CONCLUSIONS

Pendred syndrome and nonsyndromic hearing loss associated with EVA are a continuum of disease characterized as being associated with congenital, fluctuating and progressive hearing loss, and most patients have vertigo and/or goiter. However, in the present study, no genotype–phenotype correlation was found. The results obtained from the present study will facilitate accurate molecular diagnosis and better genetic counseling.

ACKNOWLEDGEMENTS

We thank the participants of the Deafness Gene Study Consortium: Drs Norihito Takeichi and Satoshi Fukuda (Hokkaido University), Drs Atsushi Namba and Hideichi Shinkawa (Hirosaki University), Drs Yumiko Kobayashi and Hiroaki Sato (Iwate Medical University), Drs Tetsuaki Kawase and Toshimitsu Kobayashi (Tohoku University), Drs Tomoo Watanabe, Tsukasa Ito and Masaru Aoyagi (Yamagata University), Drs Hiroshi Ogawa and Koichi Omori (Fukushima Medical University), Drs Kotaro Ishikawa and Keiichi Ichimura (Jichi Medical University), Drs Kyoko Nagai and Nobuhiko Furuya (Gunma University), Drs Shuntaro Shigihara, Yasuyuki Nomura and Minoru Ikeda (Nihon University School), Drs Tetsuo Ikezono and Toshiaki Yagi (Nippon Medical School), Dr Shunichi Tomiyama (Nippon Medical School Tama Nagayama Hospital), Drs Hiromi Kojima, Yuika Sakurai and Hiroshi Moriyama (Jikei University), Dr Kozo Kumakawa (Toranomon Hospital), Drs Hajime Sano and Makito Okamoto (Kitasato University), Dr Satoshi Iwasaki (Hamamatsu Medical University), Dr Kazuhiko Takeuchi (Mie University), Dr Masako Nakai (Shiga Medical Center for Children), Drs Masahiko Higashikawa and Hiroshi Takenaka (Osaka Medical College), Drs Yuko Saito, Masafumi Sakagami (Hyogo College of Medicine), Dr Yasushi Naito (Kobe City Medical Center General Hospital), Drs Keiji Fujihara, Akihiro Sakai and Noboru Yamanaka (Wakayama Medical University), Drs Kunihiro Fukushima, and Kazunori Nishizaki (Okayama University), Drs Kazuma Sugahara and Hiroshi Yamashita (Yamaguchi University), Drs Naoto Hato and Kiyofumi Gyo (Ehime University), Drs Yasuhiro Kakazu and Shizuo Komune (Kyushu University), Drs Mayumi Sugamura and Takashi Nakagawa (Fukuoka University), Dr Haruo Takahashi (Nagasaki University), Dr Yukihiko Kanda (Kanda ENT Clinic), Drs Hirokazu Kawano and Tetsuya Tono (Miyazaki Medical College), Drs Ikuyo Miyanohara andYuichi Kurono (Kagoshima University), Drs Akira Ganaha and Mikio Suzuki (Ryukyus University), for providing samples of their patients. We also thank AC Apple-Mathews for help in preparing the manuscript.

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Ganaha et al. BMC Medical Genetics 2013, 14:56
http://www.biomedcentral.com/1471-2350/14/56

BMC Medical Genetics

RESEARCH ARTICLE Open Access

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 *SLC26A* 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

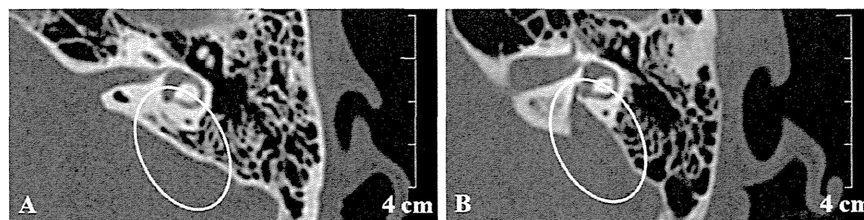

Figure 1 Computed tomography of the temporal bone showing an enlarged vestibular aqueduct. Circles show the vestibular aqueduct. The vestibular aqueduct is not identified in control subject (**A**). The enlarged vestibular aqueduct is identified in a patient with EVA (**B**).

