic 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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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).

Inheritance mode	Number (%)					
	–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).

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

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

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

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

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

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

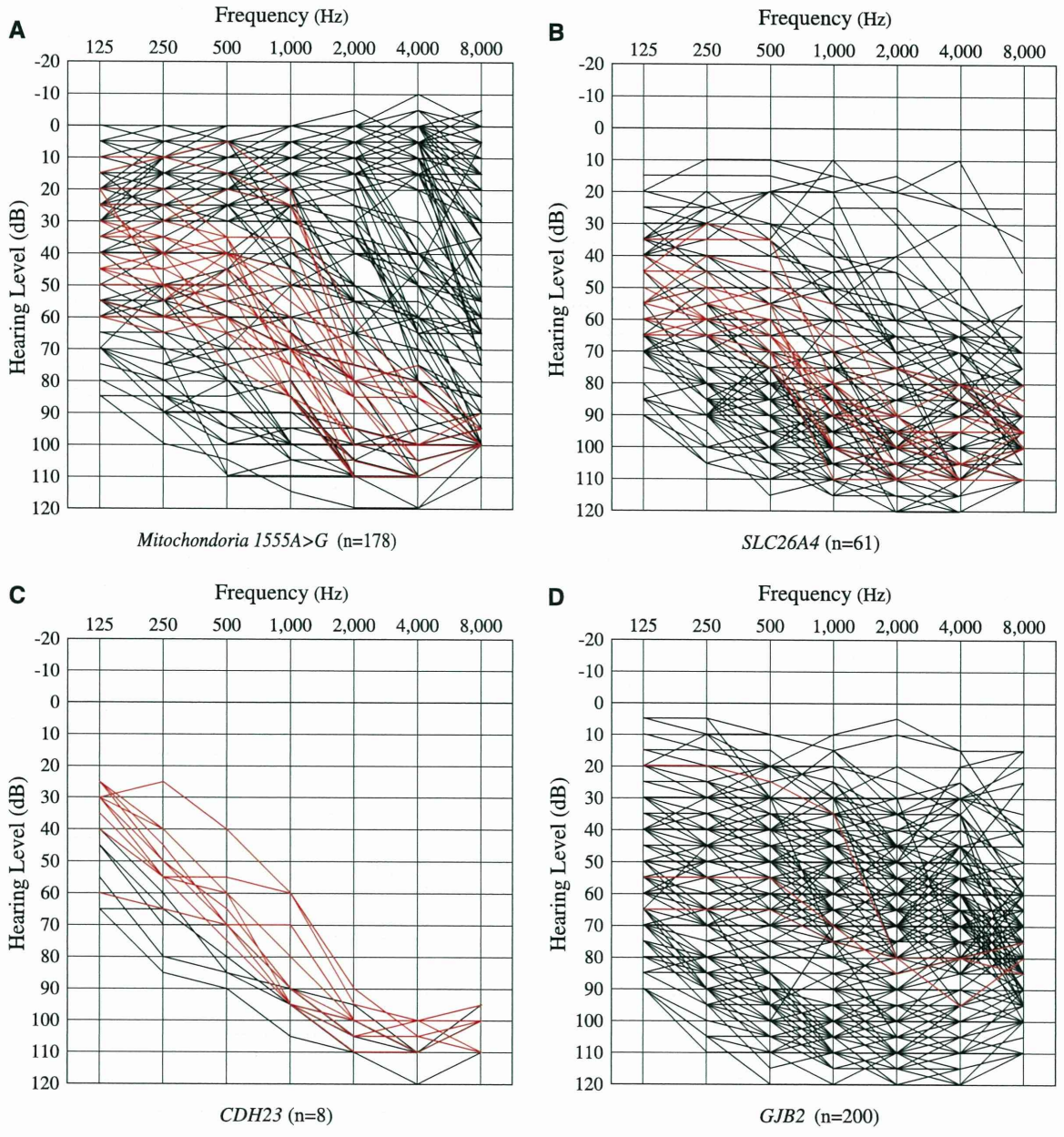

Figure 1. Overlapping audiograms of the patients with mutations. Candidates for EAS are indicated with red lines (A, mitochondrial 1555A>G; B, *SLC26A4;* C, *CDH23;* D, *GJB2*).

