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Nationwide Survey of Pediatric Cochlear Implant in Japan

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Summary

The Committee for Hearing-Impaired Infants and Children of the Oto-Rhino-Laryngological Society of Japan conducted nationwide surveys of surgery for pediatric cochlear implant in 2005 and 2006 and compared problems of preoperation, operation and postoperative auditory verbal training between the two years. This survey clarified the present problems with regard to pediatric cochlear implants and revealed that more efforts are required to develop better hearing, speech and language skills of patients.

Introduction

To investigate data of pediatric cochlear implants in Japan and to identify current problems, we conducted nationwide questionnaire surveys for all hospitals responsible for cochlear implantation. Since 1994, when cochlear implantation was first covered by the government health insurance system in Japan, we have asked all hospitals to register patients' profiles with the Oto-Rhino-Laryngological Society of Japan. However, over the last several years, the number of pediatric cochlear implants performed has increased markedly and the registration system has been found insufficient to obtain detailed data. This is the first comprehensive report on our data on pediatric cochlear implantation in Japan.

Methods

The Committee for Hearing-Impaired Infants and Children prepared the questionnaire regarding pediatric cochlear implantation in 2005. The questionnaire was sent to 94 hospitals, of which 79 hospitals (84%) sent it back. The questionnaire consisted of 3 sections concerning numbers of patients (3 questions), preoperative backgrounds (19 questions), operations (4 questions) and postoperative auditory training (10 questions). This questionnaire survey was conducted in 2005 and 2006 and changes were compared between these two years. The collected data were analyzed by the committee members. In this study, data of pediatric cochlear implantation in individuals below 6 years of age were analyzed and the results are reported here.

1. Number of patients

The numbers of pediatric cochlear implant were 189 among 399 patients (47.4%) in 2005 and 199 among 474 patients (42%) in 2006. The numbers of pediatric patients below 6 years of age were 156 (39.1%) in 2005 and 198 (41.8%) in 2006.

2. Preoperative background of patients below 6 years of age

1) Average age at operation: 47.7 months in 2005 and 37.6 months in 2006.

2) Average age at diagnosis of deafness: 12.9 months in 2005 and 14.3 months in 2006.

3) Average age at hearing aid fitting: 15.8 months in 2005 and 16.4 months in 2006.

4) How were hearing problems found?

a) Newborn hearing screening and refer: 36 patients (23.1%) in 2005 and 51 patients (25.8%) in 2006.

b) Passed newborn hearing screening but deafness found later:10 patients (6.4%) in 2005 and 16 patients (8.1%) in 2006.

c) Deafness was found without newborn hearing screening:69 patients (44.2%) in 2005 and 88 patients (44.4%) in 2006.

d) Deafness was found through one-and-a-half-year-old childrens' health examination system: 16 patients (10.3%) in 2005 and 12 patients (6.1%) in 2006.

e) Others: 25 patients (16%) in 2005 and 33 patients (16.7%) in 2006.

5) Prelingual age or postlingual age?

Numbers at prelingual age were 107 patients (68.6%) in 2005 and 141 patients (71.2%) in 2006 and numbers at postlingual age were 42 patients (26.9%) in 2005 and 46 patients (23.2%) in 2006.

6) Etiology of deafness (2005 vs. 2006):

a) Cytomegalovirus infection (5 and 11). b) Meningitis (5 and 4).

c) Waardenburg syndrome (4 and 3). d) Inner ear anomaly (5 and 5).

e) Congenital rubella (1 and 2). f) Mumps (1 and 2).

7) Gene abnormality: *GJB2* was detected in 7 patients in 2005 and 6 patients in 2006. *GJB2* (235 delc) was detected in 7 patients in 2005 and 0 patients in 2006.

8) Inner ear anomaly and cochlear nerve hypogenesis: Inner ear anomaly was present in 20 patients (12.8%) in 2005 and 26 patients (13.1%) in 2006 and cochlear

nerve hypogenesis was present in 4 patients (2.6%) in 2005 and 2 patients (1%) in 2006.

9) Auditory neuropathy: 4 patients (2.6%) in 2005 and 3 patients (1.5%) in 2006.

10) Double-handicapped children including these with cerebral palsy, mental retardation, developmental disorders and others: 25 patients (16%) in 2005 and 35 patients (17.7%) in 2006.

11) Another 9 questions were asked but the results are not described here.

3. Operations

1) Difficulties of electrode insertion: 7 patients (4.5%) in 2005 and 10 patients (5.1%) in 2006.

2) Type of device: Cochlear N24 was used in 151 patients (96.8%) in 2005 and 97 patients (49%) in 2006 and Cochlear N24 (Contour) was not used in 2005 but was used in 95 patients (48%) in 2006.

3) Complications including infection and transient facial palsy: 17 patients (10.9%) in 2005 and 9 patients (4.6%) in 2006.

4. Postoperative auditory training

a) Type of training: Auditory oral training was performed in 119 patients (76.3%) in 2005 and 171 patients (86.4%) in 2006. Visual language training was performed in 24 patients (15.4%) in 2005 and 20 patients (10.1%) in 2006.

b) Type of school: Deaf schools were attended by 101 patients (64.8%) in 2005 and 125 patients (63.1%) in 2006 and auditory oral centers for infants were attended by 29 patients (18.6%) in 2005 and 41 patients (20.7%) in 2006.

c) Average hearing level: 36.7dBHL in 2005 and 39.5dBHL in 2006.

d) Mapping: Performed by speech therapists only in 109 hospitals (69.9%) in 2005 and 145 hospitals (73.2%) in 2006. Performed with cooperation between speech therapists and otolaryngologists in 31 hospitals (19.9%) in 2005 and 28 hospital (14.1%) in 2006.

e) Others: Not described here.

Discussion and Conclusion

This nationwide survey clarifies current problems facing congenitally deaf infants and children. The first is the delayed discovery of congenital deafness in infants. The second is that surgeons are limited to only one company's device. The third is the limited numbers of postoperative auditory training preschools for patients. We need to make more efforts to achieve the best outcomes of pediatric cochlear implants.

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Risk Factors for Hearing Loss After Pediatric Meningitis in Japan

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Objectives: We sought to identify predictors for hearing loss in Japanese children with meningitis.

Methods: We analyzed 155 cases of pediatric meningitis without other entities causing hearing loss in children admitted to Saitama Children's Medical Center between 1990 and 2005 for potential risk factors for hearing loss, using multiple logistic regression. Auditory brain stem response tests were performed to evaluate hearing loss.

Results: Of 155 children, 35 (23%) developed hearing loss (21 unilaterally and 14 bilaterally). Profound hearing loss (greater than 90 dB normal hearing level) occurred in 15 patients (9.7%; 4 unilaterally and 11 bilaterally). Of 112 patients with positive cerebrospinal fluid cultures, 27 (24%) developed hearing loss and 13 (12%) showed profound loss. Of 22 patients with *Streptococcus pneumoniae* meningitis, 11 (50%) developed hearing loss and 7 (32%) showed profound loss. Of 54 patients with *Haemophilus influenzae* meningitis, 11 (20%) developed hearing loss and 4 (7.4%) showed profound loss. High serum C-reactive protein levels and cerebrospinal fluid cultures positive for *Streptococcus pneumoniae* were identified as significant risk factors for hearing loss.

Conclusions: A high serum C-reactive protein level was first identified as a risk factor for hearing impairment after pediatric meningitis.

Key Words: Asian population, child, complication, hearing loss, meningitis, risk factor.

INTRODUCTION

Hearing impairment is one of the most important sequelae of pediatric meningitis. Although the incidence has been reported principally in white populations,[1-8] the rates vary considerably among reports, and possible differences among races have not been elucidated. Moreover, only a few articles have reported risk factors or predictors for hearing impairment in pediatric meningitis.[2,3,6,7] Thus, the issue remains controversial.

In this study, we report the first review of a large consecutive series of children with meningitis in an Asian population. We also conducted statistical analyses to elucidate risk factors for hearing impairment in our series.

