

Figure 4. Distortion-product otoacoustic emissions (DPOAE) graphs of four subjects with the c.211delC mutation in the *KCNQ4* gene.

supported by the finding of Naito et al. that 20 patients with a maximum age of 73 who carried this mutation did not have progressive hearing loss. The detailed estimation of progression also does not suggest that development of profound hearing loss will occur.

Our findings from extensive audiological examination suggested that cochlear impairment induced by c.211delC mutation of the *KCNQ4* gene might start from the basal turn of the cochlea and progress to the middle turn. DPOAE in the middle frequency area was detectable in the youngest subject with high-frequency SNHL (III-1). Subject II-2 had detectable DPOAE only in the 1 kHz, at least in the right ear. The oldest subject (I-2) did not have detectable DPOAE in any frequency areas. These findings indicate that dysfunction of outer hair cells progressed from the basal turn to middle turn of the cochlea along with aging. In addition, this is supported by the results of self-recording audiometry, in which subject III-1 showed Jerger type I, indicating normal hearing, and subject I-2 showed Jerger type II (cochlear origin) hearing loss in the high-frequency area.

It is unclear whether vestibular symptoms are associated with the *KCNQ4* gene mutation. In the present study, two subjects carrying the c.211delC mutation showed recurrent vertigo and hyporeflexia in the right ear on caloric testing. However, one subject with this mutation showed normal caloric test responses without vestibular symptoms. In the two patients with vertigo, there was a unilateral decline of caloric response. Therefore, this vestibular dysfunction may not be due to the *KCNQ4* mutations, because if it were such a genetically determined vestibular response, it would usually be symmetric. Naito et al. also reported that in 20 patients carrying the c.211delC mutation, the majority did not have apparent vestibular symptoms, suggesting that this mutation is not associated with vestibular dysfunction [5].

We detected a novel missense mutation, p.H989Q, in the *TECTA* gene in two subjects with SNHL. This mutation is located in the TIL region of the zonadhesin-like domain D2 and is highly conserved in many species (from humans to fish). Alasti et al.

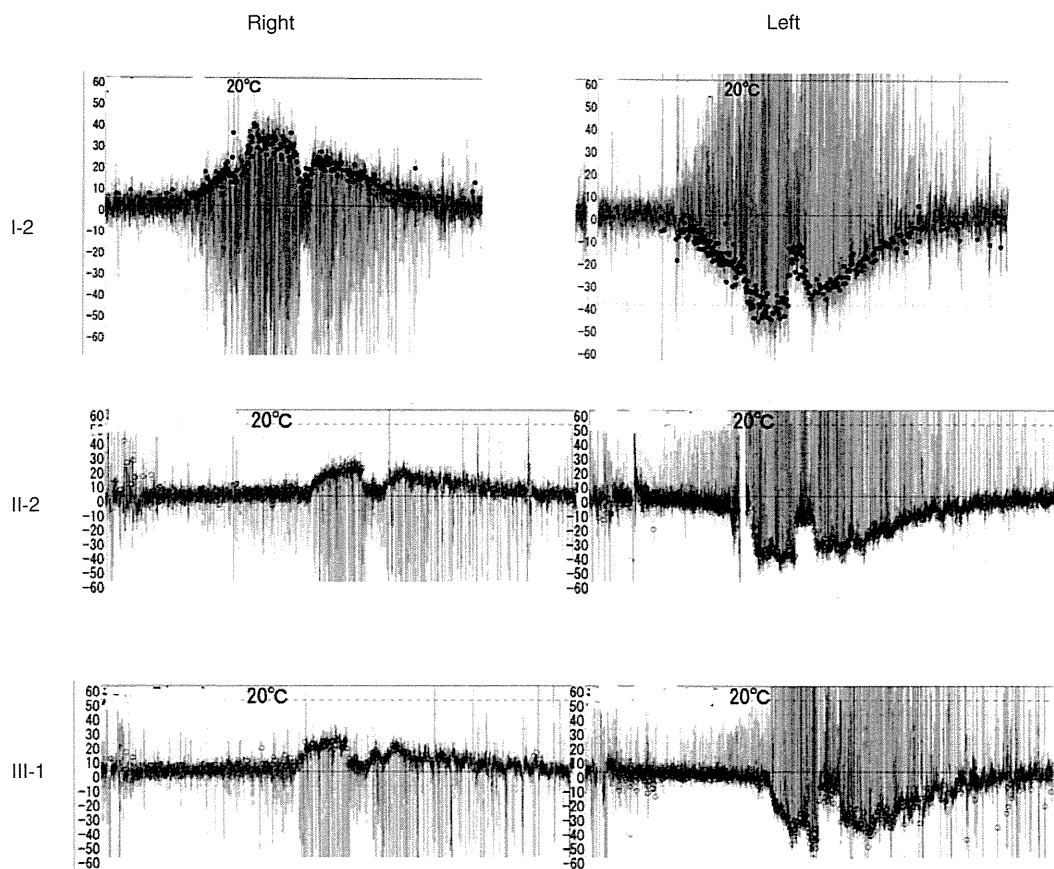


Figure 5. Caloric testing results of the three subjects with high-frequency sensorineural hearing loss (SNHL).

reported that ZA domain mutations cause progressive and high-frequency hearing loss [13]. However, our subject I-2 with high-frequency SNHL did not carry this mutation. Therefore we suspect that it was not associated with hearing loss in this family.

As technology develops and wide genome searches become more commonly performed, the detection of cases with two or more gene mutations is predicted to increase. It will be increasingly important to consider genotype-phenotype correlation of each mutation detected and to exercise due caution in determination of the causative mutation and selection of appropriate treatment.

### Conclusion

In the present study, we found a c.211delC mutation in the *KCNQ4* gene in a Japanese family with autosomal dominant inherited progressive high-frequency SNHL, therefore the existence of this mutation should be considered in such families.

### Acknowledgments

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**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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RESEARCH ARTICLE

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# Pathogenic substitution of IVS15 + 5G > A in *SLC26A4* in patients of Okinawa Islands with enlarged vestibular aqueduct syndrome or Pendred syndrome

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## Abstract

**Background:** Pendred syndrome (PS) and nonsyndromic hearing loss associated with enlarged vestibular aqueduct (EVA) are caused by *SLC26A4* mutations. The Okinawa Islands are the southwestern-most islands of the Japanese archipelago. And ancestral differences have been reported between people from Okinawa Island and those from the main islands of Japan. To confirm the ethnic variation of the spectrum of *SLC26A4* mutations, we investigated the frequencies of *SLC26A4* mutations and clinical manifestations of patients with EVA or PS living in the Okinawa Islands.

**Methods:** We examined 22 patients with EVA or PS from 21 unrelated families in Okinawa Islands. The patient's clinical history, findings of physical and otoscopic examinations, hearing test, and computed tomography (CT) scan of the temporal bones were recorded. To detect mutations, all 21 exons and the exon-intron junctions of *SLC26A4* were sequenced for all subjects. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for *SLC26A4* and calculations using the comparative CT ( $2^{-\Delta\Delta CT}$ ) method were used to determine the pathogenicity associated with gene substitutions.

**Results:** *SLC26A4* mutations were identified in 21 of the 22 patients. We found a compound heterozygous mutation for IVS15 + 5G > A/H723R in nine patients (41%), a homozygous substitution of IVS15 + 5G > A in six patients (27%), and homozygous mutation for H723R in five patients (23%). The most prevalent types of *SLC26A4* alleles were IVS15 + 5G > A and H723R, which both accounted for 15/22 (68%) of the patients. There were no significant correlations between the types of *SLC26A4* mutation and clinical manifestations. Based on qRT-PCR results, expression of *SLC26A4* was not identified in patients with the homozygous substitution of IVS15 + 5G > A.

**Conclusions:** The substitution of IVS15 + 5G > A in *SLC26A4* was the most common mutation in uniquely found in patients with PS and EVA in Okinawa Islands. This suggested that the spectrum of *SLC26A4* mutation differed from main islands of Japan and other East Asian countries. The substitution of IVS15 + 5G > A leads to a loss of *SLC26A4* expression and results in a phenotype of PS and EVA.

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## Background

Profound hearing loss affects about 1 in 300 to 1 in 1000 newborns [1-4], and about one-half of these cases can be attributed to genetic factors [5]. About 51% of these cases are due to single nucleotide polymorphisms [5]. As to inheritance pattern among monogenic probands, about 1% is X-linked, 22% is autosomal dominant, and 77% is autosomal recessive [5]. Pendred syndrome (PS) is an autosomal recessive disorder characterized by congenital sensorineural hearing loss and goiter [6]. The causative gene for PS and EVA was identified to be *SLC26A4* [7,8]. Enlarged vestibular aqueduct (EVA) is a common inner ear malformation that can be diagnosed radiographically in patients with impaired hearing (Figure 1). EVA is frequently associated with PS [9-11]. In addition to PS, *SLC26A4* mutations also cause nonsyndromic hearing loss with EVA in the absence of a thyroid phenotype [12,13].