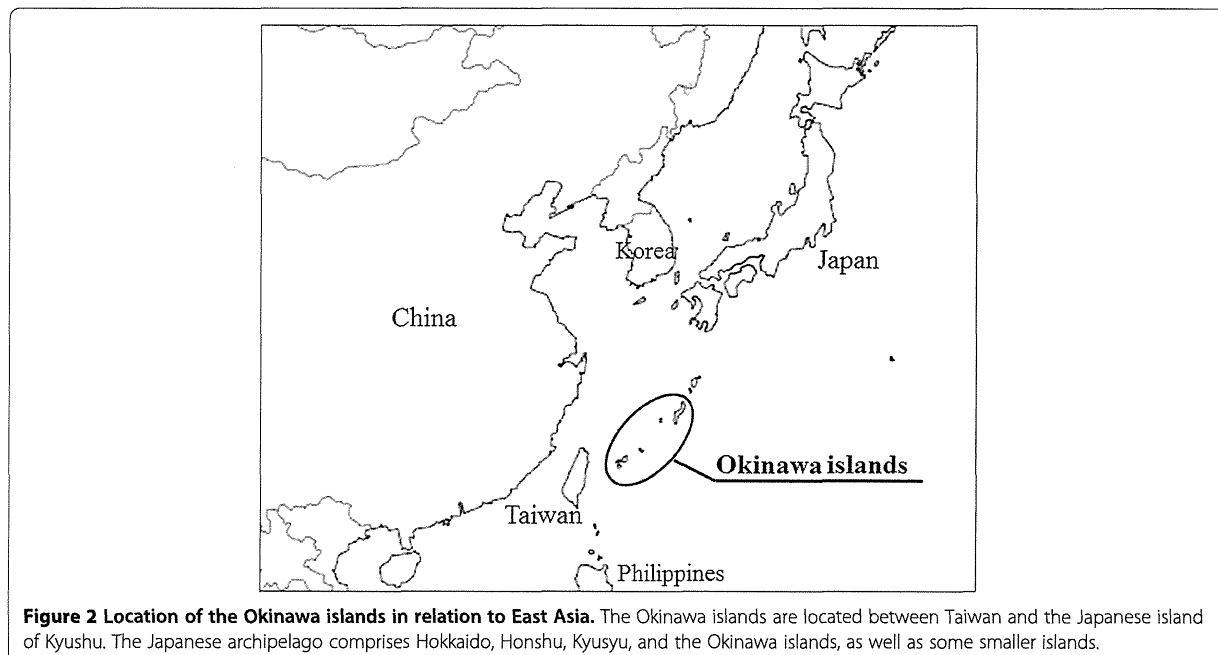

Figure 2 Location of the Okinawa islands in relation to East Asia. The Okinawa islands are located between Taiwan and the Japanese island of Kyushu. The Japanese archipelago comprises Hokkaido, Honshu, Kyusyu, and the Okinawa islands, as well as some smaller islands.

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 × l 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 $MgCl_2$, with distilled water (H_2O) 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			PTA		Vertigo	Thyroid	
			EVA	MD	VE	HL (dB)	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	TCTTGGAATGGCCTTGGAAGC	282
	Exon 17	reverse	TGAAACAGCATCACTTATGATGC	
Second-step PCR (internal primer)	Exon 15	forward	TGAAGAACCTCAAGGAGTGAAG	154
	Exon 16	reverse	TTTCTGTATTTTCCTCAGCGCT	

reaction volume was adjusted to 20 μl with distilled H_2O. 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 ΔCt 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. ΔΔCT 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

