OPEN ACCESS Freely available online

PLoS one

Simultaneous Screening of Multiple Mutations by Invader Assay Improves Molecular Diagnosis of Hereditary Hearing Loss: A Multicenter Study

Shin-ichi Usami[1]*, Shin-ya Nishio[1], Makoto Nagano[2], Satoko Abe[2], Toshikazu Yamaguchi[2], the Deafness Gene Study Consortium[¶]

1 Department of Otorhinolaryngology, Shinshu University School of Medicine, Asahi, Matsumoto, Japan, **2** Department of Clinical Genomics, Biomedical Laboratories, Inc., Matoba, Kawagoe-shi, Saitama, Japan

Abstract

Although etiological studies have shown genetic disorders to be a common cause of congenital/early-onset sensorineural hearing loss, there have been no detailed multicenter studies based on genetic testing. In the present report, 264 Japanese patients with bilateral sensorineural hearing loss from 33 ENT departments nationwide participated. For these patients, we first applied the Invader assay for screening 47 known mutations of 13 known deafness genes, followed by direct sequencing as necessary. A total of 78 (29.5%) subjects had at least one deafness gene mutation. Mutations were more frequently found in the patients with congenital or early-onset hearing loss, i.e., in those with an awareness age of 0–6 years, mutations were significantly higher (41.8%) than in patients with an older age of awareness (16.0%). Among the 13 genes, mutations in *GJB2* and *SLC26A4* were mainly found in congenital or early-onset patients, in contrast with mitochondrial mutations (12S rRNA m.1555A>G, tRNA(Leu(UUR)) m.3243A>G), which were predominantly found in older-onset patients. The present method of simultaneous screening of multiple deafness mutations by Invader assay followed by direct sequencing will enable us to detect deafness mutations in an efficient and practical manner for clinical use.

Citation: Usami S-i, Nishio S-y, Nagano M, Abe S, Yamaguchi T, et al. (2012) Simultaneous Screening of Multiple Mutations by Invader Assay Improves Molecular Diagnosis of Hereditary Hearing Loss: A Multicenter Study. PLoS ONE 7(2): e31276. doi:10.1371/journal.pone.0031276

Editor: Francesc Palau, Instituto de Ciencia de Materiales de Madrid - Instituto de Biomedicina de Valencia, Spain

Received July 21, 2011; **Accepted** January 5, 2012; **Published** February 24, 2012

Funding: This work was supported by the (then) Ministry of Health and Welfare, Japan (http://www.mhlw.go.jp/english/) (S.U.) and a Grant-in-Aid for Scientific Research from the (then) Ministry of Education, Science and Culture of Japan (http://www.mext.go.jp/english/) (S.U.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have read the journal's policy and have the following conflicts. The authors did not receive funding from the Department of Clinical Genomics, Biomedical Laboratories, Inc. They felt that for genetic analysis of patients with hearing impairment in which many gene/gene mutations are involved, Invader Assay is the appropriate choice. However, for patent reasons, the authors cannot develop this method independently. The development of this method was therefore performed in collaboration with Biomedical Laboratories. This relationship had no influence on results and the direct sequencing results were all double checked for accuracy. Although Invader Assay is more efficient, if a method other than Invader Assay had been used, the results would have been identical.

* E-mail: usami@shinshu-u.ac.jp

¶ Membership of the Deafness Gene Study Consortium is provided in the Acknowledgments.

Introduction

From a series of etiological studies, 60–70% of childhood hearing loss has been estimated to be of genetic etiology, with the rest due to environmental causes, including newborn delivery trouble, acoustic trauma, ototoxic drug use, and prenatal/postnatal infection [1]. However, until now, there has been no multicenter study based on genetic testing. Along with early discovery of hearing loss by newborn hearing screening programs and subsequent intervention programs, much attention has been paid to the determination of the hearing loss etiology. Therefore, genetic testing has become more important for highly accurate diagnosis, prediction of severity of hearing loss, estimation of associated abnormalities, selection of appropriate habilitation options, prevention of hearing loss, and better genetic counseling. Although more than one hundred loci have been mapped and 46 genes reported to be responsible for hereditary hearing loss (Hereditary Hearing Homepage; http://webh01.ua.ac.be/hhh/), many may cause similar phenotypes without any abnormality other than hearing loss. This genetic heterogeneity has made clinical application difficult, in spite of the considerable advances in discovery of deafness genes. We have previously established a screening strategy focusing on recurrent mutations and demonstrated its benefits for clinical application [2]. We carried out the current multicenter study to determine 1) whether the simultaneous screening of the multiple deafness mutations by Invader assay is applicable for clinical use, 2) whether the genetic etiology is truly prevalent among hearing loss patients and 3) whether genetic causes differ by ages.

Materials and Methods

Subjects and clinical status

As summarized in Table 1, two hundred sixty-four Japanese patients with bilateral sensorineural hearing loss from 33 ENT departments nationwide participated in the present study. We first applied the Invader assay for screening forty-seven known mutations of 13 known deafness genes, followed by direct sequencing as necessary.

Table 1. Clinical features of subjects in this study.

	Total (n = 264)	*Early onset (n = 141)*	*Late onset (n = 100)*
Severity of HL			
normal – moderate	148	58	78
severe – profound	95	70	21
unknown	21	13	1
Inheritance			
AD or Mitochondrial	38	9	24
AR or Sporadic	119	69	42
unknown	107	63	34
Other clinical features			
inner ear malformations	52	37	10
EVA	30	22	4
goiter	8	4	3
diabetes mellitus	14	3	11

HL: Hearing loss.
AD: Autosomal dominant.
AR: Autosomal recessive.
EVA: Enlarged vestibular aqueduct.
doi:10.1371/journal.pone.0031276.t001

Hearing loss was evaluated using pure-tone audiometry (PTA) classified by a pure-tone average over 500, 1000, 2000 and 4000 Hz in the better hearing ears. For children who were unable to be tested by PTA, we used an average over 500, 1000, 2000 Hz in either auditory steady-stem response (ASSR) or conditioned oriented reflex audiometry (COR), or the response threshold (dB) from auditory brainstem response (ABR). Computed tomography (CT) scans were performed to check for congenital inner ear anomalies.

Status of hearing loss in the 264 patients was: mild (21–40 dB) in 39 patients (14.7%), moderate (41–70 dB) in 84 (31.8%), severe (71–94 dB) in 39 (14.8%) and profound (>95 dB) in 56 patients (21.2%). Twenty-four subjects were classified as having normal hearing due to a specific audiogram with hearing loss only in the high or low frequency portions. With regard to onset age (the age of awareness), 141 patients had early onset deafness (below 6 y.o.), 100 had late onset deafness, and the rest had unknown onset ages.

The inheritance composition of the subjects was as follows: 38 subjects from autosomal dominant or mitochondrial inherited families (two or more generations affected); 119 subjects from autosomal recessive families (parents with normal hearing and two or more affected siblings) or subjects with sporadic deafness (also compatible with recessive inheritance or non-genetic hearing loss). None of the patients had an X-linked pattern of inheritance. The numbers of patients with other manifestations were inner ear malformations (52), enlarged vestibular aqueduct (EVA) (30), goiter (8), and diabetes mellitus (14). None of the patients had typical clinical features of Usher syndrome or BOR syndrome.

All subjects gave prior informed consent for participation in the project and the Ethical Committee of Shinshu University as well as the relevant bodies of the participating institutions of the Deafness Gene Study Consortium approved the study.

Invader assay

Invader technology is convenient for mutation genotyping, offering a simple diagnostic platform to detect single nucleotide changes with high specificity and sensitivity from unamplified genomic DNA.

We applied the Invader assay for screening forty-seven known mutations of 13 known deafness genes [*GJB2*(NM_004004.5), *SLC26A4*(NM_000441.1), *COCH*(NM_001135058.1), *KCNQ4*(NM_172163.2), *MYO7A*(NM_000260.3), *TECTA*(NM_005422.2), *CRYM* (NM_001888.3), *POU3F4*(NM_000307.3), *EYA1*(NM_172060.2), mitochondrial 12 s ribosomal RNA, mitochondrial tRNA(Leu), mitochondrial tRNA(Ser), and mitochondrial tRNA(Lys)] (Table 2). Mutations were selected on the basis of a mutation/gene database established in the Japanese deafness population. The detailed methodological protocol was described elsewhere [2]. In brief, 1.2 ul of primary probe/Invader oligonucleotides mixture (containing 0.5 umol/l wild type primary probes, 0.5 umol/l mutant primary probe, 0.05 umol/l Invader oligonucleotide, and 10 mmol/l MOPS) were poured into each well of 384-well plates. Fluorescent resonance energy transfer (FRET)/Cleavase mixture (Third Wave Technologies, Madison, WI) was added to the probe/Invader oligonucleotide-containing plates. Then, 3 ul of 5–100 fmol/l synthetic target oligonucleotides (positive control), 10 ug/ml yeast tRNA (no target control), and denatured genomic DNA samples (>15 ng/ul) were added. Next, 6 ul of mineral oil (Sigma, St. Louis, MO) were overlayed into all reaction wells and incubated at 63°C for 4 hour. After incubation fluorescence was measured by a Cyto Fluor 4000 fluorescent micro plate reader (Applied Biosystems, Foster CA). The heteroplasmy rate for mitochondrial mutations was quantified by detection of fluorescently labeled and digested PCR products through a fluorescence imaging system [2].