PATIENTS AND METHODS

Between 1990 and 2005, a total of 192 children (up to 15 years) were admitted to Saitama Children's Medical Center with a diagnosis of meningitis. Of these patients, 155 children without other causes of hearing loss, eg, inner ear anomalies, who underwent auditory brain stem response (ABR) testing during their hospital stay comprised the study subjects. Patient ages ranged from 1 day to 13 years (median, 8 months), and there were 84 male and 71 female patients. The medical records were reviewed retrospectively. Treatment was directed by the pediatricians. The standard treatment comprised administration of antibiotics (penicillins or cephems) along with mannitol or glycerol. Antiviral drugs, gamma globulins, and steroids were sometimes used, but aminoglycosides were administered only exceptionally.

Hearing loss was defined as threshold elevation of 40 dB normal hearing level or more in either ear, determined by click-evoked ABRs. This value of 40 dB was chosen to exclude mild hearing loss, which leaves little potential handicap. Candidate predictors for hearing loss, chosen after previous reports, included patient age, gender, positive cerebrospinal fluid (CSF) culture, CSF glucose level, CSF protein level, CSF white blood cell (WBC) count, serum C-reactive protein (CRP) level, and development of seizures. In an additional analysis to evaluate the influence of bacterial strains, CSF cultures positive for *Streptococcus pneumoniae* and for *Haemophilus influenzae* replaced the parameter of an overall positive CSF culture. Table 1 shows the patient demo-

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TABLE 1. PATIENT DEMOGRAPHICS

Parameter	*Values*
Age	1 d to 13 y (median, 8 mo)
Gender	84 male, 71 female
Serum CRP (mg/dL)	0.0 to 38.2 (mean, 11.1)
CSF glucose (mg/dL)	0.0 to 151 (mean, 37.3)
CSF protein (mg/dL)	6.1 to 3,200 (mean, 277)
CSF WBCs (cells/μL)	8 to 308,000 (mean, 11,600)
Seizure	29/155 (18.7%)
Positive CSF culture	112/152 (73.7%)
Positive CSF culture for *Streptococcus pneumoniae*	22/152 (14.5%)
Positive CSF culture for *Haemophilus influenzae*	54/152 (35.5%)

CRP — C-reactive protein; CSF cerebrospinal fluid; WBCs white blood cells.

graphics. Possible risk factors for hearing impairment were assessed with multiple logistic regression analysis.[9]

RESULTS

Of the 155 children, 35 (23%) developed hearing loss (21 unilaterally and 14 bilaterally). Profound hearing loss (greater than 90 dB normal hearing level) occurred in 15 patients (9.7%; 4 unilaterally and 11 bilaterally). Bacteria were isolated from the CSF cultures in 112 patients. Of these, *S pneumoniae* was isolated in 22 patients and *H influenzae* in 54. Of 112 patients with positive CSF cultures, 27 (24%) developed hearing loss and 13 (12%) showed profound loss. Of 22 patients with *S pneumoniae* meningitis, 11 (50%) developed hearing loss and 7 (32%) showed profound loss. Of 54 patients with *H influenzae* meningitis, 11 (20%) developed hearing loss and 4 (7.4%) showed profound loss. Twenty-nine children developed seizures. Other apparent neurologic complications, eg, involvement of cranial nerves other than the eighth nerve and paralyses of the extremities, were not noted.

Table 2 shows the results of multiple logistic regression analyses. Of 8 candidate factors, only a high serum CRP level was identified as a significant risk factor ($p < 0.05$), for both hearing loss and profound hearing loss. To further analyze the effect of bacterial strains, we replaced the parameter of overall positive CSF culture with that of CSF cultures positive for *S pneumoniae* and for *H influenzae* (Table 3). For overall hearing loss, a CSF culture positive for *S pneumoniae* was determined as the most significant risk factor, whereas the serum CRP level failed to reach significance. However, for profound hearing loss, the serum CRP level regained significance. A CSF culture positive for *S pneumoniae* followed, but failed to reach significance for profound loss. On the basis of these results, a high serum CRP level and a CSF culture positive for *S pneumoniae* were considered important risk factors for developing hearing loss due to pediatric meningitis.

TABLE 2. LOGISTIC REGRESSION USING PARAMETER OF OVERALL POSITIVE CSF CULTURE

Parameter	*Overall Hearing Loss*	*Profound Hearing Loss*
Age (logarithmic)	0.2239	0.8357
Gender	0.1321	0.3257
Serum CRP	0.0470*	0.0216*
CSF glucose	0.2155	0.1746
CSF protein	0.3851	0.9765
CSF WBCs	0.5868	0.6318
Seizure	0.1984	0.4202
Positive CSF culture	0.6142	0.4060

Data are p values.
*Significance ($p < 0.05$)

TABLE 3. LOGISTIC REGRESSION USING PARAMETERS OF POSITIVE CSF CULTURE FOR *STREPTOCOCCUS PNEUMONIAE* AND FOR *HAEMOPHILUS INFLUENZAE* INSTEAD OF OVERALL POSITIVE CSF CULTURE

Parameter	*Overall Hearing Loss*	*Profound Hearing Loss*
Age (logarithmic)	0.2990	0.7204
Gender	0.1879	0.5142
Serum CRP	0.0755	0.0442
CSF glucose	0.1687	0.1810
CSF protein	0.3211	0.9831
CSF WBCs	0.7218	0.7085
Seizure	0.0674	0.2044
Positive CSF culture for *Streptococcus pneumoniae*	0.0284*	0.0528
Positive CSF culture for *Haemophilus influenzae*	0.5390	0.7652

Data are p values.
*Significance ($p < 0.05$).

DISCUSSION

The incidence of hearing loss in pediatric meningitis has been reported principally in Western populations. The overall incidence of hearing impairment has ranged from 14% to 29%,[1,2,5] and that in bacterial meningitis has ranged from 7% to 31%.[1-4,6,7] The incidences reported in the present report — 23% for overall meningitis and 24% for bacterial meningitis — correspond to those in previous reports, despite racial and climate differences. The incidence of hearing loss has been greater in bacterial meningitis than in overall meningitis,[1,2] as was also confirmed by the present findings.

Streptococcus pneumoniae, *H influenzae*, and *Neisseria meningitides* are the 3 most important pathogens for pediatric meningitis. The incidence of

hearing impairment due to *S pneumoniae* meningitis ranges from 20% to 52%,[2-4,6,8] that due to *H influenzae* meningitis ranges from 22% to 41%,[1,2,4] and that due to *N meningitidis* meningitis ranges from 4% to 24%.[2-4,6] The order of preponderance in hearing involvement has remained consistent, including in the present report: *S pneumoniae* first, then *H influenzae* meningitis, and then *N meningitidis* meningitis. Other bacteria cultured in the present study included *Escherichia coli*, methicillin-resistant *Staphylococcus aureus*, other *Streptococcus* species, *Neisseria* species, *Listeria*, etc, but the small number of affected patients prevented these from being nominated as risk factors. In Japan, vaccination for *S pneumoniae* is not yet popular, and that for *H influenzae* type B has only recently been approved. Encouraging vaccination for these 2 bacteria will be important in preventing hearing loss caused by meningitis in our country.

Several risk factors or predictors for hearing impairment in pediatric meningitis have been proposed,[2,3,6-8] but the significance of risk factors has varied greatly among reports. The most common factor was a low CSF glucose level,[2,3,6,7] followed by a CSF culture positive for *S pneumoniae*.[2,6] In the present study, a CSF culture positive for *S pneumoniae* was a significant risk factor, but a low CSF glucose level failed to reach significance. Instead, a high serum CRP level was recognized as another significant risk factor — a finding not reported in previous articles. This outcome is not surprising, since the serum CRP level corresponds to the severity of overall inflammation. In our search of the English-language literature, there were no reports describing risk factors for neurologic dysfunction other than hearing impairment (eg, other cranial nerve disorders) as sequelae after meningitis.

CONCLUSIONS

Logistic regression analyses of our consecutive series of Japanese children with meningitis identified a high serum CRP level and a CSF culture positive for *S pneumoniae* as risk factors for hearing impairment after pediatric meningitis.