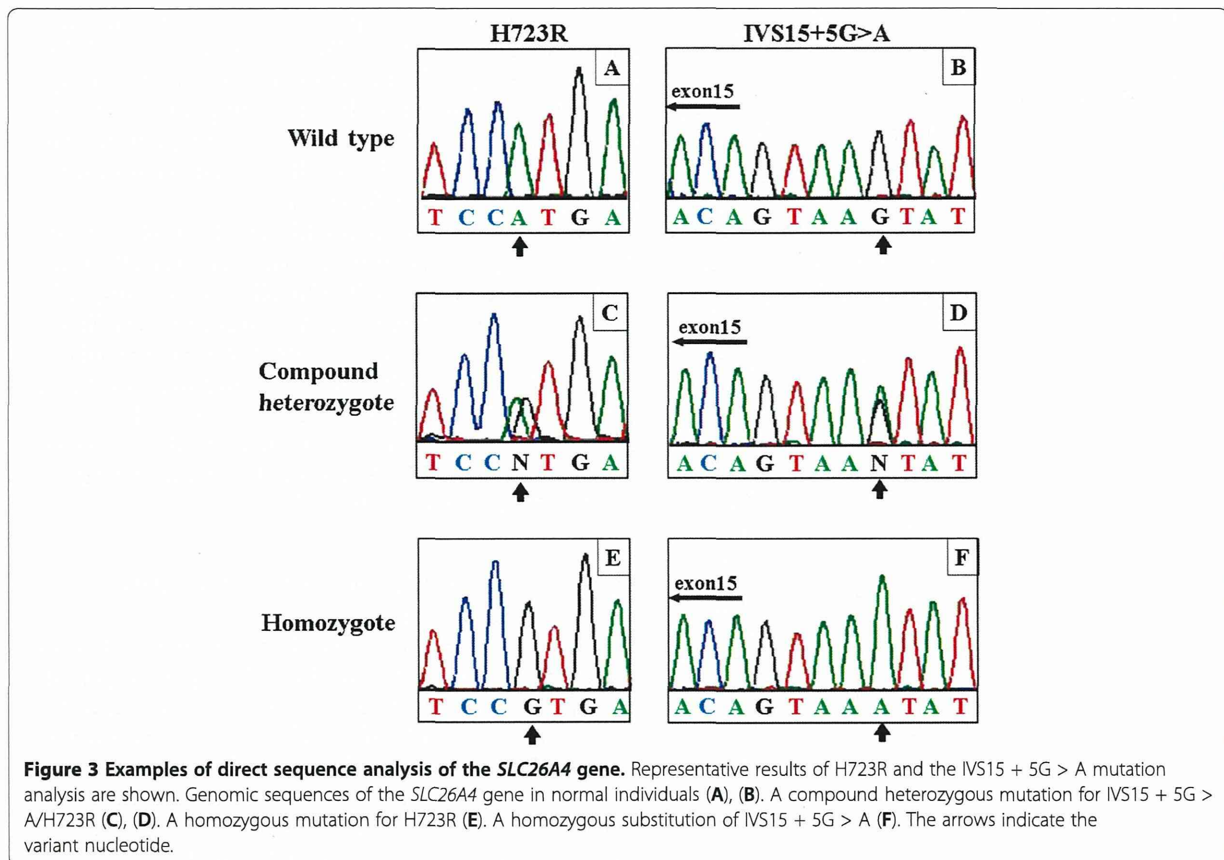

Figure 3 Examples of direct sequence analysis of the *SLC26A4* gene. Representative results of H723R and the IVS15 + 5G > A mutation analysis are shown. Genomic sequences of the *SLC26A4* gene in normal individuals (**A**), (**B**). A compound heterozygous mutation for IVS15 + 5G > A/H723R (**C**), (**D**). 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 ± 441.5 (standard deviation), 2417 ± 189.5, and 4956 ± 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 ± 79.12, 2846 ± 206.5 and 1183 ± 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.

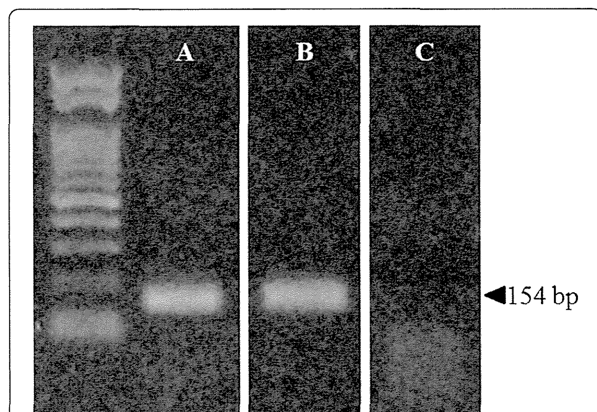

Figure 4 Expression of the *SLC26A4* gene in patients with PS or EVA. The expected RT-nested PCR amplification product of *SLC26A4* was 154 base pairs (bp) in length. Agarose gel electrophoresis shows the 154 bp band for the control subject (**A**) and the patient with IVS15 + 5G > A/H723R compound heterozygous mutation (**B**); however, there was no band for the patient with IVS15 + 5G > A homozygous substitution (**C**).

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

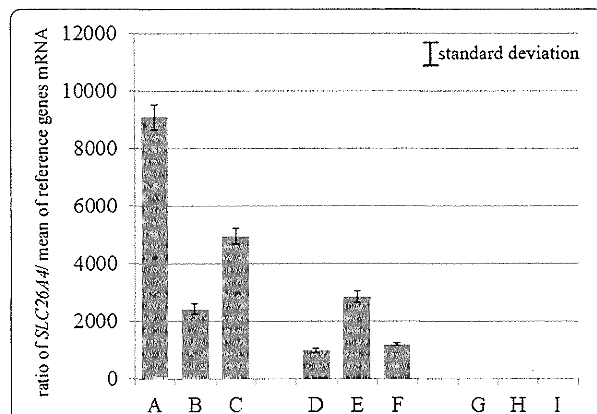

Figure 5 Relative expression of the *SLC26A4* gene in control subjects and in patients with a homozygous mutation of IVS15 + 5G > A or compound heterozygous mutation of IVS15 + 5G > A/H723R. The ratio of SLC26A4 mRNA to GAPDH mRNA is shown in three control subjects (**A**, **B**, **C**), three patients with compound heterozygous mutation of IVS15 + 5G > A/H723R (**D**, **E**, **F**), and three patients with IVS15 + 5G > A homozygous substitution (**G**, **H**, **I**). No expression of *SLC26A4* was observed in the three patients with the IVS15 + 5G > A homozygous substitution (**G**, **H**, **I**). All experiments were done in tripicate.