Direct sequencing

Dominant mutations and mitochondrial mutations are themselves diagnostic criteria for molecular diagnosis. But a hallmark of recessive mutations, in *GJB2* and *SLC26A4* for example, is the detection of two mutations in the paternal and maternal alleles. In this study, direct sequencing was further carried out as follows: 1) *GJB2* mutation analysis for all subjects, because the authors wanted to clarify whether the number of mutations on the invader panel are enough (saturated) or not. 2) *SLC26A4* mutation analysis for all the subjects with EVA, 3) *SLC26A4* mutation analysis for heterozygous patients for these genes. DNA fragments containing the entire coding region were sequenced as described elsewhere [3,4].

Results

The mutations found by Invader assay and direct sequencing in this study are summarized in Table 2 and 3.

Invader Assay

A total of 74 (28.0%) hearing-impaired subjects (n = 264) were found to have at least one deafness gene mutation. Among the deafness genes situated on the present diagnostic panel, mutations were most frequently found in the *GJB2* gene. Screening of *GJB2* showed mutations of one or both alleles of the gene in 43 (43/264; 16.2%) samples from the subjects, of which 13 cases had only a single mutation, and 30 cases were compound heterozygotes or homozygotes, confirmed by segregation analysis (Table 4). The most common mutation was c.235delC, accounting for nearly 67% (29/43) of all *GJB2* mutated patients. On the other hand, the *GJB2*: c.35delG mutation, which is known to be the most common mutation in Caucasian or other ethnic populations, was not found in this group. The second most common group of *GJB2* mutations consisted of p.[G45E; Y136X], p.V37I, and c.299_300del. These mutations were detected in more than 5 patients each, and their allele frequencies were relatively high. Three mutations (p.T86R, p.R143W, and c.176_191del) were observed in more than one

Table 2. Mutation list of Invader based genetic screening test.

Gene	*Exon*	*Codon location*	*Nucleotide change*	*Frequency of mutant alleles (n = 528)*	*Number of patients with mutations (n = 264)*
GJB2	exon 2	p.L79fs	c.235delC	43 (8.1%)	29 (10.9%)
GJB2	exon 2	p.V37I	c.109G>A	7 (1.3%)	6 (2.3%)
GJB2	exon 2	p.[G45E; Y136X]	c.[134G>A; 408C>A]	10 (1.9%)	10 (3.8%)
GJB2	exon 2	p.G59fs	c.176_191del	3 (0.6%)	3 (1.1%)
GJB2	exon 2	p.R143W	c.427C>T	4 (0.8%)	4 (1.5%)
GJB2	exon 2	p.H100fs	c.299_300del	5 (0.9%)	5 (1.9%)
GJB2	exon 2	p.T123N	c.368C>A	4 (0.8%)	4 (1.5%)
GJB2	exon 2	p.T86R	c.257C>G	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.F191L	c.570T>C	0	0
GJB2	exon 2	p.I71T	c.212T>C	0	0
GJB2	exon 2	p.A49V	c.146C>T	0	0
GJB2	exon 2	p.G12fs	c.35delG	0	0
SLC26A4	exon 19	p.H723R	c.2168A>G	22 (4.1%)	17 (6.4%)
SLC26A4	int 7/exon 8	splice site	c.919-2A>G	2 (0.4%)	2 (0.8%)
SLC26A4	exon 7	p.T410M	c.1229C>T	4 (0.8%)	3 (1.1%)
SLC26A4	exon 7	p.V306fs	c.917insG	0	0
SLC26A4	exon 19	p.T721M	c.2162C>T	0	0
SLC26A4	exon 8/int 8	splice site	c.1001+1G>A	0	0
SLC26A4	exon 9	p.A372V	c.1115C>T	0	0
SLC26A4	exon 5	p.M147V	c.439A>G	1 (0.2%)	1 (0.4%)
SLC26A4	int 5/exon 6	splice site	c.601-1G>A	0	0
SLC26A4	exon 9	p.K369E	c.1105A>G	1 (0.2%)	1 (0.4%)
SLC26A4	exon 15	p.S551fs	c.1652insT	1 (0.2%)	1 (0.4%)
SLC26A4	exon 15	p.C565Y	c.1693G>A	0	0
SLC26A4	exon 17	p.S666F	c.1997C>T	0	0
SLC26A4	exon 19	p.E704fs	2111ins GCTGG	1 (0.2%)	1 (0.4%)
SLC26A4	exon 4	p.L108fs	c.322delC	0	0
SLC26A4	exon 4	p.P123S	c.367C>T	0	0
SLC26A4	exon 10	p.N392Y	c.1174A>T	0	0
SLC26A4	exon 17	p.S610X	c.1829C>A	0	0
SLC26A4	exon 17	p.S657N	c.1970G>A	0	0
EYA1	exon 12	p.D396G	c.1187A>G	0	0
EYA1	exon 8	p.R264X	c.790C>T	0	0
EYA1	exon 7	p.Y193X	c.579C>G	0	0
COCH	exon 5	p.A119T	c.441G>A	0	0
KCNQ4	exon 5	p.W276S	c.827G>C	0	0
MYO7A	exon22	p.A886fs	c.2656_2664del	0	0
TECTA	exon 16	p.R1773X	c.5318C>T	0	0
TECTA	exon 20	p.R2121H	c.6063G>A	0	0
Mitochondrial 12S rRNA			m.1555A>G	-	5 (1.9%)
Mitochondrial tRNALeu			m.3243A>G	-	6 (2.3%)
Mitochondrial tRNASer			m.7445A>G	-	0
Mitochondrial tRNALys			m.8296 A>G	-	0
CRYM	exon 8	p.K314T	c.941 A>C	0	0
CRYM	exon 8	p.X315Y	c.945 A>T	0	0

doi:10.1371/journal.pone.0031276.t002

Table 3. Mutation list found by direct sequencing analysis.

Gene	*Exon*	*Codon location*	*Nucleotide change*	*Frequency of mutant alleles (n = 528)*	*Number of patients with mutations (n = 264)*
GJB2	exon 2	p.T8M	c.23C>G	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.K12fs	c.35insG	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.F106Y	c.317T>A	1 (0.2%)	1 (0.4%)
GJB2	exon 2	p.A171fs	c.511insAACG	2 (0.4%)	2 (0.8%)
GJB2	exon 2	p.C174S	c.522G>C	1 (0.2%)	1 (0.4%)
SLC26A4	exon 14	p.S532I	c.1595G>T	2 (0.4%)	2 (0.8%)
SLC26A4	exon 16	p.R581S	c.1743G>C	1 (0.2%)	1 (0.4%)
SLC26A4	exon 17	p.V659L	c.1975G>C	2 (0.4%)	2 (0.8%)
SLC26A4	exon 10	p.L407fs	c.1219delCT	1 (0.2%)	1 (0.4%)
SLC26A4	exon 15/int 15	splice site	c.1931+5 G>A	5 (0.9%)	4 (1.5%)

doi:10.1371/journal.pone.0031276.t003

patient. p.F191L, p.I71T, p.A49V and c.35delG mutations were not found. One pair of p.[G45E; Y136X] mutations was detected among 10 persons in a heterozygous state. Subsequent parental DNA segregation study through direct sequencing indicated two mutations were in *cis*. The p.T123N mutation was found in 4 subjects but, based on our recent study, is not likely to be a pathologic mutation [5].

The second most frequent gene with mutations was the *SLC26A4* gene (23/264; 8.7%). Five cases were homozygotes of p.H723R, one was a homozygote of p.T410M, 3 were compound heterozygotes, and 14 had only one mutation of *SLC26A4* (Table 4). Of the 19 *SLC26A4* mutations, 12 (c.917insG, p.T721M, c.1001+1G>A, p.A372V, c.601-1G>A, p.C565Y, p.S666F, c.322delC, p.P123S, p.N392Y, p.S610X, and p.S657N) were not found in any samples, but the remaining 7 *SLC26A4* mutations were confirmed in more than one subject. Especially, the p.H723R mutation was found to be in high allele frequency (4.1%). All of the patients with *SLC26A4* mutations had EVA, which has been demonstrated to be a result of the mutations of this gene. *SLC26A4* mutations were detected by Invader assay in 63.6% of the patients with EVA.

Mitochondrial m.1555A>G mutations were found in 1.9% (5/264) of the patients and the m.3243A>G mutation was identified in 2.3% (6/264).

Mutations in nine deafness genes (*COCH*, *KCNQ4*, *MYO7A*, *TECTA*, *CRYM*, *POU3F4*, *EYA1*, mitochondrial tRNA(Lys) m.8296A>G, mitochondrial tRNA(Ser) m.7445A>G) were not identified in any patients (Table 2).

Notably, 4 subjects were found to have double gene mutations. Two cases were *SLC26A4* compound heterozygous or homozygous mutations with a *GJB2* heterozygous mutation. One case was a compound heterozygous of *GJB2* with a *SLC26A4* heterozygous mutation and the remaining case was a *GJB2*

Table 4. Diagnostic efficiency of Invader assay alone and Invader assay and direct sequencing.

