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Contents

REVIEW

Value of Genetic Testing in the Otological Approach for Sensorineural Hearing Loss

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Abstract

Sensorineural hearing loss (SNHL) is one of the most common disabilities in human, and genetics is an important aspect for SNHL, especially in children. In recent 10 years, our knowledge in genetic causes of SNHL has made a significant advance, and now it is used for diagnosis and other clinical practices. Hereditary hearing loss can be classified into syndromic and nonsyndromic hearing loss. As the nonsyndromic deafness genes, more than 100 loci for deafness genes have been determined, and more than 40 genes were identified. Furthermore, more than 300 forms of syndromic hearing loss have been characterized, and each syndrome may have several causative genes. In childhood hearing loss, early educational intervention is required in addition to medical intervention for normal development of speech and language. In addition, even severe to profound hearing loss may be restored very effectively by hearing aids or cochlear implants. Because of these features of SNHL, genetic testing has exceptionally high value in the medical practice for hereditary hearing loss. Several strategies are used for genetic testing of SNHL for accurate and efficient identification of the genetic causes, and the results were used for explanation of the cause, prediction of auditory features, prevention of deafness, management of associated symptoms, determination of therapy, and genetic counseling. Identification of damaged cells in the inner ear and the underlying mechanism by genetic testing undoubtedly facilitates development and introduction of novel and specific therapies to distinct types of SNHL. (Keio J Med 58 (4) : 216–222, December 2009)

Keywords: **hereditary hearing loss, deafness gene, inner ear, cochlea**

Introduction

Sensorineural hearing loss (SNHL) is one of the most common disabilities in human, and genetics is an important aspect in research and clinical practice for SNHL. One child in 1000 is born with bilateral SNHL, and 50-70% of them have monogenic causes.[1,2] In addition, 10% of the people over 65 years have SNHL that interfere speech communication.[3] Although most of them have polygenic causes associated with aging and various environmental causes, some of them have monogenic causes. In recent 10 years, our knowledge in monogenic causes of SNHL has made a significant advance. The knowledge of genetics in SNHL was originally established in the laboratory, but it is now used for genetic testing and following clinical procedures for patients with SNHL.

Classification of Hereditary Hearing Loss

Hereditary hearing loss can be classified into syndromic and nonsyndromic hearing loss.[4] Syndromic type which is associated with distinctive clinical features accounts for 30% of hereditary congenital hearing loss, and

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Table 1 Identified deafness genes

Autosomal dominant loci and genes			
DFNA1	DIAPH1	DFNA11	MYO7A
DFNA2	Cx31/KCNQ4	DFNA13	COL11A2
DFNA3	Cx26/Cx30	DFNA15	POU4F3
DNFA4	MYH14	DFNA17	MYH9
DFNA5	DFNA5	DFNA20/26	ACTG1
DFNA6/14	WFS1	DFNA22	MYO6
DFNA8/12	TECTA	DFNA28	TFCP2L3
DFNA9	COCH	DFNA36	TMC1
DFNA10	EYA4	DFNA48	MYO1A
Autosomal recessive loci and genes			
DFNB1	Cx26/Cx30	DFNB21	TECTA
DFNB2	MYO7A	DFNB22	OTOA
DFNB3	MYO15	DFNB23	PCDH15
DFNB4	SLC26A4	DFNB28	TRIOBP
DFNB6	TMIE	DFNB29	CLDN14
DFNB7/11	TMC1	DFNB30	MYO3A
DFNB8/10	TMPRSS3	DFNB31	WHRN
DFNB9	OTOF	DFNB36	ESPN
DFNB12	CDH23	DFNB37	MYO6
DFNB16	STRC	DFNB67	TMHS
DFNB18	USH1C		
X-linked loci and genes		Mitochondrial genes	
DFN3	POU3F4	12S rRNA tRNASer(UCN)	

nonsyndromic type which is not associated with other clinical features accounts for the other 70%. Nonsyndromic hearing loss can be classified into 4 groups by the inheritance pattern, and relatively common clinical features have been noted for each inheritance pattern with a few exceptional genes, genotypes, and patients. Patients with autosomal dominant inheritance typically show progressive SNHL which begins in age 10-40, and the degree of hearing loss is various while patients with autosomal recessive inheritance most frequently show congenital and severe hearing loss. Patients with mitochondrial inheritance tend to develop progressive SNHL which begins in age 5-50, and the degree of hearing loss is various. Autosomal recessive inheritance accounts for 80% of congenital nonsyndromic hereditary hearing loss, and autosomal dominant inheritance accounts for most of the other 20%. X-linked and mitochondrial inheritance accounts for only 1-2%. After aging, the prevalence of autosomal dominant inheritance and mitochondrial inheritance increases while that of autosomal recessive inheritance decreases. The precise prevalence of each inheritance pattern is not known for adults because of the difficulty in sampling and excluding the effect of age-related hearing loss.

Deafness Genes

The first nonsyndromic deafness gene was discovered in 1993.[5] Since then, more than 100 loci for deafness genes have been determined, and more than 40 genes were identified (**Table 1**). Most of these genes play their roles within the cochlea. Thus, hereditary hearing loss almost exclusively features cochlear dysfunction.[1,2]

Although many genes are known for nonsynromic hearing loss, only a few genes including GJB2, GJB6, SLC26A4 accounts for over one third of patients with congenital hearing loss. Mutations in GJB2 account for 50% of patients with autosomal recessive hearing loss, i.e. 20% of all congenital hearing loss.[6,7] GJB2 encodes connexin 26, a gap junction protein expressed in the cochlea. Gap junctions are intercellular channels allowing recycling of potassium ions from hair cells to the stria vascularis in the cochlea and maintains a high endocochlear potential which is of critical importance for normal hearing. Mutations in GJB2 show considerable phenotypic variation, but genotype-phenotype studies showed that it is possible to predict the hearing loss associated with GJB2 mutations based on the specific genotype.[8] Combination of mutations in GJB2 and closely linked GJB6, in digenic transmission, accounts for about 8 % of deaf patients with GJB2.[9] GJB6 is a gene with sequence similarity to GJB2, is also expressed in the cochlea, and its product, connexin 30, can form gap junction with connexin 26, explaining digenic transmission of GJB2 and GJB6.

With regard to syndromic hearing loss, more than 300 forms have been characterized. In many forms, several genes that can cause the same phenotype or a closely related phenotype have been identified. In syndromic hearing loss, hearing loss is most frequently caused by dysfunction of the cochlea but the middle ear and the outer ear are also frequently involved. The most common form of syndromic hereditary SNHL is Pendred syndrome which is characterized by SNHL, bilateral dilatation of vestibular aqueduct with or without cochlear hypoplasia, and goiter. Majority of patients with Pendred syndrome have mutations in SLC26A4, and these mutations also cause nonsyndromic SNHL.[10,11] Pendred syndrome accounts for 3 % of all congenital hearing loss and mutations in SLC26A4 including those causing nonsyndromic SNHL account for 7 % of all deaf children at age of 4 years.[2] SLC26A4 encodes a chloride-iodide cotransporter and is critical for maintaining endolymphatic ion homeostasis, which is essential to normal inner ear function.