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

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

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

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

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

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

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

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

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ORIGINAL ARTICLE

Semi-quantitative evaluation of endolymphatic hydrops by bilateral intratympanic gadolinium-based contrast agent (GBCA) administration with MRI for Meniere's disease

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Abstract

Conclusion: Bilateral intratympanic administration of a gadolinium-based contrast agent (GBCA) in MRI was successfully performed and proved to be beneficial in the semi-quantitative evaluation of endolymphatic hydrops. Such image-based diagnosis will lead to re-revaluation and reclassification of the diagnostic criteria for Meniere's disease (MD). *Objective:* To visualize endolymphatic hydrops semi-quantitatively in patients with MD, by using bilateral intratympanic GBCA administration with MRI. *Patients and methods:* A total of 13 patients were evaluated, including 12 with MD and one with acute low-tone sensorineural hearing loss. Diluted gadodiamide (a kind of GBCA) was administered to the bilateral tympanic cavity by injection through the tympanic membrane. After 24 h, the endolymphatic hydrops was evaluated with a 3.0 T MR scanner. The areas enhanced by gadodiamide were measured semi-quantitatively. *Results:* Three-dimensional, fluid-attenuated inversion recovery (3D-FLAIR) MRI showed that the gadodiamide successfully penetrated the round window membrane, entering the perilymphatic space and delineating the gadodiamide-enhanced perilymphatic and gadodiamide-negative endolymphatic spaces of the inner ear. All the patients with MD showed a reduced gadodiamide-enhanced area representing the perilymphatic space, and the quantitative ratio was 0.15 to 0.85. Furthermore, endolymphatic hydrops was also demonstrated in the patient with atypical MD who had fluctuating low frequency sensorineural hearing loss without vertigo.

Keywords: *Endolymphatic hydrops, Meniere's disease, semi-quantitative analysis, gadolinium, gadolinium-based contrast agent (GBCA), MRI*

Introduction

Meniere's disease (MD) is an idiopathic disorder of the inner ear characterized by fluctuating sensorineural hearing loss (SNHL), tinnitus and aural fullness, and recurrent spontaneous episodic rotational vertigo (see Sajjadi and Paparella for review [1]). MD has been thought to be attributable to endolymphatic hydrops, but this has only been confirmed histopathologically after death. Therefore, MD has been diagnosed on the basis of clinical symptoms and is classified into typical MD with all cochlear and vestibular symptoms, and atypical MD with either cochlear symptoms (e.g. hearing loss, tinnitus, aural pressure) or vestibular symptoms (e.g. vertigo alone with aural pressure) [2]. Typical MD can further be classified into certain, definite, probable, and possible MD according to the nature of the hearing loss, tinnitus, aural fullness, and vertigo [2]. In addition, clinical diagnosis has sometimes been hampered by other conditions that closely resemble MD, such as acute low tone sensorineural hearing loss (ALSNHL) [3]. Therefore, along with clinical symptoms, clinical tests suggestive for endolymphatic hydrops are usually used for diagnosis. Functional

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testing including electrocochleography (EcochG) or glycerol test has been used to estimate endolymphatic hydrops [1]. However, even if functional testing is performed, the results are still indirect proof.

Recent advances in imaging by three-dimensional, fluid-attenuated inversion recovery (3D-FLAIR) of magnetic resonance imaging (MRI), in association with enhancement by gadolinium-based contrast agents (GBCAs), enables visualization of endolymphatic hydrops in patients with MD [4–6]. In the present study, involving patients with typical MD, atypical MD, and ALSNHL, we evaluated endolymphatic hydrops in a semi-quantitative manner, through comparison of bilateral perilymphatic spaces enhanced by a GBCA.