Previous studies revealed that the spectrum of *SLC26A4* mutations varied on the basis of ethnic background [14,15]. Tsukamoto et al. [15] demonstrated that *SLC26A4* mutations occurred in 90% of families with a history of PS and in 78% of families with a history of EVA in Japan. Among these *SLC26A4* mutations, H723R was suggested to have a founder effect in the Japanese population.

The Okinawa Islands are the southwestern-most islands of the Japanese archipelago (Figure 2). Previous studies suggested that there were substantial ancestral differences between Okinawa Islands the main islands of Japan [16]. In this study, we examined patients with EVA or PS from the Okinawa Islands to determine the frequencies and the genotypes of *SLC26A4* mutations and their clinical manifestations.

## Methods

### Subjects

From May 2008 to July 2012, 22 patients (8 males, 14 females; age range: 0–33 years; mean age: 5.8 years; median age: 8.5 years; Table 1) were diagnosed with PS or EVA in the Department of Otorhinolaryngology, Head and Neck Surgery of the University of the Ryukyus, Japan.

Prior to enrollment, all subjects provided a written informed consent. Our research protocol was approved by the Ethical Review Board of the University of the Ryukyus.

### Clinical manifestations of PS and EVA

Clinical history of 22 patients with neuro-otologic symptoms was recorded. A physical examination, including otoscopy, hearing level test, computed tomography (CT) scan of the temporal bones, and examination for thyroid goiter was conducted.

Depending on a subject's ability, hearing level was determined using auditory brainstem response, conditioned orientated response, or pure tone audiogram. Hearing level was defined as the average of the hearing threshold at 0.5, 1.0, 2.0, and 4.0 kHz. Hearing was described as: normal, < 20 dB; mild impairment, 21–40 dB; moderate impairment, 41–70 dB; severe impairment, 71–90 dB; and profound impairment, >91 dB.

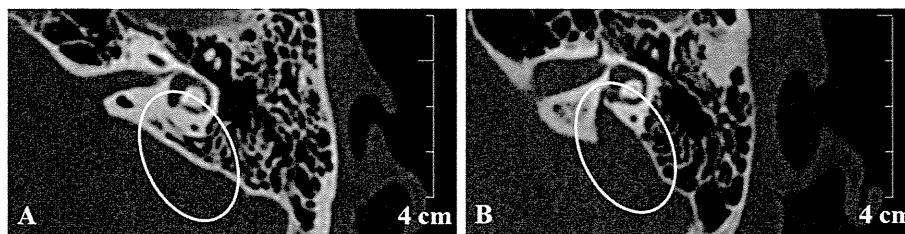
Neck palpation or echography of the neck was performed in all patients, to determine thyroid goiter. In addition, their serum levels of thyroid-stimulating hormone (TSH) and free thyroxine (FT4) were measured to evaluate thyroid function (normal values: 0.9–1.6 ng/dl and 0.5–5.0 mU/l, respectively). A perchlorate test was not performed.

High-resolution temporal bone CT was performed in all patients to determine if there were any other inner ear malformations in addition to EVA. EVA was defined as a vestibular aqueduct with a diameter of >1.5 mm at the midpoint between the common crus of the semicircular canal and the external aperture of the vestibular aqueduct on CT [17].

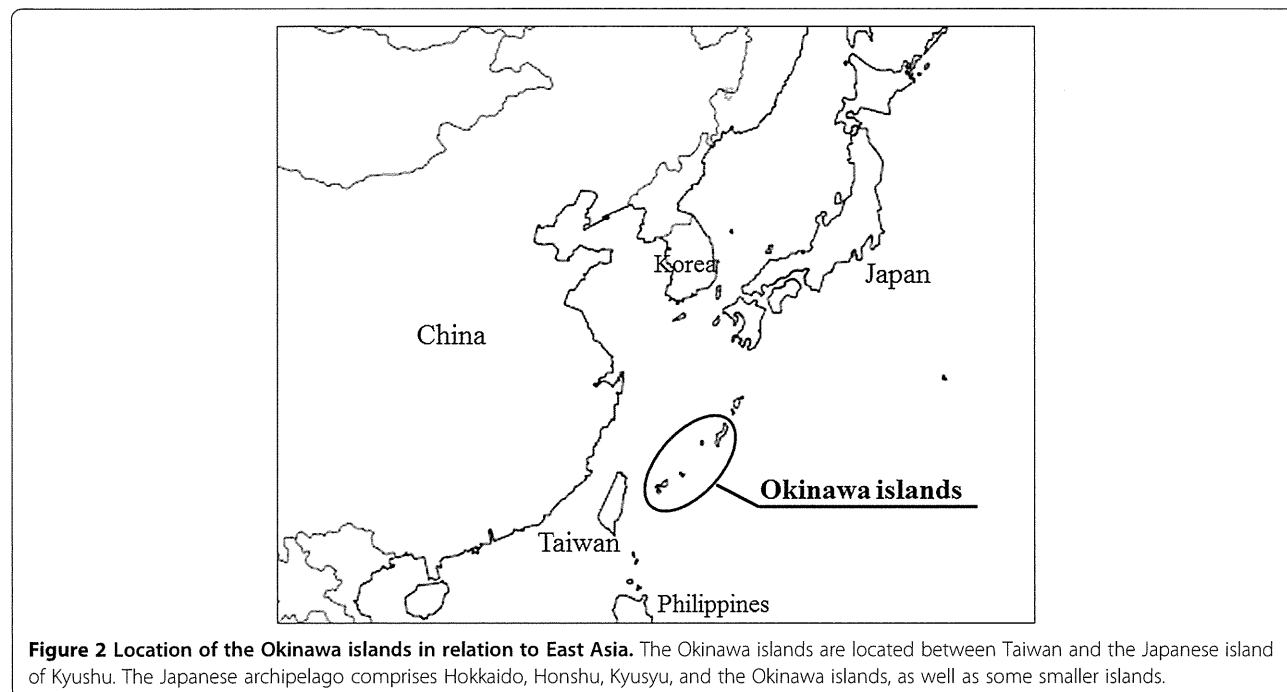
Mondini dysplasia was defined when the cochlea consisted of 1.5 turns in which the middle and apical turns had coalesced to form a cystic apex due to the absence of the interscalar septum [18,19].

Vestibular enlargement was defined when the ratio of the membranous vestibule diameter to the inner ear diameter of the lateral semicircular canal was >1.2 [20].

Vertigo was investigated based on spontaneous nystagmus, caloric vestibular test or patients' self-reporting of past episode. The spontaneous nystagmus was evaluated



**Figure 1** Computed tomography of the temporal bone showing an enlarged vestibular aqueduct. Circles show the vestibular aqueduct. The vestibular aqueduct is not identified in control subject (A). The enlarged vestibular aqueduct is identified in a patient with EVA (B).



using Frenzel's glass or infrared CCD camera (IRN-1, Morita, Kyoto, Japan).

#### ***SLC26A4* genotyping**

Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). To detect mutations, all 21 exons and the exon-intron junctions of *SLC26A4* were sequenced for all subjects. A 35 step cycle of Polymerase chain reactions (PCR) was performed as follows: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 40 s, 60°C for 40 s, and 72°C for 1 min; and a final extension at 72°C for 5 min. PCR reactions were run using a programmable thermal cycler (Verti™ 96-Well Thermal Cycler, Applied Biosystems, CA, USA).

PCR products were purified using a Wizard® SV Gel and PCR Clean-Up System (Promega, WI, USA) and directly sequenced using an ABI PRISM 3130 × 1 Genetic Analyzer (Applied Biosystems). The sequences obtained were aligned and compared using the BLAST program with known human genome sequences available in the GenBank database.

We surveyed the substitution IVS15 + 5G > A in 100 healthy objects as control.

The genotype of the IVS15 + 5G > A was detected by digestion of the PCR product with the restriction enzyme SspI (New England Biolabs, Ipswich, MA, U.S.A).

#### **Total RNA isolation and reverse-transcription**

Total RNA was isolated from leukocytes using a QIAamp RNA Blood Mini Kit (Qiagen) according to the

manufacturer's protocol. Before cDNA synthesis, residual DNA was removed by incubation with RNase-free DNase I (Ambion Inc., City, TX, USA). Then, total RNA was reverse transcribed using a TaKaRa Prime Script High Fidelity RT® Kit (TaKaRa, Tokyo, Japan) according to the manufacturer's protocol. Possible contaminating genomic DNA in RNA samples was determined by electrophoresis.