Mutations in mitochondrial DNA are rarely detected in congenital hearing loss, but its prevalence in patients with SNHL increases with aging. A1555G or A3243G mitochondrial DNA mutations are found in approximately 6 % of adult patients with SNHL without known causes, and both mutations cause cochlear dysfunc-

tion.[12,13] A3243G mitochondrial DNA mutation cause not only nonsyndromic SNHL but also syndromic SNHL such as MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) and MIDD (maternally inherited diabetes and deafness). A1555G mitochondrial DNA mutation causes rapidly progressive SNHL which leads to severe degree in patients with the onset of SNHL before age 10 and slowly progressive or nonprogressive SNHL which leads to mild to moderate degree in patients with the onset after age 10.[14] A3243G mitochondrial DNA mutation causes progressive SNHL which leads to moderate to severe degree in patients who developed hearing loss during adulthood.

Unique Clinical Aspects of Hereditary Hearing Loss

Hereditary hearing loss is unique compared to other hereditary diseases in the following three points. First, a large number of genes are involved in hereditary hearing loss, which makes it very difficult to identify causes and pathological mechanism in clinical practice. Second, without speech and language rehabilitation, hearing loss not only impedes audition but also hampers normal development of speech and language. Without speech and language, it is almost impossible to maintain good social relationship in the society of people with normal hearing. Thus, educational intervention is required in addition to medical intervention for children with SNHL. Third, congenital deaf children can learn and manage to communicate with others if early diagnosis of hearing loss followed by adequate rehabilitation can be made. Even severe hearing loss can be restored very effectively by hearing aids or cochlear implants coupled with early rehabilitative training in patients with hereditary hearing loss.[15] In most hereditary diseases, this level of functional restoration has not been possible yet. This feature lead to the worldwide implementation of universal newborn hearing screening which aims to screen neonates for hearing loss immediately after birth or before hospital discharge so that intervention can be initiated to prevent delayed language acquisition. Because of these unique clinical aspects of hereditary hearing loss, genetic testing of SNHL has high value in the otological approach to this disorder. Identification of genetic causes provides a key to understand the mechanism of hearing loss, leads to better management of hearing loss, and facilitates functional recovery by effective rehabilitation.

Strategy for Genetic Testing of Hearing Loss

Genetic testing of SNHL is conducted in several institutes worldwide including our institute, and the strategy is various among different institutes. In our institute, it consists of the following 3 steps; 1) identification of candidate patients who are suspected of having hereditary hearing loss, 2) identification of candidate genes to be tested, and 3) identification of causative mutations in the suspected genes.

Our criteria for candidate patients are patients presenting with bilateral hearing loss without known causes except for heredity. Unilateral hearing loss is included only when hearing loss is associated with specific types of anomaly in the inner ear, middle ear, or outer ear.

Candidate genes for syndromic hearing loss are determined by clinical diagnosis of syndromic hearing loss based on associated clinical symptoms. Usually, only one or a few candidate genes are responsible for each syndrome. Syndrome may be classified into subclasses based on the different expression of phenotypes, and diagnosis of subclasses may further narrow down candidate genes. On the other hand, it is very difficult to determine candidate genes for nonsyndromic hearing loss, and often impossible because of a large number of causative genes for a relatively undistinguishable phenotype, i.e. SNHL. Part of deafness genes for nonsyndromic hearing loss demonstrates unique auditory features or other clinical features in CT imaging of inner ear, electrophysiological testing, or inheritance pattern. For those genes, we are making an algorithm indicating the genes which should be tested and the order of the genetic tests based on clinical features and the results of genetic tests. After all the clinical examinations and tests for hearing loss, we determine the candidate genes and the order of genetic analysis according to the established algorithm (**Fig. 1**). This strategy is named systematic genetic testing for deafness, and tentative algorithm is currently used in our institutes to evaluate the sensitivity, specificity, and efficiency for clinical use.

Identification of causative mutations is mostly done with direct sequencing of the candidate genes using DNA extracted from blood samples. All exons and its flanking short sequences in introns are sequenced and analyzed for mutations. For large genes in which pathological mutations are mostly distributed within the restricted regions, sequencing may be done for the restricted region. In contrast, for several large genes with ubiquitous distribution of pathological mutations over entire region, screening by degenerate HPLC are first conducted, and sequencing analysis can be done only for the regions which showed abnormal screening results. For a few genes in which mutations are limited to only one or two frequent changes, restriction fragment length polymorphism PCR analysis is performed to detect the specific mutations. With the astonishing progress in the speed of sequencing machines, sequencing of whole human genome will be practically available in several years, first in laboratories, then in clinics. This may fundamentally change the way of genetic testing for SNHL.

Feedback to Patients

Discovery of many deafness genes had a significant

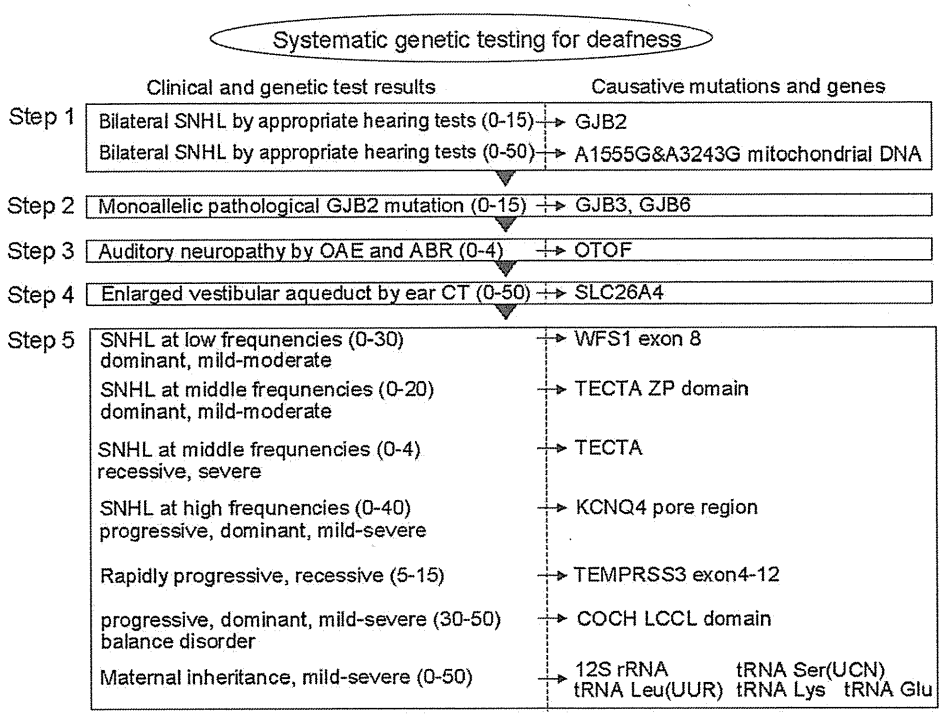

Fig. 1 Our original algorithm for systematic genetic testing for deafness in patients who are suspected of nonsyndromic SNHL (sensorineural hearing loss). Based on the clinical or genetic test results shown in the left column, candidate mutations or genes listed in the right column are determined. Corresponding mutations or genes for each clinical or genetic category are indicated by horizontal arrows. Genetic tests start from Step 1. If causative mutations are not determined or an indicated category does not fit for a patient, genetic tests proceed to the next step until causative mutations are determined or no appropriate category is found. Genes examined for specific exons, regions or domains are described with short explanatory tags, and those examined for all exons are described without explanation. Numbers in parenthesis indicate periods of age at onset of SNHL.