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 heterozyote 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.

Acknowledgements
This work was supported in part by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant-in-Aid for Young Scientists (B) Number 23791914, by Special Account Budget for Education and Research from the Japan Ministry of Education and by research grants from the Japan Ministry of Health, Labor, and Welfare.

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Received: 18 January 2013 Accepted: 17 May 2013
Published: 24 May 2013

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doi:10.1186/1471-2350-14-56
Cite this article as: Ganaha *et al.*: Pathogenic substitution of IVS15 + 5G > A in *SLC26A4* in patients of Okinawa Islands with enlarged vestibular aqueduct syndrome or Pendred syndrome. *BMC Medical Genetics* 2013 14:56.

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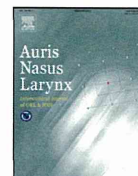

Long term speech perception after cochlear implant in pediatric patients with *GJB2* mutations

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ARTICLE INFO

Article history:
Received 4 September 2012
Accepted 24 January 2013
Available online 9 March 2013

Keywords:
GJB2 hearing impairments
Cochlear implant
Genetic testing
Hereditary hearing loss

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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http://dx.doi.org/10.1016/j.anl.2013.01.006

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 postoperative 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 ± 12.9 (range, 0–31) preoperatively. The averaged IT-MAIS score at 2 years after CI increased up to 33.6 ± 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

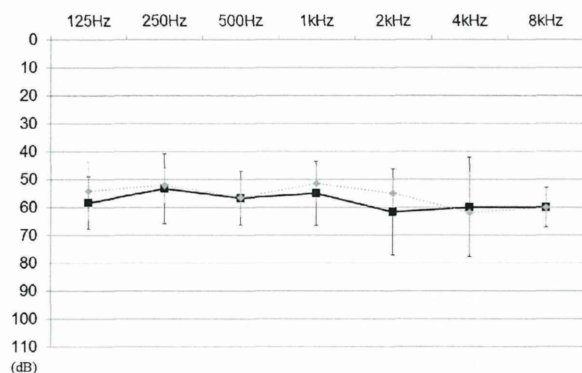

Fig. 1. Results of the average level of preoperative aided hearing thresholds at each frequency. Diamond dots and solid line: control group; square dots and solid line: *GJB2* group; bars: indicate two standard deviations.

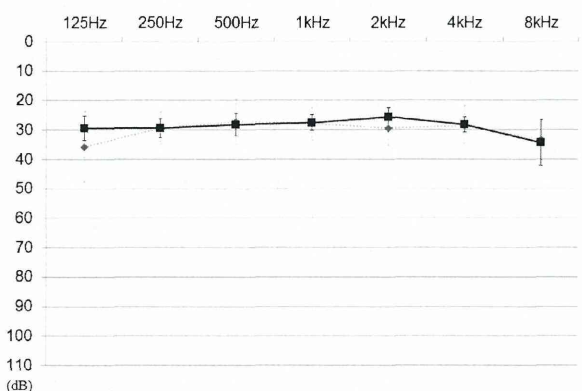

Fig. 2. Results of the average level of postoperative hearing thresholds with CI at each frequency. Diamond dots and solid line: control group; square dots and solid line: *GJB2* group; bars: indicate two standard deviations.

was 30.4 ± 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.

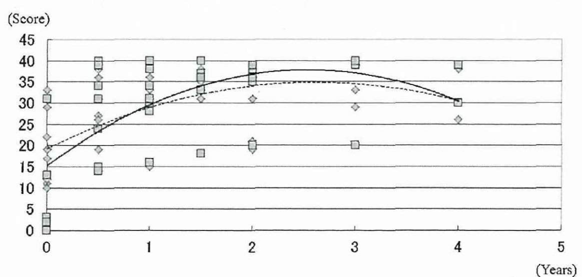

Fig. 3. Results of the difference of IT-MAIS scores from 0 years (=preoperative) to 4 years after CI. Diamond dots: scores in the control group; square dots: scores in the *GJB2* group; dotted line: trend line in the control group; solid line: trend line in the *GJB2* group.

