Table 1. Characteristics of patients with VKH disease and of controls

	Patients/controls		
	total subjects	≤50 years old	>50 years old
Number	85/85	49/49	36/36
Gender – male:female, n	37:48/37:48	22:27/22:27	15:21/15:21
Mean age ± SD, years	47.1±14.0/47.1±14.0	37.0±8.3/37.0±8.3	60.8±6.6/60.8±6.6
Mean duration from ocular symptom onset to initial treatment ± SD, days	8.2±5.9/–	8.4±5.8/–	8.0±5.9/–

(1) no impairment (the average of hearing thresholds at 500, 1,000, 2,000 and 4,000 Hz equates to a 25-dB hearing level, dB HL, or less); (2) mild (the average of hearing thresholds is 26–40 dB HL); (3) moderate (the average of hearing thresholds is 41–60 dB HL); (4) severe (the average of hearing thresholds is 61–80 dB HL); and (5) profound (the average of hearing thresholds is 81 dB HL or more).

The pattern of the pure tone audiogram was categorized into 4 types using the classification scheme of Mazzoli et al. [2003]. Low frequencies were defined as ≤500 Hz, mid frequencies as >500 and ≤2,000 Hz, and high frequencies as >2,000 and ≤8,000 Hz. The audiogram types were defined as follow: (a) low frequency, a >15-dB HL difference between the poorer low-frequency thresholds and those at higher frequencies; (b) mid frequency, a >15-dB HL difference between the poorest thresholds in the mid frequencies and those at higher and lower frequencies; (c) high frequency, a >15-dB HL difference between the mean of the 500- and 1,000-Hz thresholds and the mean of the 4,000- and 8,000-Hz thresholds; and (d) flat, a <15-dB HL difference between the mean of the 250- and 500-Hz thresholds, the mean of the 1,000- and 2,000-Hz thresholds, and the mean of the 4,000- and 8,000-Hz thresholds. Asymmetric hearing loss was defined as a loss of hearing sensitivity with a difference of ≥15 dB HL between the ears at ≥2 frequencies.

Distortion Product Otoacoustic Emissions

DPOAE measurements were performed using an ER-33 instrument (Rion). An acoustic probe enclosed in a soft rubber covering and containing a miniature microphone and a speaker was placed – but not hermetically sealed – in the distal portion of the external auditory canal. The stimuli applied were a 65-dB sound pressure level (dB SPL) tone of f_1 and a 55-dB SPL tone of f_2 ($f_2 > f_1$; $f_2/f_1 = 1.22$). The DPOAE response levels were measured at the frequency $2f_1-f_2$. The noise floor levels were also calculated at each frequency. DPOAE were recorded for 3 frequency pairs at 2,000, 3,000 and 4,000 Hz. The DPOAE response levels and the noise floor levels for each of the frequency pairs were used to assess pass-fail results. A 'DPOAE pass' result was defined as a DPOAE level for the 3 frequency pairs at least 6 dB SPL above the noise floor level.

Treatment Procedure

Patients with complete or incomplete VKH disease were treated with high-dose systemic corticosteroids. An initial daily dose of prednisolone (200 mg/day) was given by intravenous infusion for 2 days, and thereafter tapered off to 150, 100 and 80 mg/day for 2 days. Intravenous prednisolone was then changed to oral prednisolone (60 mg/day), which was also tapered off gradually over a period of 6 months or more by the treating ophthalmologists according to the severity of the ocular inflammation.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 12.0; SPSS Inc., Chicago, Ill., USA). Statistical differences were analyzed using the Mann-Whitney U test and the Kruskal-Wallis test, with $p < 0.05$ considered statistically significant.

Results

Subject Profiles

The patient and control profiles are summarized in table 1. The study population comprised 85 patients, consisting of 37 males and 48 females ranging in age from 18 to 70 years, with a mean age ± SD of 47.1 ± 14.0 years. The duration from the onset of ocular symptoms to initial treatment ranged from 1 to 23 days, with a mean duration ± SD of 8.2 ± 5.9 days. The patients were divided into groups of 50 years or under (n = 49) and over 50 years (n = 36). There were no differences in gender distribution or in duration from the onset of ocular symptoms to initial treatment between the two groups.

The control group comprised 85 subjects, consisting of 37 males and 48 females ranging in age from 18 to 70 years. They showed normal pure tone thresholds at all frequencies, normal tympanic membranes on otoscopic examination, normal peak amplitudes with –100 to +50 daPa tympanic peak pressure in response to impedance audiometry, and pass results for DPOAE.

Characteristics of Hearing Loss in VKH Patients

The characteristics of hearing loss in the VKH patients are summarized in table 2. Hearing loss was detected by audiometry in 76 (89.4%) of the 85 patients,

Table 2. Characteristics of hearing loss in Vogt-Koyanagi-Harada disease

	Total subjects	≤50 years old	>50 years old	p
Hearing loss detected by audiometry	76 (89.4%)	43 (87.8%)	33 (91.7%)	0.565
Symptoms				
Hearing loss	23 (27.1%)	13 (26.5%)	10 (27.8%)	0.565
Tinnitus	29 (34.1%)	15 (30.6%)	14 (38.9%)	0.565
Affected ear				
Bilateral	59 (77.6%)	31 (72.1%)	28 (84.8%)	0.189
Symmetry	50 (65.8%)	29 (67.4%)	21 (63.6%)	0.731
Asymmetry	9 (11.8%)	2 (4.7%)	7 (21.2%)	0.028
Unilateral	17 (22.4%)	12 (27.9%)	5 (15.2%)	0.189
Total number of affected ears	135	74	61	
Severity				
Normal-mild	107 (79.3%)	69 (93.2%)	38 (62.3%)	<0.001
Moderate	23 (17.0%)	4 (5.4%)	19 (31.1%)	<0.001
Severe	5 (3.7%)	1 (1.4%)	4 (6.6%)	0.112
Profound	0 (0%)	0 (0%)	0 (0%)	
Audiogram pattern				
High frequency	96 (71.1%)	44 (59.5%)	52 (85.3%)	0.001
Flat	36 (26.7%)	28 (37.8%)	8 (13.1%)	0.001
Low frequency	3 (2.2%)	2 (2.7%)	1 (1.6%)	0.678
Mid frequency	0 (0%)	0 (0%)	0 (0%)	
DPOAE	75	42	33	
Pass	17 (22.7%)	15 (35.7%)	2 (6.1%)	0.002
Fail	58 (77.3%)	27 (64.3%)	31 (93.9%)	0.002

Values denote numbers with percentages in parentheses unless specified otherwise. Statistical differences were analyzed using the Mann-Whitney U test to compare groups.

whereas 23 (27.1%) of the 85 patients complained of hearing loss and 29 (34.1%) complained of tinnitus. Among the total number of subjects, the rate of objective hearing loss was significantly higher than either that of subjective hearing loss ($p < 0.001$) or tinnitus ($p < 0.001$). Fifty-nine (77.6%) of the 76 patients presented with bilateral and 17 (22.4%) with unilateral hearing loss (9 involving the right ear and 8 the left ear), so that a total of 135 ears were diagnosed with VKH disease-associated hearing loss; 107 (79.3%) of the 135 ears showed normal hearing to mild hearing loss according to the WHO classifications of severity, with no patient showing profound hearing loss; 96 (71.1%) of the 135 ears indicated a high-frequency audiogram pattern, and 58 (77.3%) of 75 ears received a fail result for DPOAE.

Hearing Thresholds: Patients versus Controls

Figure 1 shows the mean pure tone thresholds ± SD for air conduction in the audiograms of the affected ears and of the control group. In both patient groups, we found that hearing thresholds in the affected ear were significantly higher than those in the control group at all frequencies ($p < 0.001$). In particular, the hearing thresholds were significantly elevated at high frequencies compared with those at low-to-mid frequencies ($p < 0.001$).

Hearing Loss Features: Patients Aged 50 Years or under versus Those over 50 Years

When we compared the features of auditory disturbance between the two groups of patients, the severity of hearing loss ($p < 0.001$), the rate of high-frequency audiogram patterns ($p < 0.001$) and DPOAE fail results ($p = 0.002$) were significantly higher in patients aged over 50 years than in those aged 50 years or under (table 2). When we compared hearing thresholds in the affected ear of both groups of patients, thresholds in the over-50-year-old group were found to be significantly higher than those in the group aged 50 years or under at all frequencies ($p < 0.01$).

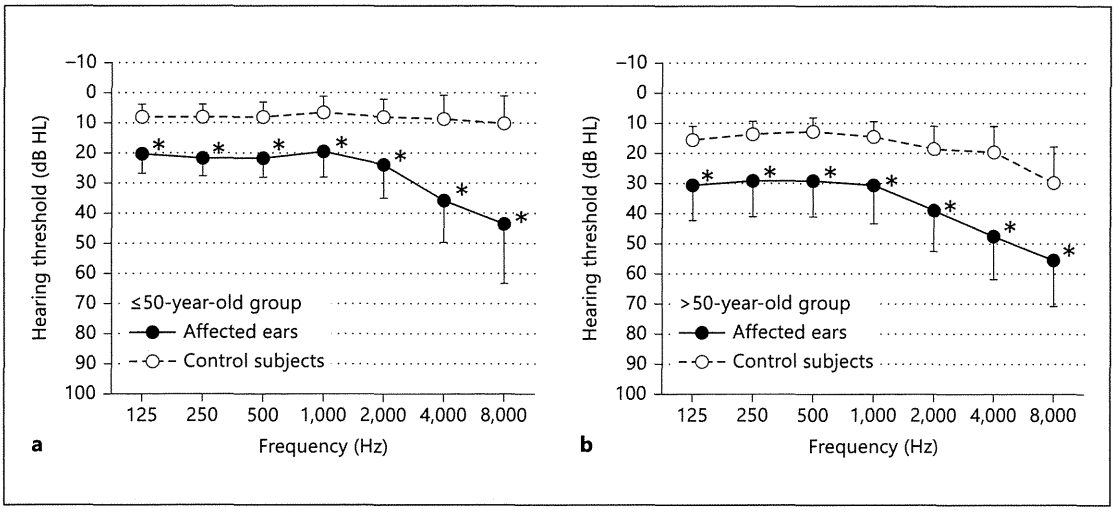

Fig. 1. Mean pure tone thresholds for air conduction in audiograms of the group 50 years of age and under (**a**) and of the group over 50 years of age (**b**) at initial presentation. Error bars: SD for each frequency. Statistical differences were analyzed using the Mann-Whitney U test. * $p < 0.001$.