impact on the otological approach to patients with SNHL. First, explanation of the cause of SNHL to deaf patients or parents of deaf children has become possible in many cases. Without definite explanation, patients tend to visit other hospitals seeking for explanation and repeat redundant tests or treatments and feel anxiety about what is related to deafness of themselves or their children and whether other disability is also present but not detected. These lead to delay of rehabilitation which should be initiated immediately after diagnosis of hearing loss for effective acquisition of language and speech.[16] Thus, early and definite explanation by genetic tests facilitates rehabilitation.

Second, identification of causative mutations helps doctors to predict auditory features such as audiogram of the patients and prognosis of their hearing, especially in children who cannot cooperate with subjective hearing tests. This provides valuable information in making adequate planning of clinical follow-up, estimation of hearing levels for fitting hearing aids, and selection of occupation by patients.[17]

Third, prevention of deafness can be done by avoiding use of specific drugs or specific activities in genetically susceptible patients. As an example, patients with A1555G mitochondrial DNA mutation should avoid aminoglycosides which induce or aggravate SNHL by even one injection in subjects with this mutation.[18] Another example is that detection of SLC26A4 suggests dilatation of vestibular aqueduct even in neonates who are usually not tested for inner ear anomaly by CT or MRI. Patients with this mutation should have temporal bone CT and patients who are found to have dilatation of vestibular aqueduct should avoid activities in which physical shock on their head is likely to occur. This is because such a shock tends to cause aggravation of SNHL in these patients.

Fourth, identification of causative mutations in patients with syndromic hearing loss enables prevention or early detection of associated symptoms. These examples include diabetes mellitus in patients with A3243G mitochondrial DNA mutation and goiter in patients with SLC26A4 mutations. Early detection and management of these associated symptoms help to prevent disorders related to the associated symptoms such as diabetic retinopathy for diabetes mellitus, and facilitate early recovery from symptoms such as hypothyroidism. Because

occurrence of associated symptoms may delay more than 10 years after the onset of SNHL, many patients and even doctors who see those patients cannot notice the association of the symptoms with SNHL, and unnecessary or even harmful tests tend to be done for the diagnosis. Thus, it would be worthwhile to understand the associated symptoms and prepare the risk of manageable disorders at the time of diagnosis of SNHL. In addition, genetic tests may be valuable in substituting more stressful tests. For an example, renal biopsy and/or skin biopsy are currently necessary for diagnosis of Alport syndrome which is a hereditary nephritis associated with SNHL, and this procedure usually requires hospitalization and has a certain physical risk. Mutations in COL4A3, COL4A4, COL4A5, and MYH9 are known causes of Alport syndrome, but genetic tests of these genes are currently rarely available as a clinical test mainly because of an extremely high cost. Several laboratories in the world including my laboratory offer these tests as a research basis. With remarkable advances in genetics, increase of sensitivity and specificity and decrease of costs for genetic analysis are in progress. In the near future, diagnosis of Alport syndrome may be first done by clinical genetic tests, and renal and skin biopsy may be avoided in many patients.[19]

Fifth, identification of causative mutations clarifies the cell types and nature of damages which are responsible for SNHL, which is especially important for indication of cochlear implant surgery. Because spiral ganglion neurons which are necessary for successful cochlear implants are well preserved in most types of hereditary hearing loss, identification of mutations in the deafness genes usually indicates good indication for cochlear implant surgery. This is most helpful in babies who cannot corporate detailed audiological tests for evaluation of SNHL.

Identification of causative mutations is also important for clinical management of patients with auditory neuropathy. Auditory neuropathy is a distinct type of SNHL which features normal outer hair cell function and abnormal activities of auditory neurons, and a relatively frequent cause of congenital SNHL (~15 %). Development of speech and language cannot be expected by hearing aids in congenital auditory neuropathy because of poor speech recognition inherent in this disorder. Either inner hair cells or spiral ganglion neurons are affected, but current clinical tests cannot distinguish these two types. Because normal spiral ganglion neurons are necessary for success of cochlear implants, pathology underlying SNHL needs to be determined in order to evaluate the indication of cochlear implant surgery. Recent studies have shown that mutations in OTOF cause auditory neuropathy by inner hair cell dysfunction and that spiral ganglion neurons are normal in patients with these mutations.[20] In agreement with the pathological mechanism of mutations in OTOF, results of cochlear implants have been successful.[21] According to the recent studies, OTOF mutations may account for majority of congenital auditory neuropathy.[22] Thus, the genetic test for OTOF mutations in patients with auditory neuropathy is of high clinical importance.

Sixth, identification of causative mutations significantly helps to provide adequate genetic counseling which primarily concerns planning of pregnancy and delivery with the information of a recurrence risk. Prenatal genetic diagnosis of nonsyndromic SNHL is not conducted in most countries because of ethical issues. For syndromic SNHL which is associated with severe symptoms other than SNHL, prenatal diagnosis may be considered.

Future Expectation of the Use of Genetic Testing in Therapeutics

Although hearing aids or cochlear implants can significantly restore hearing in patients with SNHL, quality of restored hearing is quite different from original or normal hearing. These instruments are made to help remaining functions of the damaged inner ear, but future therapeutics aims at complete recovery of the inner ear. Because current clinical diagnostic modalities cannot identify which parts or cells in the inner ear is damaged, therapeutic approach targeting at specific parts or cells in the inner ear has not been used. Identification of damaged cells in the inner ear and the underlying mechanism by genetic testing undoubtedly facilitates development and introduction of novel and specific therapies to distinct types of SNHL.