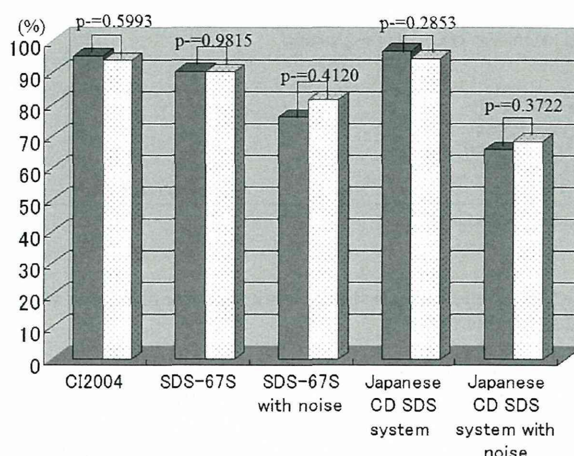

Fig. 4. Results of speech perception skills examined by using CI 2004, SDS-67S, and Japanese CD SDS system (TY-89). Longitudinal axis indicates the correct answer rate (%). Gray bars: control group; dotted bars: *GJB2* group.

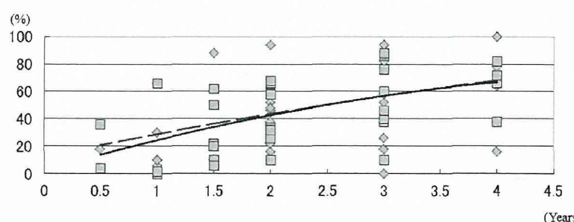

Fig. 5. Results of the development of articulation from 0.5 to 4 years after CI. Diamond dots: accuracy rates in the control group; square dots: accuracy rates in the *GJB2* group.

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

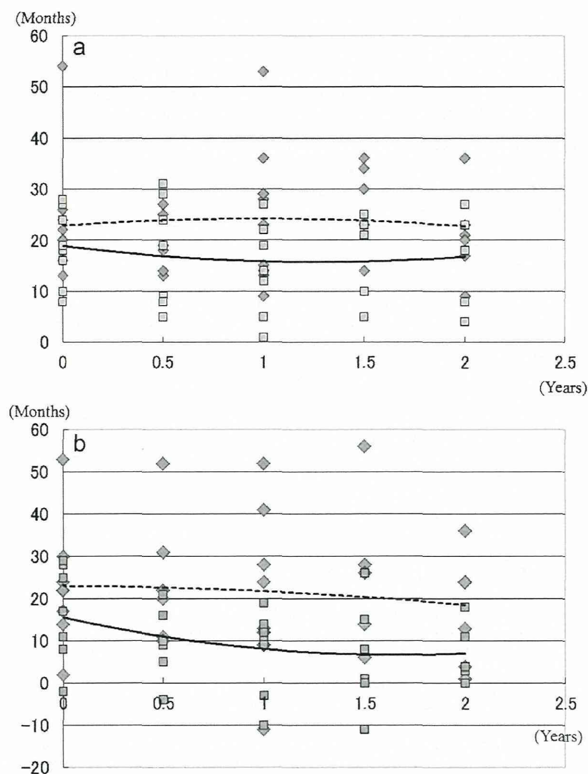

Fig. 6. Results of the developmental course of language perception (a) and production (b) in the control and *GJB2* groups examined by Enjoji Scale of Infant Analytical Development test. Diamond dots: scores in the control group; square dots: scores in the *GJB2* group; dotted line: trend line in the 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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新生児聴覚スクリーニング

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【要　旨】
新生児聴覚スクリーニング（NHS）は，2001年より厚生労働省のモデル事業として始まり耳音響放射（OAE）あるいはAABR（自動ABR）を用いて全国的に実施されている．2007～2009年の期間について，日本耳鼻咽喉科学会が調べたところ，実施率は約60％である．スクリーニングでreferとされたもののうち，両側の難聴は約25％であった．この中に高度難聴も軽～中等度難聴も含まれる．NHSの実施されなかった35％の新生児は，その大半がNHSの機器を所有しない個人産科医院での出生であった．現在のNHSは有料であるが，米国や台湾のように無料にしない限り，実施率の向上は困難であろう．現在の問題点を「不都合な現実」として関連する多くの問題点を解説した．近年の研究で先天性難聴の約半数近くは難聴遺伝子変異であることがわかっている．これによる新たな社会問題が生まれている．周産期難聴については，米国の周産期難聴のハイリスクファクターは日本の現実に合わせて新たにmajor factor6疾患，minor factor7疾患に分けて著者らの考えを解説した．