Patients and methods

Subjects

Ten patients with 'definite' MD and one with 'possible' MD who met the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) criteria, one patient with atypical MD (who had fluctuated low frequency sensorineural hearing loss without vertigo), and one patient with acute low-tone sensorineural hearing loss (ALSNHL) participated in this study.

MRI

Gadodiamide (Omniscan, Daiichi Pharmaceutical Co. Ltd, Tokyo) was diluted eightfold with saline, and 0.4–0.6 ml of the diluted gadodiamide was administered to the bilateral tympanic cavity by injection through the tympanic membrane using a 23 G needle. The injection was carried out under a microscope. The patient then lay down in the supine position for 60 min. After 24 h, the endolymphatic hydrops was evaluated by MRI. We used a 3.0 T MR scanner (Trio, Siemens, Erlangen, Germany) with a receive-only eight-channel phased-array coil. It can perform T1-weighted three-dimensional (3D) magnetization prepared rapid gradient echo (MP-RAGE). The parameters for MP-RAGE were: TR 1500 ms, TE 3 ms, matrix size of 320 × 290 × 320; 72 axial 0.8 mm thick slice, 0.8 mm × 0.8 mm × 0.8 mm isotropic voxels, heavily T2-weighted 3D-TSE sequence, and 3D fluid-attenuated inversion recovery (FLAIR) with variable flip angle echo train (SPACE). The parameters for heavily T2-weighted SPACE were: TR 1350 ms, TE 199 ms, echo train length (ETL) 93, matrix size of 320 × 288 × 278, 56 axial 0.8 mm thick slice, and voxel size of 0.6 × 0.4 × 0.8 mm. In addition to the methods described previously, we used 3D-FLAIR with higher in-plane spatial resolution. The scan parameters for the 3D-FLAIR sequence were as follows: repetition time of 10 000 ms, echo time of 666 ms, inversion time of 2500 ms, single slab 3D turbo spin echo with variable flip angle distribution, echo train length of 173, matrix size of 320 × 320, 52 axial 0.8 mm thick slices to cover the labyrinth with a 20 cm square field of view, acceleration factor of two using the parallel imaging technique, and generalized autocalibrating partially parallel acquisitions. Voxel size was 0.7 × 0.8 × 0.8 mm. The number of excitations was one and the scan time was 9 min.

The multi-planar reconstruction (MPR) image was created from 3D-FLAIR images by imaging analysis software (Aquarius Net Viewer). The areas enhanced by gadodiamide in the cochlea and vestibule were traced and measured on the image in the plane perpendicular to the modiolus. Then, the affected side/contralateral side ratios were calculated (Figure 1). Semi-quantitative comparison of endolymphatic space in the vestibule was also calculated using Dicom Viewer software (EV Insite).

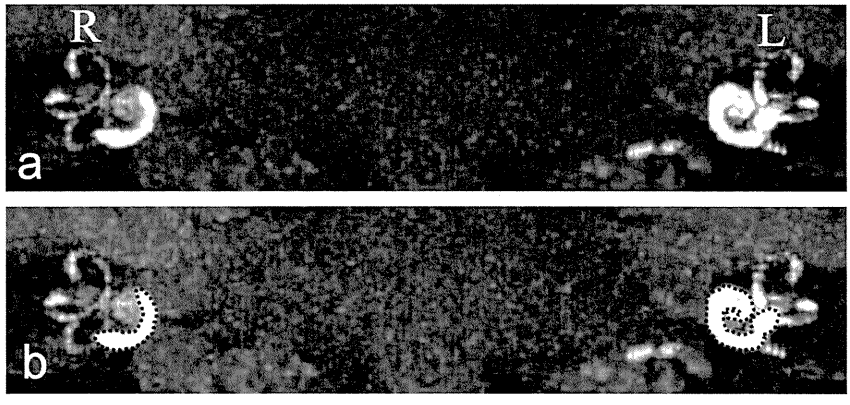

Figure 1. The areas enhanced by gadodiamide in cochlea and vestibule were measured using multi-planar reconstruction (MPR) image by imaging analysis software (dotted lines), and the affected side/unaffected side ratios were calculated.