As one of such therapies, we have established novel therapeutic approaches targeting at cochlear fibrocytes which are essential for normal hearing and involved in various type of SNHL including certain types of hereditary SNHL, age-related SNHL, noise-induced SNHL, and Meniere's disease. A rat model of SNHL due specific to cochlear fibrocytes was made by treatment with a mitochondrial toxin, 3-nitropropionic acid (3-NP), at a round window of inner ear.[23] Histological and molecular analysis in this model revealed caspase-mediated apoptosis in the cochlear fibrocytes.[24] As the therapy during acute phase of SNHL due to damages on cochlear fibrocytes, we used a general administration of caspase inhibitor, Z-VAD-FMK, to inhibit apoptosis.[25] This chemical, when administered before 3-NP treatment, almost completely inhibited 3-NP induced apoptosis of cochlear fibrocytes without obvious side effects and significantly improved the hearing level. Administration of Z-VAD-FMK after 3-NP treatment also showed significant inhibition of apoptosis and improvement of hearing. As the therapy during chronic phase of SNHL due to damages on cochlear fibrocytes, we used transplantation of bone marrow-derived mesenchymal stem cells into the inner ear in this animal model.[26] Histological examination of the transplanted rats demonstrated that transplanted stem

cells survived, migrated to the damaged area, and apparently substituted the damaged cochlear fibrocytes. Those stem cells made a connection with the surrounding fibrocytes and expressed connexins which are essential for reestablishment of potassium recycling pathway mediated by cochlear fibrocytes within the cochlea. Evaluation of hearing by auditory brainstem responses in the transplanted rats revealed significant improvement of hearing compared to control rats. These animal experiments indicate that therapeutic strategy for genetic SNHL may be personalized, based on the cause of SNHL, using chemicals targeting at specific molecules or stem cells targeting at specific tissues for regenerative therapy. In addition, novel therapies developed for genetic SNHL may be applicable to other types of SNHL with similar pathological features.

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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 OPA1and 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

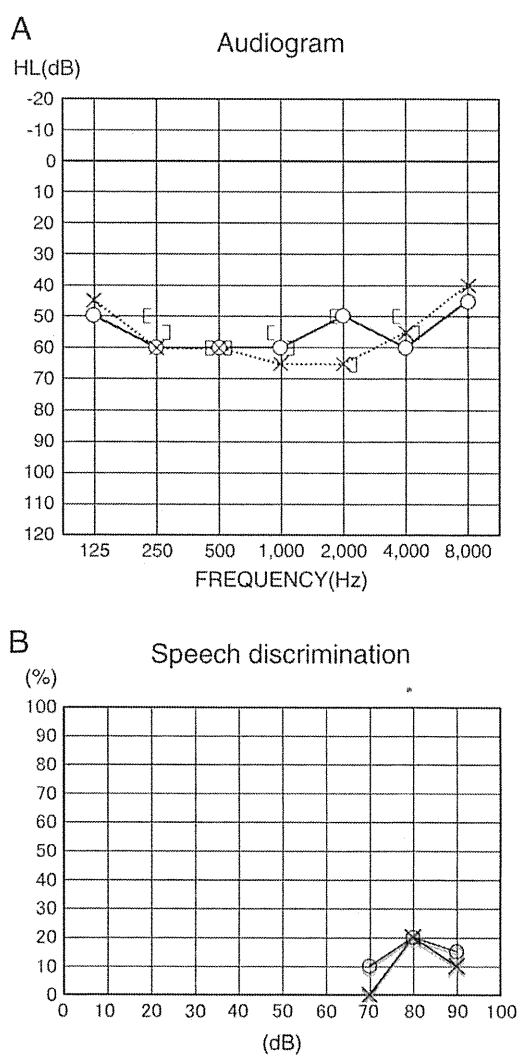

Fig. 1. Pure-tone (A) and speech (B) audiograms of a patient with an *OPA1* mutation. O = right air conduction hearing level; X = left air conduction hearing level; [= right bone conduction hearing level;] = left bone conduction hearing level.

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 either ear (Fig. 3A). Short-tone burst-evoked VEMP analysis revealed a biphasic VEMP waveform in the right ear; however, the latency of n23, which is the second wave of VEMP, was delayed. No VEMPs were evoked in the left ear (Fig. 3B).

4.3. Neuroimaging studies

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

4.4. OPA1 predicted structure

The distance between Cα of R445 of OPA1 and the GTP binding pocket is 18 Å (Fig. 5). The electric field around R445 is negatively charged due to its proximity to D450, D442, and E444. Under physiological conditions, positively charged R445 is structurally stable, and thus the mutation p.R445H reduces the electrostatic stability and indirectly distorts the structure of the GTPase catalytic

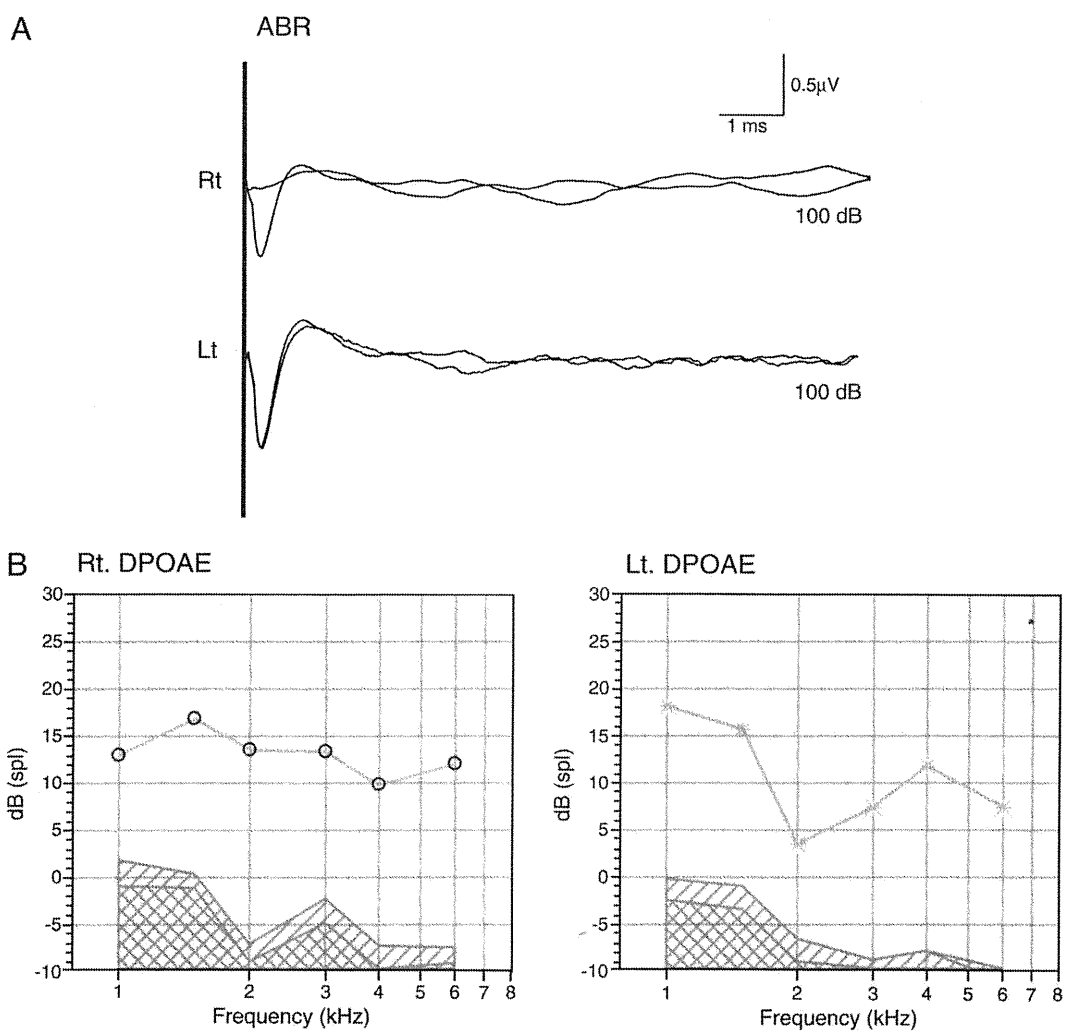

Fig. 2. (A) ABR tests revealed no ABR waveforms in this patient. (B) DPOAE recordings were normal for this patient. Residual noise levels are shown by the shaded area.