【キーワード】
新生児聴覚スクリーニング，精密聴力検査，ABR，人工内耳，言語発達

Ⅰ．新生児聴覚スクリーニングの歴史と現状

2001年からわが国でも導入された新生児聴覚スクリーニングは，すでに10年が過ぎた．それ以前は先天性難聴は2～3歳で発見されたが，現在は60％は生後1～2ヶ月で発見されるようになった[1]．新生児聴覚スクリーニングのコンセプトは優れており，早期発見がされる難聴児が増えている．しかし，地域格差，専門家の不足，療育施設が少ないなど現実には問題が多い．著者らは新生児聴覚スクリーニング導入後の新たな問題を「不都合な現実」と呼んでいる．具体的には，産科でのスクリーニングデータの取り扱いの間違った判断，ABR（auditory brainstem response，聴性脳幹反応）が正しく判読されていない，ABRが変化する生理学的反応であることが知られていない，一部のろう学校教師の人工内耳に対しての理解が不足しているなどさまざまな問題がある[2]．

新生児聴覚スクリーニングは2001年より5年間厚生労働省のモデル事業として医療機関に援助があり無料で始まった．しかしその後の実施は都道府県に任され，有料で行われている．使い捨てのイヤフォンと電極が数千円するためであるが，産科では5,000円から10,000円を設定している．全国の実施率はまだ60％程度にすぎないが，米国や台湾では100％近い高率であるのは無料であるからである．

新生児聴覚スクリーニングは以下の2つの方

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法のどちらかで行われている．すなわち，①自動ABR（automated auditory brainstem response：AABR）．スクリーニングレベルは35～40dB．機器の価格は約400万円．②耳音響放射（oto acoustic emission）はTransient OAEとDistortion Product OAEの2つがあり，スクリーニングレベルは20dB前後．機器の価格は300万円前後．

AABR, OAEともスクリーニングレベルが低いため，表示される結果のPass（合格）は信頼性が高いが，Refer（不合格）となった場合でも，真の難聴はその一部に過ぎない．次のステップの精密聴力検査で初めて難聴の有無がわかる．

精密聴力検査のABRを行うと，半数以上は正常である．ABRの閾値が高いとしても軽度，中等度，高度，重度の難聴のいずれもあり得るため，新生児聴覚スクリーニングでReferとされたとしてもその時点では確定的なことはいえない．表1に，新生児聴覚スクリーニングの現実として，検査装置が備えられていない場合の約40%と，備えられている約60%に分けて問題と課題を示した．

個人の産科医院で備えられていないところの方が多く，その理由は機器の価格が高いものであることが挙げられる．しかし東京の大学病院や総合病院の産科でも実施していないところが少なくなく，見逃されている．先天性難聴は1,000の出生に対して1～2人という低率であるということと，先天性代謝スクリーニングが尿や血液で済むのにもかかわらず，AABRではイヤフォンをつける，電極をはる，OAEではプローブを耳に入れるなど，ある程度の技術が必要なため人材養成が必要であり，面倒がられるため避けている病院もある．しかし，著者らの外来では，スクリーニングのなかった産科医院や病院で生まれた新生児の親が心配して受診することが少なくない．結果的には聴力に問題のある場合もない場合もある．

表1．新生児聴覚スクリーニングの現実

1．スクリーニング装置が備えられていない（全国の約40%）場合

① 個人医院
② 都市の総合病院
③ 大学病院

課題：①先天性難聴の発見が1～2歳と遅れる
②母子手帳の項目に含まれていない
③母子手帳の代謝スクリーニングに含まれていると誤解される

2．スクリーニング装置がある場合（60%）

① 任意検査のため希望しない（検査費用約1万円と高い）
② Referの表示だけで難聴と診断する
（スクリーニングレベルは耳音響放射が25dB，自動ABRは40dBと低い）

課題：①無料，全員検査が必要（米国95%，台湾100%）
②referの要精密聴力検査は確定診断ではない
③DPOAE・referは正常である可能性が否定できない
④AABR・referはAuditory Neuropathyを否定できない

母子手帳に，2012年4月より新生児聴覚スクリーニングを受けたか否かが印刷されることになった．それ以前の母子手帳には記載がなく，先天性代謝スクリーニングの項目に含まれているものと誤解している母親が少なくなかった．今後，母子手帳に印刷された後どうなるであろうか．実施している医療機関で結果を記載しても，そうでない個人病院では聴覚検査のために耳鼻咽喉科に受診をすすめるであろうか．今後産科医と母親がどのように行動するかが注目される．

新生児聴覚スクリーニング後，refer（不合格）と判定された乳児は，耳鼻咽喉科学会で認定された全国150の機関に精密聴力検査のために紹介される．著者らのところには総合病院の産科，新生児科から紹介されてくる．このルート以外に他の耳鼻科を受診した後にセカンドオピニオンを求め，インターネットで調べて著者の外来に受診することも少なくない．検査は他覚的聴力検査のABRを中心に，DPOAEやティンパノメトリーも行う．滲出性中耳炎の合併が少なくないからである．著者は必ず伝声管で名前を呼びかけ音に対する反応を観察するが，これは簡単ですぐできるよい方法で，両親は目の前で音に対する反応を観察でき安心できる（図19）．行動反応聴力検査も必ず行う．ABRは脳幹の誘発電位にあるが，1回の検査で確定することはできない．とくに生後1年の間は軽度あるいは正常化することがあるからである．また，CMV感染のように初め正常であったものが悪化することもある（表2）．ABRは高い信頼がおける反応であるが，ABRが2～6kHzの範囲からなるクリック音で誘発されるため，オージオグラムのすべての周波数をカバーするわけではない．そのため周波数別に検査が可能なASSR（Auditory