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ORIGINAL ARTICLE

Vestibular function of patients with profound deafness related to *GJB2* mutation

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Abstract

Conclusion: GJB2 mutations are responsible not only for deafness but also for the occurrence of vestibular dysfunction. However, vestibular dysfunction tends to be unilateral and less severe in comparison with that of bilateral deafness. *Objectives:* The correlation between the cochlear and vestibular end-organs suggests that some children with congenital deafness may have vestibular impairments. On the other hand, *GJB2* gene mutations are the most common cause of nonsyndromic deafness. The vestibular function of patients with congenital deafness (CD), which is related to *GJB2* gene mutation, remains to be elucidated. The purpose of this study was to analyze the relationship between *GJB2* gene mutation and vestibular dysfunction in adults with CD. *Methods:* A total of 31 subjects, including 10 healthy volunteers and 21 patients with CD, were enrolled in the study. A hearing test and genetic analysis were performed. The vestibular evoked myogenic potentials (VEMPs) were measured and a caloric test was performed to assess the vestibular function. The percentage of vestibular dysfunction was then statistically analyzed. *Results:* The hearing level of all CD patients demonstrated a severe to profound impairment. In seven CD patients, their hearing impairment was related to *GJB2* mutation. Five of the seven patients with CD related to *GJB2* mutation demonstrated abnormalities in one or both of the two tests. The percentage of vestibular dysfunction of the patients with CD related to *GJB2* mutation was statistically higher than in patients with CD unrelated to *GJB2* mutation and in healthy controls.

Keywords: *Vestibular evoked myogenic potentials, caloric test*

Introduction

Since a correlation between the peripheral auditory and vestibular systems has been identified both anatomically and phylogenetically, a subgroup of children with congenital deafness (CD) may be associated with vestibular and balance impairments [1–3]. Interestingly, the vestibular disturbance in these children gradually disappears as they grow up, probably because of a compensatory mechanism of the central nervous system. However, there have been only a few reports that conducted a detailed analysis of the vestibular function in adults with CD.

CD has been reported in approximately one child per 1000 births [1]. In more than half of these cases, the disease is caused by gene mutation. In particular, mutation in the *GJB2* gene, which encodes Cx26 in the gap junction, is known to be a most common cause (up to 50% of such cases) [2,3]. Gap junction channels enable the neighboring cells to exchange small signaling molecules. Immunohistochemical studies have revealed that Cx26 exists not only in the cochlea but also in the vestibular organs [4]. K^+ cycling involving gap junction protein Cx26 in the vestibular labyrinth, which is similar to that in the cochlea, is thought to play a fundamental role in the endolymph homeostasis and sensory transduction [5]. These findings suggest that mutations in the *GJB2* gene may thus cause vestibular dysfunction.

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In this study, the relationship between *GJB2* gene mutation and vestibular dysfunction in adults with CD was investigated to confirm whether or not there are any abnormalities associated with the vestibular function.

Material and methods

Subjects

The subjects in this prospective study included 21 patients with CD and 10 healthy volunteers. The patients were excluded from the study if they were being treated with ototoxic drugs or if they had a cytomegalovirus infection, bacterial meningitis, external and middle ear pathological findings, or other risk factors for inner ear damage. No participants had syndromic deafness due to pigmentary retinopathy, nephropathy, goiter, or any other diseases. Patients with vestibular dysfunction due to head trauma, brain tumor, Menière's disease, or other conditions were also excluded from the study. All subjects underwent an otoscopic examination and were found to have a normal tympanic membrane. Audiometric testing was performed in a double-walled, sound-treated booth. All patients gave their informed consent in writing and the study was approved by the Ethics Committee of Juntendo University School of Medicine.

Genetic analysis

DNA was extracted from peripheral blood leukocytes of the subjects. The coding region of *GJB2* was amplified by PCR using the primers *GJB2*-2F 5′-GTGTGCATTCGTCTTTTCCAG-3′ and *GJB2*-2R 5′-GCGACTGAGCCTTGACA-3′. The PCR products were sequenced using the PCR primers and sequence primers *GJB2*-A 5′-CCACGC-CAGCGCTCCTAGTG-3′ and *GJB2*-B 5′-GAA-GATGCTGCTGCTTGTGTAGG-3′. These were visualized using an ABI Prism 310 Analyzer (PE Applied Biosystems, Tokyo, Japan).

Vestibular evoked myogenic potentials

The vestibular evoked myogenic potentials (VEMPs) were measured as described in a previous report [6]. Both sound stimuli of clicks (0.1 ms, 95 dBnHL) and short tone burst (500 Hz; rise/fall time, 1 ms, 95 dBnHL) were presented to each side of the ear through the headphones using a Neuropack evoked-potential recorder (Nihon Kohden Co. Ltd, Tokyo, Japan). The surface electromyographic activity was recorded with the patient in the supine position from symmetrical sites over the upper half of each sternocleidomastoid (SCM) muscle with a reference electrode on the lateral end of the upper sternum. During recording, the subjects were instructed to lift their head up or to turn the contralateral side to induce hypertonicity of the SCM. Thereafter, the electromyographic signals from the stimulated side of the SCM muscle were amplified.

Caloric test

The caloric test in the current study was performed as described elsewhere [7]. Briefly, 2 ml of ice-water (at 4°C) was irrigated in the external auditory meatus to induce a thermal gradient across the horizontal semicircular canal of one ear. The duration of horizontal and vertical nystagmus was recorded. The results were compared between the right and left ears.

Statistical analysis

The data are expressed as the mean ± SD. Statistical analyses were conducted using a non-repeated measures analysis of variance (ANOVA). Significant effects were further analyzed by post hoc multiple comparison tests using the Student-Newman-Keuls test. A value of $p < 0.05$ was considered to indicate statistical significance.

Results

Hearing test

The pure-tone averages of 0.5, 1.0, and 2.0 kHz are shown in Table I. The hearing impairments of CD patients ranged from severe (71–95 dB) to profound (>95 dB). The hearing levels of all controls were at the normal level (<30 dB; data not shown).

Genetic analysis

GJB2 mutations were found in nine CD patients (Table I). All three mutations have been described previously in association with deafness. Among these mutations, 235delC mutation was found in eight patients. One nonsense mutation (Y136X) and one frameshift mutation (176-191del) were also identified. In six patients with a homozygous *GJB2* mutation and one patient with a compound heterozygous

Table I. Results of hearing level, genetic analysis, and vestibular function of subjects with congenital deafness (CD)

Case no.	Hearing level (dB) Left	Right	Sex	Age (years)	Mutation in *GJB2*	VEMPs	Caloric test
Patients with *GJB2*-related CD							
1	86	98	M	26	Homo 235delC	Right decreased	Left CP
2	106	108	M	25	Homo 235delC	Right decreased	Normal
3	108	106	M	28	Homo 235delC	Right decreased	Normal
4	108	106	M	37	Homo 235delC	Normal	Right CP
5	100	106	M	32	Homo 235delC	Normal	Right poor/left CP
6	80	91	M	25	Homo 235delC	Normal	Normal
7	115	108	M	25	Y136X/235delC	Normal	Normal
Patients without *GJB2*-related CD							
8	98	98	F	24		Left decreased	Bilateral CP
9	98	115	M	26		Normal	Bilateral CP
10	97	97	M	20		Normal	Normal
11	111	108	M	31		Normal	Normal
12	100	104	F	34		Normal	Normal
13	98	95	M	21		Normal	Normal
14	91	91	M	24		Normal	Normal
15	99	101	F	26		Normal	Normal
16	99	95	F	23		Normal	Normal
17	80	68	M	27		Normal	Normal
18	96	95	M	27		Normal	Normal
19	85	73	M	23		Normal	Normal
Patients with heterozygous *GJB2* mutation							
20	73	100	M	25	Hetero 235delC	Normal	Normal
21	97	98	M	25	Hetero 176-191del16	Normal	Normal

CP, canal paresis; Poor, nystagmus was obviously weak.

mutation (case nos 1–7); their profound deafness was thought to be caused by a *GJB2* mutation. No *GJB2* mutation was identified in any of the controls.