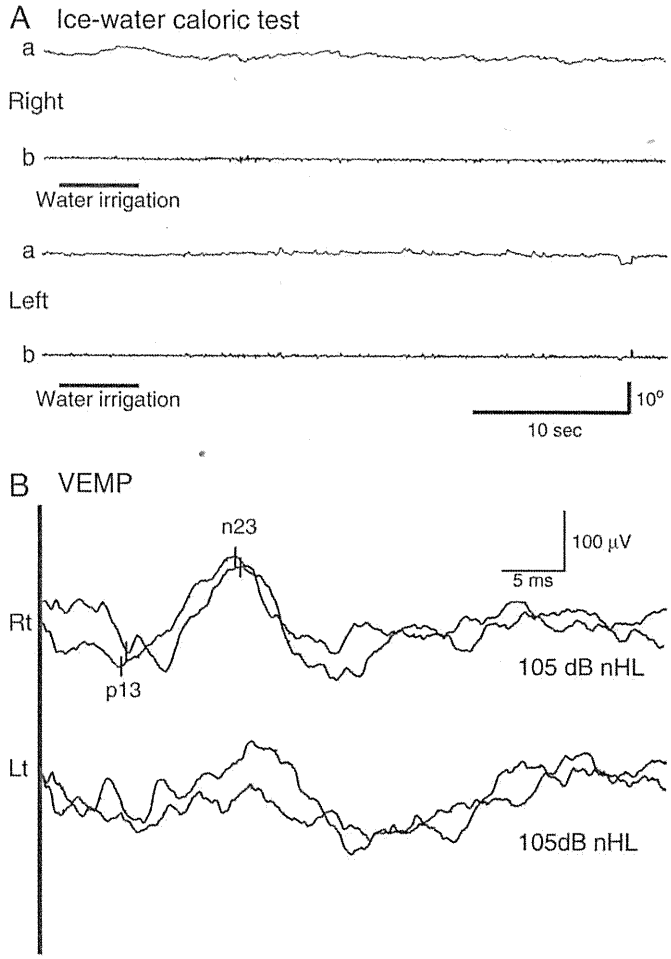

Fig. 3. (A) Horizontal record of electronystagmograph on ice-water caloric test. Time constants: a, 3.0 s; b, 0.03 s. No nystagmus were elicited in both side of ears. (B) Air-conducted VEMPs. Electromyographic responses of the right (Rt) and left (Lt) SCM to right ear stimulation. A biphasic VEMP waveform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