	Total (n = 264)	*Early onset (n = 141)*	*Late onset (n = 100)*
Invader assay alone			
GJB2 homozygote/compound heterozygote	30 (11.4%)	29 (20.6%)	1 (1.0%)
GJB2 heterozygote	13 (4.9%)	7 (5.0%)	6 (6.0%)
SLC26A4 homozygote/compound heterozygote	9 (3.4%)	9 (6.4%)	0 (0%)
SLC26A4 heterozygote	14 (5.3%)	10 (27.1%)	2 (2.0%)
Mitochondria A1555G	5 (1.9%)	2 (1.4%)	2 (2.0%)
Mitochondria A3243G	6 (2.2%)	1 (0.7%)	5 (5.0%)
Total	74 (28.0%)*	55 (39.0%)*	16 (16.0%)
Invader assay and direct sequencing			
GJB2 homozygote/compound heterozygote	33 (12.5%)	31 (21.9%)	2 (2.0%)
GJB2 heterozygote	13 (4.9%)	7 (5.0%)	5 (5.0%)
SLC26A4 homozygote/compound heterozygote	18 (6.8%)	18 (12.7%)	0 (0%)
SLC26A4 heterozygote	7 (2.7%)	4 (2.8%)	2 (2.0%)
Mitochondria A1555G	5 (1.9%)	2 (1.4%)	2 (2.0%)
Mitochondria A3243G	6 (2.2%)	1 (0.7%)	5 (5.0%)
Total	78 (29.5%)**	59 (41.8%)**	16 (16.0%)

*Three cases carried double mutations (cases 1 to 3 in Table 5).
**Four cases carried double mutations shown in Table 5.
doi:10.1371/journal.pone.0031276.t004

Table 5. Double mutation cases found in simultaneous mutation screening.

Genotype	*Patients Number*
GJB2:p.[V37I];[V37I]; Mitochondria m.1555A>G	1 (0.4%)
GJB2:c.[235delC];p.[R143W]; *SLC26A4*:p.[M147V]	1 (0.4%)
GJB2:p.[V37I]; *SLC26A4*:p.[H723R];[H723R]	1 (0.4%)
GJB2:p.[F106Y]; *SLC26A4*:p.[H723R]; c.[1931+5G>A]	1 (0.4%)
Total	4 (1.5%)

doi:10.1371/journal.pone.0031276.t005

homozygous mutation with a mitochondrial 1555A>G mutation (Table 5).

The detection rate of mutations was 40.4% for the patients with congenital or early-onset hearing loss, i.e. in those with an awareness age of 0~6 years. The rate in congenital hearing loss patients also increased when restricting to the patients with moderate or more severe hearing loss (>50 dB; 40.7%) or severe hearing loss (>70 dB; 44.3%) (Fig. 1). In contrast, the detection rate was only 16.0% in the patients with an older age of onset/awareness (Fig. 1). Among the 13 included genes, mutations is *GJB2* and *SLC26A4* were mainly found in congenital patients or early-onset patients, in contrast with mitochondrial mutations, such as 12S rRNA m.1555A>G or tRNA(Leu(UUR)) m.3243A>G, which were predominantly found in older-onset patients (Table 4). The p.V37I mutation in the *GJB2* gene was also found in older-onset patients (data not shown).

With regard to the relationship between radiographic findings and genetic testing, the mutation detection rate was elevated when restricting to the patients with inner ear anomaly (50.0%) and EVA (63.6%) (Fig. 2).

Direct sequencing

Direct sequencing identified 9 mutations in 15 cases which were not included in the Invader assay panel and improved the mutation detection/diagnostic rate obtained by Invader assay analysis (28.0%/18.6%) to 29.5%/22.7%. (Fig. 1). Combining direct sequencing with invader screening enhanced the diagnostic rate notably but not the mutation detection rate. In detail, direct sequencing identified additional mutations in three cases with single *GJB2* mutations by Invader assay that were finally diagnosed as compound heterozygous mutations of *GJB2* (p.[T86R]; c.[511insAACG], p[T8M];[V37I] and c.[35insG];[235delC]).

In 7 cases only a single *SLC26A4* mutation was found by invader assay, and additional mutations were found by direct sequencing (two cases of p.[H723R];c[1931+5G>A] and one each cases of p.[R581S];[H723R], p.[V659L];[H723R], p.[S532I]; c.[2111insGCTGG], p.[T410M]; c.[1931+5G>A] and p.[K396E];[S532I]). Two cases carried EVA but without any mutations found in Invader assay, c[1931+5G>A]; [1931+5G>A] and p.[V659L];c[1219 delCT] compound heterozygous mutations were found by direct sequencing. With the combination of Invader assay and direct sequencing, and restriction to patients with EVA, the mutation detection rate was elevated to 17/22 cases (77.3%, Fig. 2). Fifteen of them carried homozygous or compound heterozygous *SLC26A4* mutations.

Discussion

We previously reported that simultaneous detection of common deafness gene mutations has excellent sensitivity and accuracy [2]. In this study, using samples from patients at 33 institutions nationwide from northern to southern Japan, we confirmed that the Invader assay based on the Japanese deafness gene mutation database works efficiently in the clinical base to detect the responsible gene mutations from the patients with

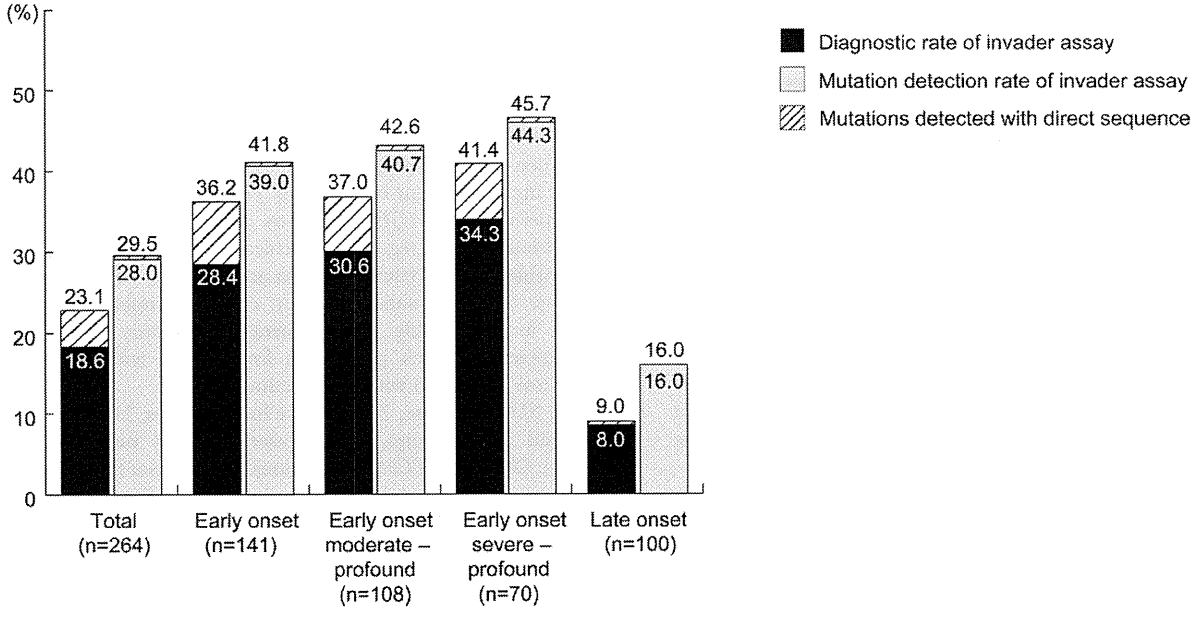

Figure 1. Detection rate by onset/awareness age and severity of hearing loss. Diagnostic rates and detection rates of this simultaneous multiple mutations screening and direct sequencing for biallelic mutations in autosominal recessive genes or mitochondrial mutations increased when restricted to congenital/early-onset hearing loss, and moderate or severe hearing loss. Combined direct sequence and invader screening enhanced the diagnostic rate but not the mutation detection rate.
doi:10.1371/journal.pone.0031276.g001

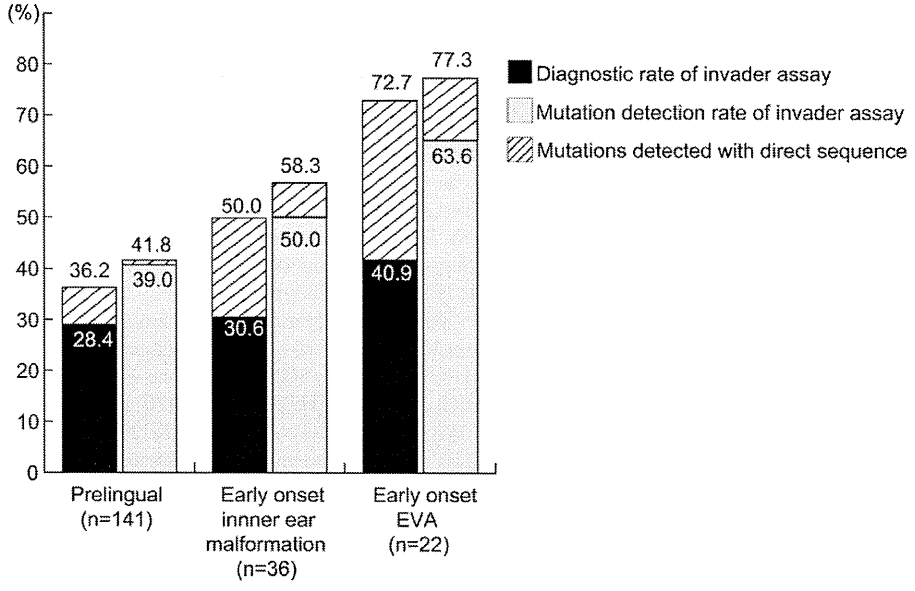

Figure 2. Radiographic findings and detection rate. Detection rate was elevated when subjects were restricted to those with inner ear anomaly or EVA. Combined direct sequence and invader screening enhanced the diagnostic rate but not the mutation detection rate.
doi:10.1371/journal.pone.0031276.g002