Vestibular function

No patients or controls had any subjective symptoms of vertigo. Table I shows the results of the vestibular function in all CD patients. Abnormal responses of VEMPs and the caloric test in CD with a *GJB2*-related mutation were observed in three patients each (case nos 1–5). Three patients with a homozygous *GJB2* mutation showed asymmetrical responses in VEMPs (case nos 1–3). Three patients with a homozygous *GJB2* mutation showed asymmetrical responses in the caloric test (case nos 1, 4, and 5). One of them showed both VEMPs and the caloric test asymmetrical responses (case no. 1). One patient with a homozygous *GJB2* mutation and one patient with compound heterozygous *GJB2* mutation showed normal responses in both VEMPs and the caloric test (case nos 6 and 7). It is notable that five of the six patients with a homozygous 235delC mutation showed no abnormalities in either test. Two heterozygous patients (case nos 20 and 21) showed normal responses in both tests.

Two CD patients with no *GJB2* mutation exhibited abnormal findings for the vestibular tests (case nos. 8 and 9). One patient showed a unilateral reduction in VEMPs and bilateral canal paresis (case no. 8). Bilateral canal paresis was also observed in another patient (case no. 9).

All the controls with normal hearing showed normal responses in both the VEMPs and the caloric test (data not shown).

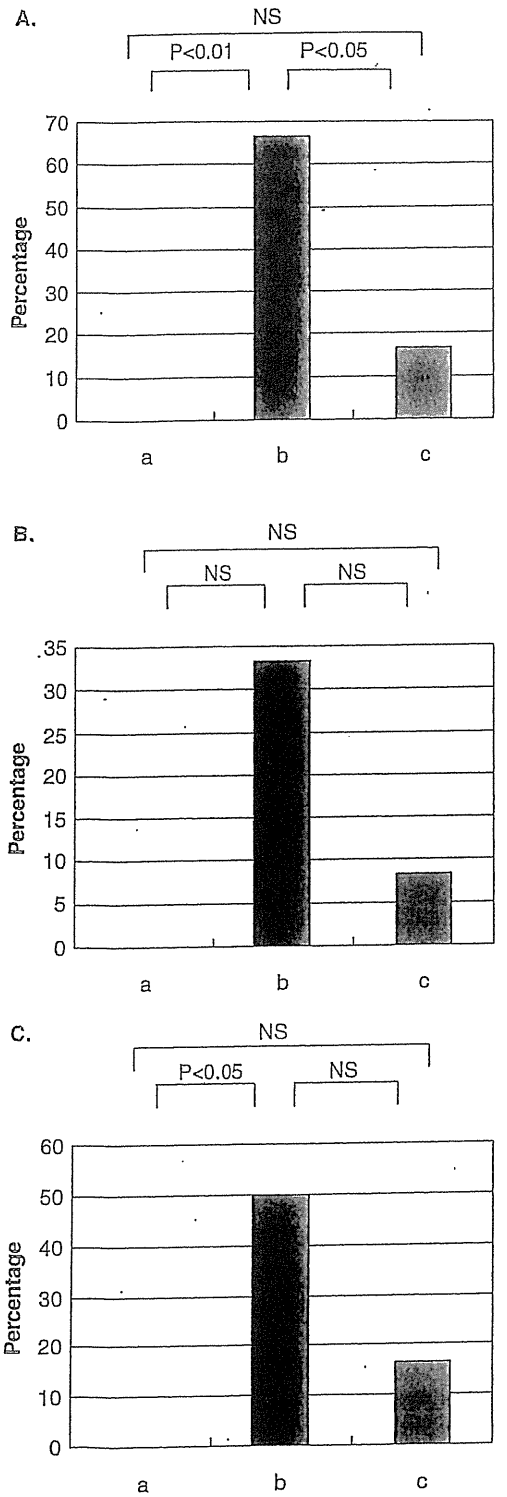

Figure 1. Comparison of the incidence of abnormality in the vestibular tests among the three groups. (A) Percentage showing abnormality in VEMPS and/or caloric test. (B) Percentage showing abnormality in VEMPs. (C) Percentage showing abnormality in the caloric test. a, Controls; b, *GJB2*-related CD subjects; c, CD subjects without *GJB2* mutations.

Statistical analysis of vestibular function in the three groups

Figure 1 shows a comparison of the controls, patients with CD related to a *GJB2* mutation, and those with CD without a *GJB2* mutation. The CD patients with *GJB2* heterozygous mutation were excluded from this statistical analysis, since their symptoms of hearing impairment are not necessarily caused by the *GJB2* mutation alone. Vestibular dysfunction showing an abnormality in VEMP and/or the caloric test significantly increased in patients with *GJB2*-related CD in comparison with those with CD without *GJB2* mutation ($p < 0.05$) and the controls ($p < 0.01$), whereas no difference was observed between CD without a *GJB2* mutation and the controls (Figure 1A). No differences in the incidence of abnormality in VEMPs were observed among the three groups (Figure 1B). The incidence of abnormalities in the caloric test in patients with *GJB2*-related CD differed significantly from that in the controls, but the other two comparisons were not significant (Figure 1C).

Discussion

In this study, vestibular tests were performed in CD patients with or without a *GJB2* mutation by measuring the VEMPs and using the caloric test. Only one report has previously investigated the vestibular function of patients with *GJB2*-related CD [8]. The authors noted that five of the seven patients showed no VEMP responses bilaterally and that only one case had a unilateral pathological response in the caloric test, which led to the conclusion that CD with a *GJB2* mutation is associated with severe saccular dysfunction. However, in the present study, there were no patients showing the absence of both VEMP and a caloric response. Todt et al. [8] showed the existence of *GJB2* mutations that do not cause CD (polymorphisms), thus suggesting a considerable bias. Furthermore, patients with low-grade hearing loss were included in their study. In contrast, all of the *GJB2* mutations detected in the present study are known to cause CD in the Asian population [9]. In addition, the present study included only patients with severe to profound hearing loss, which would therefore clarify the correlation between CD and *GJB2* mutations. Among the seven patients with *GJB2*-related CD, five (71.4%) showed abnormal responses in either or both tests. The incidence was apparently and significantly higher than that in patients with CD without a *GJB2* mutation (2/13: 15.4%). Moreover, the incidence in the controls significantly differed from that in patients with CD related to a *GJB2*

mutation but not in those with CD without *GJB2* mutation. Therefore, these findings support the hypothesis that *GJB2* mutations play a critical role in the disturbance of the vestibular function.

GJB2 mutations cause profound deafness and the associated mechanism has been discussed in several studies [10,11]. A recent study showed that *GJB2* is indispensable in the normal development of the organ of Corti and normal hearing on the basis of the study in *Gjb2* dominant-negative mutant mice [12]. Despite the widespread expression of Cx26 in both the cochlear and vestibular organs [4], the vestibular function impairment of the patients with a *GJB2* mutation is not as severe as the hearing dysfunction observed in the present study. Two hypotheses have been proposed to explain this inconsistency between hearing and balance function. One hypothesis is based on the fact that two temporal bone studies performed in patients with *GJB2*-related hearing impairment in the previous study revealed that one patient had mild vestibular hydrops and saccular degeneration, while another patient had a dysplastic neuroepithelium of the saccule [13,14]. This suggests that a *GJB2* mutation can cause morphological dysplasia in not an entire organ, but in part of the vestibular organs. This is contrast to the cochlea of these patients, which showed nearly total dysplasia of the organ of Corti. These histopathological studies support the results of the vestibular dysfunction of patients with *GJB2*-related CD in the present study. The other hypothesis is based on the presence of several connexins such as Cx26, Cx30 (encoded by *GJB6*), Cx31 (encoded by *GJB3*), and Cx32 (encoded by *GJB1*) in the inner ear. A previous study showed all of these connexins to be distributed in the vestibular organs [15]. Cx30 gene knockout mice had hair cell loss in the saccule, which was restored by the over-expression of the Cx26 gene [16]. Therefore, the specific loss of Cx30 causes vestibular dysfunction, which can be compensated by other types of connexins. The present clinical study in which a complete defect of Cx26 resulted in a definitive but partial dysfunction of vestibular end organs can be explained by the compensation of other connexins normally expressed in the vestibule. Further studies are required to clarify the relationship between connexins and the vestibular function.