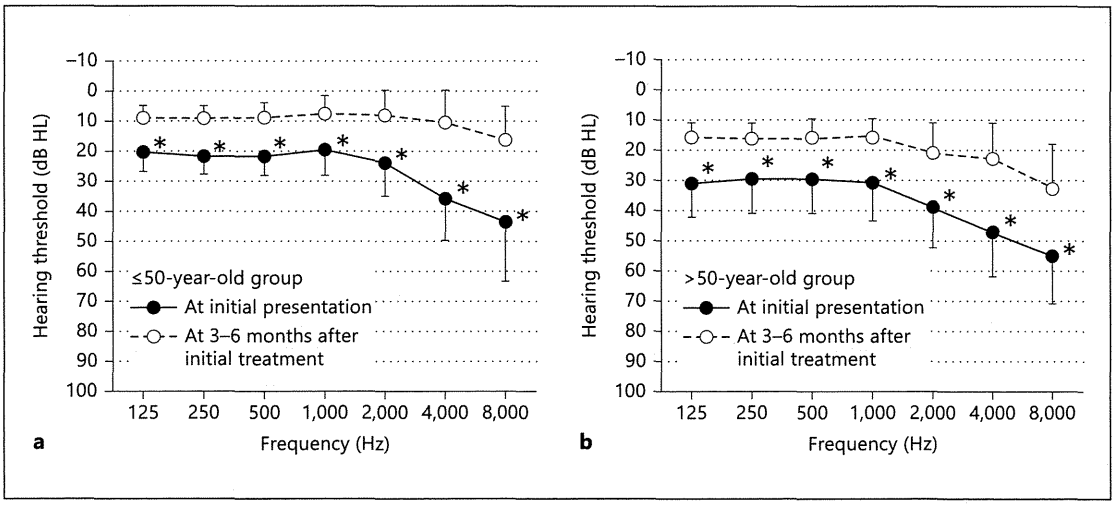

Fig. 2. Mean pure tone thresholds for air conduction in audiograms of the group 50 years of age and under (**a**) and of the group over 50 years of age (**b**) 3–6 months after the initial treatment. Error bars: SD for each frequency. Statistical differences were analyzed using the Mann-Whitney U test. * $p < 0.001$.

Efficacy of High-Dose Systemic Corticosteroid Therapy

Figure 2 shows the mean pure tone thresholds ± SD for air conduction in the audiograms of the affected ears 3–6 months after initial treatment. In both patient groups, we found that hearing thresholds at all frequencies after high-dose corticosteroid therapy were significantly better than those at initial presentation ($p < 0.001$).

The rate of recovery was 74.8% among the total subjects, 77.0% in the group aged 50 years or under, and 72.1% in the group aged over 50 years. The relationship between hearing outcome in VKH disease and each category was then assessed (table 3). Age, duration from onset of symptoms to initial treatment, and hearing threshold at initial presentation were significantly related to better hearing results ($p < 0.001$, $p = 0.008$ and $p = 0.001$).

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Table 3. Factors related to hearing outcome in VKH disease

	Recovery (n = 101)	No recovery (n = 34)	p
Mean age ± SD, years	44.0±13.4	57.2±9.4	<0.001
Gender – male:female, n	44:57	16:18	0.724
Mean duration from symptom onset to initial treatment ± SD, days	6.6±4.2	11.4±6.5	0.008
Symptom of hearing loss, n	30 (29.7%)	8 (23.5%)	0.490
Symptom of tinnitus, n	38 (37.6%)	11 (32.4%)	0.582
Audiogram pattern, n			
High frequency	73 (72.3%)	23 (67.6%)	0.857[1]
Flat	26 (25.7%)	10 (29.4%)	
Low frequency	2 (2.0%)	1 (2.9%)	
DPOAE, n			
Pass	14 (13.9%)	3 (8.8%)	0.710[1]
Fail	42 (41.6%)	16 (47.1%)	
Mean hearing threshold at initial presentation ± SD, dB HL	28.8±11.7	36.5±10.0	0.001

Statistical differences were analyzed using the Mann-Whitney U test.
[1] Statistical differences in audiogram pattern and DPOAE were analyzed using the Kruskal-Wallis test.

Discussion

The clinical features of VKH disease include anterior uveitis, exudative retinal detachment and depigmented fundal lesions, as well as the presence of neurological, auditory or dermatologic manifestations. The diagnosis of VKH disease requires both ocular and extraocular symptoms and signs [Read et al., 2001]. However, only 20–40% of VKH patients develop neurological and auditory findings, such as meningismus and tinnitus, at the acute ophthalmic stage [Mondkar et al., 2000; Kitamura et al., 2005; Miyanaga et al., 2007]. The integumentary findings, such as alopecia, poliosis and vitiligo, usually appear in patients with VKH disease only in the convalescent stage, some 3–6 months after the onset of uveitis [Mondkar et al., 2000]. Therefore, with the present diagnostic criteria, accurate diagnosis of VKH disease in the acute ophthalmic stage is sometimes difficult, making physicians hesitant to begin high-dose systemic corticosteroid therapy. Although the diagnostic criteria include only tinnitus as an auditory finding, otologic complaints other than tinnitus, such as hearing loss and/or vertigo, have been reported [Kitamura et al., 2005; Ondrey et al., 2006].

The presence of age-related hearing loss must be excluded to accurately discuss hearing loss. Thus, we used the ISO 7029 standard [International Organization for Standardization, 2000], which provides age- and gender-specific normative data on hearing thresholds and takes into account age-related hearing loss across the population as a whole. It is useful for evaluating any degree of hearing loss, whether or not the loss is clinically relevant. In the current study, 89.4% of the total number of subjects were found to be suffering from hearing loss on the basis of audiometric findings, while only 34.1% complained of tinnitus. While hearing thresholds in the affected ear were significantly higher than those in the controls at all frequencies ($p < 0.001$), most of the patients showed only normal hearing to mild hearing loss based on the WHO classifications of severity [World Health Organization, 2003]. This might explain the higher rate of hearing loss detected by audiometry than either that of subjective hearing loss ($p < 0.001$) or tinnitus ($p < 0.001$). These results suggest that history taking alone is insufficient to detect auditory manifestations, and that some auditory examinations, other than for tinnitus, should be performed to evaluate this disease.

In patients aged over 50 years, a differential diagnosis between presbycusis and auditory manifestations associated with VKH disease is difficult. Presbycusis is the most common hearing problem in older people. In general, people aged over 50 years tend to lose their hearing slowly as they age. Presbycusis is estimated to effect 30–83% of adults aged 65 years and more [Helzner et al., 2005]. Most cases of presbycusis include high-frequency hearing loss and are typically a bilateral, symmetric phenomenon, although those affected do not always complain of hearing loss [Nelson and Hinojosa, 2006]. In the current study, hearing loss in patients with VKH disease was of-

ten mild, with bilateral symmetric high-frequency audiogram patterns. In particular, there was a strong tendency for bilateral high-frequency audiogram patterns to be observed in patients aged over 50 years. Similarly, most of these patients (61.1%) did not complain of audiological disturbances, although their hearing thresholds were significantly higher than those in patients aged 50 years or under at all frequencies ($p < 0.01$). These features appear to be similar to those of presbycusis and make it difficult to evaluate auditory manifestations associated with VKH disease.

We found that most VKH disease patients with hearing loss scored a fail result on the DPOAE, and hearing thresholds were significantly elevated at high frequencies compared with those of low-to-mid frequencies ($p < 0.001$), which may indicate more serious damage at the base of the cochlea than at the apex. In the cochlea, melanin or melanocytes are located in the stria vascularis, auditory receptors or hair cells, vestibular organ and endolymphatic sac [Barrenäs and Axelsson, 1992]. Although the functions of melanin and melanocytes in the inner ear remain unclear, it has been reported that melanin has semiconductive properties, responding to phonic, acoustic and electric stimulation, and the ability to convert energy states into molecular rotation and vibration, as well as the reverse [McGinness et al., 1974]. Furthermore, melanin in the cochlea functions as an intracellular calcium buffer and as a depot of essential metal ions that control the activity of various enzymes and metabolic processes [McGinness et al., 1974; Steel and Barkway, 1989]. Melanocytes also are required for the maintenance of normal function of the stria vascularis and cochlea, the development of endocochlear potentials, and the conservation of the ion and fluid gradients between endolymph and perilymph, all of which are critical for hair cell survival [Hilding and Ginzberg, 1977; Steel and Barkway, 1989]. Thus, melanin-containing cells in the inner ear are thought to protect the cochlea from various stresses such as loud noise, mechanical trauma and ototoxic drug administration [Steel and Barkway, 1989]. Meanwhile, reduced levels and/or activities of pigment cells may result in audiological abnormalities [Steel and Barkway, 1989; Barrenäs and Axelsson, 1992; Ardic et al., 1998]. Melanin-containing cells are considered to be concentrated more in the basal region than in the apical region, so the basal region is considered to be more vulnerable to various stresses in pigmentary abnormalities [Wästerström et al., 1986]. Several prominent cutaneous pigmentary abnormalities characterized by a loss of functional melanocytes have also demonstrated higher-frequency hearing loss [Ardic et al., 1998; Aydogan et al., 2006], which supports our present results on VKH disease.