#### **Quantitative nested real-time PCR**

Nested real-time quantitative (q) PCR was performed to investigate the level of *SLC26A4* expression in the blood.

#### **First-step PCR (conventional PCR)**

A conventional PCR assay was performed in a 10 µl reaction mixture that included 2 µl of cDNA, 0.5 units of DNA Taq polymerase (TaKaRa), 2.5 mM deoxynucleotide triphosphates (dNTPs), 1 µM forward and reverse primers for first-step PCR (Table 2), 10 × buffer, and 1.875 mM MgCl<sub>2</sub>, with distilled water (H<sub>2</sub>O) for the final reaction volume of 10 µl. A 33 step cycle of PCR were performed as follows: 94°C for 5 min, 33 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 40 s, and a final extension at 72°C for 5 min.

#### **Second-step PCR (quantitative nested PCR)**

Following the first PCR, a second PCR was performed using a set of internal primers (Table 2). The reaction mixture contained 1 µl of the first PCR product (diluted 10-fold), 10 µl of SYBR Premix Ex Taq, and 0.2 µM of the internal forward and reverse primers; the final

**Table 1 Summary of clinical features of 22 patients**

Age (years)			CT			HL (dB)	PTA	Vertigo	Thyroid	
			EVA	MD	VE		Conductive hearing loss		Goiter	Thyroid function
1	3	R	+	+	+	SO	unknown	-	-	normal
		L	+	+	+	SO	unknown			
2	14	R	+	+	-	105	+	-	+	normal
		L	+	+	-	96	+			
3	21	R	+	+	+	73	+	+	+	normal
		L	+	+	+	91	+			
4	21	R	+	-	-	81	+	+	+	normal
		L	+	-	-	85	+			
5	28	R	+	+	+	96	+	+	+	normal
		L	+	+	+	SO	+			
6	33	R	+	+	-	101	+	+	+	normal
		L	+	+	+	106	+			
7	1	R	+	+	-	SO	unknown	-	-	normal
		L	+	+	+	SO	unknown			
8	1	R	+	-	-	SO	unknown	-	-	normal
		L	+	-	-	103	unknown			
9	2	R	+	+	-	101	unknown	-	-	normal
		L	+	+	-	100	unknown			
10	12	R	+	-	-	95	+	-	+	normal
		L	+	-	-	100	+			
11	29	R	+	+	+	85	+	-	-	
		L	+	+	+	110	+			
12	0	R	+	-	-	55	unknown	+	-	normal
		L	+	-	-	73	unknown			
13	3	R	+	-	+	85	unknown	+	-	normal
		L	+	+	+	58	+			
14	5	R	+	+	+	95	+	+	-	normal
		L	+	+	+	93	+			
15	5	R	+	+	+	103	+	-	-	normal
		L	+	+	+	100	unknown			
16	6	R	+	-	-	81	+	+	-	normal
		L	+	-	-	91	+			
17	7	R	+	-	-	83	+	-	-	normal
		L	+	-	+	81	+			
18	14	R	+	+	+	96	+	-	+	normal
		L	+	+	+	91	+			
19	16	R	+	-	+	91	+	-	+	normal
		L	-	-	+	21	-			
20	26	R	+	-	-	98	+	+	-	normal
		L	+	-	+	103	+			
21	5	R	+	+	+	85	+	-	-	normal
		L	+	+	-	97	+			
22	10	R	+	-	-	53	+	-	-	normal
		L	-	-	-	15	-			

EVA enlarged vestibular aqueduct, MD Mondini malformation, VE vestibular enlargement, PTA pure tone audiogram, HL hearing level, SO scale out, NA no available data.



**Table 2 Primer sequences used for nested real-time PCR**

Nested PCR assay		Sequence		PCR product size (bp)
First-step PCR (external primer)	Exon 14	forward	TCTTGGAAATGGCCTTGAAGC	282
	Exon 17	reverse	TGAAACAGCATCACTTATGATGC	
Second-step PCR (internal primer)	Exon 15	forward	TGAAGAACCTCAAGGAGTGAAG	154
	Exon 16	reverse	TTTCTGATTTTCTCAGCGCT	

reaction volume was adjusted to 20  $\mu$ l with distilled H<sub>2</sub>O. A Light Cycler real-time quantitative PCR system (Roche, Basel Switzerland) was used for amplification and detection of the PCR products. A 40 step cycle of thermal cycler program was performed as follows: denaturation at 95°C for 5 min; 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 40 s; followed by recording the fluorescence values after each elongation step and melting curve analysis with denaturation at 95°C for 5 s, annealing at 65°C for 1 min, and redenaturation by increasing the temperature to 95°C. The second-step PCR products were separated by 1.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized by UV transillumination. For this analysis, we used three control subjects with no mutations (wild type), three patients compound heterozygous for IVS15 + 5G > A/H723R, and three patients homozygous for IVS15 + 5G > A.

#### Validation of comparative CT ( $2^{-\Delta\Delta CT}$ ) method and calculations for quantifying *SLC26A4* mRNA

We used the CT ( $2^{-\Delta\Delta CT}$ ) method by assuming approximately equal amplification efficiencies for both target and reference genes. This prerequisite was verified by performing a validation experiment using both *SLC26A4* and a housekeeping gene. Calculations were made using the comparative CT ( $2^{-\Delta\Delta CT}$ ) method. *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase), *PGK-1* (phosphoglycerate kinase 1), and *ACTB* (actin beta) were used as internal reference genes for PCR normalization with regard to the amount of RNA added to the reverse transcription reactions. Normalized results were expressed as the mean ratio of *SLC26A4* mRNA to *GAPDH* mRNA, *PGK-1* mRNA, and *ACTB* mRNA. To evaluate relative transcript levels, the threshold cycle value (Ct) of each sample was used to calculate and compare the  $\Delta$ Ct of each sample to that of the control subject and patients with a compound heterozygous for IVS15 + 5G > A/H723R, and a homozygous for IVS15 + 5G > A.  $\Delta\Delta$ CT was also calculated to compare the transcript levels in the control subject, and patients with a compound heterozygous for IVS15 + 5G > A/H723R, and a homozygous for IVS15 + 5G > A. The transcript levels were calculated in each genotype with three subjects and each subject was calculated in triplicate.

## Results

### Mutation analysis for *SLC26A4*

By direct DNA sequence analysis, *SLC26A4* mutations were observed in 21 of 22 patients. Among the 21 patients with mutations, a compound heterozygous mutation for IVS15 + 5G > A/H723R was identified in nine patients (Figure 3C, D), a homozygous mutation for H723R was identified in five patients (Figure 3E), and a homozygous substitution of IVS15 + 5G > A was identified in six patients (Figure 3F). A compound heterozygous substitutions for IVS15 + 5G > A/T527P was identified in one subject. We could not identify any *SLC26A4* mutations in one subject (Table 3). We could not find the substitution IVS15 + 5G > A in 100 control objects.

### Clinical characteristics

Table 1 summarizes the clinical characteristics of all 22 subjects. High-resolution temporal bone CT scans revealed that bilateral EVA was present in 20 patients and unilateral EVA was present in other two. Mondini dysplasia and vestibular enlargement was observed in 17 ears (17/44; 39%) and 22 ears (22/44; 50%), respectively.

Hearing loss grades in the affected ears ranged from moderate to profound in the patients with EVA (Table 1). The hearing levels of the two unaffected ears were normal and mild hearing loss, respectively. Table 4 shows the hearing level distributions based on genotypes. No significant differences were expected in the distributions for hearing level among the five genotype groups due to the small sample of only 22 patients.