Although there was a statistically significant difference in the objective examination of the vestibular function among patients with *GJB2*-related CD, those with CD without a *GJB2* mutation, and healthy controls, none of these subjects had any vestibular symptoms regardless of the presence or absence of a *GJB2* mutation. The peripheral vestibular dysfunction predicted in individuals with the *GJB2* mutation may be compensated by the central vestibular system in young patients with deafness, as shown in the present study. However, aging is known to affect both the peripheral and central vestibular system [17]. In patients with a *GJB2* mutation, the vestibular symptoms may progress with aging. Another problematic point regarding patients with CD related to *GJB2* mutations is cochlear implantation, which has been reported to cause vestibular dysfunction, such as a reduction of the caloric responses [18] and a decrease in the VEMP responses [19]. It is thought that the mechanical damage caused by the insertion of the electrode may induce vestibular dysfunction [20]. In the present study, four patients with *GJB2*-related deafness showed unilateral vestibular dysfunction, while only one of them had bilateral dysfunction. Therefore, it should be emphasized that the assessment of the vestibular function in patients with *GJB2*-related CD is important to determine which side of the ear should be selected to insert the cochlear implant.

Conclusions

A *GJB2* mutation is responsible not only for deafness but also for vestibular dysfunction. However, such vestibular dysfunction is likely to be unilateral and less severe in patients with a *GJB2* mutation than in those with bilateral deafness.

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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Vestibular dysfunction in a Japanese patient with a mutation in the gene *OPA1*

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ABSTRACT

OPA1 mutations are known to cause autosomal dominant optic atrophy (ADOA), and some types of *OPA1* mutations also cause auditory neuropathy. In the present study, we evaluated the vestibular dysfunction that accompanied auditory neuropathy in a patient with an *OPA1* mutation. A caloric test failed to elicit nystagmus or dizziness in either ear. Vestibular evoked myogenic potentials (VEMPs) in the right ear were characterized by a normal biphasic waveform. In contrast, no VEMPs were evoked in the left ear. Model building suggested that the *OPA1* mutation, p.R445H, indirectly distorts the catalytic structure of the GTPase reaction center and decreases GTPase activity. The patient complained of instability while walking or moving but thought these symptoms were caused by visual dysfunction. This is the first report of a detailed evaluation of vestibular dysfunction in a patient with an *OPA1* mutation. This case suggests that vestibular dysfunction may be involved in motor instability in patients with an *OPA1* mutation, even when patients do not complain of vestibular symptoms. Based on this case, we suggest that vestibular evaluation should be performed in auditory neuropathy patients carrying an *OPA1* mutation, even if the patients are free of symptoms of vestibular dysfunction.

1. Introduction

Autosomal dominant optic atrophy (ADOA; OMIM #165500) is a dominantly inherited optic neuropathy resulting in progressive loss of visual acuity, color vision deficits, a centrocecal scotoma, and optic nerve pallor [1]. ADOA is the most common form of optic atrophy, with an estimated prevalence of 1 in 50,000 individuals [2]. Although several types of loci are known to cause ADOA, it has been reported that as many as 89% of cases may be associated with a mutation in the gene *OPA1* (3q28-29) [3]. *OPA1* encodes a dynamin-related GTPase that is located in the mitochondrial intermembrane space and plays a key role in controlling the balance of mitochondrial fusion and fission. In most cases, ADOA occurs without additional neurological symptoms. However, there are several known cases of optic atrophy associated with sensorineural hearing loss, and the Arg445His (p.R445H) mutation of *OPA1* has been reported in patients with ADOA and moderate progressive hearing loss [4]. In patients having the p.R445H mutation, progressive hearing impairment begins in childhood, and audiological examinations show features of auditory neuropathy, for which the primary lesion is located in the inner hair cells, the auditory nerve, or the synapses between them [4,5]. Recently, a detailed analysis of OPA1 protein expression in the inner ear was reported in rat, and OPA1 protein was detected in the inner hair cells, outer hair cells, and spiral ganglia in the cochlea, as well as the hair cells and ganglia in the vestibular organ [6]. Although there have been several reports of auditory function in patients with this *OPA1* mutation, the analysis of vestibular function has not yet been reported in any *OPA1* mutation. In this paper, we report the results of examinations for auditory and vestibular function in a patient who presented with both hearing impairment and vestibular dysfunction due to an *OPA1* mutation that leads to distortion of the catalytic structure of the OPA1 protein.

2. Materials and methods

2.1. Auditory function tests

2.1.1. Audiometric tests

The patient underwent standard pure-tone air- and bone-conducted audiometry (125–8000 Hz) and speech discrimination testing using an audiometer (AA-75, Rion Co., Tokyo, Japan) and the 67-S Japanese word list.

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2.1.2. DPOAEs

DPOAES were recorded and analyzed using the ILO-92 system (Otodynamics Ltd, Herts, UK). DPOAE primary tones f1 and f2 were presented at 70 dB SPL. The f2:f1 ratio was kept at 1.22, and the frequency of f2 was changed in one-third octave steps from 708 to 6299 Hz. The levels of 2f1–f2 DPOAE were recorded. DPOAE values were plotted on a DP-gram, which expresses the emission level as a function of the f2 frequency.

2.1.3. Auditory brainstem responses (ABRs)

ABRs were recorded using the Neuropack system (Nihon Kohden, Tokyo, Japan) with an electrode montage of vertex (CZ) to the ipsilateral (stimulated) ear lobe and ground to forehead (Fz). The amplifier band pass was 100–1000 Hz. Alternating-polarity click stimuli were presented monaurally at a rate of 20 Hz at 100 dB nHL. Average responses to 1024 clicks were collected in each of two experiments.

2.2. Vestibular function tests

2.2.1. Electronystagmography

The patient underwent an electronystagmography test battery consisting of spontaneous, optokinetic, positional, postural, and caloric-induced nystagmus recordings. Nystagmus was recorded using an electronystagmograph recorder (Rion, Tokyo, Japan). Caloric testing using 20 °C and ice-cold water (5 cm^3, 5 s) was used to irrigate the external auditory meatus to induce a thermal gradient across the lateral semicircular canal.

2.2.2. Vestibular evoked myogenic potentials (VEMPs)

The sternocleidomastoid (SCM) muscle was chosen as the target to record VEMPs using the Neuropack system (Nihon Kohden, Tokyo, Japan). Surface electromyographic activity was recorded from symmetrical sites over the upper half of each SCM, with a reference electrode over the sternal attachment site of the contralateral SCM. The patient was laid supine on a bed and asked to raise and orient his head contralateral to the tested ear to maximally activate the SCM ipsilateral to the stimulation. Responses to 200 short-tone bursts (105 dB nHL, 500 Hz) were recorded at 100-ms intervals over a band pass of 500–1500 Hz.

2.3. Neuroimaging studies

2.3.1. High-resolution computed tomography (HRCT)

The protocol for HRCT included scanning with a multi-slice computed tomography scanner (Sensation 64; Siemens Medical Solutions, Inc., Malvern, PA, USA). Images were acquired with direct axial sequences using a spiral scan procedure with a 1.0-mm collimation. Data were reconstructed with a slice thickness of 1.0 mm using a bone algorithm.

2.3.2. Magnetic resonance imaging (MRI)

The patient was scanned on a 1.5-T MRI machine (Signa EXITE 1.5T, General Electric, Fairfield, CT, USA) with surface and head coil. Axial three-dimensional fast imaging employing steady-state acquisition (FIESTA, repetition time, 9.3 ms/echo time, 3.3 ms; scan thickness 1.0 mm) was performed. The axial images were reconstructed in the oblique sagittal plane traversing the internal auditory canal (IAC), producing cross-sectional images that visualize the neural structures of the IAC.