Treatment of VKH disease is based on the early administration of high-dose systemic corticosteroids, followed by gradual tapering and maintenance for at least 6 months, and this regimen is associated with less intraocular inflammation, a decreased frequency of ocular recurrences and dermatologic features, and better visual outcomes [Rubsamen and Gass, 1991; Moorthy et al., 1995; Mondkar et al., 2000; Chee et al., 2009; Lai et al., 2009]. Clinicians should balance the benefits of uveitis control with the risk of side effects [Lai et al., 2009]. Although several reports have demonstrated the effectiveness of high-dose systemic corticosteroids for the ocular and neurologic manifestations of VKH disease [Solaro and Messmer Uccelli, 2000], no study has specifically examined the effectiveness of systemic steroids for the otologic manifestations. Their effectiveness has also been suggested in the treatment of hearing loss associated with other autoimmune inner ear diseases [Niparko et al., 2005], which may indicate that similar results can be expected for VKH disease. In the current study, we found that hearing thresholds at all frequencies after high-dose corticosteroid therapy were significantly better than those at initial presentation ($p < 0.001$), and the rate of patients who returned to within-normal pure tone thresholds at all frequencies was 74.8%. These results demonstrate that, as with autoimmune inner ear diseases, high-dose systemic corticosteroids are effective in the treatment of the auditory manifestations of VKH disease. As ophthalmologists make the decision whether to treat with high-dose systemic corticosteroids according to the severity of ocular inflammation, but not based on auditory findings in VKH patients, our study lacked a control group not receiving steroids. Although the rates of spontaneous recovery and recurrence remain clinical problems, as with ocular symptoms and signs, hearing loss associated with acute VKH disease is reversible and shows a relatively good short-term prognosis. In particular, young patients who presented with mild hearing loss and received early treatment showed significantly better hearing outcomes. Meanwhile, it is thought that presbycusis is exacerbated under the influence of hearing loss associated with VKH disease in patients aged over 50 years. However, some elderly patients showed an improvement in hearing after high-dose systemic corticosteroids therapy even when initially thought to be suffering from presbycusis. Thus, an accurate diagnosis of, and prognosis for, hearing loss associated with VKH disease in the elderly is difficult when using pure tone audiograms alone.

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In conclusion, hearing loss associated with VKH disease was observed in a significantly larger number of patients than was tinnitus. Most of the patients showed mild hearing loss and a bilateral, symmetric high-frequency audiogram pattern, while exhibiting no auditory symptoms. Although the diagnostic criteria include only tinnitus, and not hearing loss, as an auditory sign, audiometry should be performed for the detection of asymptomatic hearing loss. High-dose systemic corticosteroids are thought to be effective for the auditory manifestations of VKH disease, which generally show a relatively good short-term prognosis. However, it is important to evaluate hearing loss in VKH disease at the acute ophthalmic stage and administer early treatment.

Disclosure Statement

We have no conflicts of financial interest to declare.

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Effect of Vestibular Dysfunction on the Development of Gross Motor Function in Children with Profound Hearing Loss

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Key Words
Vestibular evoked myogenic potential · Caloric test · Rotational test · Gross motor development

Abstract
Objective: To evaluate the function of the superior and inferior vestibular nerve systems in children with profound sensorineural hearing loss, and to assess the influence of dysfunction of each vestibular nerve system on the development of gross motor function. ***Study Design:*** Retrospective study. ***Setting:*** A tertiary referral center. ***Methods:*** Eighty-nine children (age range: 20–97 months) with profound sensorineural hearing loss who were due to undergo cochlear implant surgery were recruited. Function of the superior vestibular nerve system was evaluated by the damped rotation test and the caloric test, whereas functions of the inferior vestibular nerve systems were evaluated by the vestibular evoked myogenic potential (VEMP) test. Gross motor development was assessed using the age of acquisition of head control and independent walking. ***Results:*** Among the children able to complete the vestibular function tests, abnormalities were found in 20% (16 of 84 children) in the damped rotation test, 41% (31 of 75 children) in the caloric test and 42% (26 of 62 children) in the VEMP test. Children who showed abnormal responses in the vestibular function tests showed significantly delayed acquisition of head control ($p < 0.05$) and independent walking ($p < 0.05$) in comparison with children with normal responses. The children who showed abnormal responses in all 3 vestibular tests showed the greatest delay in acquisition of gross motor function in comparison with the other groups. ***Conclusions:*** Children with profound hearing loss tend to have dysfunction in the superior as well as the inferior vestibular nerve systems. Both the superior and inferior vestibular nerve systems are important for the development of gross motor function in children.

Introduction

The development of balance and gross motor functions such as head control and independent walking are intimately related and dependent on inputs from the vestibular, visual, proprioceptive and motor systems [Kaga, 1999; Suarez et al., 2007]. During the early stages of development, children primarily depend on the visual system to maintain balance. As they grow older, they progressively begin to use somatosensory and vestibular information until these systems reach full maturity around the age of 10 years [Kaga, 1999; Wallacott et al., 2004;

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Suarez et al., 2007]. Since vestibular function plays an important role in the development of balance and locomotion, impairment of the vestibulospinal system in infancy may lead to delayed achievement of gross motor milestones [Eviatar et al., 1979; Kaga, 1999; Kaga et al., 2008].

A close relationship exists between the cochlea and the peripheral vestibular end organs with respect to embryology, physiology and anatomy [Jin et al., 2006; Cushing et al., 2008a], hence they may be similarly affected by embryological factors, or by viral or bacterial infections. Therefore, children with profound sensorineural hearing loss may also exhibit peripheral vestibular impairments [Shinjo et al., 2007; Cushing et al., 2008a; Kaga et al., 2008; Jacot et al., 2009]. It has been reported that children with profound hearing loss tend to display balance dysfunction and delayed acquisition of gross motor skills, such as head control, sitting and walking, compared with children with normal hearing [Potter and Silverman, 1984; Butterfield, 1986; Crowe and Horak, 1988; Suarez et al., 2007; Cushing et al., 2008a]. The incidence of vestibular dysfunction in children with profound hearing loss has been reported to be between 31 and 75% [Diepeveen and Jensen, 1968; Jin et al., 2006; Cushing et al., 2008a; Zagólski, 2008].

Several previous studies have investigated the relationship between vestibular function and gross motor development in children with profound hearing loss [Kaga et al., 1981; Potter and Silverman, 1984; Crowe and Horak, 1988; Suarez et al., 2007; Cushing et al., 2008a]. Kaga et al. [1981] showed that the age of acquiring head control and independent walking in children with vestibular dysfunction was significantly delayed compared with normal controls [Kaga et al., 1981]. Rine et al. [2000] reported delayed gross motor development in children with vestibular dysfunction. In these studies, vestibular function in infants and children was evaluated using the rotational test and/or the caloric test, which reflect function in the lateral semicircular canal and superior vestibular nerves [Kaga, 1999; Suarez et al., 2007; Cushing et al., 2008a]. Vestibular evoked myogenic potentials (VEMPs) in response to air-conducted sound have recently been used to evaluate vestibular function [Colebatch and Halmagyi, 1992; Murofushi et al., 1996, 1998; Welgampola and Colebatch, 2005]. Physiological and clinical studies have suggested that VEMPs are generated by activation of the saccule and the inferior vestibular nerves [McCue and Guinan, 1994; Murofushi et al., 1995, 1996]. Combined use of VEMP and rotational and/or caloric tests has enabled examination of the inferior and superior vestibular nerve systems separately [Murofushi et al., 1996, 1998; Iwasaki et al., 2005]. Although VEMPs have been studied mainly in adults, it has been shown that the VEMP can be recorded from infants and children in almost the same way as adults [Kelsch et al., 2006].

In the present study, we assessed vestibular function in children with profound hearing loss, before they underwent cochlear implantation, using VEMPs as well as caloric and rotational testing, and compared the results with the development of gross motor function. The purposes of the present study were to evaluate the function of the superior and inferior vestibular nerve systems in children with profound hearing loss, and to investigate the effect of each vestibular nerve system on the development of gross motor function.

Methods

We enrolled 101 consecutive new children (20–97 months old) who presented at the University of Tokyo Hospital between January 2003 and June 2010 with profound hearing loss and who subsequently underwent cochlear implant surgery. We excluded 11 children with acquired hearing loss (4 due to meningitis, 2 due to severe neonatal infections, and 5 who had passed the newborn hearing screening test). We also excluded 1 child with extremely low birth weight. We did not exclude 8 children with cytomegalovirus (CMV) infection or 2 with Waardenburg syndrome because they did not show any neurological abnormalities except for hearing loss. As a result, 89 children (45 male, 44 female; age range 20–97 months, mean age 40 months) were included. The data of individual children are listed in the online supplementary table 1 (see www.karger.com/doi/10.1159/000346344 for all online suppl. material). All these patients underwent high-resolution computed tomography of the temporal bone and magnetic resonance imaging of the brain. Screening for the connexin 26 *(GJB2)* mutation in the peripheral blood and screening for CMV DNA in the umbilical cord blood were performed in 31 and 28 patients, respectively. The etiologies of hearing loss in the 89 children are listed in table 1. Classification of the inner ear malformation [Sennaroglu and Saatci, 2002] in 19 patients is listed in the online supplementary table 2.

This study was approved by the ethics committee in the Faculty of Medicine at the University of Tokyo and was conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from the parents of each participant.

Evaluation of the Etiologies of Hearing Loss

High-resolution computed tomographic scans (slice thickness 1.0 mm), screening for CMV DNA in the umbilical cord blood, and screening for the *GJB2* mutation were performed. High-resolution computed tomographic scans were checked by both otolaryngologists and radiologists. Both homozygous and heterozygous mutations of *GJB2* were classified as positive. There was no patient selection protocol with regard to the performance of these tests, which may cause a bias in the etiology results. A family history of significant perinatal problems was checked by having parents complete questionnaires (open-ended questions). These procedures were approved by the local ethics committee.

Table 1. Etiologies of hearing loss in 89 children

	Number %	Mean age at evaluation, months
Inner ear malformation	19 (21%)	43 (25–87)
GJB2 mutation	13 (15%)	27 (20–33)
Congenital CMV infection	8 (9%)	38 (24–63)
Waardenburg syndrome	2 (2%)	51 (27–75)
Unknown	47 (54%)	42 (24–97)
Total	89 (100%)	40 (20–97)

Age ranges are indicated in parentheses.

Vestibular Function Tests

Damped Rotation Test

The children were held upright on their mother's knees on a rotational chair with their heads bending down 30°. The rotational chair was accelerated to a maximum rotational velocity of 200°/s with a maximum acceleration of 300°/s^2 and then decayed to 0°/s by a deceleration of −4°/s^2. The test was conducted twice in both clockwise and counterclockwise directions. Eye movements were recorded by electronystagmography. Since calibration for accurate velocity measurements could not be performed in most children, we calculated the number of beats of per-rotatory nystagmus. The number of beats was measured and compared with age-matched controls according to the results of the damped rotation test in normal children reported by Kaga et al. [1981] for children up to 6 years old. If the number of per-rotatory nystagmus beats was more than 2 standard deviations smaller than the average value at each age, as reported by Kaga et al. [1981], it was considered abnormal. For children older than 6 years, the normal limit of the number of per-rotatory nystagmus beats was set as 23. This value is based on the number of per-rotatory nystagmus beats in 15 normal children between the ages of 7 and 9 years (31 ± 3.9 beats) recorded in this laboratory.