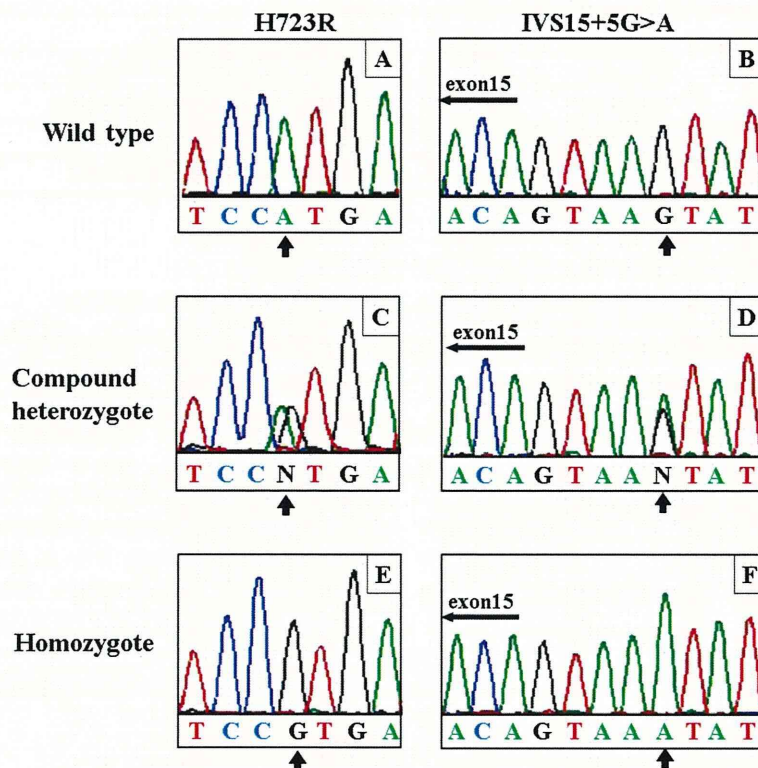
Neck examinations revealed thyroid goiters in 8 of 22 patients. Overall, 0% (0/11) and 73% (8/11) of the patients younger and older than 10 years of age, respectively, had a thyroid goiter. Their serum FT4 and TSH levels were within the normal ranges. There is no relation between occurrence of goiter and mutation genotypes.

### *SLC26A4* expression in patients with IVS15 + 5G > A

Electrophoretic separation of the real-time PCR products did not exhibit any bands in patients with the homozygous substitution for IVS15 + 5G > A (Figure 4C).

Because the *SLC26A4* expression levels were not high in blood samples, we investigated its expression using nested real-time qPCR for three control subjects, three





**Figure 3** Examples of direct sequence analysis of the *SLC26A4* gene. Representative results of H723R and the IVS15 + 5G > A mutation analysis are shown. Genomic sequences of the *SLC26A4* gene in normal individuals (A), (B). A compound heterozygous mutation for IVS15 + 5G > A/H723R (C), (D). A homozygous mutation for H723R (E). A homozygous substitution of IVS15 + 5G > A (F). The arrows indicate the variant nucleotide.

patients with the compound heterozygous mutation for IVS15 + 5G > A/H723R, and three patients with the homozygous substitution for IVS15 + 5G > A. The control subjects had normal hearing without any malformations of the inner or middle ear and no family history of hearing loss. After obtaining a written informed consent, blood samples were collected from each subject and were subjected to Real-time PCR with SYBR Green and the expression level was evaluated using the comparative CT ( $2^{-\Delta\Delta CT}$ ) method. The relative *SLC26A4* expression levels in the control no.1, control no.2 and control no.3 with no *SLC26A4* mutations were  $9089 \pm 441.5$  (standard deviation),  $2417 \pm 189.5$ , and  $4956 \pm 260.4$  respectively. In patient no.12, patient no.14 and patient no.16 with a compound heterozygous mutation for IVS15 + 5G > A/H723R were  $979.5 \pm 79.12$ ,  $2846 \pm 206.5$  and  $1183 \pm 33.93$  respectively. In patient no.1, patient no.2 and patient no.4 with a homozygous substitution for IVS15 + 5G > A were  $1.96 \times 10^{-4} \pm 7.66 \times 10^{-5}$ ,  $5.76 \times 10^{-5} \pm 3.37 \times 10^{-6}$  and  $4.35 \times 10^{-5} \pm 8.09 \times 10^{-6}$  respectively (Figure 5).

Based on the results of both electrophoresis and RT-nested qPCR, no *SLC26A4* expression was observed in patients with homozygous substitution of IVS15 + 5G > A.

## Discussion

### Correlations between *SLC26A4* genotypes and hearing phenotypes

Hearing loss in patients with EVA and PS is usually apparent at the pre- or perilingual stage [6,21]. Hearing loss in EVA and PS is sensorineural with some mixed hearing loss in the low-frequency range [22-27]. The hearing level sometimes deteriorates suddenly and may be followed by a partial recovery, such as with fluctuating hearing loss [28,29]. In our study, hearing loss was detected at the pre- or perilingual stage in all cases except for two cases of unilateral EVA. However, in all cases, hearing levels eventually deteriorated to severe or profound loss (Table 1) and were permanent with or without hearing fluctuation or stepwise hearing deterioration. No significant differences were observed in the hearing levels among the five genotypes (Table 4).

### Correlations between *SLC26A4* genotypes and thyroid phenotype

*SLC26A4* encodes for the 86 kDa transmembrane protein pendrin [7,30]. In the thyroid, this protein acts as co-transporter of chloride and iodine in the thyroid

**Table 3 Distribution of SLC26A4 genotypes of 22 patients**

	Age at onset of hearing loss (years)	Age at genetic test (years)	Sex	Allele 1	Allele 2
1	0	3	M	IVS15 + 5G > A	IVS15 + 5G > A
2	2	14	F	IVS15 + 5G > A	IVS15 + 5G > A
3	3	21	F	IVS15 + 5G > A	IVS15 + 5G > A
4	2	22	F	IVS15 + 5G > A	IVS15 + 5G > A
5	0	23	M	IVS15 + 5G > A	IVS15 + 5G > A
6	0	29	F	IVS15 + 5G > A	IVS15 + 5G > A
7	0	1	F	H723R	H723R
8	1	1	F	H723R	H723R
9	4	2	M	H723R	H723R
10	0	12	F	H723R	H723R
11	5	29	M	H723R	H723R
12	0	0	M	IVS15 + 5G > A	H723R
13	2	3	M	IVS15 + 5G > A	H723R
14	0	5	F	IVS15 + 5G > A	H723R
15	1	5	F	IVS15 + 5G > A	H723R
16	0	6	F	IVS15 + 5G > A	H723R
17	2	7	F	IVS15 + 5G > A	H723R
18	2	14	F	IVS15 + 5G > A	H723R
19	7	16	F	IVS15 + 5G > A	H723R
20	5	26	M	IVS15 + 5G > A	H723R
21	1	5	M	H723R	T527P
22	7	10	F	ND	ND

ND not determined.

[31,32]. In PS patients, a mutation in *SLC26A4* results in reduced pendrin-induced chloride and iodide transport and, ultimately, goiter [33].

Goiter usually develops around the end of the first decade of life or during young adulthood, although the time of onset and severity vary considerably among patients [12,34], and even within families [35]. Despite an impaired incorporation of iodide, most patients with PS are clinically and biochemically euthyroid [21,34,36].

To our knowledge, no previous studies have investigated correlations between *SLC26A4* genotypes and the thyroid phenotype. In the present study, PS was diagnosed in 8 of 11 patients older than 10 years of age, but not in any of the 11 patients who were younger than 10 years of age. This indicates that it is difficult to diagnose PS before the age of 10 years.

Thyroid function was normal in all of the 21 patients we examined, as demonstrated by their normal serum concentrations of FT4 and TSH. There were no significant differences in serologic thyroid test results and goiter status among patients with homozygous substitution for IVS15 + 5G > A, the H723R homozygous mutation, or compound heterozygous mutation for IVS15 + 5G > A/H723R. Therefore, our results indicate that serologic testing of FT4 and TSH levels is not useful to distinguish between individuals with PS or EVA.

#### Distributions of SLC26A4 mutations in EVA and PS patients in Okinawa Islands

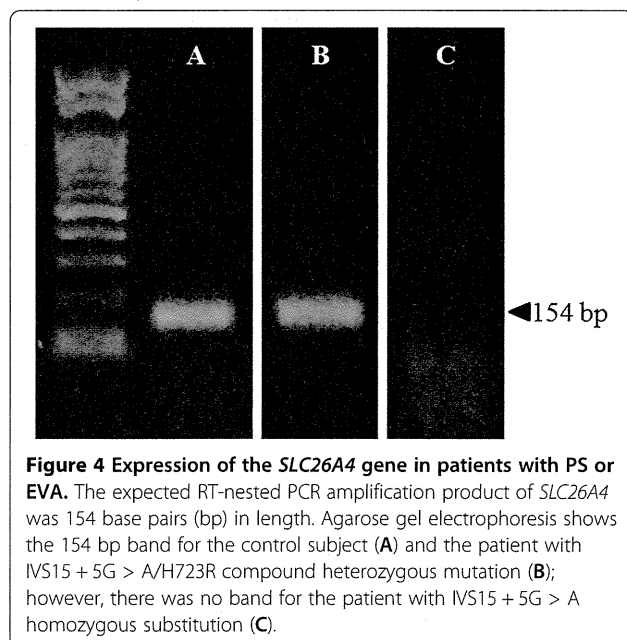
It was previously reported that the spectrum of *SLC26A4* mutations varied based on ethnic background [35,36]. H723R and IVS7-2A > G are prevalent alleles that account for the majority of the observed *SLC26A4* mutations in East Asian populations [35]. In the Japanese population, H723R was the most common mutation [15,36,37]. In Chinese and Taiwanese populations, IVS7-2A > G was the most common mutation [38-40], whereas in the Korean population, H723R and IVS7-2A > G were the most frequent and accounted for 60.2% (47/78) and 30.7% (24/78) of the mutated alleles, respectively [41].