2.4. Homology modeling of OPA1 and ligand fitting

The crystal structure of the GTPase domain of rat dynamin 1 (PDB ID: 2AKA) was used as a template in homology modeling because the GTPase domain of rat dynamin 1 is closely related to that of OPA1 in both function and structure (32% amino acid sequence identity). A program package for protein engineering and drug design, BIOCES[E] (NEC Corp., Tokyo, Japan) [7], was used for a series of molecular modeling. This package runs on an OCATANE2 (Silicon Graphics Inc., Fremont, CA, USA). The GTP molecule of Ras-GTP (PDB ID: 5P21) was fitted into the corresponding active site of the OPA1 model using DALI (http://ekhidna.biocenter.helsinki.fi/dali_server/) [8]. The p.R445H mutation structure was superimposed on the native structure (backbone atoms only) and displayed using UCSF Chimera (http://www.cgl.ucsf.edu/chimera/) [9].

3. Case report

The patient is a 28-year-old man who first presented with sudden optic atrophy at the age of 17 years. Clinical history of vision disorder and the result of genetic test have been reported [10]. In brief, he received a detailed examination for visual function at age 21. His best corrected visual acuity was 20/200 in both eyes. He had atrophy of the optic disks, central scotoma, and generalized bilateral dyschromatopsia. As a result, the patient was diagnosed with ADOA, and a genetic examination revealed a heterozygous G-to-A substitution in the second nucleotide of codon 445 in *OPA1*, resulting in an Arg-to-His amino acid substitution (p.R445H). He had no apparent family history of either optic atrophy or hearing impairment. At that time, he was also found to have a slight bilateral hearing impairment. The patient

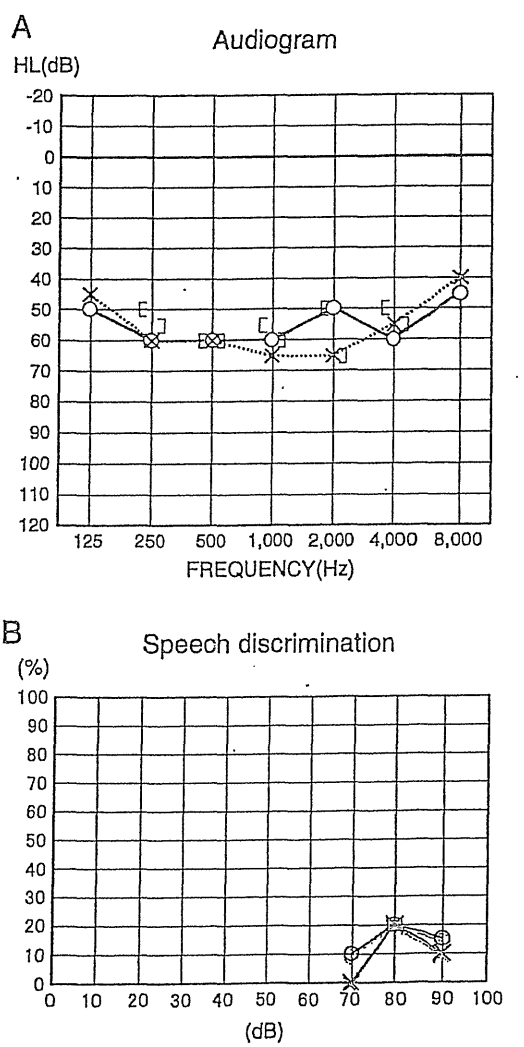

Fig. 1. Pure-tone (A) and speech (B) audiograms of a patient with an *OPA1* mutation. O = right air conduction hearing level; X = left air conduction hearing level; [=right bone conduction hearing level;] = left bone conduction hearing level.

developed progressive hearing impairment, and had particular difficulty understanding speech. He came to our department for a hearing evaluation at age 28. Although he did not initially complain of balance disorders, he stopped riding a bicycle at age 17 years because of difficulty controlling balance and also started to feel unsteady walking at that time. He thought the unsteadiness resulted from his visual dysfunction.

4. Results

4.1. Auditory function test results

Direct otoscopic observation revealed normal findings in both ears. A bilateral sensorineural hearing loss of approximately 60 dB was shown by pure-tone audiometry (Fig. 1A). The maximum speech discrimination scores were 20% in both ears (Fig. 1B), which were significantly worse than expected based on the results of pure-tone audiometry. Although no differences were observed between left and right ears, the patient reported better hearing discrimination in the right ear (Fig. 1). ABRs were absent bilaterally even at 100 dB nHL (Fig. 2A), but high-amplitude DPOAEs were present at all frequencies tested in both ears (Fig. 2B).

4.2. Vestibular function test results

No spontaneous, positioning, or pressure-induced nystagmus was found by electronystagmography. Neither 20 °C nor ice-water caloric stimulation of the labyrinth elicited nystagmus or dizziness in eithe ear (Fig. 3A). Short-tone burst-evoked VEMP analysis revealed biphasic VEMP waveform in the right ear; however, the latency c n23, which is the second wave of VEMP, was delayed. No VEMPs wer evoked in the left ear (Fig. 3B).

4.3. Neuroimaging studies

There were no abnormal findings by HRCT. In particular, no inner ea malformation or internal auditory canal stenosis was observed (Fig. 4 D). By MRI, both the cochlear nerves and vestibular nerves wer detected from brainstem to the inner ear in both ears in axial FIEST slices (Fig. 4B, E). However, the diameter of the right cochlear nerve wa 0.82 mm whereas that of the left cochlear nerve was 0.69 mm, and th diameter of the right facial nerve was 1.06 mm whereas that of the le facial nerve was 1.02 mm in oblique sagittal reconstructions through th IAC (Fig. 4C, F). Thus, the cochlear nerves on both sides are considere hypoplasia according to reported criteria [11].

4.4. OPA1 predicted structure

The distance between Cα of R445 of OPA1 and the GTP bindin pocket is 18 Å (Fig. 5). The electric field around R445 is negativel charged due to its proximity to D450, D442, and E444. Unde physiological conditions, positively charged R445 is structurall stable, and thus the mutation p.R445H reduces the electrostati stability and indirectly distorts the structure of the GTPase catalyti