Caloric Test

The caloric test was performed using 4°C ice water. Horizontal and vertical eye movements were recorded using electronystagmography. We measured the duration of induced nystagmus and compared it with age-matched controls since calibration of eye movements was difficult in most children. The duration of induced nystagmus in 112 normal control children was 94.7 ± 20.7 s for the age range 13–24 months, 103.8 ± 28.4 s for 25–36 months, 109.2 ± 28.4 s for 37–48 months, 98.1 ± 20.3 s for 49–60 months, 105 ± 28.4 for 61–72 months, and 123.3 ± 35.1 s for >72 months. If the duration of induced nystagmus was more than 2 standard deviations smaller than the average value at each age, it was considered abnormal. Therefore, normal limits were set as 53.3 s for 13–24 months, 54.3 s for 25–36 months, 52.4 s for 37–48 months, 57.4 s for 49–60 months, 48.1 s for 61–72 months and 35.1 s for >72 months.

Vestibular Evoked Myogenic Potentials

Each subject was placed in the supine position. The active electrode was placed over the upper half of the sternocleidomastoid muscle (SCM), the reference electrode on the upper sternum and the ground electrode on the midline of the forehead. Subjects were instructed to raise their heads off the pillow to activate the SCM. In children who could not follow this instruction, the examiner helped them to raise their body with their head hanging down to induce contraction of the SCM. Electromyographic activity in the SCMs was monitored to confirm sufficient normal muscle activity (>150 μV). Sound stimuli of 500-Hz tone bursts (95 dB nHL) were presented to each ear through calibrated headphones (DR-531, Elega Acoustic Co. Ltd., Tokyo, Japan). Electromyographic signals from the SCM on the stimulated side were amplified using Neuropack Sigma (Nihon Koden, Tokyo, Japan). The stimulation rate was 5 Hz, the band-pass filter intensity was 20–2000 Hz, and the analysis time was 50 ms. VEMPs in response to 50 stimuli were averaged twice. VEMPs were considered to be present when there was a reproducible short-latency biphasic wave (p13–n23) [Sheykholeslami et al., 2005; Kelsch et al., 2006]. We calculated the asymmetry ratio for the amplitude of VEMPs (VEMP AR) with the following formula using the peak-to-peak amplitude of p13–n23 (μV) on the right side *(Ar)* and that on the left side *(Al)*:

$$\text{VEMP AR (\%)} = 100 \cdot |(Ar - Al)/(Ar + Al)|.$$

VEMP AR (%) <33.3 was considered to indicate a significant asymmetry [Jin et al., 2006; Shinjo et al., 2007].

Gross Motor Development

To assess gross motor development, we interviewed parents about the age at which the children started to acquire head control and to walk by themselves. We also checked the ages given against the relevant data recorded in the Maternity Health Record Book provided by the Japanese government.

If the age of acquiring head control was >5 months and the age of independent walking was >18 months, the development of the gross motor function was considered to be delayed according to the modified version of DENVER II for Japanese children published by the Japanese Society of Child Health (Nihon Shoni Iji Shuppansha, Tokyo, Japan).

Statistics

For comparison of two groups, the Mann-Whitney U test was used. For comparing multiple groups, the nonparametric Kruskal-Wallis test was used. Variables that showed a significant difference in this test were then compared in pairs using the nonparametric Steel-Dwass multiple-comparison method. Values were expressed as means ± SD. A p value <0.05 was considered significant.

Results

Vestibular Function in Children with Profound Hearing Loss

A summary of the results of the damped rotation test, caloric test and VEMPs in the children with profound hearing loss is shown in table 2. Since these vestibular tests need a certain amount of cooperation, they could not be completed in some children. Among the 89 children recruited, 51 were able to complete all 3 vestibular tests

Table 2. Results of vestibular function testing

	Normal	Unilateral dysfunction	Bilateral dysfunction	Total
Inner ear malformation				
Rotation test	9 (50%)	0 (0%)	9 (50%)	18 (100%)
Caloric test	4 (27%)	4 (27%)	7 (47%)	15 (100%)
VEMP	6 (40%)	2 (13%)	7 (47%)	15 (100%)
GJB2 mutation				
Rotation test	13 (100%)	0 (0%)	0 (0%)	13 (100%)
Caloric test	13 (100%)	0 (0%)	0 (0%)	13 (100%)
VEMP	10 (83%)	0 (0%)	2 (17%)	12 (100%)
Congenital CMV infection				
Rotation test	3 (60%)	1 (20%)	1 (20%)	5 (100%)
Caloric test	4 (67%)	1 (17%)	1 (17%)	6 (100%)
VEMP	2 (33%)	1 (17%)	3 (50%)	6 (100%)
Others				
Rotation test	42 (88%)	0 (0%)	6 (13%)	48 (100%)
Caloric test	23 (56%)	8 (20%)	10 (24%)	41 (100%)
VEMP	18 (62%)	2 (7%)	9 (31%)	29 (100%)
All children				
Rotation test	67 (80%)	1 (1%)	16 (19%)	84 (100%)
Caloric test	44 (59%)	13 (17%)	18 (24%)	75 (100%)
VEMP	36 (58%)	5 (8%)	21 (34%)	62 (100%)

Table 3. Relationship between superior and inferior vestibular function tests

	Rotation test/caloric test			Total
	normal	asymmetry	bilateral dysfunction	
VEMP				
Normal	26	7	1	36
Asymmetry	1	3	1	5
Bilateral dysfunction	9	0	11	20
Total	36	10	15	61

whereas the other 38 children were only able to complete 1 or 2 of the tests.

The damped rotation test was completed in 84 of the 89 children (94%). Among these 84 children, 16 (19%) showed reduced or absent per-rotatory nystagmus on both clockwise and counterclockwise rotations, whereas 67 children (80%) showed normal responses during rotation in both directions. One child (1%) showed reduced per-rotatory nystagmus in the clockwise rotations only (patient No. 36 in the online suppl. table 1).

Caloric testing was completed in 75 of the 89 children (84%). Among them, 18 children (24%) showed reduced or absent nystagmus induced in both ears, whereas 44 children (59%) showed normal responses in both ears. Thirteen children (17%) showed abnormal responses in one ear only.

VEMP testing was completed in 62 of the 89 children (70%). Among them, 21 children (34%) showed no responses on either side whereas 36 children (58%) showed normal responses on both sides. Five children (8%) showed responses on one side only.

Most children with the *GJB2* mutation showed normal responses bilaterally in the damped rotation test, caloric test and VEMP test. On the other hand, more than half of the children with inner ear malformations and congenital CMV infection showed abnormal responses in these 3 vestibular function tests (table 2).

The relationship between the results of the damped rotation test, caloric test and VEMP test are shown in table 3. Since both the damped rotation and caloric tests reflect the function of the superior vestibular nerve system, we combined the results of these two tests. If cases showed abnormal responses in either of these two tests, we classified them as having abnormal superior vestibular function. Cases which showed abnormal VEMP responses were classified as having abnormal inferior vestibular function. Among the 61 children who were able to complete all 3 vestibular tests, 26 (43%) showed normal responses in both the superior and inferior vestibular function tests whereas 15 children (25%) showed abnormal responses in both of these tests. Ten children (16%) showed abnormalities in the superior vestibular function tests while sparing inferior vestibular function. On the other hand, 10 children (16%) showed abnormalities in the inferior vestibular dysfunction tests while sparing superior vestibular nerve function.

Gross Motor Development in Children with Profound Hearing Loss

The distribution of ages at which children with profound hearing loss started acquiring head control and independent walking are shown in figure 1 and table 4. We were unable to obtain information regarding the age of acquisition of head control in 10 children, and the age of independent walking in 13 children.

The age at which the children acquired head control was delayed to later than 5 months of age in 24 (30%) of 79 children. The age at which children began to walk independently was delayed to later than 18 months of age in 20 (26%) of 76 children.

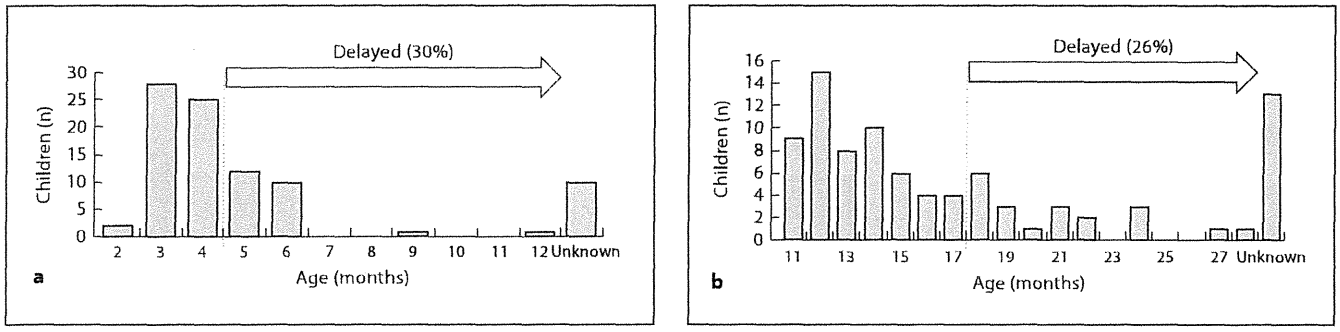


Fig. 1. Distribution of the age of acquiring head control and independent walking in children with profound hearing loss. **a** Distribution of the age of acquiring head control. Ages greater than 5 months were considered to be delayed. **b** Distribution of the age of independent walking. Ages greater than 18 months were considered to be delayed.