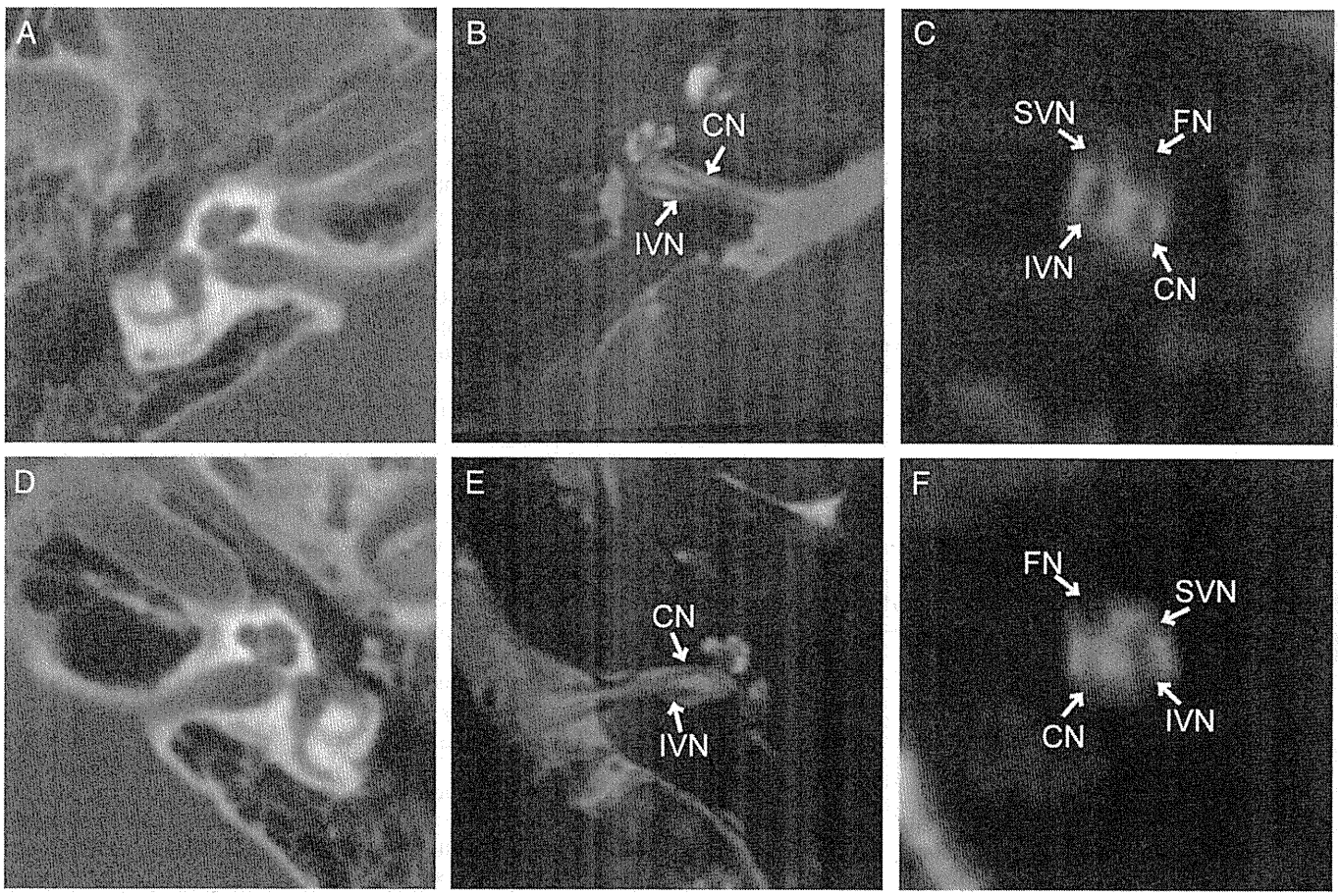

Fig. 4. Images showing the HRCT (A, D), axial MRI (FIESTA; B, E), and oblique sagittal reconstructions (C, F). The facial nerve (FN), cochlear nerve (CN), superior vestibular nerve (SVN), and inferior vestibular nerve (IVN) can be recognized in both sides of the internal auditory canal. However, the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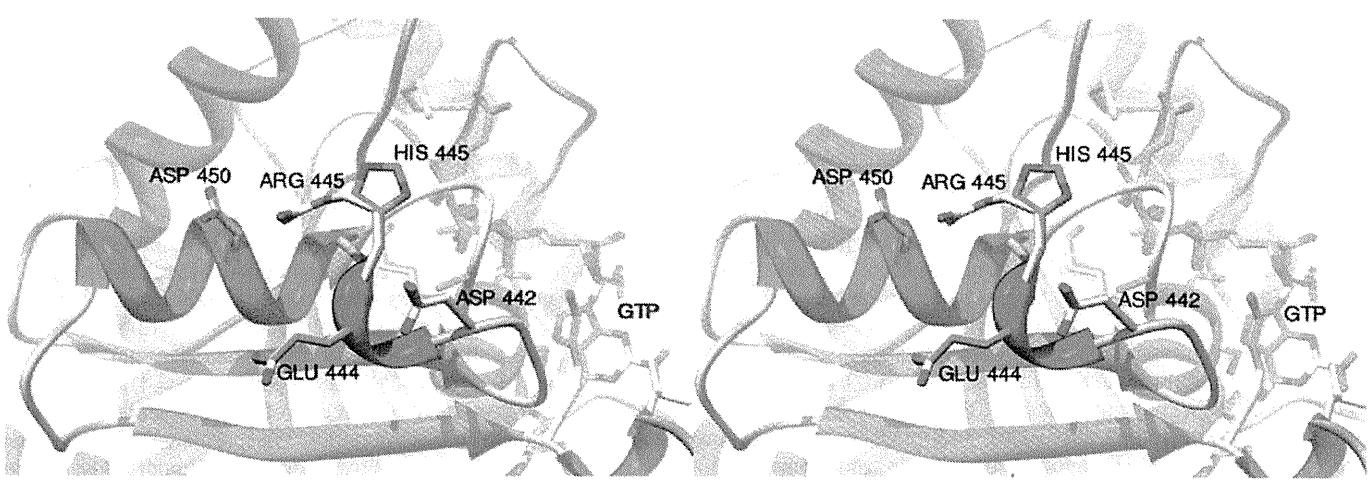

Fig. 5. Stereo view of the GTPase domain of predicted structure of human OPA1 with arginine at position 445 replaced by histidine. The electric field around R445 is negatively charged due to the proximity of D450, D442, and E444. Positively charged R445, under a physiological environment, is structurally 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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Risk factors for elevation of ABR threshold in NICU-treated infants

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ABSTRACT

Objective: Several risk factors for hearing impairment among infants treated in the neonatal intensive care unit (NICU) have been reported, but there have been few studies that show the correlation strength between the risk factors in NICU-treated infants and hearing impairment in childhood. The aim of this study was to clarify the relationship between risk factors in NICU-treated infants and a deteriolation of auditory brainstem response (ABR) threshold in their childhood.
Methods: One hundred one NICU-treated infants with ABR threshold of 50 dBnHL or more underwent 2nd ABR test at 20 months after delivery. Multiple regression analysis was performed with ABR threshold change as an objective variable and risk factors as explanatory variables.
Results: Two ABR tests of the 101 infants resulted in that 7 showed an elevation of ABR threshold by 20 dB, 70 showed a drop of ABR threshold by 20 dB, and 24 showed no significant change. Multiple regression analysis revealed that the factors contributing to the elevation of ABR threshold were congenital diaphragmatic hernia, severe respiratory disease, and a high C-reactive protein (CRP) level.
Conclusions: In the infants treated in NICU, an incidence of ABR threshold of 50 dBnHL or more was 9.0%, and 6.9% of the infants with the ABR threshold abnormality showed a significant elevation of ABR threshold in their childhood. Factors significantly related to an elevation of ABR threshold were a history of congenital diaphragmatic hernia, severe respiratory disease, and elevation of CRP. In infants with such factors, periodical examination of hearing is required.

1. Introduction

Advances in perinatal medicine have increased the survival rate of infants who are admitted to the neonatal intensive care unit (NICU). However, many infants discharge from the NICU have medical problems including communicative and/or cognitive difficulties due to hearing impairment and brainstem dysfunction. The occurrence of these auditory impairments is known to be related to risk factors [1], such as low birth weight (<1500 g), severe birth asphyxia, assisted ventilation for >5 days, administration of ototoxic drugs [2], intrauterine infection, central nervous system abnormalities, hyperbilirubinemia, and congenital syndromes [3]. However, the correlation strength between the risk factors in NICU-treated infants and hearing deterioration in their childhood has not been fully determined. Elucidation of the correlation strength can facilitate diagnosis of hearing impairment in potentially diseased infants by means of intensive hearing tests focusing on limited subjects with risk factors that are closely related to hearing deterioration. This approach would be cost-effective.

Therefore, we aimed to clarify the correlation strength between risk factors in NICU-treated infants and hearing deterioration that appeared in childhood (over 1-year old). In the present study, we defined an elevation of ABR threshold by >20 dB between two testing sessions as a significant change indicating the existence of hearing deterioration.

2. Subjects

A total of 1121 neonates were treated in the NICU of the National Center for Child Health and Development (Tokyo, Japan) from March 2002 to October 2005 and underwent an auditory brainstem response (ABR) test before discharge from the NICU for the purpose of hearing screening. Initial examination revealed that 125 infants had ABR threshold of 50 dBnHL or more in 1 or both ears. Of these 125 infants, 101 were eligible for this study after

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excluding 10 infants with conductive hearing loss (3 for auditory canal stenosis and atresia, 7 for otitis media with effusion) and 14 infants who were lost to follow-up.

Most infants underwent the initial ABR test within 6 months after delivery, but some infants with a poor performance status could not do so until 8 months.

3. Methods

3.1. ABR examination

The initial ABR test was conducted with a Neuropak (Nihon Koden, Tokyo, Japan) during natural sleep in the NICU before discharge and was performed at 1–33 weeks after delivery. Click stimuli were presented to alternating ears 1000 times at a rate of 9.5 Hz through headphones. The analysis time was 10 ms. To determine the hearing threshold, stimuli started from 105 dBnHL and were decreased to 30 dBnHL in steps of 20 dB. In addition, 1000 clicks were presented at 0.1 ms intervals and the threshold was defined by detection of the V wave. The infants were classified into three groups by comparison between the two ABR testing sessions: infants with a decrease of the ABR threshold by ≥20 dB in at least 1 ear (improved group), those with an increase of the threshold by ≥20 dB (worsened group), and others (unchanged group).

3.2. Distortion products otoacoustic emission (DPOAE) test

We conducted a DPOAE test in 64 of the 101 infants in which the ABR threshold was 50 dBnHL or more during stay in NICU, excluding those in whom it could not be done due to behavioral problems. An OAE analyzer (model ER32, Grason-Stadler, USA, $L1/L2 = 65/55$ dBSPL) was employed, and infants who responded to noise levels of 2, 3, and 4 kHz with >3 dB amplitude in the DP gram were regarded as having a positive test.