various onset/awareness ages. We detected mutations in 29.5% overall, and the 41.8% detection rate for congenital or early onset sensorineural hearing loss was especially remarkable. A series of epidemiological studies have demonstrated that genetic disorders are common causes of congenital deafness and it is estimated that 60–70% of the etiology may be caused by genetic factors [1]. Genetic testing is crucial to diagnose the etiology, but more than 100 genes are estimated to be involved and such genetic heterogeneiety has hampered the genetic testing for deafness as a routine clinical test. The present detection rate; i.e., 41.8%, is a strikingly good rate for a clinical application, and it is expected that clinical deafness mutation screening will greatly improve medical management and facilitate extensive genetic counseling for hearing impairment. Additional direct sequencing, as well as a new version of the screening panel which includes novel identified mutations, will likely improve the detection rate. For the older ages of onset, the detection rate was comparatively low (16.0%). Probably this is due to the panel mainly including responsible genes for congenital deafness but not the responsible genes for late onset hearing loss. An alternative explanation may be that environmental factors may be involved in this group of deafness patients.

The present study confirmed that mutations in three genes, *GJB2*, *SLC26A4*, and the mitochondrial 12 s rRNA, are so far the major known causes of hereditary hearing loss nationwide in Japanese [6], and thus much attention should be paid to these genes when performing genetic testing of hearing loss patients.

The most frequently found were mutations in the *GJB2* gene. This gene is so far the most common responsible gene for congenital deafness worldwide [7]. The detection rates (17.4% for all, 27.0% for congenital) are in accordance with our previous data of 15% in the overall deafness population and 25% in congenital deafness patients [5]. The mutation spectrum found in this study is also in accordance with our previous results [2,4,5]. In *GJB2* screening, 46 (17.4%) samples from deafness subjects had mutations of one or both alleles of the *GJB2* gene. As expected from the above reports, the c.235delC mutation was found to be the most prevalent mutation in our screening, accounting for 10.9% (29 of 264) of the hearing-impaired persons. Fourteen patients were c.235delC homozygotes and 11 were compound heterozygotes having c.235delC, confirmed by segregation analysis, and 4 patients were c.235delC heterozygotes without a second mutation. Direct sequencing identified novel mutations (p.T8M, c.35insG, p.F106Y, p.C174S and c.512insAACG) in the patients with a single mutation detected by Invader assay (Table 3).

Many benefits of *GJB2* gene genetic testing have been pointed out. There have been general rules that inactivating mutations (deletion mutations and stop mutations) show more severe phenotypes compared to those caused by non-inactivating mutations (missense mutations) [5,8,9]. As well as a highly accurate diagnosis, these genotype-phenotype correlation data could provide prognostic information to help decide the strategy of intervention with hearing, i.e., whether a child should receive cochlear implantation or hearing aids. For the patients with severe phenotypes who have *GJB2* mutations, genetic information would aid decision-making regarding cochlear implantation, because their hearing loss is of cochlear origin and they therefore are good candidates for implantation. In fact, cochlear implantation has resulted in remarkable improvement in auditory skills and development of speech production for patients with profound hearing loss associated with *GJB2* mutations [10].

In the *SLC26A4* gene, 7 cases were homozygotes, 11 cases were compound heterozygotes, and 7 cases had only one mutation (Table 4). Of the 19 *SLC26A4* mutations, 12 were not found in any samples, but the remaining 7 mutations were all confirmed in more than one patient. Especially, the p.H723R mutation was found to be in high allele frequency (4.1%). Direct sequencing identified novel mutations (c.1931+5G>A, p.S532I, p.R581S, p.V659L) in the patients with a single mutation by Invader assay and c.1219delCT mutation in a patient with EVA (Table 3).

As in our previous study [2], *SLC26A4* mutations were found only in the patients with EVA, suggesting a phenotype of hearing loss with EVA can be a diagnostic indicator of this category of disease.

Fluctuation and progressiveness of hearing loss are characteristic of hearing loss associated with EVA [11,12] and the early detection of *SLC26A4* mutations enables prediction of these clinical symptoms. Genetic testing is also useful in estimating associated abnormalities (goiter), selection of appropriate habilitation options, and better genetic counseling. In some cases, goiter is evident during the teen years [12]. In this study, 8 patients had hearing loss and goiter and 4 of them carried homozygous or compound heterozygous *SLC26A4* mutations.

In recessive mutations such as *GJB2* and *SLC26A4*, detection of two mutations in the paternal and maternal alleles is a hallmark. In the present "two step" screening method Invader assay is first performed followed by direct sequencing. As seen in Figs. 1 and 2, most of the mutations were successfully detected by the first Invader screening and the additional direct sequencing improved the "diagnostic" rate. This is very important to find the first mutation for identifying the responsible gene and the results indicate this screening is technically efficient. Difficult cases of a heterozygous state without a second mutation are also seen [4,5,13,14]. As previously reported, in a substantial proportion of patients our Invader techniques and additional direct sequencing revealed only one mutant *GJB2* or *SLC26A4* allele causing deafness by recessive pattern. We believe that there is one more occult mutation somewhere because the frequency of heterozygous patients was much higher than that of mutation frequency in the control population. Another explanation may be the high frequency of carriers in the population. But given the carrier frequency in normal controls, the number of heterozygous deafness cases was greater than would be expected. Second mutations may be present in the same gene or genes in the same chromosomal region. Recent statistical analysis has shown that one allele mutation of *GJB2* and *SLC26A4* is more likely to be a pathological status than a carrier status [15] and indeed, patients with one *SLC26A4* mutation are associated with EVA, therefore it is strongly likely that there is a second mutation within this gene. Another possibility is that mutations in the regulatory region may be involved in phenotypic expression [16].

The m.1555A>G mutation in the mitochondrial 12SrRNA gene, which was found in 5 4 subjects, was mainly found in those with older onset age. This mutation has been reported to be associated with aminoglycoside injection and found in 3% of the patients who visited the outpatient clinic [17,18]. The current findings are compatible with our previous report that this mutation is a frequently encountered cause for postlingual deafness in patients who received cochlear implantation [18]. This mutation was also found in the congenital or early onset age group as well, in line with our previous study [2]. It is likely that there is a considerably large high-risk population worldwide and a rapid screening method as well as careful counseling should be established to prevent aminoglycoside-induced hearing loss in this group.

The m.3243A>G mutation in the tRNA(Leu (UUR)) gene was found in 6 patients in the older-onset group. This mutation was first reported at a high frequently in the patients with clinical manifestations of MELAS [19], and has also been found in diabetes mellitus patients [20]. It is known to be commonly associated with hearing loss patients (especially with diabetes mellitus) [21]. The hearing loss is adult onset, symmetric high frequency involved [22]. In this study, all 6 patients with this mutation were associated with diabetes mellitus and progressive hearing loss. Five patients had maternally inherited hearing loss (the mother also had hearing loss), but one subject was a sporadic case (the mother did not have hearing loss from the anamnestic evaluation) and therefore is unlikely to be a mitochondrial candidate from clinical evaluation. The present multigene screening is also unexpectedly efficient for such atypical cases.

Heteroplasmy is one of the significant factors determining the expression of mitochondrial disease. The Invader assay is comparatively accurate at detecting the heteroplasmic rate [2], and the present two patients with the 3243 mutation showed 3% and 24% heteroplasmic rates.

In contrast to the three genes discussed above, mutations of the *COCH, KCNQ4, MYO7A, TECTA, CRYM, POU3F4 and EYA1* genes were not found in the present deaf subjects in line with our previous study [2]. This is likely due to them being very rare and usually independent mutations found in only one family. Although analysis for these mutations should be performed to identify the molecular nature of deafness as the first deafness screening step, a different strategy may be necessary for screening for them.

In conclusion, the simultaneous examination of the multiple deafness mutations by Invader assay followed by direct sequencing if necessary, will enable us to detect deafness mutations in an efficient and practical manner for clinical use. This screening strategy will facilitate more precise clinical genetic diagnosis, appropriate genetic counseling and proper medical management for auditory disorders. Against this background, since 2008 the Ministry of Health and Welfare of Japan has allowed this screening to be performed as an advanced medical technology.

A Japanese summary of this article has been provided as Supporting Information (Japanese summary S1).

Supporting Information

Japanese Summary S1 Simultaneous Screening of Multiple Mutations by Invader Assay. The present method of simultaneous screening of multiple deafness mutations by Invader assay followed by direct sequencing will enable us to detect deafness mutations in an efficient and practical manner for clinical use.
(PDF)

Acknowledgments

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 Univer-

sity), Drs. Akira Ganaha and Mikio Suzuki (Ryukyus University), for providing samples of their patients. We also thank A. C. Apple-Mathews for help in preparing the manuscript.

Author Contributions

Conceived and designed the experiments: SU. Performed the experiments: SN MN SA TY. Analyzed the data: SN MN SA TY. Contributed reagents/materials/analysis tools: SN MN SA TY. Wrote the paper: SU. Collection of DNA samples and clinical data: The Deafness Gene Study Consortium.