Table 4. Age of acquiring head control and independent walking

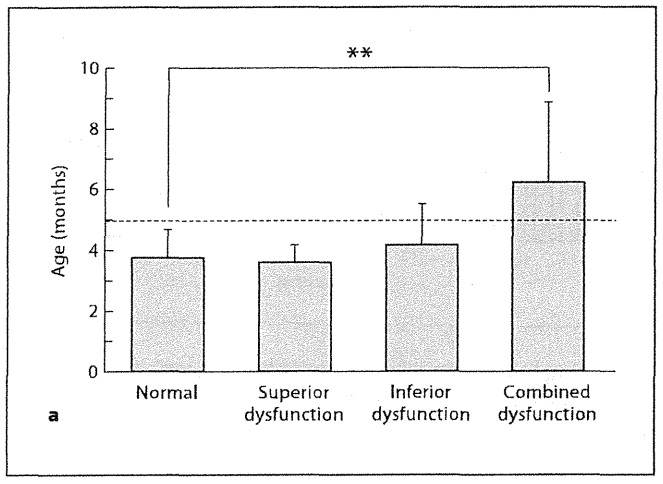
	Head control			Independent walking			Total
	normal	delayed	unknown	normal	delayed	unknown	
Inner ear malformation	8 (42%)	8 (42%)	3 (16%)	8 (42%)	7 (37%)	4 (21%)	19 (100%)
GJB2 mutation	11 (85%)	1 (8%)	1 (8%)	13 (100%)	0	0	13 (100%)
Congenital CMV infection	6 (50%)	1 (13%)	1 (13%)	4 (50%)	2 (25%)	2 (25%)	8 (100%)
Others	30 (4%)	14 (29%)	5 (10%)	31 (63%)	11 (22%)	7 (14%)	49 (100%)
All children	55 (62%)	24 (27%)	10 (11%)	56 (33%)	20 (22%)	13 (15%)	89 (100%)

In most children with the *GJB2* mutation, the age of acquiring head control and independent walking was within normal limits (11 of 12 children with available information for head control; all children with available information for independent walking; table 4). On the other hand, approximately half of the children with inner ear malformations showed delayed head control (8 of 16 children) and delayed independent walking (7 of 15 children). In children with CMV infection, the age of independent walking was delayed in one third (2 of 6 children) whereas the age of acquiring head control was delayed in one seventh of them (1 of the 7 children).

Vestibular Function and the Development of Gross Motor Function

To estimate the effect of vestibular dysfunction on the development of gross motor function, we compared the age of acquiring head control and independent walking with the results of each of the vestibular function tests (table 5). The age of acquiring both head control and independent walking was significantly delayed in children who showed abnormal responses bilaterally in comparison with those who showed normal responses bilaterally ($p < 0.05$ in the rotation test, caloric test and VEMP test for both head control and independent walking). On the other hand, there were no significant differences in the age of acquiring head control and independent walking between the children who showed asymmetric responses and those with normal responses in the caloric and VEMP testing ($p > 0.05$ for both tests).

To clarify the effect of dysfunction of the superior and inferior vestibular nerve systems on the development of gross motor function, we classified the children according to the involvement of the superior and the inferior vestibular nerve systems into the following 4 groups: (1) normal group, i.e. children who showed normal responses bilaterally in both the superior vestibular function tests (caloric testing and damped rotation test) and the inferior vestibular function test (VEMPs) (n = 26); (2) superior dysfunction group, i.e. those with abnormal re-



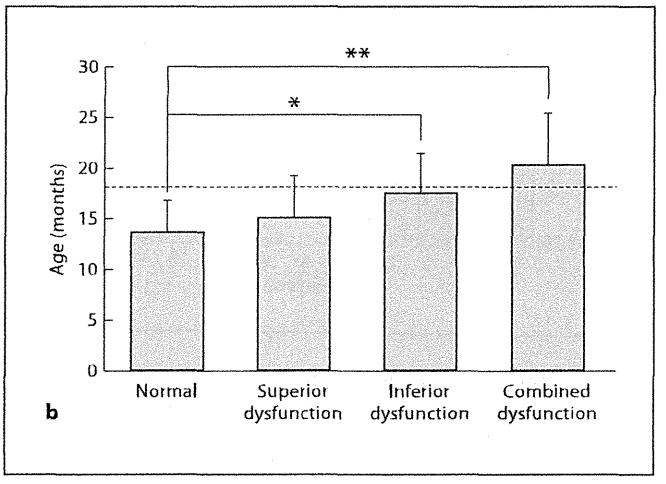


Fig. 2. Comparison of the age of acquiring head control and independent walking in children with profound hearing loss classified according to the involvement of the superior and inferior vestibular nerve systems. * $p < 0.05$, ** $p < 0.01$. **a** The age of acquiring head control in children with normal vestibular function, those with involvement of the superior vestibular nerve system sparing the inferior vestibular nerve system (superior dysfunction), those with involvement of the inferior vestibular nerve system sparing the superior vestibular nerve system (inferior dysfunction) and those with involvement of both the superior and inferior vestibular nerve systems (combined dysfunction). **b** The age of independent walking in the normal, superior, inferior and combined dysfunction groups.

Table 5. Age (months) of gross motor development in relation to vestibular function test results

	Normal	Asymmetry	Bilateral dysfunction
Head control			
Rotation test	3.8±1.1 (59)	3 (1)	5.6±2.3 (14)
Caloric test	3.7±1.0 (37)	4.0±1.0 (12)	5.2±2.2 (17)
VEMP	3.7±0.8 (31)	4.8±1.1 (5)	5.3±2.2 (19)
Independent walking			
Rotation test	14.6±3.5 (59)	14 (1)	19.1±4.8 (12)
Caloric test	14.5±3.8 (39)	14.0±2.1 (12)	18.4±5.0 (15)
VEMP	13.7±2.7 (29)	15.8±4.6 (4)	18.8±4.7 (17)

Data are shown as means ± SD; numbers of children are indicated in parentheses.

sponses bilaterally in either of the superior vestibular function tests in the presence of normal VEMP responses bilaterally (n = 9); (3) inferior dysfunction group, i.e. those with abnormal VEMP responses bilaterally in the presence of normal superior vestibular function tests (n = 3), and (4) combined dysfunction group, i.e. those with abnormal responses bilaterally in both the superior and inferior vestibular function tests (n = 11). Ten patients who showed unilateral vestibular dysfunction in either the damped rotation test or the caloric test (patients No. 12, 14, 15, 38, 50, 51, 57, 62, 64 and 69 in the online suppl. table 1) and 5 patients who showed abnormal VEMP responses on one side (patients No. 12, 14, 38, 77 and 83) were excluded from this analysis. Among them, 2 children (patients No. 14 and 38) showed dysfunction on the same sides in both caloric and VEMP tests, whereas the other children showed dysfunction on different sides in these tests. The age of acquiring head control was significantly delayed in the combined dysfunction group in comparison with the normal group ($p < 0.01$), whereas there were no significant differences among the normal group, the superior dysfunction group and the inferior dysfunction group ($p > 0.1$; fig. 2a). The age of independent walking was significantly delayed in the combined dysfunction group and in the inferior dysfunction group compared with the normal group ($p < 0.01$ and $p < 0.05$, respectively), whereas there were no significant differences between the normal group and the superior dysfunction group ($p > 0.8$) or between the inferior dysfunction group and the combined dysfunction group ($p > 0.5$; fig. 2b).

Discussion

In the present study, we have shown that approximately 40% of children with profound hearing loss have dysfunction of the superior vestibular nerve system, approximately 40% have dysfunction of the inferior vestibular nerve system, and approximately 20% have dysfunction of both vestibular nerve systems. Acquisition of head control and independent walking in children with bilateral vestibular dysfunction was significantly delayed in comparison with those with normal vestibular function.

In previous studies, vestibular function in infants and children has been evaluated by rotation and caloric tests, which reflect the function of the lateral semicircular canals and superior vestibular nerve [Diepeveen and Jensen, 1968; Kaga et al., 1981; Kaga, 1999; Buchmann et al., 2004; Shinjo et al., 2006; Jacot et al., 2009]. Kaga et al. [1981] examined vestibular function using the damped rotation test in 22 children with congenital deafness and found hypoactivity of the vestibulo-ocular reflexes in 12 children (55%). Shinjo et al. [2007] assessed vestibular function in 20 children with severe hearing loss using the damped rotation and caloric tests, and reported that abnormalities were found in 85% of these children with caloric testing and in 30% with the rotation test. Jacot et al. [2009] examined 224 children with profound hearing loss, using the caloric and rotation tests. They showed that 50% of the children tested have unilateral or bilateral vestibular dysfunction. In the present study, 41% of the children tested showed abnormal caloric responses and 20% showed abnormal responses in the damped rotation test. This prevalence of vestibular dysfunction was lower compared to that of previous studies [Kaga et al., 1981; Shinjo et al., 2007; Jacot et al., 2009]. This discrepancy might be caused by differences between the patient groups since the number of children with the *GJB2* mutation was relatively higher in our study compared to those previous studies [Buchman et al., 2004; Shinjo et al., 2007]. In the present study, most children with the *GJB2* mutation showed normal responses bilaterally in the damped rotation test, caloric test and with VEMPs. This result is consistent with previous reports showing that vestibular function is rarely affected in patients with the *GJB2* mutation [Todt et al., 2005; Tsukada et al., 2010]. On the other hand, more than half of the children with inner ear malformations and congenital CMV infection showed abnormal vestibular function in at least 1 of the 3 kinds of vestibular function tests used in the present study.

VEMPs in response to air-conducted sound have been used to evaluate vestibular function, especially that of the saccule and inferior vestibular afferents [Welgampola and Colebatch, 2005]. Combined use of VEMPs and the caloric test has enabled examination of the superior and inferior vestibular nerve systems separately [Murofushi et al., 1998; Iwasaki et al., 2005]. VEMPs have been extensively studied primarily in adult subjects since VEMPs require neck contraction during recording. However, several recent studies have shown that VEMPs can be recorded from infants and children in almost the same way as adults [Tribukait et al., 2004; Jin et al., 2006; Kelsh et al., 2006; Shinjo et al., 2007]. Tribukait et al. [2004] recorded VEMPs in 39 deaf children between the ages of 15 and 17 years and reported that VEMPs were absent bilaterally in 22% and asymmetric in 19%. Shinjo et al. [2007] recorded VEMPs in 20 children with profound deafness with ages ranging from 2 to 7 years and reported that 20% of patients showed no responses bilaterally and 30% showed asymmetric responses. In the present study, we attempted to record VEMPs from children by helping them to raise their heads during the recording. Furthermore, we used a 95-dB nHL tone burst instead of 90-dB nHL clicks, which were used in the study by Kelsch et al. [2006], as a stimulus for eliciting VEMPs, since it has been shown that tone bursts are superior to clicks in eliciting VEMP responses [Murofushi et al., 1999; Viciana and Lopez-Escamez, 2012]. Of the children tested in this study, with an age range of 2–8 years, 70% were able to generate sufficient neck muscle activity (>150 μV) to successfully complete VEMP testing. Among these children, 8% showed asymmetric VEMP responses and 34% showed no VEMP responses on either side, indicating that approximately 40% of these children with profound hearing loss have dysfunction of the inferior vestibular system on at least one side. This finding is compatible with the finding in previous studies in terms of the percentage of children showing inferior nerve system dysfunction [Tribukait et al., 2004; Shinjo et al., 2007].