Steady-State Response）の結果を参考にする．オージオグラム上に結果だけがプロットされる．しかし波形が記録されるわけではない．まずABR，次いでASSR，そして聴性行動反応や発達の変化を合わせて合理的に考えなければ正しい診断はできない[3]．

難聴が明らかになった場合，生後6か月までに補聴器を装用させ教育を開始する．

難聴児の教育には，①聴覚口話法，②聴覚口話に手話併用，③手話法に分かれる．表3に現状をまとめた．なぜこのように分かれるのであ

表2．ABRによる精密聴力検査の現実

＊全国で150機関と少ない

1．ABRの多様性と行動反応

①ABRの正常化と行動反応の改善（ダウン症に多い）
②ABRにより中等度難聴の疑い
③ABR無反応（高度難聴と診断して良いか）

課題：①ABRは成長とともに変化することが知られていない
②1回の検査で済むとみなす医師が多い
③ABR正常でも成長とともに悪化することがある（CMV，LVAS）

2．DPOAEとABRの関係

超出生低体重児におけるAuditory Neuropathy Spectrum Disorders症例の増加
3つのタイプがある（不変，重度難聴化，正常化）

課題：Auditory Neuropathy Spectrum Disorderであっても
聴覚正常例と難聴症例の両方が存在する

表3．難聴児の療育の現実

1．聴覚口話法の教育施設の不足
①通える希望施設が定員一杯
②私的施設の登場

2．公立ろう学校の手話併用とアンチ人工内耳感情

3．難聴は病気でないという思想と手話の重視

4．中等度難聴児に対する補聴器交付制度を欠く
（例外的な地域があり，耳鼻科医による地方自治体の交付運動の増加）

表4．人工内耳手術をめぐる現実

1．新生児聴覚スクリーニングで発見された場合
手術年齢：**1歳6ヶ月～2歳**

2．新生児聴覚スクリーニングがなかったために発見が遅れた場合
手術年齢：**2～4歳**

3．普通小・中学校就学児の増加
バリアフリーに基づく公的聴覚補償なし（大学はある場合がある）

4．全国ろう学校の生徒における人工内耳装用児の増加
（約5,000人中1,000人と20%を占める）

5．人工内耳手術の適応の拡大
他疾患合併児（発達障害，脳性麻痺他）も対象

ろうか．難聴児の将来に対する療育・教育思想の違いがあり，それぞれの教育法によって形成される人間像は異なる．重大なことはいずれの教育法を選んでも後でやり直しがきかないことである．

新生児聴覚スクリーニングの導入によって，難聴児の早期発見・早期教育は著しく影響を受けてきた（表4）．著者らの場合は新生児聴覚スクリーニング以前は人工内耳手術の年齢は3～4歳であったが，現在は2歳前後で手術を行っている[4]．その結果，小学校入学時の言語性IQをWPPSI検査でみると，発見年齢の早い方が，より高い言語力を身につけることがわかる（図1）[5]．

新生児聴覚スクリーニングは価値が高い方法である．結果的に以前に比べ高い言語力を身につけることにつながり，成人後は自立し社会によりスムーズに共生することに発展する可能性が高いからである．人工内耳装用下で医師として活躍する人も現れ，大きな希望につながっている[6,7]．

Ⅱ．新生児聴覚スクリーニングと不都合な現実

2001年に始まった新生児聴覚スクリーニングは5年間のモデル事業を経て，その実施は地方に任され，最初の計画とは異なり，希望者に対して有料で行われている．そのために生じた問題と，既に10年が過ぎた今日，何が問題かを「不都合な現実」として厚生労働科学研究費の

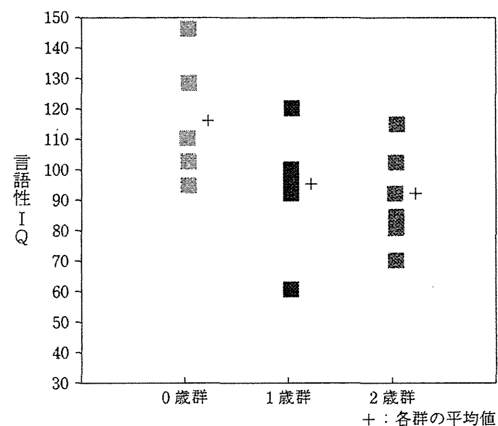

図1．人工内耳装用児の療育開始年齢と6歳時点でのWPPSI検査言語性IQ[5]