Ancestral differences have been reported between people from Okinawa Islands and those from the main islands of Japan based on single-nucleotide polymorphism genotypes [16]. We analyzed *SLC26A4* mutations among 22 patients with EVA or PS from 21 unrelated families. H723R have been reported as the most common mutation found in the main islands of Japan. As with H723R mutation, IVS15 + 5G > A substitution was

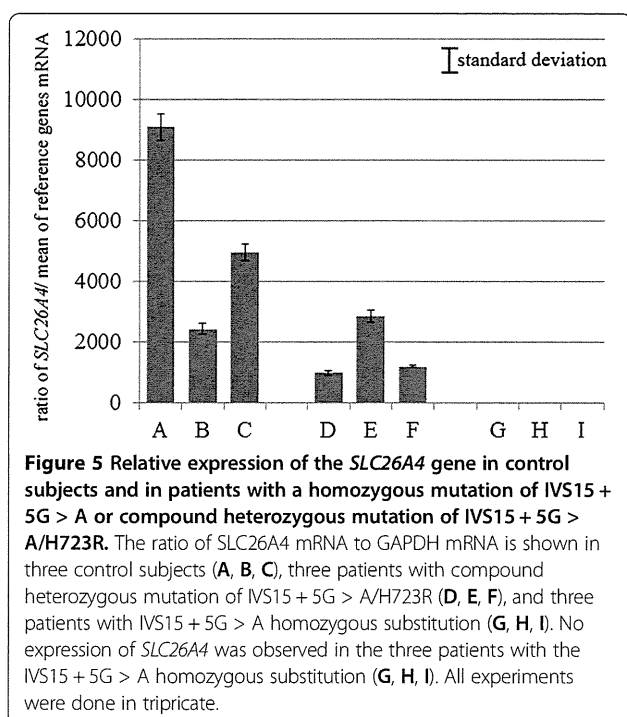
**Table 4 Clinical features in different genotype groups**

Genotype	Hearing level					CT		Vertigo
	Normal	Mild	Moderate	Severe	Profound	MD	VE	
IVS15 + 5 G > A homozygous (n = 6)	0	0	0	3	9	6/12	6/12	4/6
H723R homozygous (n = 5)	0	0	0	1	9	4/10	3/10	0/5
IVS15 + 5 G > A/H723R (n = 9)	0	1	2	4	11	5/18	11/18	4/9
IVS15 + 5G > /T527P (n = 1)	0	0	0	1	1	2/2	1/2	0/1
No mutation (n = 1)	1	0	1	0	0	0/2	0/2	0/1
Subtotal	1	1	3	9	30	17/44	21/44	8/22
Total	44							

Normal: ≤20 dB; Mild: 21–40 dB; Moderate: 41–70 dB; Severe: 71–90 dB; Profound: >91 dB.  
 MD Mondini malformation, VE Vestibular enlargement, CT computed tomography.



also identified most frequently in 15 of 22 of our Okinawa patients. The substitution of IVS15 + 5G > A in one allele have been reported only 10 cases in Asian populations [36,42-45]. Thus, IVS15 + 5G > A was the characteristic *SLC26A4* gene mutation among patients in Okinawa Islands, indicating a difference in the spectrum of *SLC26A4* mutations among patients in Okinawa Islands compared with patients in other



populations. These results suggest that this *SLC26A4* mutation may have originated from a common ancestor.

#### Pathogenic effect of IVS15 + 5G > A substitution

The heterozygous substitution of IVS15 + 5G > A has been assumed to cause aberrant splicing [36,42-45]. However, Yang et al. [42] could not find any abnormal RT-PCR products related to the size for *SLC26A4* sequence analysis in patients with splice mutation. Because its pathogenicity was only implicated on the basis of uncommon polymorphisms, the pathogenic potential of IVS15 + 5G > A still remains unknown.

Substitutions near the canonical splice sites are difficult to classify as pathogenic or non-disease causing. Because such substitutions affect proper RNA splicing but some substitutions do not cause any effect [46-48]. Thus, it is important to determine the pathogenic effect of a particular substitution near the donor site by mRNA analysis [48]. We investigated *SLC26A4* expression in patients with compound heterozygous mutation for IVS15 + 5G > A/H723R and homozygous substitution for IVS15 + 5G > A by RT-PCR and RT-real time PCR by targeting genes around these mutations. No aberrant PCR products were detected in the patient with heterozygous substitution of IVS15 + 5G > A (Figure 4B), which suggests that IVS15 + 5G > A does not cause aberrant splicing, as also argued by Yang et al. However, in patients with the homozygous substitution of IVS15 + 5G > A, *SLC26A4* was not expressed, as shown in Figure 4. In addition, for patients with the heterozygous substitution, *SLC26A4* expression was reduced from the normal control level. These findings suggest that IVS15 + 5G > A disrupts pre-mRNA splicing and causes the loss of *SLC26A4* expression. The patients in Yang et al. [42] were heterozygote so that Yang et al. [42] most likely amplified the non-mutated allele. Taken together, our results indicate that the substitution of IVS15 + 5G > A is a loss-of-function mutation caused by a loss of *SLC26A4* expression.

#### Conclusions

We found no correlations between the type of *SLC26A4* mutation and hearing levels or the thyroid phenotype. Moreover, thyroid testing using serum FT4 and TSH levels was not useful for distinguishing between individuals with PS and EVA.

The substitution of IVS15 + 5G > A in the *SLC26A4* was unique and the most common in PS and EVA patients from Okinawa Islands. This supports that the spectrum of *SLC26A4* mutations differs by geographic area in East Asia. Our qPCR results for *SLC26A4* indicate that the substitution of IVS15 + 5G > A should be a pathogenic mutation that leads to a loss of *SLC26A4* expression and results in a phenotype of PS and EVA.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

AG diagnosed the patients, collected clinical data, performed the experiments, and wrote the manuscript. TK, KY, and SU carried out data analysis. KN, TT, and MS edited the manuscript and supervised the project. All authors read and approved the final manuscript.

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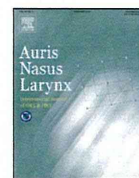
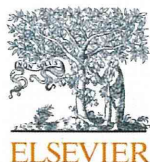
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## Long term speech perception after cochlear implant in pediatric patients with *GJB2* mutations

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### ABSTRACT

**Objectives:** To determine the long term effect of cochlear implant (CI) in children with *GJB2*-related deafness in Japan.

**Methods:** Genetic testing was performed on 29 children with CI. The speech perception in 9 children with *GJB2* gene-related deafness fitted with CI was compared with those in matched 10 children who were diagnosed as having no genetic loci. The average follow-up period after CI was 55.9 months and 54.6 months, respectively.

**Results:** A definitive inherited hearing impairment could be confirmed in 12 (41.4%) of the 29 CI children, including 10 with *GJB2*-related hearing impairment and 2 with *SLC26A4*-related hearing impairment. The results of IT-MAIS, word or speech perception testing under the noise, and development of speech perception and production testing using the Enjoji scale were slightly better for the *GJB2* group after CI than for the control group without statistical significant difference.

**Conclusion:** The long-term results of this study show that CI is also effective in the development of speech performance after CI in Japanese children with *GJB2*-related hearing impairments as HL due to other etiologies.

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### 1. Introduction

Recent progress in the research on hereditary hearing loss is remarkable. Since 1992, more than 125 genetic loci have been reported to be involved in nonsyndromic hearing loss (HL) [1], and over 67 of those loci are involved in autosomal recessive nonsyndromic HL [2]. Among these, the *GJB2* gene encoding the connexin (Cx) 26 protein (chromosomal 13q11-12) is the most common, of which about 100 different *GJB2* mutations have been reported globally [3]. It is reported to account for between 20 and 50% of all recessive nonsyndromic cases [4].

On the other hand, the benefits of cochlear implantation (CI) for spoken language, reading skills, and cognitive development have been clearly demonstrated [5,6]. Recently, the outcomes of CIs in patients with *GJB2* mutations have also been reported. Several studies have shown that patients with *GJB2* mutations (OMIM 121011) usually exhibit excellent speech perception and language

performance after CI, when compared with those without identifiable *GJB2* mutations [7–11]. However, other studies have demonstrated that when the control group is appropriately matched with regard to age at implantation and length of post CI, there is no significant difference when comparing those with *GJB2*-related deafness to those without it [12–15]. Results analyzing post-CI speech performance in patients with *GJB2* mutations are still controversial.