In the present study, both the ages of acquiring head control and independent walking were significantly delayed in children with vestibular dysfunction in comparison with those with normal vestibular function. All the children were able to walk independently within 30 months. A few previous studies have shown that gross motor development is delayed in children with bilateral vestibular dysfunction [Kaga et al., 1981; Rine et al., 2000]. Kaga et al. [1981] reported that the age of acquiring head control and independent walking in children with bilateral vestibular dysfunction was significantly delayed

when compared with normal controls. They also reported that all children of preschool age with vestibular dysfunction were able to achieve head control, independent walking and running, suggesting the substitution of vestibular function by other sensory inputs such as visual and somatosensory cues [Kaga et al., 1981; Wallacott et al., 2004]. The development of gross motor function is affected by various factors including the functioning of the visual, vestibular, proprioceptive and motor systems [Kaga, 1999; Wallacott et al., 2004; Suarez et al., 2007]. It has been shown that a substantial proportion of children with profound hearing loss show balance dysfunction, especially when visual and/or somatosensory information is disturbed [Suarez et al., 2007; Cushing et al., 2008b]. Since the relative importance of visual, vestibular and somatosensory inputs to head stabilization and balance control has been shown to change dynamically during preschool ages [Berger et al., 1987; Assaiante and Ambrad, 1992], it is possible that the contribution of visual and somatosensory inputs steadily increases with age in children with vestibular dysfunction. Several studies have shown that children with bilateral vestibular dysfunction show postural instability in conditions with reduced visual and/or somatosensory cues [Enbom et al., 1991; Cushing et al., 2008b].

The contribution of the superior and inferior vestibular nerve systems to the development of gross motor function has not been studied previously. We classified children with profound hearing loss into 4 groups according to the results of 3 vestibular tests (normal function, superior dysfunction, inferior dysfunction, combined dysfunction) and compared the gross motor development among these groups. The age at acquisition of both head control and independent walking in the combined dysfunction group was the latest among the 4 groups, suggesting that the inferior as well as the superior nerve systems play an important role in gross motor development. Furthermore, the age of acquiring independent walking was significantly delayed in the inferior dysfunction group as well as the combined dysfunction group in comparison with the normal group, whereas it was not significantly different between the superior dysfunction group and the normal group. The inferior vestibular nerve system, which has an input to neck and leg muscles, may have a greater influence on the acquisition of independent walking than the superior vestibular nerve system.

In conclusion, we have shown that a substantial proportion of children with profound hearing loss have dysfunction of the inferior as well as the superior vestibular nerve system and that they show delayed acquisition of gross motor function. Since the development of gross motor function varies according to the extent of the involvement of each vestibular nerve system, it is preferable to evaluate both the superior and inferior vestibular function separately in order to form an individualized treatment plan for each child with profound hearing loss.

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Disclosure Statement

We have no conflicts of financial interest in this paper.

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ORIGINAL ARTICLE

Mutation spectrum and genotype–phenotype correlation of hearing loss patients caused by *SLC26A4* mutations in the Japanese: a large cohort study

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Mutations in *SLC26A4* cause a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss associated with enlarged vestibular aqueduct. Identification of these mutations is important for accurate diagnosis, proper medical management and appropriate genetic counseling and requires updated information regarding spectrum, clinical characteristics and genotype–phenotype correlations, based on a large cohort. In 100 patients with bilateral enlarged vestibular aqueduct among 1511 Japanese hearing loss probands registered in our gene bank, goiter data were available for 79, of whom 15 had Pendred syndrome and 64 had nonsyndromic hearing loss. We clarified the mutation spectrum for the *SLC26A4* mutations and also summarized hearing levels, progression, fluctuation and existence of genotype–phenotype correlation. *SLC26A4* mutations were identified in 82 of the 100 patients (82.0%). Of the Pendred syndrome patients, 93% (14/15) were carriers, as were 77% (49/64) of the nonsyndromic hearing loss patients. Clinical characteristics of patients with *SLC26A4* mutations were congenital, fluctuating and progressive hearing loss usually associated with vertigo and/or goiter. We found no genotype–phenotype correlations, indicating that, unlike in the case of *GJB2* mutations, the phenotype cannot be predicted from the genotype. Our mutation analysis confirmed the importance of mutations in the *SLC26A4* gene among hearing loss patients with enlarged vestibular aqueduct and revealed the mutation spectrum, essential information when performing genetic testing.
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Keywords: congenital hearing loss; DFNB4; enlarged vestibular aqueduct; goiter; Pendred syndrome; *SLC26A4*

INTRODUCTION

Based on our genetic screening, *SLC26A4* is the second most common responsible gene in Japanese deafness patients.[1] Mutations in the *SLC26A4* gene are known to be responsible for a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss with enlarged vestibular aqueduct (EVA). The prevalent association of *SLC26A4* mutations in these patients (90% in Pendred syndrome and 78.1% in nonsyndromic hearing loss associated with EVA) indicates the importance of this gene in the pathophysiology of this category of hearing impairment.[2] More than 160 mutations have been found in *SLC26A4* (Pendred/BOR Homepage, http://www.medicine.uiowa.edu/pendredandbor), and different mutational spectrums among different ethnic groups have been reported.[2] The identification of *SLC26A4* mutations enables more appropriate genetic counseling and proper medical management for these patients. For such clinical application, updated information regarding mutation spectrum, clinical characteristics and genotype–phenotype correlations based on a large cohort is needed. In addition to our previous reports,[1–7] the present study was performed using a large cohort of patients to collect updated data and summarize these data to enable more precise decision making by ear, nose and throat clinicians.

MATERIALS AND METHODS

Subjects

Data on 1511 independent probands and 1545 family members were collected from 33 ear, nose and throat departments nationwide in Japan and registered in our gene bank. All subjects or next of kin, caretakers or guardians on behalf of the minors/children gave prior written informed consent for participation in the project, and the Shinshu University Ethical Committee as well as the respective Ethical Committees of the other participating institutions of the Deafness Gene Study Consortium (Hokkaido University, Hirosaki University, Iwate Medical University, Tohoku University, Yamagata University, Fukushima Medical University, Jichi Medical University, Gunma University, Nihon University, Nippon Medical School, Nippon Medical School Tama Nagayama

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Hospital, Jikei University, Toranomon Hospital, Kitasato University, Hamamatsu Medical University, Mie University, Shiga Medical Center for Children, Osaka Medical College, Hyogo College of Medicine, Kobe City Medical Center General Hospital, Wakayama Medical University, Okayama University, Yamaguchi University, Ehime University, Kyushu University, Fukuoka University, Nagasaki University, Kanda ENT Clinic, Miyazaki Medical College, Kagoshima University and Ryukyus University) approved the study.

Computerized tomography scan was used to diagnose EVA (according to the criteria of EVA: a diameter of >1.5 mm at the midpoint between the common crus and the external aperture), and they were clinically well characterized by repeated auditory examinations.

The 100 subjects (51 males and 49 females) from among the 1511 probands who met the criteria of bilateral EVA and who ranged in age from 0 to 59 years with a mean age of 13.9 years at the time of examination were enrolled in the current study. Fifteen subjects had Pendred syndrome and 64 had nonsyndromic hearing loss.

The controls were 192 unrelated Japanese healthy individuals with normal hearing evaluated by auditory testing.

Mutation analysis

To identify *SLC26A4* mutations, a DNA fragment containing all the exons of *SLC26A4*, including flanking intronic sequences, was sequenced as described elsewhere.[4] New variants were tested in 192 unrelated normal hearing controls.

Possible pathologic mutations were defined as (1) mutations found to be homozygotes or compound heterozygotes (and determined by segregation study); (2) variants that were not found, or were very few, in the 192 control subjects; and (3) amino acids that were well conserved among various species.

Clinical evaluations

Hearing levels were determined by pure-tone audiometry in adults. For the young patients, conditioned orientation response audiometry or auditory steady-state response was used. Clinical data, including hearing loss progression, fluctuation, episodes of tinnitus and vestibular dysfunction (vertigo, dizziness) and goiter, were collected by anamnestic evaluation. For genotype–phenotype correlation analysis, one-way analysis of variance (Tukey's honest significant difference (HSD) test), Kruskal–Wallis test and multivariate statistics (multiple regression analysis and logistic regression analysis) were used.

RESULTS

SLC26A4 mutation spectrum

There were a total of 39 *SLC26A4* mutations found in the probands with bilateral EVA (Table 1). These mutations were either homozygous, compound heterozygous or heterozygous with no other mutations being detectable. There were two nonsense mutations (p.S610X, p.L727X), three deletion frameshift mutations (c.322delC, c.917delT, c.1219delCT) and three insertion frameshift mutations (c.139insC, c.1652insT, c.2111ins GCTGG). Seven splice site mutations were found (c.416-1G>A, c.600+1G>T, c.601-1G>A, c.919-2A>G, c.1001+1G>A, c.1002-9A>G and c.1707+5G>A).

There were 24 missense mutations (p.P76S, p.T94I, p.P123S, p.M147V, p.P297Q, p.K369E, p.A372V, p.N392Y, p.G396E, p.T410M, p.A434T, p.G439R, p.S448L, p.T527P, p.I529S, p.S532I, p.C565Y, p.R581S, p.S657N, p.V659L, p.S666F, p.T721M, p.H723R and p.H723Y). To evaluate the evolutionary conservation of the amino acids affected by these missense mutations, we made an alignment of the *SLC26A4* amino acid sequence of four mammalian species: human, rat, cow and mouse. On the basis of this alignment, all missense mutations had changed evolutionary conserved amino acids. Of these mutations, nine variants had not been reported. We checked the 192 control subjects with normal hearing, but with the exception of p.H723R in 1 case, no mutations were detected.