References

1. Morton CC, Nance WE (2006) Newborn hearing screening: a silent revolution. N Engl J Med 354: 2151–2164.
2. Abe S, Yamaguchi T, Usami S (2007) Application of deafness diagnostic screening panel based on deafness mutation/gene database using Invader Assay. Genetic Testing 11(3): 333–340.
3. Usami S, Abe S, Weston MD, Shinkawa H, Van Camp G, et al. (1999) Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by PDS mutations. Hum Genet 104: 188–192.
4. Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, et al. (2003) GJB2 deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet 112: 329–333.
5. Tsukada K, Nishio S, Usami S (2010) A large cohort study of GJB2 mutations in Japanese hearing loss patients. Clin Genet 78: 464–470.
6. Usami S, Wagatsuma M, Fukuoka H, Suzuki H, Tsukada K, et al. (2008) The responsible genes in Japanese deafness patients and clinical application using Invader assay. Acta Otolaryngol 128: 446–454.
7. Smith RJ, Bale JF, Jr., White KR (2005) Sensorineural hearing loss in children. Lancet 365: 879–890. Review.
8. Snoeckx RL, Huygen PLM, Feldmann D, Marlin S, Denoyelle F, et al. (2005) GJB2 mutations and degree of hearing loss: a multicenter study. Am J Hum Genet 77: 945–957.
9. Oguchi T, Ohtsuka A, Hashimoto S, Oshima A, Abe S, et al. (2005) Clinical features of patients with GJB2 (connexin 26) mutations: severity of hearing loss is correlated with genotypes and protein expression patterns. J Hum Genet 50: 76–83.
10. Fukushima K, Sugata K, Kasai N, Fukuda S, Nagayasu R, et al. (2002) Better speech performance in cochlear implant patients with GJB2-related deafness. Int J Pediatr Otorhinolaryngol 62: 151–157.
11. Abe S, Usami S, Shinkawa H (1997) Three familial cases of hearing loss associated with enlargement of the vestibular aqueduct. Ann Otol Rhinol Laryngol 106: 1063–1069.
12. Suzuki H, Oshima A, Tsukamoto K, Abe S, Kumakawa K, et al. (2000) Clinical characteristics and genotype-phenotype correlation of hearing loss patients with SLC26A4 mutations. Acta Otolaryngol 127: 1292–1297.
13. Azaiez H, Chamberlin GP, Fischer SM, Welp CL, Prasad SD, et al. (2004) GJB2: the spectrum of deafness-causing allele variants and their phenotype. Hum Mutat 24: 305–311.
14. Tsukamoto K, Suzuki H, Harada D, Namba A, Abe S, et al. (2003) Distribution and frequencies of PDS (SLC26A4) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: a unique spectrum of mutations in Japanese. Eur J Hum Genet 11: 916–922.
15. Kimberling WJ (2005) Estimation of the frequency of occult mutations for an autosomal recessive disease in the presence of genetic heterogeneity: application to genetic hearing loss disorders. Hum Mutat 26: 462–470.
16. Yang T, Vidarsson H, Rodrigo-Blomqvist S, Rosengren SS, Enerback S, et al. (2007) Transcriptional control of SLC26A4 is involved in Pendred syndrome and nonsyndromic enlargement of vestibular aqueduct (DFNB4). Am J Hum Genet Jun; 80(6): 1055–1063.
17. Usami S, Abe S, Kasai M, Shinkawa H, Moeller B, et al. (1997) Genetic and clinical features of sensorineural hearing loss associated with the 1555 mitochondrial mutation. Laryngoscope 107: 483–490.
18. Usami S, Abe S, Akita J, Namba A, Shinkawa H, et al. (2000) Prevalence of mitochondrial gene mutations among hearing impaired patients. J Med Genet 37: 38–40.
19. Goto Y, Nonaka I, Horai S (1990) A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature Dec 13;348(6302): 651–653.
20. Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, et al. (1994) A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. N Engl J Med Apr 7;330(14): 962–968.
21. den Ouweland JM, Lemkes HH, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, et al. (1992) Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet 1992 Aug;1(5): 368–371.
22. Oshima T, Ueda N, Ikeda K, Abe K, Takasaka T (1996) Bilateral sensorineural hearing loss associated with the point mutation in mitochondrial genome. Laryngoscope Jan; 106(1 Pt 1): 43–48.

Acta Oto-Laryngologica, 2011; 131: 1232–1236

informa
healthcare

SHORT COMMUNICATION

Different cortical metabolic activation by visual stimuli possibly due to different time courses of hearing loss in patients with *GJB2* and *SLC26A4* mutations

HIDEAKI MOTEKI[1], YASUSHI NAITO[2], KEIZO FUJIWARA[2], RYOSUKE KITOH[1], SHIN-YA NISHIO[1], KAZUHIRO OGUCHI[3], YUTAKA TAKUMI[1] & SHIN-ICHI USAMI[1]

[1]*Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto,* [2]*Department of Otorhinolaryngology, Kobe City Medical Center General Hospital, Kobe and* [3]*Positron Imaging Center, Aizawa Hospital, Matsumoto, Japan*

Abstract

Conclusion. We have demonstrated differences in cortical activation with language-related visual stimuli in patients who were profoundly deafened due to genetic mutations in *GJB2* and *SLC26A4*. The differences in cortical processing patterns between these two cases may have been influenced by the differing clinical courses and pathogenesis of hearing loss due to genetic mutations. Our results suggest the importance of hearing during early childhood for the development of a normal cortical language network. *Objectives.* To investigate the cortical activation with language-related visual stimuli in patients who were profoundly deafened due to genetic mutations in *GJB2* and *SLC26A4*. *Methods:* The cortical activity of two adult patients with known genetic mutations (*GJB2, SLC26A4*) was evaluated with fluorodeoxyglucose-positron emission tomography (FDG-PET) with a visual language task and compared with that of normal-hearing controls. *Results:* A patient with a *GJB2* mutation showed activation in the right auditory association area [BA21, BA22], and the left auditory association area [BA42] even with visual language task; in contrast, a patient with an *SLC26A4* mutation showed no significant activation in the corresponding area.

Keywords: *FDG-PET, visual language task, functional brain imaging*

Introduction

Functional brain imaging is an effective method for investigating the cortical processing of language, which has provided much evidence for the plasticity of the central auditory pathway following a profound loss of hearing [1–4]. Many previous studies showed that there is a capacity of the auditory cortex for cross-modal plasticity after auditory deprivation of the brain. Cerebral glucose metabolism in the primary auditory and related cortices in individuals with prelingual deafness was shown to decrease in younger patients, but to increase as they aged and, in fact, recover fully or even exceed the normal level of activation [5–7]. Children with prelingual deafness can acquire spoken language by cochlear implantation, but its efficacy decreases with age. The development of the auditory cortex is believed to depend on the patient's auditory experience within 'critical periods' in the early lifetime. Adults who had severe congenital hearing loss in their childhood may take advantage of hearing with cochlear implants if they had exploited residual hearing with hearing aids. It has been shown that low glucose metabolism in the temporal auditory cortex predicts a good cochlear implant outcome in prelingually deafened children, which suggests that low metabolism in the auditory cortex may indicate its potential of plasticity for spoken language acquisition [7].

Correspondence: Shin-ichi Usami, MD PhD, Department of Otorhinolaryngology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Tel: +81 263 37 2666. Fax: +81 263 36 9164. E-mail: usami@shinshu-u.ac.jp

(Received 9 March 2011; accepted 29 May 2011)

ISSN 0001-6489 print/ISSN 1651-2251 online
DOI: 10.3109/00016489.2011.593719

Meanwhile, several etiological studies suggest that at least 60% of congenital hearing loss has genetic causes. Recent advances in molecular genetics have made genetic diagnosis possible [8]. The identification of the mutation responsible for hearing loss may provide some information as to cochlear damage, and help predict the time course and manifestations of hearing loss. Genetic testing can therefore be useful in decision-making regarding cochlear implantation and other necessary treatment.

Evaluation of brain function and diagnosing accurate etiology of hearing loss may be the keys to personalizing post-cochlear implantation habilitation programs and predicting the outcomes thereof.

In this study, we used 18 F-fluorodeoxyglucose (FDG) positron emission tomography (PET) to measure cortical glucose metabolism with a visual language task before cochlear implantation in profoundly deaf patients whose etiologies were identified by genetic testing.

Material and methods

Genetic diagnosis

Genetic screening was performed in two cases using an Invader assay to screen for 41 known hearing loss-related mutations [9] and direct sequencing for *GJB2* and *SLC26A4* mutations [10,11].

FDG-PET scanning and image analysis

FDG-PET scanning and image analysis were performed using the method described by Fujiwara et al. [12]. During the time period between the intravenous injection of 370 MBq 18 F-FDG (the dose was adjusted according to the body weight of each subject) and the PET scanning of the brain, the patients were instructed to watch a video of the face of a speaking person reading a children's book. The video lasted for 30 min, and several still illustrations taken from the book were inserted (for a few seconds each) to help the subjects to follow the story. The subjects were video-recorded to confirm that they were watching the task video. PET images were acquired with a GE ADVANCE NXi system (General Electric Medical Systems, Milwaukee, WI, USA). Spatial preprocessing and statistical analysis were performed with SPM2 (Institute of Neurology, University College of London, UK) implemented in Matlab (Mathworks, MA, USA). The cortical radioactivity of each deaf patient was compared with that of a control group of normal-hearing adults by a t test in the basic model of SPM2. The statistical significance level was set at $p < 0.001$ (uncorrected).