In this study, in order to know whether the long term effect of CI is better in children with *GJB2*-related deafness or not, we have studied the speech perception outcome of CI in children with *GJB2* gene mutations, and compared them to those in matched children without inherited hearing loss.

### 2. Materials and methods

#### 2.1. Subjects

We have performed CI in 301 cases in our clinic since 1997. Genetic testing was performed in 29 children with CI, and definitive *GJB2*- and *SLC26A4*-related hearing impairment was confirmed in 10 (34.5%) and 2 (6.9%) children with CI, respectively.

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**Table 1**  
Clinical information of cases in the 2 groups.

Group	Control	GJB2	P value
Number of cases	10	9	
Sex (male:female)	3:7	2:7	
Age at CI (months)	36.7	37.4	0.5996
Post CI (months)	54.6	55.9	0.6736
Pre-CI education	Auditory-verbal/oral	Auditory-verbal/oral	

CI: cochlear implantation.

Finally, 19 children whose selection criteria were as follows were enrolled for this study.

1. Their age at CI was 6 years or less
2. Their guardian accepted gene mutation analysis
3. There was no any other apparent cause of deafness such as inner ear anomaly, central disorders/learning difficulties, or cytomegalovirus (CMV) infection

We divided them into two groups: the first, a control group consisting of 10 children who were diagnosed as having no genetic loci, while the second was the actual *GJB2* study group consisting of 9 children with *GJB2* gene-related deafness. Detail of their clinical information is shown in Table 1. HL was diagnosed at a different age in each child, but showed 90 dB or more severe HL before the age of 6 on auditory brainstem response (ABR) test. Preoperative imaging studies (CT and MR) showed no abnormal findings in any of the children in each group. None of the children showed any cognitive delay. The average age at CI in the two groups was 36.7 months (ranging from 21 to 67 months old; 3 male and 7 female) and 37.4 months (ranging from 22 to 63 months old; 2 male and 7 female), respectively. Thus, there is no significant difference between the two groups (Student's *t*-test,  $t = -0.5339$ ,  $P = 0.5996$ ). Their average follow-up period after CI was 54.6 months (ranging from 24 to 110 months) and 55.9 months (ranging from 47 to 62 months), respectively (Student's *t*-test,  $t = -0.4278$ ,  $P = 0.6736$ ). All the cases in this study had an intensive auditory-verbal education without visual information since childhood. Both the CI operation and the (re)habilitation after CI took place in the same clinic.

All patients were fitted with a CI system from either nucleus multichannel cochlear implant system (Cochlear Corporation, Englewood, CO, U.S.A.) or Combi40+ cochlear implant system (MED-EL, Innsbruck, Austria). All electrode arrays were inserted in all patients. There were no perioperative complications in any of the patients.

We examined the hearing level (both with CI and with hearing aids), the Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS), speech perception skills, and development of articulation in the two groups before and after CI several times in the post-operative period ranging from 6 months to 4 years. The best results from this period were used in evaluating the hearing level and the speech perception skills in the two groups. The speech perception skills were evaluated using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89) tested at 70 dB SPL (sound pressure level) using an open-set questionnaire. We also examined the development of speech perception and production by using the Enjoji Scale of Infant Analytical Development (Enjoji Scale), which was developed in Japan and is now established as one of the standard developmental examinations for evaluating the development of children from birth to about the age of 6 [16]. In this examination, the development of a child can be assessed by checking his or her performance on the chart, in which standard developmental items at each month are described in the three fields including motor, social and language skills. The results allow us to clearly assess to

what extent a child is successfully developing in each of the three fields and the six subdivided categories. These tests were conducted up to 2 years after CI.

## 2.2. Mutation detection

15 ml peripheral venous blood using standard procedure was sent to the Institute of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan for Genomic DNA extraction. All subjects underwent mutation screening for 47 common mutations of 10 hearing loss related genes in Japan by using invader assay [17,18].

Written informed consent was obtained from the guardians of all the subjects and the study was approved by the ethical committee of our institute (approval number: 07122106). The differences between in the two groups were analyzed statistically using the paired *t*-test and the unpaired Student's *t*-test. All the acceptance criterion for a significant addition to the explained variance was set at *P* values under 0.05.

## 3. Results

A definitive *GJB2*-related hearing impairment was confirmed in 9 (32.2%) of the 29 children with CI. Table 2 shows the details of detected *GJB2* gene-mutations. *GJB2* c.235delC was observed in 3 cases, while six children each had one distinct mutation as listed in the table.

Fig. 1 shows the preoperative aided hearing thresholds. The preoperative hearing level was over 90 dB in all the cases, and the average level of preoperative aided hearing thresholds was nearly 60 dB in the two groups presenting no significant difference between the two groups.

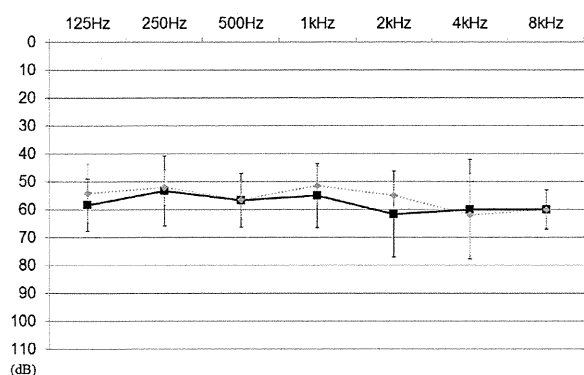
Fig. 2 shows the postoperative hearing thresholds with CI. After CI, the hearing level improved to 25–30 dB in both groups, thus there was no significant difference between the groups.

Fig. 3 shows the results of the IT-MAIS for the two groups. Preoperative scores were worse in the *GJB2* group than in the control group, however, these improved from 1 year to 3 years after CI. The averaged IT-MAIS score in the *GJB2* group was  $9.8 \pm 12.9$  (range, 0–31) preoperatively. The averaged IT-MAIS score at 2 years after CI increased up to  $33.6 \pm 7.8$  (range, 20–39), and this improvement was statistically significant (paired *t*-test,  $P = 0.017$ ). The averaged IT-MAIS score in the control group at 2 years after CI

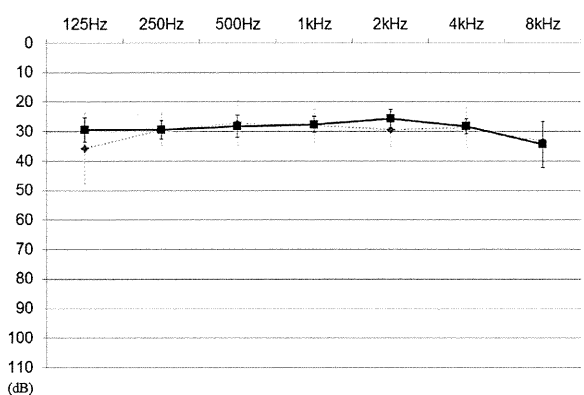
**Table 2**  
Mutations with *GJB2* gene in 9 cases.

Mutation	Number of cases
GJB2 c.[235delC];[235delC]	3
GJB2 c.[511insAACG];p.[T86R]	1
GJB2 c.[235delC];[299-300delAT]	1
GJB2 p.[G45E;Y136X];[R143W]	1
GJB2 c.[176-191del16];[299-300delAT]	1
GJB2 c.[235delC];p.[G45E;Y136X]	1
GJB2 c.[235delC];p.[R143W]	1





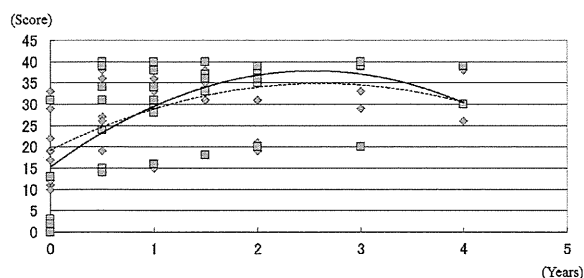
**Fig. 1.** Results of the average level of preoperative aided hearing thresholds at each frequency. Diamond dots and solid line: control group; square dots and solid line: *GJB2* group; bars: indicate two standard deviations.