3.3. Behavioral audiometric evaluation

Behavioral audiometric evaluation was performed in 101 infants in whom the ABR threshold was 50 dBnHL or more and in whom no disjunction was detected between ABR threshold and hearing threshold.

3.4. Associated risk factors

The risk factors shown in Table 2 were investigated.

Abnormalities seen in brain CT or MRI included cerebral calcification, hydrocephalus, periventricular leukomalacia and abnormal myelinization. Craniofacial malformation included premature craniosynostosis such as Crouzon's disease and Apert syndrome. EEG abnormalities included spike & sharp waves, slow dominant rhythm and diffuse slowing. C-reactive protein (CRP) elevation was determined when the maximum value measured within about 1 month after birth was more than 1 mg/dL. History of birth asphyxia (Apgar score < 4 at 1 min and ≤6 at 5 min), severe respiratory disease (pneumonia or pneumothorax), mechanical ventilation (infants requiring a ventilator for more than 5 days), hypotension (decreased urine output due to hypotension and use of vasopressors), congenital diaphragmatic hernia, and heart disease (coarctation of the aorta, patent ductus arteriosus, ventricular septal defect, etc.) were assessed. Use of ototoxic drugs such as aminoglycosides (gentamycin, amikacin, and vancomycin) and use of muscle relaxants such as pancronium bromide before the second ABR were also assessed. Chromosomal aberration such as 21-trisomy was also checked.

3.5. Statistical analysis

Multiple regression analysis was performed with a change of the ABR threshold as an objective variable and the above-mentioned risk factors as explanatory variables. A probability (p) value of less than 0.05 was considered statistically significant.

3.6. Institutional board

The study was conducted in accordance with ethical principles described in the Declaration of Helsinki and was approved by the Ethics Committee of the National Center for Child Health and Development.

4. Results

4.1. Incidence of ABR threshold elevation in NICU infants

The initial ABR test revealed that 101 of the 1121 infants had ABR threshold of 50 dBnHL or more. Of those 101 infants, 57 infants (5.1%) showed ABR threshold of 50 dBnHL or more bilaterally and 44 infants (3.9%) had a threshold of 50 dBnHL or more in 1 ear, and 20 infants (1.8%) showed ABR threshold of 90 dBnHL or more bilaterally. In the second ABR test, 28 infants (2.5%) had ABR threshold of 50 dBnHL or more bilaterally, 9 (0.8%) had ABR threshold of 50 dBnHL or more in 1 ear and 19 infants (1.7%) had a threshold of 90 dBnHL or more in both ears. When compared with the initial test, 7 of the 101 infants showed an elevation of the ABR threshold by ≥20 dB, 70 showed a decrease of the threshold by ≥20 dB, and 24 showed a change of less than 20 dB (treated as 'no change'). In the 70 infants with ABR threshold decrease, 65 infants showed a normal threshold of ABR (30 dBnHL) bilaterally in the second test. Consequently, at the second test, 65 infants had ABR threshold of 30 dBnHL in both ears and the other 36 infants had ABR threshold of 50 dBnHL or more in at least 1 ear.

Nineteen (46%) of the 41 infants with ABR threshold of 70 dBnHL or more in the initial test showed an improvement: the ABR threshold in the second test was less than 70 dBnHL (Table 1). Also, 51 (85%) of the 60 infants with ABR threshold of 50–70 dBnHL in the initial test showed ABR threshold of less than 50 dBnHL in the second test, whereas 5 (8.3%) of the 60 infants with ABR threshold of 50–70 dBnHL in the initial test showed a deterioration: the ABR threshold in the second test was 70 dBnHL or more.

Table 1
Change in ABR threshold.

		Second result				
		<50 dBnHL	≧50, <70 dBnHL	≧70, <90 dBnHL	≧90 dBnHL	
Initial result	<50 dBnHL	0 (cases)	0	0	0	0
	≧50, <70 dBnHL	51	4	1	4	60
	≧70, <90 dBnHL	10	1	4	2	17
	≧90 dBnHL	6	2	0	16	24
		67	7	5	22	101

Table 2
Risk factors for ABR threshold change.

	Improved ABR threshold ($n=70$)	Unchanged ABR threshold ($n=24$)	Worsend ABR threshold ($n=7$)	p-Value	Correlation coefficient
Congenital diaphragmatic hernia	0	2	3	0.000*	0.42
CRP elevation	7	10	4	0.002*	0.33
Severe respiratory disease	4	4	2	0.003*	0.29
Hypotension	28	13	2	0.082	0.19
Birth weight average (g)	2003 ± 851	1882 ± 919	2560 ± 628	0.206	0.18
Birth weeks average (weeks)	34.2 ± 4.6	33.9 ± 5.5	37.7 ± 2.0	0.305	0.14
EEG abnormality	15	10	0	0.147	0.12
Abnormality seen in brain CT or MRI	21	13	2	0.352	0.09
Use of muscle relaxants	14	7	5	0.560	0.08
Multiple pregnancy	6	4	0	0.602	0.05
Craniofacial malformation	4	0	0	0.669	0.04
Mechanical ventilation	7	17	5	0.802	0.03
Heart disease	0	3	0	0.807	0.02
Ototoxic drugs	18	16	5	0.865	0.02
Asphyxia	14	11	2	0.891	0.02
Chromosomal aberrations	8	4	2	0.889	0.01

* $P_A < 0.01$.

4.2. ABR threshold change and risk factors

Among the 7 infants with an elevated ABR threshold, 3 had congenital diaphragmatic hernia. Of those 3 infants, only 1 received extracorporeal membrane oxygenation therapy (ECMO) and the other 2 received high frequency oscillatory ventilation. All 3 had persistent pulmonary hypertension of the newborn (PPHN). Including infants who needed ventilation because of tracheo-esophageal fistula or postoperative pneumothorax, 5 of the 7 infants had a history of respiratory support and 6 had received ototoxic drugs. On brain CT or MRI, 2 infants showed brain atrophy and 1 had chronic subdural hematoma, while 1 had an abnormal electroencephalogram.

Among the 68 infants with a decrease of the ABR threshold, 25 (37%) were presumed to have central nervous system abnormalities due to changes on the electroencephalogram or brain CT scans, 22 (32%) had received ventilation, 18 (26%) had received ototoxic drugs, and 10 (15%) had an elevated CRP level.

Of the 26 infants who showed no significant change of ABR threshold (within 20 dB), 18 (69%) had a presumed central nervous disease due to abnormalities on their electroencephalograms or brain CT scans. Nineteen infants (73%) had been on a ventilator, 17 (65%) had received ototoxic drugs such as vancomycin or gentamycin, and 10 (38%) had an elevated CRP level.