This study was approved by the Ethics Committee of Shinshu University School of Medicine and written consent was obtained from each participant.

Control group

The control group consisted of six normal-hearing right-handed adult subjects. The average (mean ± standard deviation) age of the normal-hearing subjects was 27.5 ± 3.8 years. The pure-tone average hearing levels were within 20 dB HL for all.

Case 1

A right-handed 22-year-old female with a *GJB2* mutation (235 delC homozygous) had hearing impairment that was noticed by her parents when she was 2 years old. She had used hearing aids ever since, but with insufficient hearing amplification. She used lip-reading and some sign language, and her speech was not intelligible to hearing people. Computed tomography (CT) findings of the middle and inner ear were normal. Her average pure-tone hearing levels were 102.5 dB for the right ear and 95 dB for the left ear (Figure 1A).

Case 2

A right-handed 26-year-old male with an *SLC26A4* mutation (H723R homozygous) had hearing impairment that was noticed by his parents when he was 2 years old, from which time he had used hearing aids bilaterally. He did not use lip-reading or sign language during the acquisition age for language. He obtained spoken language with hearing aids but had progressive hearing loss, and sometimes suffered vertigo attacks. His pronunciation was clear, and his speech was almost completely intelligible. CT findings exhibited an enlarged vestibular aqueduct on each side. His average pure-tone hearing levels were 106.2 dB for the right ear and 100 dB for left ear (Figure 1B).

Results

Figure 2 shows transaxial PET images of each participant's brain. The visual stimuli resulted in bilateral activation of the superior temporal gyrus, including Heschl's gyrus in case 1 with *GJB2* mutation (Figure 2A, white arrowhead). In contrast, in case 2 with *SLC26A4* mutation, the activation of the superior temporal gyrus was much lower than in case 1 (Figure 2B, white arrowhead).

Figure 3 shows supra-threshold clusters in each case. In case 1, activation higher than normal controls

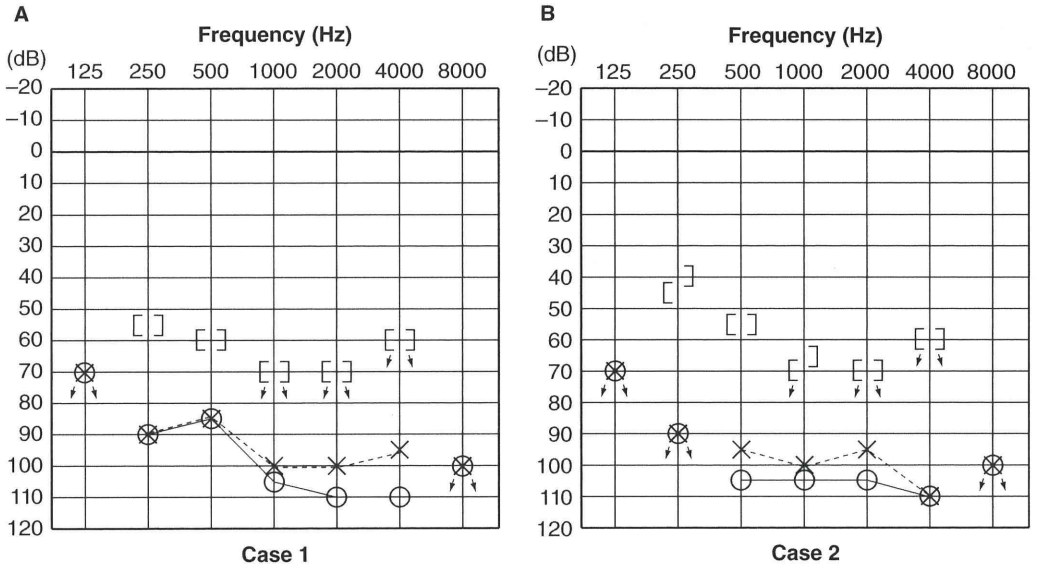

Figure 1. Pure-tone audiograms: (A) a 22-year-old female with a *GJB2* mutation; (B) a 26-year-old male with an *SLC26A4* mutation. There were no clear differences in hearing thresholds in these two cases.

was observed in the right auditory association area [BA21, BA22], and the left auditory association area [BA42] ($p < 0.001$). In case 2, the right superior frontal gyrus [BA9], and the middle temporal gyrus [BA20], showed higher activation than normal controls ($p < 0.001$).

Discussion

More than half of congenital hearing loss has been estimated to be from genetic causes, and phenotypes are affected by genetic mutations. There have been no reports of the influence of phenotype on brain function associated with hearing. This is the first report on evaluation of cortical processing of language in patients with genetic mutations as a main etiology of hearing loss. The auditory association area was activated bilaterally in case 1 (*GJB2* mutation), but not activated in case 2 (*SLC26A4* mutation). A previous study indicated that the temporal lobe is activated during speech-reading in normal subjects [13] and another study found that the temporal lobe is not activated when reading fluent speech from a talking face [14]. For the present study we used a

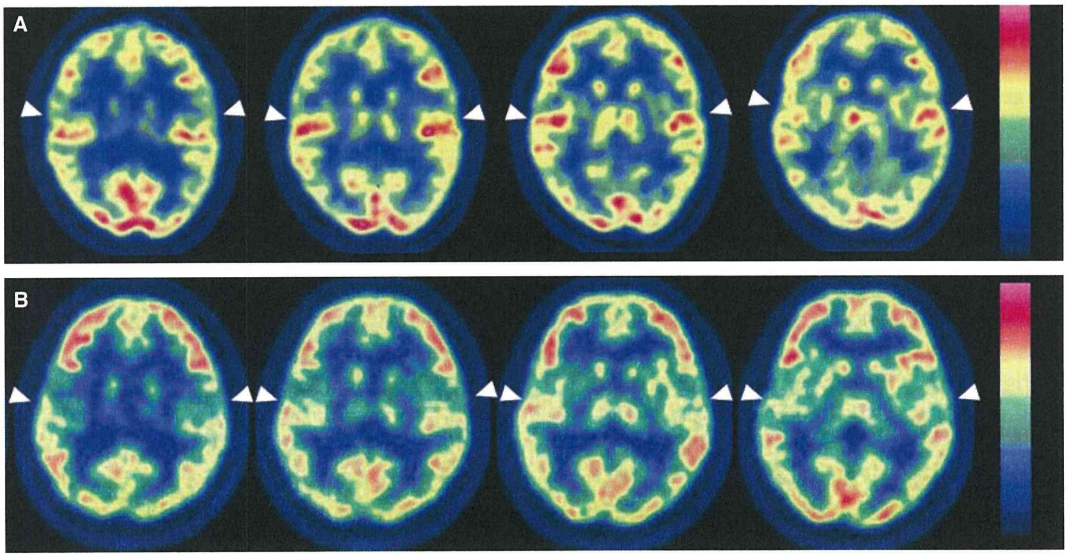

Figure 2. Transaxial PET images of each participant's brain: activation (arrowheads) of the superior temporal gyrus with visual language stimuli in each case. (A) Case 1 (*GJB2* mutation). The superior temporal gyri were strongly activated bilaterally. (B) Case 2 (*SLC26A4* mutation). The superior temporal gyri exhibited less activation than in case 1.

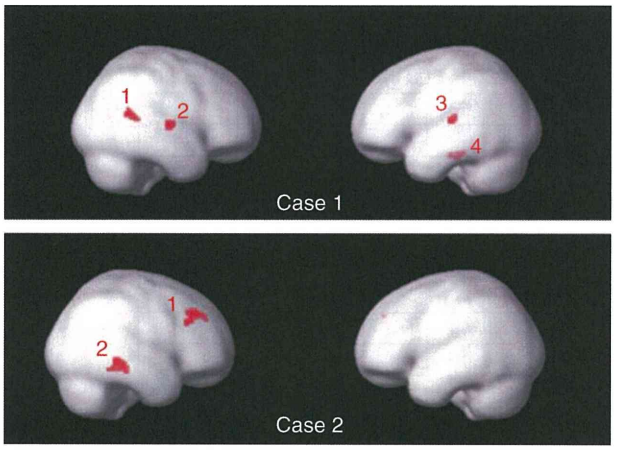

Figure 3. Cortical activation by language-related visual stimuli in the two profoundly deafened cases. Case 1 (*GJB2* mutation) showed significant activation in the right middle temporal gyrus [BA21] (1), superior temporal gyrus [BA22] (2), and left superior temporal gyrus [BA42] (3), and left cerebellum (4), while case 2 (*SLC26A4* mutation) exhibited significant activation in the right superior frontal gyrus [BA9] (1), and middle temporal gyrus [BA20] (2) (SPM2, $p < 0.001$, uncorrected).

fluent speech-reading task, similar to that described by Hall et al. [14]. Fujiwara et al. in a FDG-PET study using the same methods and task as the present study, showed that subjects with better spoken language skills had less temporal lobe activation [12].