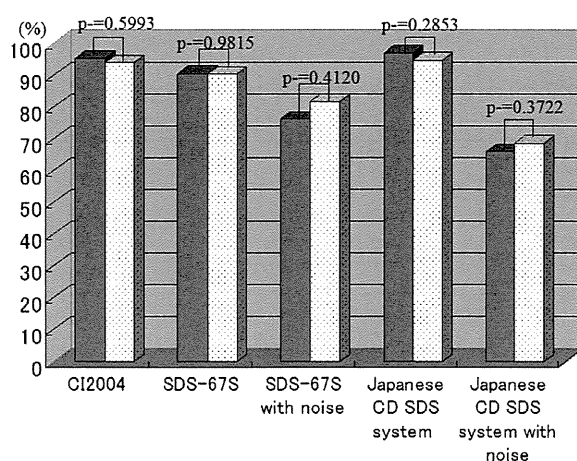
**Fig. 2.** Results of the average level of postoperative hearing thresholds with CI at each frequency. Diamond dots and solid line: control group; square dots and solid line: *GJB2* group; bars: indicate two standard deviations.

was  $30.4 \pm 7.6$  (range, 19–38). There was no significant difference in the scores between the two groups at 4 years after CI.

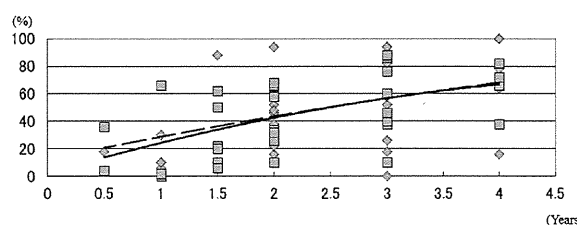
Fig. 4 shows the results of speech perception skills in the two groups after CI. Longitudinal axis indicates the results (%) when tested at 70 dB SPL using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89) in the two groups. There was no significant difference between the two groups, but the percentage of correct answers (%) examined under the noise tended to be better in the *GJB2* group.



**Fig. 3.** Results of the difference of IT-MAIS scores from 0 years (=preoperative) to 4 years after CI. Diamond dots: scores in the control group; square dots: scores in the *GJB2* group; dotted line: trend line in the control group; solid line: trend line in the *GJB2* group.



**Fig. 4.** Results of speech perception skills examined by using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89). Longitudinal axis indicates the correct answer rate (%). Gray bars: control group; dotted bars: *GJB2* group.



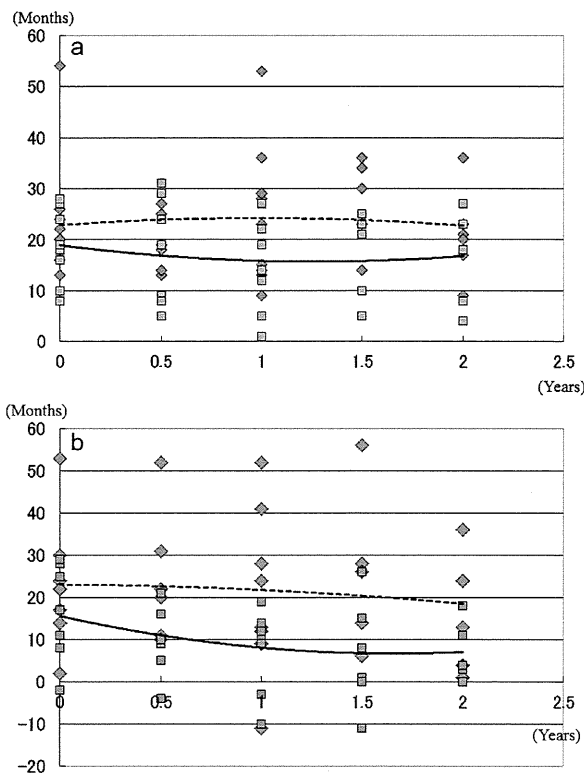
**Fig. 5.** Results of the development of articulation from 0.5 to 4 years after CI. Diamond dots: accuracy rates in the control group; square dots: accuracy rates in the *GJB2* group.

Fig. 5 shows the results of development of articulation in the two groups after CI. There was no significant difference in the scores between the two groups.

Fig. 6 shows the results in the development of speech perception (Fig. 6a) and production (Fig. 6b) in the two groups after CI. Values of month in the ordinate were calculated by subtracting the developmental months assessed by the Enjoji Scale from the actual age at each period, thus, smaller values indicate better development of speech perception and production. Postoperative language perception and production in the *GJB2* group tended to be slightly better, especially at one and half years after surgery, but there was no significant difference in these scores.

#### 4. Discussion

The incidence of HL is approximately 0.1% among newborns, and hereditary HL is identified in at least 60% of patients with congenital HL, for whom the proportion of syndromic and non-syndromic is 30% and 70%, respectively [19]. The most common trait of nonsyndromic HL is autosomal recessive, which accounts for about 80% of cases [20], and *GJB2* is the gene most frequently associated with hereditary HL. The incidence of *GJB2* mutations in the Japanese population with HL is 14.2% overall and 25.2% in patients with congenital hearing loss [21], and 35 of the 119 cases (29.4%) with non-syndromic deafness [22]. In children with CI, 135 hearing-impaired patients (270 alleles) were tested, and *GJB2* mutations for the c.235delC were found in 39 alleles of 270 alleles (14%). Especially the homozygous of c.235delC was detected in 26



**Fig. 6.** Results of the developmental course of language perception (a) and production (b) in the control and *GJB2* groups examined by Enjoji Scale of Infant Analytical Development test. Diamond dots: scores in the control group; square dots: scores in the *GJB2* group; dotted line: trend line in the control group; solid line: trend line in the *GJB2* group.

alleles (9.6%), single heterozygous of c.235delC was detected in 1 allele (0.4%) and compound heterozygous of c.235delC was found in 12 alleles (4.4%) [23].

In this study, a definitive inherited hearing impairment could be confirmed in 11 (37.9%) of the 29 CI children, including 9 with *GJB2*-related hearing impairment, 2 with *SLC26A4*-related hearing impairment. These percentages are quite high and remind us of the importance of performing the mutation detection for CI patients.

The *GJB2* group underwent the IT-MAIS, word or speech perception testing under the noise, and development of speech perception and production testing using the Enjoji scale. The finally achieved performances in the two groups were not significantly different, but the averaged IT-MAIS score at 2 years after CI was significantly better in the *GJB2* group than in the control group. This result may indicate that the necessary period to achieve the actual age development was shorter in the *GJB2* group than in the control group, and the difference may become smaller as they acquire language through CI in longer term. Matsushiro evaluated 4 CI children with *GJB2* gene mutation and reported that the postoperative IT-MAIS score at 6 months was significantly higher in comparison with that of other prelingual CI patients [24]. In this study, children such as those having inner ear anomaly or cytomegalovirus infection, whose postoperative performance after CI is not necessarily good, were excluded from the control group. Considering that these children may also be candidates for CI in general, we can expect CI is efficient for Japanese children with *GJB2* gene mutation as well as for those reported previously [8,23,24].

*GJB2* and *GJB6*, mapping to the *DFNB1* locus and encoding the gap-junctions Cx 26 and 30, respectively [25]. Cx 26 and 30 are widely expressed in the cochlea at the level of the organ of Corti's supporting cells and connective tissues, and have an important role in forming homomeric or heteromeric hemichannels [26,27]. Mutations in Cx26 are presumed to result in altered potassium recirculation, leading to an accumulation of potassium in the cochlear endolymph and causing hair cell dysfunction and deafness [28]. In other words, mutations in the Cx26 protein mainly lead to the impairment of the endolymph potassium concentrations, which are required for auditory signal transduction, but may not lead to severe damage or decreasing the number of hair cells. It is generally assumed that the results of CI are poorer for inner ear malformation and in cases with neural and/or central damage than in cases with disorders within the inner ear causing the hair cells damage because the auditory pathway including the first neuron, spiral ganglion cells, may well be preserved in the latter. We speculate that the reason why the *GJB2* group had better results in this study is perhaps due to a comparatively good survival and preservation of electrical excitability of the cochlear spiral ganglion cells and the auditory nerve, which is important in the successful CI results [29].

There are some specific reports which support the present results and our speculations. In a rat model, Cx26 was shown to be expressed in nonsensory epithelial and connective tissue cells, but not in the inner or outer hair cells or cochlear nerve fibers [30]. Anatomically, Cx26 mutations result in a dysgenesis of the stria vascularis and hair cells in the organ of Corti, but with minimal neural degeneration and a normal population of spiral ganglion cells in both the apical and basal turns of the cochlea. [31] In the electrophysiological study, children with *GJB2*-related HL had greater similarities between low- and high-frequency residual hearing and between neural activity electrically evoked at apical and basal regions of the cochlea than children with non-*GJB2*-related HL [32]. These results may suggest more consistent spiral ganglion survival along the length of the cochlea in *GJB2*-related HL, which appears to involve a decreasing gradient of spiral ganglion survival from the apex to the base of the cochlea.