Sequencing identified mutations in 82 of the 100 patients (82.0%). Mutations were detected in 93% of those with Pendred syndrome (14/15) and 77% (49/64) of those with nonsyndromic hearing loss. Of these, 15/100 (15.0%) were homozygous, 51/100 (51.0%) were compound heterozygous and 16/100 (16.0%) were heterozygous (Table 2).

The most frequent mutation was p.H723R that accounted for 36.0%, and the second was c.919-2A>G found in 7.0%, followed by c.1707+5G>A (4.0%). Frequency of the other 36 mutations was very low (0.5–2.0%).

Clinical findings

Table 2 shows the clinical details for the 100 subjects.

The subjects had an average hearing level of 80.9 dB (7.5–112.5 dB), with hearing loss that was mild in 5, moderate in 22, severe in 37, profound in 19 and unknown in 12. Regarding onset age of hearing loss, 45 patients were congenital, 18 were prelingual (1–3 years old), 20 were from 4 to 14 years and 17 were unknown. These results clearly indicated that early onset is dominant in patients with EVA. Also, 70 patients (70%) showed progressive hearing loss and 56 patients (56.0%) felt fluctuation of hearing. With regard to the 79 patients for whom data on vertigo were available, 41 patients complained of vertigo and 38 did not. Of the 79 patients for whom data on goiter were available, 15 had goiter and 64 did not, with an onset age from 12 to 33 years. As to family history, all families were recessive inheritance or sporadic cases.

Genotype–phenotype correlations for diagnostic age, fluctuation, vertigo, tinnitus and goiter are summarized in Figure 1.

We defined nonsense or frameshift mutations as truncating (T) and missense mutations as nontruncating (NT) and classified the genotypes as truncating/truncating (T/T), truncating/nontruncating (T/NT) or nontruncating/nontruncating (NT/NT). Significant differences were not found between the groups in any of the clinical features (Tukey's HSD test was used for diagnostic age and Kruskal–Wallis test was used for fluctuation, vertigo, tinnitus and goiter, all tests indicated $P>0.05$; Figure 1). Figure 2 shows the relationship between hearing loss severity and the mutation (T or NT) that also showed no significant differences (Tukey's HSD test, $P>0.05$). ~~We also performed multivariate statistics (multiple regression analysis and logistic regression analysis) and we only found that the age of the patients correlate with the hearing loss severity but genotype of *SLC26A4* mutations did not indicate any significant effect to the hearing loss severity ($P>0.05$).~~

DISCUSSION

The present large cohort study revealed a high prevalence (82%; 82/100) of *SLC26A4* mutations in sensorineural hearing loss patients with EVA in Japanese. The frequency (8.7%) is the second most common next to *GJB2* that is found in 16.2% of overall and 25.6% of congenital hearing loss patients.[1]

Our mutation analysis results confirmed the previous reports that indicated the importance of this gene among hearing loss patients with EVA. This study also added novel mutations and summarized updated data for the precise molecular diagnosis.

First, the high prevalence (82%) of *SLC26A4* mutations in EVA patients is compatible with the high prevalence of *SLC26A4* mutations reported in eastern Asians; that is, 97.9% in Chinese,[8] and 92% in Koreans.[9] These frequencies are higher than those reported in Caucasoid populations (20% in Americans,[10] 40.0% in French[11] and 28.4% in Spanish[12]). It is still an open question whether other genes are involved in the EVA patients without *SLC26A4* mutations.

Table 1 Possible pathogenic variants found in enlarged vestibular aqueduct (EVA) subjects ($n = 100$)

			Frequency (n = 100)				
Nucleotide change	*Amino acid change*	*Exon*	*Homozygote*	*Compound heterozygote*	*Heterozygote*	*Allele frequency (in 200 alleles)*	*References*
c. 139insC		1		1		0.50	This study
c. 266C>T	p. P76S	2		1		0.50	Suzuki *et al.*[5,6]
c. 281C>T	p. T94I	3		1		0.50	Wang *et al.*[7,8]
c. 322delC		4		1		0.50	Tsukamoto *et al.*[2,4]
c. 367C>T	p. P123S	4		1		0.50	Tsukamoto *et al.*[2,4]
c. 416-1G>A		Intron 4		2		1.00	Tsukamoto *et al.*[2,4]
c. 439A>G	p. M147V	5		2		1.00	Tsukamoto *et al.*[2,4]
c. 600+1G>T		Intron 5		1		0.50	This study
c. 601-1G>A		Intron 5		1		0.50	Tsukamoto *et al.*[2,4]
c. 890C>A	p. P297Q	7		1		0.50	This study
c. 917delT		7			1	0.50	Tsukamoto *et al.*[2,4]
c. 919-2A>G		Intron 7	1	11	1	7.00	Coucke *et al.*[1999]
c. 1001+1G>A		Intron 8		2		1.00	Coyle *et al.*[1998]
c. 1002-9A>G[a]		Intron 8		1		0.50	This study
c. 1105A>G	p. K369E	9		1		0.50	Usami *et al.*[2,3]
c. 1115C>T	p. A372V	9		1		0.50	Usami *et al.*[2,3]
c. 1174A>T	p. N392Y	10		3		1.50	Park *et al.*[14,16]
c. 1187G>A	p. G396E	10		1		0.50	This study
c. 1219delCT		10		1		0.50	This study
c. 1229C>T	p. T410M	10	1	1		1.50	Coyle *et al.*[1998]
c. 1300G>A	p. A434T	11			1	0.50	This study
c. 1315G>A	p. G439R	11		1		0.50	Suzuki *et al.*[5,6]
c. 1343C>T	p. S448L	11		1		0.50	Wang *et al.*[7,8]
c. 1579A>G	p. T527P	14		2		1.00	Suzuki *et al.*[5,6]
c. 1586T>G	p. I529S	14		1		0.50	Wang *et al.*[7,8]
c. 1595G>T	p. S532I	14		2		1.00	Usami *et al.*[3,17]
c. 1652insT		15		3	1	2.00	Tsukamoto *et al.*[2,4]
c. 1694G>A	p. C565Y	15		1		0.50	Tsukamoto *et al.*[2,4]
c. 1707+5G>A		Intron 15	1	6		4.00	Park *et al.*[8,9]
c. 1743G>C	p. R581S	16		2		1.00	Iwasaki *et al.*[5,18]
c. 1829C>A	p. S610X	17		1		0.50	Tsukamoto *et al.*[2,4]
c. 1970G>A	p. S657N	17		1		0.50	Tsukamoto *et al.*[2,4]
c. 1975G>C	p. V659L	17		3		1.50	Wang *et al.*[7,8]
c. 1997C>T	p. S666F	17		1		0.50	Tsukamoto *et al.*[2,4]
c. 2111ins GCTGG		19		1	1	1.00	Usami *et al.*[2,3]
c. 2162C>T	p. T721M	19		1	1	1.00	Usami *et al.*[2,3]
c. 2168A>G	p. H723R	19	11	40	10	36.00	Usami *et al.*[2,3]
c. 2168C>T	p. H723Y	19	1			1.00	This study
c. 2180T>A	p. L727X	19		1		0.50	This study

[a]c. 1002-9A>G, uncertain pathogenicity.

Mutations in *FOXI1*,[13] a modulatory gene of *SLC26A4*, were not found in our series of patients (data not shown). As seen in previous mutation screening reports, we encountered a significant number of heterozygous cases without a second mutation even after direct sequencing of the coding region of the gene. It is highly likely that there is one more occult mutation somewhere because patients with heterozygous mutation are associated with EVA.

Second, it is evident that the mutation spectrum found in the Japanese population is quite different from that in Caucasoid populations, but similar to the mutation spectrum reported in the Asian populations, especially Koreans.[8–12,14] There are two frequent mutations in east Asians, namely p.H723R and c.919-2A>G. p.H723R is most prevalent in the Japanese and Korean populations,[8] whereas c.919-2A>G is most common in the Chinese.[7]

The existence of a genotype–phenotype correlation is still controversial.[6,12,15] Mutations in *SLC26A4* can cause a broad phenotypic spectrum, from typical Pendred syndrome to nonsyndromic hearing loss associated with EVA. In the present study, various features of the phenotype were compared with the genotypes. We defined nonsense or frame shift mutations as truncating (T) and missense mutations as non-truncating (NT) and classified the genotypes as truncating/truncating (T/T), truncating/non-truncating (T/NT), or non-–truncating/non-truncating(NT/NT). S,–However, statistical differences were not found between the groups in any of the clinical features (χ^2 tests, $P > 0.05$; Figure 1).