To summarize these reports, the patients with hearing aids with better spoken language skills have less temporal lobe activation with a visual language task. Otherwise, Nishimura et al. [15] reported a sign language activation of the bilateral auditory association areas in a congenitally deafened subject. However, detailed clinical data for the subject – including his hearing levels, time course of hearing loss, and the cause of deafness – were not described. The different visual language activation patterns in the auditory cortices revealed in the current two profoundly deafened subjects with different genetic etiologies and hearing loss progressions may, thus, add further knowledge of the cross-modal plasticity brought about in the superior temporal association areas by lack of hearing.

The differences in cortical processing patterns between cases 1 and 2 – who both had hearing loss of cochlear origin – may have been influenced by the differing clinical courses of hearing loss. *GJB2* is currently known to be the most prevalent gene responsible for congenital hearing loss worldwide. Patients with severe phenotypes who have *GJB2* mutations are good candidates for implantation, because their hearing loss is of cochlear origin and non-progressive [16,17]. *SLC26A4* is known as a commonly found gene and is associated with enlarged vestibular aquaduct [11]. This phenotype includes congenital and progressive hearing loss, usually associated with vertigo [18]. In most cases hearing remains in low frequencies, enabling the understanding of spoken language with hearing aids. Cochlear implantation has resulted in remarkable improvements in auditory skills and speech perception for patients with profound hearing loss associated with *SLC26A4* mutations as well as *GJB2*.

Comparing case 1 (*GJB2* mutation) with case 2 (*SLC26A4* mutation), the crucial importance of the use of hearing aids during childhood up to age 6 years for acquisition of better hearing is evident. In case 1, even though she was able to hear sound with the use of hearing aids, she was unable to recognize enough speech language due to insufficient hearing amplification during the critical periods in her childhood. She therefore used lip-reading and some sign language in addition to hearing aids. Increased metabolism was observed by FDG-PET in the auditory association area, where no significant activation was found in the normal-hearing controls. In contrast, in case 2, a 26-year-old patient with an *SLC26A4* mutation, there was no significant activation in the corresponding area. He obtained rather hearing ability and spoken language by hearing aids with residual hearing at lower frequencies during his childhood. His hearing was supposed to be better than case 1, because 1) he did not use lip-reading or sign language during the acquisition age for language from anamnestic evaluation; 2) his pronunciation was clear, indicating better hearing (at least 40–50 dB) during the acquisition age for language; 3) from an etiological point of view, patients with *SLC26A4* mutation usually have mild to moderate hearing loss during childhood and this shows a progressive nature [18]. He had progressive hearing loss in the natural history as a phenotype of *SLC26A4* mutation. The difference in activation patterns in the cases with *GJB2* and *SLC26A4* mutations was clearly demonstrated by statistical processing with SPM, as well as in the PET scans. These results suggest the importance of hearing during early childhood for the development of a normal cortical language network, and that reorganization had occurred in the auditory cortex of the patient with a *GJB2* mutation; i.e. processing visual aspects of language in the superior temporal gyri. This implies that cross-modal plasticity as a consequence of the lack of hearing during the critical period for spoken language acquisition in early childhood was influenced by the time course of hearing loss characterized by genetic mutations.

Previous studies have suggested that auditory areas presented high accumulation of FDG with deafness of early onset, and plastic changes in auditory cortices

were strongly affected by the duration of auditory deprivation [1,5,6,19,20]. Since low activation of the auditory cortices with visual stimuli suggests the subject's lesser dependence on visual communication methods and substantial residual plasticity in his auditory cortices, case 2 with an *SLC26A4* mutation may be determined to be an appropriate candidate for cochlear implantation.

Accurate diagnosis of hearing loss and early cochlear implantation are important for successful spoken language development. The approach using PET could help those involved in the habilitation and education of prelingually deafened children to decide upon the suitable mode of communication for each individual.

Both of the patients received cochlear implantation after PET examination. Further follow-up of these cases may indicate that efficacy of the combination of genetic diagnosis and functional brain imaging helps to predict long-term outcomes of cochlear implantation. Examination of more cases is necessary to define the relationship of the varying cortical activation patterns with each genetic mutation.

Acknowledgments

We thank A.C. Apple-Mathews for help in preparing the manuscript. We also thank Masanori Sakaguchi MD and radiologic technologists, Kouichi Anraku and Hiroyuki Fujimoto, for excellent technical assistance.

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

References

[1] Okazawa H, Naito Y, Yonekura Y, Sadato N, Hirano S, Nishizawa S, et al. Cochlear implant efficiency in pre- and postlingually deaf subjects. A study with H2(15)O and PET. Brain 1996;119:1297–306.

[2] Naito Y, Hirano S, Honjo I, Okazawa H, Ishizu K, Takahashi H, et al. Sound-induced activation of auditory cortices in cochlear implant users with post- and prelingual deafness demonstrated by positron emission tomography. Acta Otolaryngol 1997;117:490–6.

[3] Naito Y, Tateya I, Fujiki N, Hirano S, Ishizu K, Nagahama Y, et al. Increased cortical activation during hearing of speech in cochlear implant users. Hear Res 2000;143:139–46.

[4] Tateya I, Naito Y, Hirano S, Kojima H, Inoue M, Kaneko K, et al. Inner ear hearing loss modulates ipsilateral temporal lobe activation by monaural speech stimuli. Neuroreport 2003;14:763–7.

[5] Catalán-Ahumada M, Deggouj N, De Volder A, Melin J, Michel C, Veraart C. High metabolic activity demonstrated by positron emission tomography in human auditory cortex in case of deafness of early onset. Brain Res 1993;623: 287–92.

[6] Deggouj N, Devolder A, Catalan M, Melin J, Michel C, Gersdorff M, et al. Positron emission tomography in deaf patients at rest. Adv Otorhinolaryngol 1995;50:31–7.

[7] Lee DS, Lee JS, Oh SH, Kim SK, Kim JW, Chung JK, et al. Cross-modal plasticity and cochlear implants. Nature 2001; 409:149–50.

[8] Usami S, Wagatsuma M, Fukuoka H, Suzuki H, Tsukada K, Nishio S, et al. The responsible genes in Japanese deafness patients and clinical application using Invader assay. Acta Otolaryngol 2008;128:446–54.

[9] Abe S, Yamaguchi T, Usami S. Application of deafness diagnostic screening panel based on deafness mutation/ gene database using Invader Assay. Genetic Testing 2007; 11:333–40.

[10] Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet 2000;37:41–3.

[11] Usami S, Abe S, Weston MD, Shinkawa H, Van Camp G, Kimberling WJ. Non-syndromic hearing loss associated with enlarged vestibular aqueduct is caused by PDS mutations. Hum Genet 1999;104:188–92.

[12] Fujiwara K, Naito Y, Senda M, Mori T, Manabe T, Shinohara S, et al. Brain metabolism of children with profound deafness: a visual language activation study by 18 F-fluorodeoxyglucose positron emission tomography. Acta Otolaryngol 2008;128:393–7.

[13] Calvert GA, Bullmore ET, Brammer MJ, Campbell R, Williams SC, McGuire PK, et al. Activation of auditory cortex during silent lipreading. Science 1997;276:593–6.

[14] Hall DA, Fussell C, Summerfield AQ. Reading fiuent speech from talking faces: typical brain networks and individual differences. J Cogn Neurosci 2005;17:939–53.

[15] Nishimura H, Hashikwa K, Doi K, Iwaki T, Watanabe Y, Kusuoka H, et al. Sign language 'heard' in the auditory cortex. Nature 1999;397:116.

[16] Fukushima K, Sugata K, Kasai N, Fukuda S, Nagayasu R, Toida N, et al. Better speech performance in cochlear implant patients with GJB2-related deafness. Int J Pediatr Otorhinolaryngol 2002;62:151–7.

[17] Tsukada K, Nishio S, Usami S-i. A large cohort study of GJB2 mutations in Japanese hearing loss patients. Clin Genet 2010;78:464–70.

[18] Suzuki H, Oshima A, Tsukamoto K, Abe S, Kumakawa K, Nagai K, et al. Clinical characteristics and genotype–phenotype correlation of hearing loss patients with SLC26A4 mutations. Acta Otolaryngol 2007;127:1292–7.

[19] Nishimura H, Doi K, Iwaki T, Hashikawa K, Oku N, Teratani T, et al. Neural plasticity detected in short- and long-term cochlear implant users using PET. Neuroreport 2000;11:811–15.

[20] Giraud AL, Truy E, Frackowiak R. Imaging plasticity in cochlear implant patients. Audiol Neurootol 2001;6:381–93.