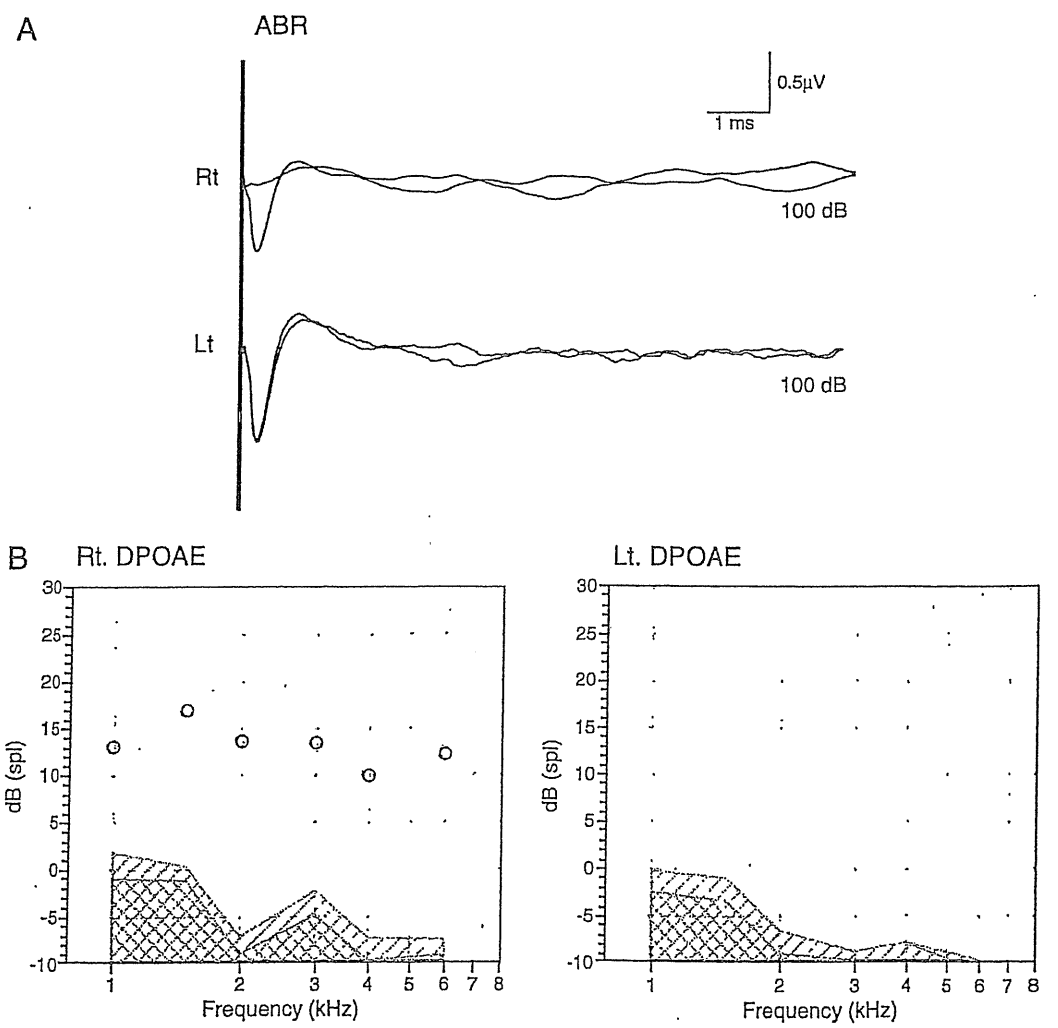

Fig. 2. (A) ABR tests revealed no ABR waveforms in this patient. (B) DPOAE recordings were normal for this patient. Residual noise levels are shown by the shaded area.

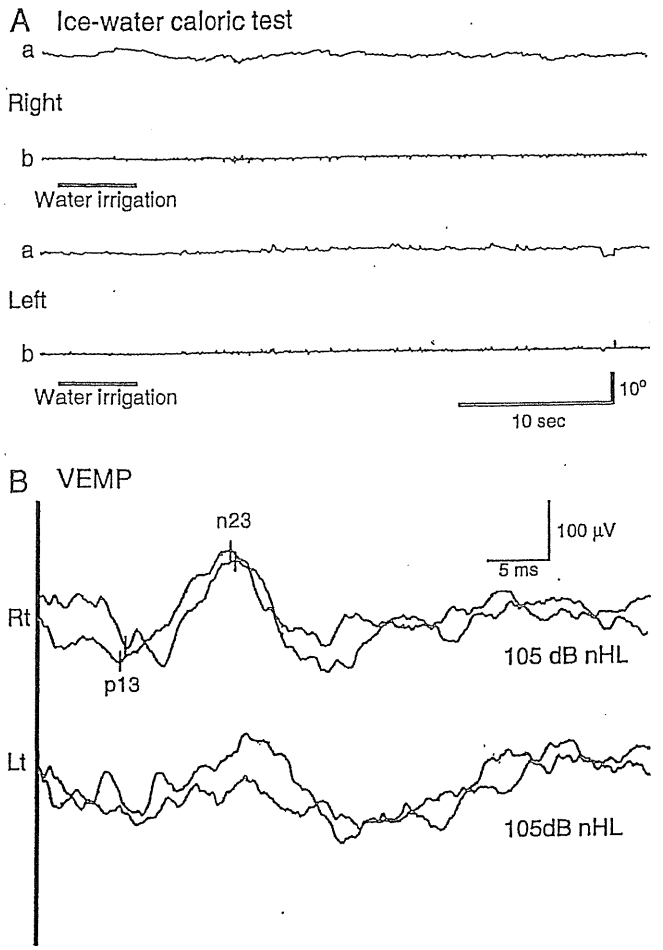

Fig. 3. (A) Horizontal record of electronystagmograph on ice-water caloric test. Time constants: a, 3.0 s; b, 0.03 s. No nystagmus were elicited in both side of ears. (B) Air-conducted VEMPs. Electromyographic responses of the right (Rt) and left (Lt) SCM to right ear stimulation. A biphasic VEMP waveform was revealed in the right ear; however, a latency of n23 was delayed. In contrast, no VEMPs were evoked in the left ear.

domain. In addition, salt bridges between R445 and D450 in the α3-helix and strong electrostatic interactions between R445 and D442/E444 are observed. The α3-helix is a key structure that constructs the common wire frame of the G-protein core fold [7,9]. Thus, the p.R445H mutation indirectly distorts the catalytic structure of the GTPase reaction center and decreases GTPase activity.

5. Discussion

Several reports have described hearing impairments associated with an *OPA1* mutation [4,12–16]. As with the case we present here, these hearing impairments were reported to result from auditory neuropathy. Common features in these patients include moderate hearing threshold elevation and a severe speech discrimination disability. No vestibular symptoms or function test results have yet been reported. To our knowledge, this is the first report of a detailed vestibular analysis in a patient with an *OPA1* mutation. Moreover, inner ear neuroimaging studies, including HRCT or 3-D MRI, have not yet been reported in patients with *OPA1* mutations. This report provides the first evidence of cochlear nerve atrophy in the IAC in a patient with an *OPA1* mutation.

OPA1 encodes a dynamin-related GTPase that is located in the mitochondrial intermembrane space and plays a key role in controlling the balance of mitochondrial fusion and fission [17]. Furthermore, release of cytochrome *c* from mitochondria and caspase-dependent activation of the apoptosis cascade have been observed in the down-regulation model of expression by RNA interference in HeLa cells [17]. The *OPA1* p.R445H mutation is reportedly associated with various neurological disturbances, including ataxia, peripheral neuropathy, ptosis, and cognitive impairment [18]. In cases involving the heterozygous p.R445H mutation, ADOAs associated with deafness have been reported [4], and these sensorineural hearing losses show audiological features compatible with auditory neuropathy. In normal rats, expression of OPA1 protein is seen in the inner hair cells, outer hair cells, and spiral ganglia in the cochlea, and in the vestibular hair cells and ganglia [6]. OPA1 protein expression has also been observed in membranous or submembranous compartments of vestibular ganglion cells and at the level of the calyx synapse, which typically envelopes type 1 hair cells in the vestibular epithelium [6]. Bilateral vestibular dysfunction in our present patient is probably caused by dysfunction of these parts of the vestibular organs.

An abnormality in the OPA1 protein may cause mitochondrial dysfunction, leading to insufficient energy production. Homozygous mutant mice are not viable and show impaired development as early E8.5. [19]. This study also reported that heterozygous mutants show a reduction in OPA1 protein level (about 50% compared with wild-type littermates) due to rapid degradation of the mutant polypeptide [19]. Skin fibroblasts obtained from patients carrying the heterozygous *OPA1* p.R445H mutation show hyperfragmentation of the mitochondrial network, decreased mitochondrial membrane potential, and an ATP synthesis defect [4]. Our three-dimensional structure study suggests that the p.R445H mutation reduces the electrostatic interactions and therefore the stability of the protein and indirectly distorts the structure of the GTPase catalytic center, thereby decreasing GTPase activity. According to these findings, we suggest that the OPA1 p.R445H mutation leads to severely insufficient energy production by decreasing GTPase activity in the mitochondria. This deficiency could, in turn, affect critical energy-dependent functions such as axoplasmic transport in both cochlear and vestibular nerve fibers as well as optic nerve fibers.

This patient had almost normal VEMP results in the right ear but no response in the left ear. Although the mechanisms underlying these different responses are unclear, asymmetrical hearing impairments have been reported in patients with the *OPA1* p.R445H mutation [12,13]. There was no response to caloric stimulation in either ear. The VEMP consists of myogenic potentials obtained as a response to tone-burst stimuli and is used to test the saccule and inferior vestibular nerve of the vestibular system. The caloric test, on the other hand, is used to evaluate the function of the lateral semicircular canals and the superior vestibular nerve [20]. In the right ear, there was no response in the caloric test but fare VEMPs. *OPA1* is expressed in sensory epithelia in both the saccule and the lateral semicircular canal [6]. Atrophy of the superior vestibular nerve was not detected by MRI scan. The mechanisms underlying different responses for the caloric test and VEMPs in the right ear are uncertain. In the present case, the patient reported slightly better hearing in the ear that also had good VEMP responses (the right ear). It is well established that ADOA is a progressive atrophy disease. If the main mechanism for nerve atrophy in ADOA is the same in both the eye and the inner ear, we speculate that nerve atrophy in the inner ear may develop gradually from the superior vestibular nerve to the inferior vestibular nerve in patients with the *OPA1* mutation. It has been reported that VEMPs are less affected than horizontal semicircular canal function during caloric testing in bilateral vestibulopathy [21]. We found only two reports with results of both caloric testing and VEMP analysis in auditory neuropathy patients with causes other than an *OPA1* mutation [20,22], and these revealed normal caloric responses and abnormal VEMPs in all patients ($n=4$) with auditory neuropathy. We revealed a different profile in a patient with auditory neuropathy due to an *OPA1* mutation. We speculate that the vestibule is also an organ that is sensitive to the mitochondrial dysfunction associated with the *OPA1* mutation.