現在5～7歳に達した聴覚・言語障害で乳幼児期に受診した症例の1/3しか新生児聴覚スクリーニングを受けていない

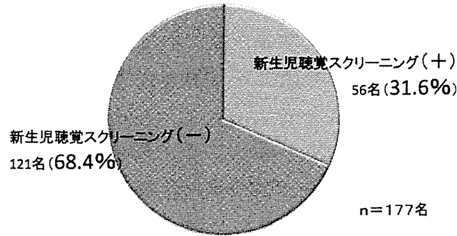

図2．東京医療センターの幼小児難聴・言語障害クリニックにおける新生児聴覚スクリーニングを経た症例の割合

スクリーニングの無い医療機関

1. 産科医院
2. 大学病院（東京/神奈川の例）
   T大病院，S大病院，J大病院，Y市大病院，T病院，S総合病院

①ただし，NICUでは行われている

②母親が難聴を疑い新たな病院を探すが，精密聴力検査が遅れ，療育も遅れる

図3．新生児聴覚スクリーニングを実施していない施設

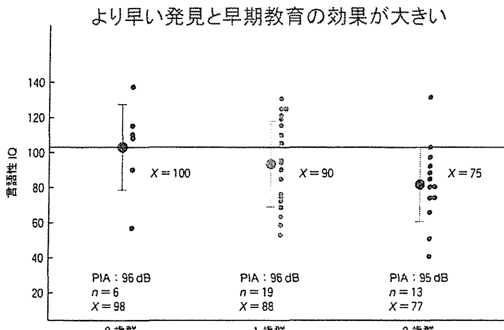

図4．先天性難聴幼児の発見・補聴教育開始年齢と小学校入学時の言語性IQの比較
（富士見台聴こえとことばの教室）

	症例数	平均年齢・標準偏差（歳）
新生児聴覚スクリーニングでrefer	23	2.4 ± 0.6 最低年齢 1歳7ヶ月 最高年齢 3歳10ヶ月
新生児聴覚スクリーニングの機会無し	33	3.3 ± 1.4 最低年齢 1歳9ヶ月 最高年齢 4歳8ヶ月
新生児聴覚スクリーニングでpass しかし後に難聴判明	5	3.7 ± 0.5 最低年齢 3歳1ヶ月 最高年齢 4歳4ヶ月

図5．新生児聴覚スクリーニングと人工内耳手術児の年齢の比較
（東京医療センター／幼小児難聴・言語障害クリニック）

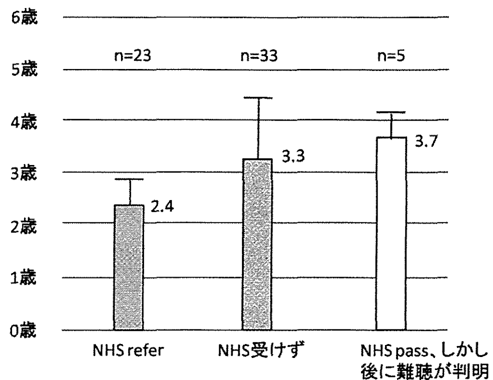

図6．新生児聴覚スクリーニングと人工内耳手術児の年齢の比較
（富士見台聴こえとことばの教室）

支援を受けて，平成22年～24年の3年間取り上げ，その対策を検討した．

1．幼小児難聴・言語障害クリニックにおける新生児聴覚スクリーニングを経た症例の割合について（図2, 3）

新生児聴覚スクリーニングの実施率について平成24年度に5～7歳に達した症例で，初診時，聴覚・言語障害を主訴に受診した症例は177例で，そのうち新生児聴覚スクリーニングを受けていたのは31.6%に過ぎないことがわかった．受けなかった約70%は，ほとんどが新生児聴覚スクリーニングの機器を持たない個人の産科で出生したことがわかった．機器を持つ個人の産科で出産したが，有料であるために希望しなかった症例もあった．

2．先天性難聴児の発見と補聴教育開始年齢と小学校入学時の言語性IQの比較（図4）

難聴発見年齢が0歳の6例，1歳の19例，2歳の13例．難聴幼児通園施設で補聴器の指導下で教育を受け，就学時での言語性IQをWPPSI検査を行い比較した．その結果，平均値で比較すると発見年齢0歳はVIQ100，1歳はVIQ90，2歳はVIQ75であり，より早い発見と早期教育の効果が大きいことがわかった．