Most genotype-phenotype correlation studies have indicated that HL of the subjects with *GJB2* mutations shows a non-progressive pattern [33,34], however, some studies indicated a progressive pattern. [23,35,36]. Considering that early CI is well known to be one of the most important factors for the better postoperative performance for children with congenital HL, even in children with progressive hearing loss due to *GJB2* mutation, we might be able to prepare for early CI for those children if we were aware of it. The early screening of *GJB2* mutation for newborns with severe to profound HL might be advisable.

## 5. Conclusions

Despite the limits imposed by the small sample size, this study points to the importance of routine genetic assessments. The long-term results of this study also show that CI is also effective in the development of speech performance after CI in Japanese children with *GJB2*-related hearing impairments as HL due to other etiologies. If a child through genetic assessment is diagnosed as having a *GJB2*-related hearing impairment, CI can provide considerable benefits.

## Conflict of interest

None.

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## 難聴の遺伝子診断と次世代シーケンス解析

— 保険収載された遺伝子診断からターゲットリシーケンシングとエクソーム解析

Genetic diagnosis of deafness

— From SNPs based invader analysis to the target resequencing and exome analysis



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◎先天性難聴は、新生児 1,000 名に 1 名に認められる比較的頻度の高い先天性疾患である。疫学調査の結果より先天性難聴の 60~70% に遺伝子が関与することが推測されているため、正確な診断のためには遺伝子診断が重要であるが、遺伝的異質性が高く 100 程度の遺伝子が関与するため、効率的な遺伝子診断手法が必要である。2012 年 4 月の診療報酬改定により日本人難聴患者に高頻度に認められる原因遺伝子変異を網羅的にスクリーニングする検査が保険収載され、日常の診断ツールとして遺伝子診断が利用可能となった。本稿では保険収載された遺伝子診断とその有用性について概説するとともに、保険収載された遺伝子診断を補完する検査として実施している次世代シーケンサーを用いたターゲットリシーケンシング解析およびエクソーム解析の現状について概説する。



遺伝学的検査, 保険診療, 先天性難聴, 次世代シーケンサー, Usher 症候群

先天性難聴は新生児 1,000 名に 1 名に認められる頻度の高い先天性疾患であり、他の先天性疾患と比較しても非常に頻度の高い疾患のひとつであるといえる。疫学調査の結果より先天性難聴あるいは小児期発症の難聴の 60~70% に遺伝子が関与することが推測されているため(図 1)<sup>1)</sup>、先天性難聴の原因として最も可能性が高いのが遺伝子変異であり、正確な診断のためには遺伝子診断が必要不可欠である。

遺伝性難聴は、①難聴以外の症状を伴う“症候群性難聴”と、②難聴のみを症状とする“非症候群性難聴”に大別される。“症候群性難聴”は遺伝性難聴の約 30% を占めるとされており、難聴以外に筋肉骨格系、腎尿路系、神経系、視覚障害、色素異常、代謝異常や種々の奇形を伴う 400 以上の疾患群が報告されている。これら“症候群性難聴”に関しては難聴以外の随伴症状より確定診断が比較的容易である。また、いくつかの症候群に関してはその原因遺伝子が明らかとなっている。一

方、遺伝性難聴の約 70% は難聴のみを症状とする“非症候群性難聴”である。分子遺伝学的解析手法の進歩により、“非症候群性難聴”の原因遺伝子は 2013 年現在で 54 種類報告されている<sup>2)</sup>が(表 1)、難聴以外の随伴症状を伴わないことより、臨床症状だけから原因遺伝子を特定することは困難である。

### ● 保険収載された“先天性難聴の遺伝子診断”

前述のように、先天性難聴の大部分に遺伝子が関与することが知られているが、遺伝的異質性が高く 100 程度の遺伝子が関与するため、効率的な遺伝子診断手法が必要となる。著者らは日本人難聴患者の遺伝子解析を精力的に行ってきたが、その結果、日本人難聴患者より見出される難聴の原因遺伝子変異部位は欧米人難聴患者に見出される部位と大きく異なっており、民族に特有の変異が存在することが明らかとなってきた<sup>3-7)</sup>。これら日本人難聴患者に特徴的な原因遺伝子変異を網羅的かつ効率的にスクリーニングを行うことが難聴

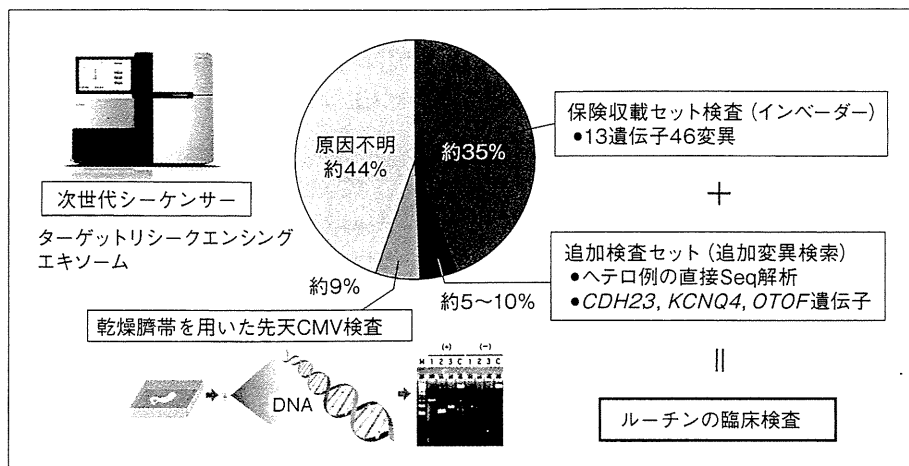


図 1 難聴遺伝子解析の現況

平成 24 年度より保険収載されたインベーター法を用いた 13 遺伝子 46 変異のスクリーニング検査では、おおよそ 35%の原因が特定可能である。現在、スクリーニング検査により原因遺伝子変異が同定されなかった症例を対象に、①追加変異解析の検査セット、②先天性 CMV 感染症の検査、③次世代シーケンサーを用いた解析を追加で行っている。※追加の検査を希望される施設は著者までご連絡いただきたい。

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の原因を特定するためには効率的であるため、日本人難聴患者に高頻度で認められる 13 遺伝子 46 変異をインベーター法により網羅的にスクリーニングする検査を開発し、その臨床応用に関する検討を行ってきた。全国 33 施設と行った共同研究では、難聴患者の 35% (発症年齢が 6 歳以下の先天性難聴に限ると 44.3%) の検出率が得られ、スクリーニング検査として有用であることが明らかとなった<sup>8)</sup>。この方法を用いた“先天性難聴の遺伝子診断”が 2008 年 7 月より先進医療として承認を受けて臨床応用が開始された。さらに、先進医療での実績数と有用性が評価され、2012 年 4 月の診療報酬改定により“遺伝学的検査(先天性難聴)”として保険収載され、日常の診断ツールとして遺伝子診断が利用可能となった。

### 難聴の遺伝子診断のメリット

難聴では原因遺伝子変異の種類により臨床像がすこしずつ異なるため、遺伝子診断により原因遺伝子変異を特定することで、原因遺伝子ごとにサブタイプ分類が可能となり、難聴のタイプや重症度、進行性や変動の有無、随伴症状の予防、発症・増悪の予防が可能となる。また、これら臨床像の予測に基づき、より適切な医療を提供するこ

とが可能となる。

#### 1. 難聴のタイプ・重症度の予測

遺伝子変異の種類や変異の部位によってコードする蛋白質に及ぼす影響が異なるため、最終的な表現型である難聴のタイプが異なってくることが知られている<sup>49)</sup>。

新生児聴覚スクリーニングの普及により、難聴のスクリーニング検査が生後 1 週間以内に可能となってきたが、乳幼児の正確な聴力評価には聴性脳幹反応 (ABR) や聴性定常反応 (ASSR) などの聴覚検査を繰り返す必要がある。しかし、これら聴覚検査に加えて遺伝子診断を組み合わせることで、より多くの情報が得られ難聴のタイプや重症度に関して予測可能となるため、より早期に、より正確な診断が可能となる。

また、原因遺伝子種類により聴力像が異なることが知られているため、遺伝子診断を行うことによりあらかじめ聴力像を予測することが可能である。とくに *CDH23*, *KCNQ4*, ミトコンドリア遺伝子 1555 A>G 変異による難聴の場合には高音障害型の感音難聴となるが、近年高音障害型感音難聴に対する新しい治療法として、残存聴力活用型人工内耳が臨床応用されており、遺伝子診断に基づいて人工内耳の種類を使い分けるオーダー