Table 2 shows the results of multiple regression analysis with change of ABR threshold as the objective variable and risk factors as the explanatory variables. The risk factors contributing significantly to ABR threshold change were a history of congenital diaphragmatic hernia (standardized coefficient = 0.41; $p = 0.001$), an elevated CRP level (standardized coefficient = 0.33; $p = 0.002$), and a history of severe respiratory disease such as pneumonia or pneumothorax (standardized coefficient = 0.28; $p = 0.005$). Other risk factors did not have a significant influence.

4.3. DPOAE and ABR threshold changes

When DPOAE testing was done in 64 infants (98 ears) with ABR threshold of 50 dBnHL or more during their stay in the NICU, 29 infants (50 ears) were negative and 37 infants (48 ears) were positive.

Among the 29 infants with negative DPOAE, 22 ears of 14 infants showed a decrease in the second ABR threshold by ≥20 dB and 8 ears of 5 infants showed an elevation in the threshold by ≥20 dB. Among the 14 infants in whom the ABR threshold decreased, 5 had central nervous system abnormalities such as delayed myelinization. Five of those 14 infant had chromosomal aberrations such as 21-trisomy.

Among those 37 infants with positive DPOAE, 10 had abnormalities on brain MRI or electroencephalography, including delayed myelinization and periventricular leukomalacia in 1 infant each. In addition, among those 37 infants, 38 ears of 31 infants showed a decrease in the ABR threshold by ≥20 dB and 1 ear of 1 infant showed elevation of the threshold by ≥20 dB.

Table 3 shows a list of the infants who had positive DPOAE with ABR threshold of 70 dBnHL or more in the initial test. ABR threshold in the second test was unchanged and worse in 5 and 1 of the 6 infants, respectively. Of the 6 infants, 4 had been on a ventilator, 3 had received ototoxic drugs, and 2 and 1 had a history of hypotension and an elevated CRP level, respectively. Five of the 6 infants had suffered from hyperbilirubinemia (8.28–14.70 mg/dl) and had received phototherapy.

5. Discussion

ABR threshold elevation in the second test was seen in 7 of 101 NICU-treated infants with ABR threshold of 50 dBnHL or more in the initial test. The ABR threshold elevation was significantly correlated with congenital diaphragmatic hernia, severe respiratory disease, and a high C-reactive protein (CRP) level.

All of the 7 infants who had ABR threshold elevation were diagnosed as having definite hearing loss at 20 months after delivery by a battery of audiological tests, and 5 of the 70 infants who had a decrease in ABR threshold were also diagnosed as having definite hearing loss in at least 1 ear at 20 months after delivery. Hence, this indicates that this study did not show risk factors correlated to hearing loss but showed risk factors correlated to ABR threshold elevation. The ABR threshold elevation is considered to reflect a part of progressive hearing losses [4,5], a part of delayed-onset hearing losses [6,7] or others. The 7 infants with ABR threshold elevation probably had progressive hearing loss because their ABR threshold in the initial ABR test (before discharge from the NICU) had already been high (50 dBnHL or more).

It has been reported that NICU infants show a 10- to 20-fold increase of risk factors for impaired hearing compared with healthy newborns because of poor performance status and long-term hospitalization [8]. Possible causes of hearing impairment include congenital factors such as genetic or anatomic abnormalities and acquired factors such as use of ototoxic drugs and excessive noise in the NICU, but the contribution of each factor

Table 3
Six infants with auditory neuropathy.

Case no.	Gestational age (month)	Birth weight (g)	Initial threshold		ABR	DPOAE		Brain CT or MRI	EEG	Hypotension	CRP elevation	Mechanical ventilation	Ototoxic drugs	Hyperbilirubinemia	Diagnosis
			Right	Left		Right	Left								
1	32	2152	90	90	(+)	(+)	(+)	Abnormal	w.n.l.	(−)	(−)	(+)	(−)	(−)	Hydrocephalus
2	26	709	110	110	(+)	(+)	(+)	Abnormal	Abnormal	(−)	(+)	(+)	(+)	(+)	Periventricular leukomalacia
3	28	974	70	30	(+)	(+)	(+)	w.n.l.	w.n.l.	(+)	(−)	(−)	(+)	(+)	Congenital lung disease
4	39	1850	105	105	(−)	(−)	(+)	w.n.l.	Abnormal	(−)	(−)	(−)	(−)	(+)	Beals syndrome, West syndrome
5	43	1398	110	110	(+)	(+)	(+)	Abnormal	w.n.l.	(−)	(−)	(+)	(−)	(+)	Periventricular leukomalacia
6	40	2675	110	110	(+)	(+)	(+)	Abnormal	Abnormal	(+)	(−)	(+)	(+)	(+)	Multiple carboxylase deficiency

w.n.l.: within normal limit.

remains unclear because a combination of several factors may contribute to hearing impairment. The results of the present study revealed that a history of severe respiratory disease, elevated CRP, and congenital diaphragmatic hernia were significant predictors of an elevation of ABR threshold. Severe respiratory disease can reduce oxygenation of the brain and oxygenation of the inner ear, which impair cochlear oxygenation, and cochlear hypoxia is presumed to be associated with delayed-onset hearing loss [9]. It has been reported that approximately 26–50% of infants with congenital diaphragmatic hernia suffer from delayed-onset hearing loss [7], and it has been suggested that aminoglycosides or PPHN with ECMO may be involved [10]. Yoshikawa et al. also reported a correlation with elevated CRP and ABR threshold elevation and concluded that the variable may predict hearing deterioration [11].

In the present study, ABR threshold decrease was seen in 60% of the infants who had shown ABR threshold of 50 dBnHL or more before discharge from the NICU. This suggests that abnormalities such as auditory canal stenosis or residual mesenchymal tissue due to immaturity at birth influence the initial ABR test results, so that the infants can show spontaneous improvement with growth [12]. Another reason may also be related to the decrease in ABR threshold: incomplete myelinization frequently seen infants with Down's syndrome causes immature auditory pathways [13], resulting in high threshold of ABR seen in the initial test. The incomplete myelinization mechanism can explain the cases with an elevated ABR threshold and positive DPOAE test; approximately 80% of the cases eventually showed ABR threshold decrease.

Yoon et al. [14] called for a protocol in which infants with risk factors for delayed or progressive hearing loss would be followed by periodical infant hearing tests until the age of 5 years, while infants without risk factors who show normal results of the initial hearing test would be examined again at 1 year. It was also proposed by JCIH that hearing of normal infants and hearing of NICU infants should be monitored by different methods [15]. Since NICU infants are more likely to show delayed intellectual development, they often have communication disorders that may mask hearing deterioration. Therefore, in infants with hearing deterioration-related risks clarified by this study, we should provide periodic medical evaluation and management of language and intellectual development in addition to hearing elevation [16,17].

6. Conclusion

Among 1121 infants treated in the NICU, 101 (9.0%) showed ABR threshold of 50 dBnHL or more before discharge from the NICU. Seven of those 101 infants showed an elevation of ABR threshold by ≥20 dB when ABR testing was repeated at 20 months. Risk factors for elevation of ABR threshold are a history of congenital diaphragmatic hernia, severe respiratory disease, and a high CPR level.

Conflict of interest

Authors declare no conflict of interest.

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