In conclusion, we have presented a case of vestibular dysfunction accompanied with auditory neuropathy in a patient with an *OPA1*

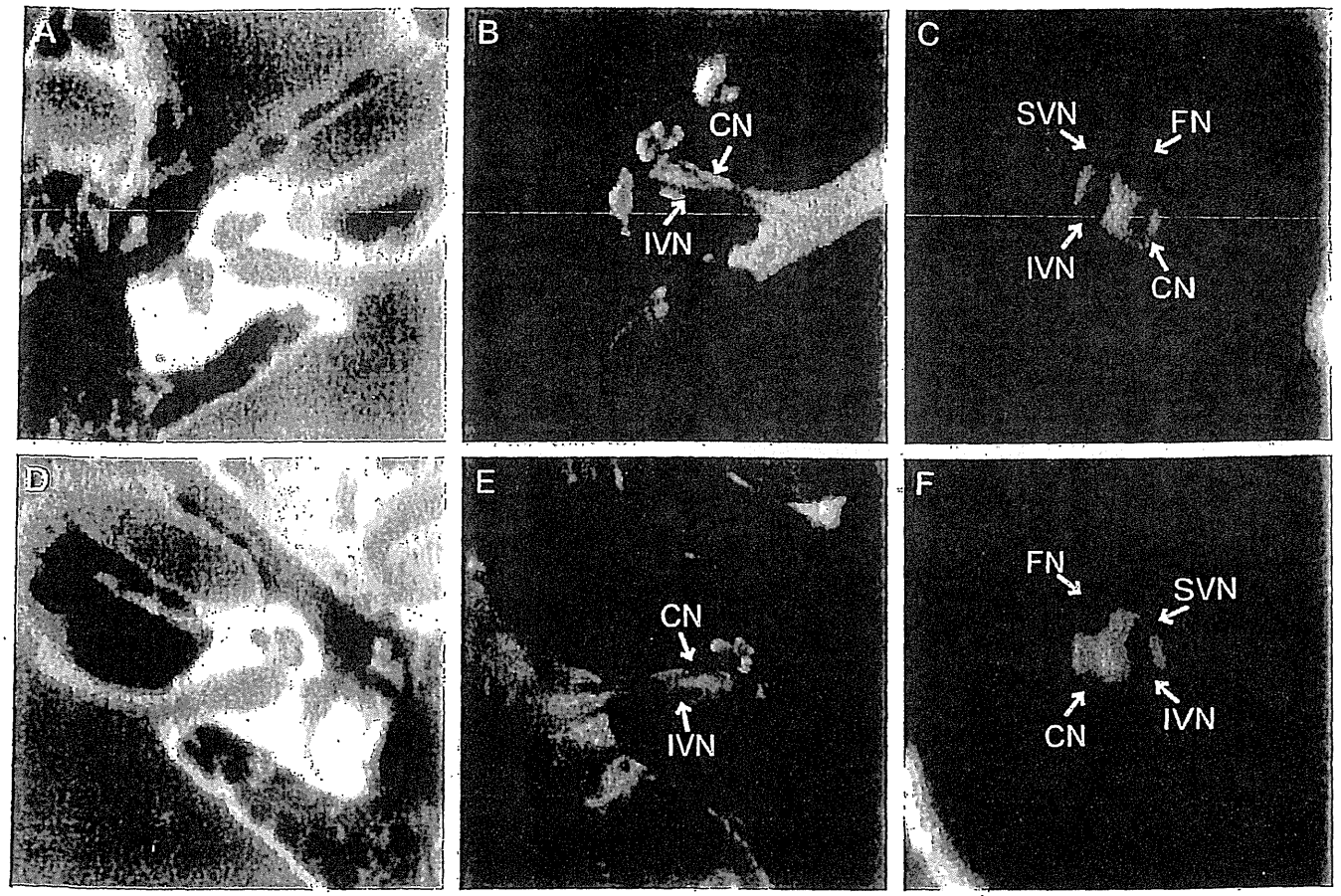

Fig. 4. Images showing the HRCT (A, D), axial MRI (FIESTA; B, E), and oblique sagittal reconstructions (C, F). The facial nerve (FN), cochlear nerve (CN), superior vestibular nerve (SVN), and inferior vestibular nerve (IVN) can be recognized in both sides of the internal auditory canal. However, the cochlear nerves in both ears were narrower than the vestibular nerves in axial FIESTA slices. Moreover, the cochlear nerves on both sides were smaller than the adjacent facial nerves in oblique sagittal reconstructions.

mutation. In a standard evaluation, this patient's balance disorder could easily have been overlooked because he attributed it to his visual dysfunction. Based on this case, we suggest that vestibular evaluation should be performed in auditory neuropathy patients carrying an *OPA1* mutation, even if the patients do not complain of balance dysfunction.

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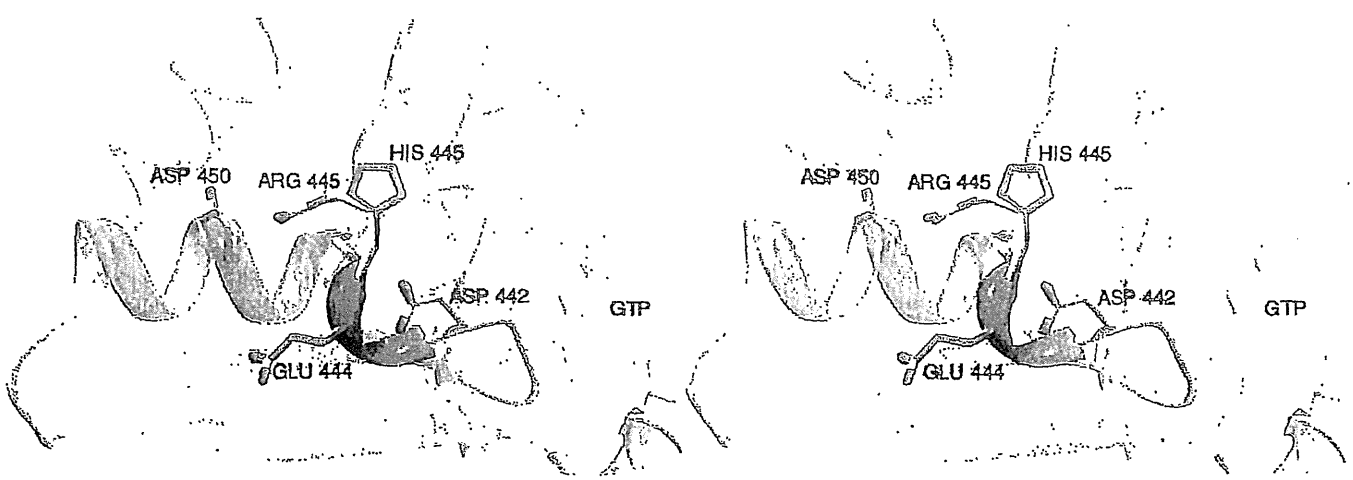

Fig. 5. Stereo view of the GTPase domain of predicted structure of human OPA1 with arginine at position 445 replaced by histidine. The electric field around R445 is negatively charged due to the proximity of D450, D442, and E444. Positively charged R445, under a physiological environment, is structurally stabilized, and thus the mutation p.R445H reduces the electrostatic stability and indirectly distorts the GTPase catalytic structure. Image produced using the UCSF Chimera package supported by NIH P41 RR-01081.

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