Concerning the relationship between the severity of hearing loss and individual *SLC26A4* mutations, several functional studies have demonstrated the property of transporter function.[16–18] Furthermore,

Table 2 Phenotypes and genotypes of affected EVA subjects

ID	*Age*	*Mutation allele 1/allele 2*	*Age of awareness*	*Progression*	*Fluctuation*	*Tinnitus*	*Vertigo*	*Goiter*	*Threshold (Rt) (dB)*[a]	*Threshold (Lt) (dB)*[a]	*Hearing level in the low frequencies*[b]
77	12	p. [917delT];[=]	12	+	+	+	+	−	58.75	45	49.375
237	7	p. [T721M];[H723R]	0	+	−	−	+	−	112.5	68.75	83.75
334	23	p. [A372V];[H723R]	0	NA	NA	+	NA	NA	96.25	83.75	81.9
695	4	p. [K369E];[H723R]	0	+	−	NA	NA	−	100	90	89.4
752	18	p. [1652insT];[=]	1	−	−	+	+	+	98.75	102.5	96.3
1045	25	p. [H723R];[H723R]	0	+	NA	−	+	+	78.75	90	85.6
1306	3	p. [919-2A>G];[H723R]	0	NA	NA	NA	NA	NA	NA	NA	NA
1365	20	p. [T721M];[=]	2	NA	NA	NA	NA	NA	96.25	105	96.9
1379	10	p. [1001+1G>A];[H723R]	0	+	+	−	−	NA	66.25	46.25	57.5
1432	6	p. [H723R];[=]	0	+	−	−	−	NA	102.5	105	100.0
1625	16	p. [919-2A>G];[H723R]	0	+	+	NA	+	NA	100	95	88.1
1795	NA	p. [H723R];[=]	NA	NA	N/A	NA	NA	NA	NA	NA	NA
1820	12	p. [H723R];[H723R]	5	+	+	−	−	NA	72.5	73.75	61.3
1957	7	p. [S666F];[H723R]	3	+	+	NA	NA	−	95	101.25	93.8
1961	12	p. [C565Y];[H723R]	0	+	N/A	NA	NA	NA	108.75	110	103.8
2010	12	p. [416-1G>A];[H723R]	9	+	+	−	−	+	80	91.25	81.3
2202	4	p. [P297Q];[T527P]	3	+	−	−	−	−	77.5	76.25	73.8
2331	31	p. [H723R];[H723R]	0	+	+	+	+	+	90	100	87.5
2449	1	p. [139insC];[322delC]	0	NA	NA	−	+	−	100	85	92.5
2462	52	p. [M147V];[H723R]	2	+	+	−	−	−	98.75	95	88.1
2498	0	p. [919-2A>G]; [1001+1G>A]	0	+	+	NA	−	−	86.25	86.25	83.8
2538	10	p. [H723R];[H723R]	3	+	+	−	−	+	81.25	55	66.9
2621	3	p. [R581S];[H723R]	0	+	+	−	−	−	91.25	91.25	90.0
2695	13	p. [T527P];[H723R]	2	+	+	+	+	−	62.5	61.25	63.1
2728	3	p. [919-2A>G];[H723R]	1	+	+	−	−	−	97.5	97.5	93.8
2798	15	p. [H723R];[H723R]	4	+	+	NA	+	+	52.5	96.25	66.3
2804	2	p. [1707+5G>A];[H723R]	0	+	+	−	−	−	78.75	78.75	82.5
3072	44	p. [G439R];[H723R]	6	+	+	+	+	−	110	108.75	105.0
3074	21	p. [H723R]; [=]	2	+	+	+	+	+	105	106.25	99.4
3298	6	p. [919-2A>G];[H723R]	0	+	+	+	+	−	73.75	110	86.9
3301	4	p. [416-1G>A];[H723R]	0	+	+	+	+	−	65	72.5	68.1
3442	6	p. [919-2A>G];[H723R]	NA	+	NA	+	+	−	81.25	50	60.0
3450	14	p. [H723R];[H723R]	0	+	+	+	+	−	110	73.75	87.5
3561	6	p. [H723Y];[H723Y]	4	NA	NA	NA	NA	NA	83.75	65	71.3
3994	59	p. [601-1G>A];[H723R]	10	+	+	+	+	+	96.0	94	91.3
3996	8	p. [H723R];[1652insT]	0	+	−	+	−	−	100	110	98.1
3999	8	p. [H723R];[1652insT]	0	+	+	−	+	−	30	50	40.0
4050	5	p. [M147V];[H723R]	1	+	+	+	+	−	107.5	85	93.8
4097	3	p. [N392Y];[1002-9A>G]	0	−	−	−	−	−	106.25	85	93.1
4098	26	p. [N392Y];[919-2A>G]	2	−	+	+	+	−	110	37.5	71.3
4102	5	p. [N392Y];[H723R]	0	+	+	+	+	−	95	78.75	83.1
4131	10	p. [H723R];[=]	8	+	+	−	−	−	81.25	60	70.6
4144	21	p. [H723R];[H723R]	4	+	NA	+	+	−	93.75	105	95.6
4232	15	p. [V659L];[H723R]	NA	−	+	+	+	−	60	92.5	69.4
4299	4	p. [S532I];[2111ins GCTGG]	3	−	+	−	+	−	17.5	70	42.5
4305	14	p. [A434T];[=]	0	+	−	+	−	−	110	110	105.0
4320	10	p. [G396E];[S532I]	NA	+	+	+	−	−	72.5	80	72.5
4338	6	p. [R581S];[H723R]	0	+	+	+	+	−	78.75	52.5	64.4
4380	10	p. [1707+5G>A];[H723R]	2	+	+	−	−	−	96.25	81.25	84.4
4386	21	p. [H723R];[H723R]	NA	+	+	+	+	+	77.5	93.75	85.0
4398	4	p. [1652insT];[H723R]	2	+	+	+	+	−	70	97.5	86.9
4434	8	p. [T410M];[1707+5G>A]	1	+	+	−	+	−	92.5	100	91.3
4469	11	p. [H723R]; [=]	0	+	NA	−	−	−	20	21.25	16.9
4485	40	p. [H723R]; [=]	10	+	+	+	+	−	56.25	65	58.8

Table 2 (Continued)

ID	Age	Mutation allele 1/allele 2	Age of awareness	Progression	Fluctuation	Tinnitus	Vertigo	Goiter	Threshold (Rt) (dB)[a]	Threshold (Lt) (dB)[a]	Hearing level in the low frequencies[b]
4486	20	p. [1707 + 5G>A]; [1707 + 5G>A]	4	+	+	+	+	+	72.5	95	78.1
4490	25	p. [T410M];[T410M]	0	−	−	+	+	+	87.5	92.5	90.0
4508	29	p. [H723R];[H723R]	5	+	+	−	−	−	85	110	91.9
4518	26	p. [H723R];[919-2A>G]	0	+	+	+	+	−	105	97.5	98.1
4530	5	p. [H723R];[919-2A>G]	0	+	+	−	+	−	67.5	86.25	71.9
4545	12	p. [1707 + 5G>A];[H723R]	4	+	+	+	+	+	86.25	28.75	53.1
4549	13	p. [V659L];[1219delCT]	NA	+	+	+	+	−	38.75	50	38.1
4663	0	p. [1707 + 5G>A];[H723R]	0	−	+	NA	NA	−	68.75	68.75	99.2
4696	0	p. [V659L];[H723R]	0	+	−	NA	NA	−	NA	NA	97.5
4362	26	p. [H723R]; [=]	6	+	−	−	−	−	70	68.75	63.8
4513	34	p. [H723R]; [=]	NA	+	+	+	NA	−	71.25	53.75	61.3
4645	23	p. [919-2A>G]; [=]	14	+	−	+	−	−	96.25	105	93.8
723	NA	p. [H723R]; [=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
724	NA	p. [2111ins5bp]; [=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
742	NA	p. [H723R]; [=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
1975	3	p. [H723R];[H723R]	0	NA	NA	NA	NA	NA	80	70	62.5
2082	2	p. [H723R];[H723R]	0	−	−	−	−	−	NA	NA	NA
4735	9	p. [H723R];[919-2A>G]	0	+	+	+	+	−	107.5	110	103.8
195	20	p. [=];[=]	2	+	+	+	+	−	83.75	83.75	81.9
670	8	p. [=];[=]	3	+	−	+	−	−	26.25	107.5	62.5
1755	16	p. [=];[=]	NA	NA	NA	NA	NA	NA	NA	NA	NA
2607	5	p. [=];[=]	0	−	+	−	−	−	97.5	105	98.8
3851	33	p. [=];[=]	0	+	+	+	−	+	103.75	103.75	100.6
4194	11	p. [=];[=]	NA	+	+	−	−	−	67.5	80	76.3
4215	5	p. [=];[=]	0	+	+	−	−	−	98.75	93.75	93.8
4216	55	p. [=];[=]	NA	+	+	+	+	NA	51.25	78.75	68.8
4258	30	p. [=];[=]	28	NA	−	+	−	−	17.5	7.5	13.8
4281	6	p. [=];[=]	2	−	−	−	−	−	57.5	61.25	63.1
4324	37	p. [=];[=]	6	−	−	−	−	−	10	27.5	22.5
4352	3	p. [=];[=]	0	+	+	−	−	−	86.25	88.75	88.1
4357	6	p. [=];[=]	4	+	+	+	−	−	71.25	72.5	67.5
4397	5	p. [=];[=]	0	−	−	−	−	−	102.5	105	100.6
4402	8	p. [=];[=]	0	+	−	−	−	−	100	90	88.8
4450	12	p. [=];[=]	NA	+	+	+	−	−	NA	NA	NA
4462	8	p. [=];[=]	7	+	−	+	−	−	63.75	20	41.3
4488	1	p. [=];[=]	0	−	−	NA	−	−	97.5	97.5	95.0
4671	2	p. [H723R];[600 + 1G>T]	0	+	−	−	+	−	NA	NA	NA
3253	NA	p. [I529S];[H723R]	NA	NA	NA	NA	NA	NA	NA	NA	NA
4949	0	p. [L727X];[H723R]	0	+	−	−	−	−	NA	NA	51.7
J27	NA	p. [H723R];[S448L]	NA	NA	NA	NA	NA	NA	NA	NA	90.6
3309	5	p. [919-2A>G];[P76S]	0	+	+	+	+	−	106.25	106.25	101.3
J15	0	p. [P123S];[H723R]	0	NA	NA	NA	NA	NA	NA	NA	NA
FUK2004	1	p. [H723R];[T94I]	0	NA	NA	N/A	NA	NA	NA	NA	85.0
1299	NA	p. [S610X];[S657N]	0	NA	NA	NA	NA	NA	NA	NA	NA
SNS5500	42	p. [919-2A>G];[919-2A>G]	4	+	+	+	+	+	70	81.3	64
SNS5503	37	p. [H723R];[1707 + 5G>A]	5	+	+	+	+	+	67.5	70	NA

Abbreviation: EVA, enlarged vestibular aqueduct.
[a]Average of 500, 1000, 2000 and 4000 Hz.
[b]Average of 125, 250 and 500 Hz.

retention of improperly folded Pendrin mutants in the endoplasmic reticulum has been suggested as the major pathological mechanism for Pendred syndrome.[19,20] In this study, we compared not only the difference between the T and NT mutations, but also compared the individual mutations and severity of hearing. However, there were no correlations (data not shown). Indeed, there was great variation regarding hearing loss severity even with the same mutations. For example, in the patients homozygous for the most prevalent mutation, p.–H723R, hearing level at low frequency varied from 61 to 99 dB (Table 2). In addition, many reports have described intrafamilial