Audiology Japan 54, 649～664, 2011

総　　説

乳幼児難聴の聴覚医学的問題
「治療における問題点」

山岨達也
東京大学大学院　医学系研究科　外科学専攻　感覚運動機能講座　耳鼻咽喉科学分野

要旨：乳幼児難聴では早期発見・早期支援が重要であり，新生児聴覚スクリーニングを広く行うことに大きな意義があるが普及率は高くない。スクリーニング未施行例や進行性難聴例では介入が遅れる傾向にある。高度難聴のみでなく軽度から中等度難聴でも早期発見・早期介入が重要であり，看過された場合はコミュニケーションに支障をきたし，言語発達，情緒，社会性の発達などに影響が生じる。補聴効果に限界があると予想される高度難聴の場合はコミュニケーションモードの選択を視野に入れた対応が求められ，療育上人工内耳が選択肢と考えられる場合には速やかに人工内耳医療を専門とする医療施設に紹介することが重要である。小児における人工内耳の術後成績には手術年齢，難聴の原因，重複障害の有無，コミュニケーションモードなど多くの因子が影響する。手術適応決定にはこれらの因子を含め考慮すべき多くの因子があり，多職種によるチーム医療での対応が求められる。乳幼児難聴の臨床上の特徴は患児のみならず保護者も対象とし，その経過が長期にわたる事とダイナミックな発達的変化を含む事である。聴力検査一つをとっても高い専門性が求められ，児の生活上の困難や保護者のニーズを把握するには聴覚医学だけでなく発達医学や心理学の知識も必要である。適切な時期の適切な判断が児の将来の発達に影響することを念頭に置いて治療にあたることが肝要である。

ーキーワードー
新生児聴覚スクリーニング，補聴器，人工内耳，重複障害

はじめに

乳幼児の聴覚障害に対する治療における問題は多岐に渡り，例えば難聴発見の遅れ，不正確な診断または診断の遅れ，診断後の対応の遅れや誤り，不適切な治療の選択などが挙げられる。乳幼児の聴覚障害が適切に対応されない場合，コミュニケーションに支障をきたし，言語発達が遅れ，情緒や社会性の発達にも影響が生じうる。難聴は重度であれば１歳前後までに気付かれることが多いが，軽・中等度の場合は言語発達の遅れにより２歳以降に発見されて診断や療育の開始が３歳以降になることもしばしば見られる。

難聴発見の遅れは新生児聴覚スクリーニング（newborn hearing screening：NHS）が普及したためかなり減少してきているが，NHSの施行率およびその後の対応には地域差も多く，いまだに大きな問題である。難聴の検査や診断における問題点については当誌に総説が掲載されている[1,2]が，難聴の不正確な診断または診断の遅れがその後の治療に影響する事は言うまでもなく，諸検査の限界と意義の十分な理解が重要である。聴覚障害については，聴性脳幹反応（auditory brainstem response：ABR），聴性定常反応（auditory steady-state response：ASSR），耳音響放射（otoacoustic emissions：OAE）などの他覚的検査のほか，年齢に応じて聴性行動反

応聴力検査（behavioral observation audiometry：BOA），条件詮索反応聴力検査（conditioned orientation response audiometry：COR），遊戯聴力検査を行うことが重要であり，診断後は裸耳の他補聴器（hearing aid：HA）装用下での評価も重要となる。

難聴診断後の治療法の選択・対応は難聴児の聴取能，言語力の発達に大きく影響する。しかしながら個々の症例に対する具体的な対応について十分に理解している耳鼻咽喉科医師が多くない現状にある。NHSや人工内耳（cochlear implant：CI）の導入以前に，耳鼻咽喉科医師・医療機関で難聴の診断が確定した難聴児の療育やHA調整を地域の療育施設に依存する体制が（一部の地域を除いて）構築されてしまった経緯があるため，診断において十分な知識を有する医師においても，難聴児の治療や療育について経験・知識が乏しく，実際の対応に関する理解が不足する傾向が生まれた。小児のCIが広く行われるようになった現在においても小児難聴の専門外来を開設している医育機関は限られ，小児難聴について研修する教育体制が十分に整っていない状態といえる。

早期に十分な教育を行う施設の問題も挙げられる。現在我が国において難聴児を指導する主な施設は，文部科学省管轄の聴覚特別支援学校，厚生労働省管轄の難聴幼児通園施設や医療機関の言語訓練部門などが挙げられる。いずれも0歳から療育を行っているが，その介入の密度には大きな差がある。積極的に支援を行っている施設では，例えば0−1歳児に対して，個人指導，母親指導，グループ指導をそれぞれ週1回以上行い，ビデオ指導を月1回以上，発達チェック表による家庭指導を月1回，両親講座，家族参観，家庭訪問などを年複数回，同時に聴覚の評価を週1回以上，HAの評価を月1回以上，達成度評価，発達評価を年数回行うなど，細やかな指導がなされている。一方，概ね週1回程度の指導（親子ふれあい遊び，歌とリズム，絵本，屋外遊びなどの活動を通してコミュニケーションの実際を学ぶ，など）と適宜施行する聴力検査とHAフィッティング，家庭訪問などに留まる施設もある。

さらに難聴児の療育を担当する施設・言語聴覚士などの療育担当者の間で教育方針が大きく異なることも問題を複雑にしている。例えばCIが一般的な医療になりつつあるとはいえ，CIにかなり否定的な意見を持つ施設や療育担当者も存在し，CIの積極的な適応にあると考えられる児の家族に対しても十分な情報を与えず，十分な効果を受けられる機会が奪われていることもしばしば経験する。難聴児療育の一つの大きな目標は言語力を高めることであり，聴覚入力，視覚入力のいずれの療育方法であっても濃密な教育と症例に応じたコミュニケーションモードの判断が求められるはずである。CIでは難聴児の聴覚が健聴児と同じレベルにまで獲得されるわけではないが，補聴効果の十分でない場合にはより多くの聴覚情報が得られる可能性が高い。療育施設として長期的な療育目標の中からコミュニケーションモードの選択とそれに伴う聴覚入力手段の選択を適宜検討し，保護者に対し積極的な情報提供をすべきであるが，それが十分なされない状況がまだ残っている。

難聴発見時期・療育開始時期の影響

1）NHSの影響

新生児の聴覚障害の約半数は，極低出生体重児，重症仮死，高ビリルビン血症（交換輸血施行例），子宮内感染（風疹，サイトメガロウィルス（CMV）など），家族性，先天異常症候群などのハイリスク児であるが，残りの半数は出生時に異常を示さない児であり，通常の健診等では聴覚障害の早期発見が難しいことがある。早期に支援を開始するためには早期発見が必須であり，そのためには全新生児を対象としたNHSを行うことが重要となる。

米国では2000年に，生後入院中に最初のNHSを行って生後1か月までにはNHSの過程を終え，生後3か月までに精密診断を実施し，生後6か月までに療育を開始する（1-3-6ルール）という聴覚障害の早期発見・早期療育ガイドラインを出した[3]。これは生後6カ月までの難聴発見・聴覚補償教育開始の重要性を指摘したYoshinaga-Itanoらの研究[4]に大きな影響を受けている。本邦では平成12年度より年間5万人規模の新生児聴覚検査モデル事業が予算化され，平成13年度より岡山県など4県で開始，平成16年度までに17都道府県・政令都市で実施された。このモデル事業は平成16年度で終了となり，「新生児聴覚検査事業」は平成17年度から創設され

た「母子保健医療対策等総合支援事業」の対象事業として実施された（平成19年度からは対象事業ではなくなった）。日本産婦人科医会による平成17年度の調査では分娩取り扱い施設の約60％が新生児聴覚検査を行っている。また難聴幼児通園施設および聾学校教育相談における０～１歳児の60％以上がNHSにより発見された児であり，平成18年においては全出生児の約60％以上がNHSを受けたと推定されている。ただし県別の検査施行率には県間で大きな差が見られる（新生児聴覚スクリーニングマニュアルHP；http://www.jaog.or.jp/japanese/jigyo/JYOSEI/shinseiji_html/shi-top.html）。この事業によりNHSの重要性は広く認識されるようになり一定の普及をしてきているが，NHSの浸透はまだ十分ではなく，いまだ難聴発見が大幅に遅れる症例も散見される。最近我々が経験した，就学時まで難聴が発見されなかった特異な一例について示す。

この症例は初診時６歳８ヶ月の男児で３人兄弟の末っ子である。妊娠・分娩時に異常は無く，NHSは未施行であった。喃語様の発声はあったものの有意味語の表出はなかったにも拘らず，乳幼児健診で聴覚障害などの異常を指摘されなかった。就学時健診ではじめて言語発達遅滞を指摘され，近医総合病院耳鼻咽喉科を受診した。聴力検査では両側聾であり，ABRで両側無反応のため，当科を紹介受診した。初診時，外耳・鼓膜は正常で，遊戯聴力検査にて右耳は聾，左耳に残聴を認めた。WISC-III知能検査では動作性IQは正常域，言語性IQは測定不能であった。画像診断では内耳奇形（蝸牛は低形成で，前庭は嚢胞性）と内耳道狭窄を認めた。左耳へのHAの仮装用では利得40dB程度での装用が可能であり（図１ａ），フィッティングが可能と判断し，療育先を紹介した。９歳３カ月の時点では，HA装用は常用にいたっているが，聴取能は極めて不良である（図１ｂ）。コミュニケーションモードは視覚中心で言語発達は緩慢であり，語彙はいくつかあるものの文字として入っているものはわずかで語彙検査では３歳未満に相当した。11歳時点でのコミュニケーション能力はジェスチャーとキュードスピーチでわずかに可能な程度である。本児に対しても０歳時からの早期療育を開始していれば，少なくとも視覚入力を併用して，より早期での言語獲得は可能であったと思われる。就学時まで高度難聴が見逃される事は極めて稀ではあるが，このような不幸な事例がいまだ存在する事は注意が必要である。

２）軽度・中等度難聴の影響

難聴支援や療育の開始時期については，難聴が軽度～中等度であっても高度であっても早期ほど良いと考えられる。軽度から中等度難聴児は一見聴こえも発育も悪くなく見えるために発見が遅れやすい傾向にある。しかし部分的な聴覚の感覚遮断状態にあるため，放置されると言語発達に深刻な影響が予想

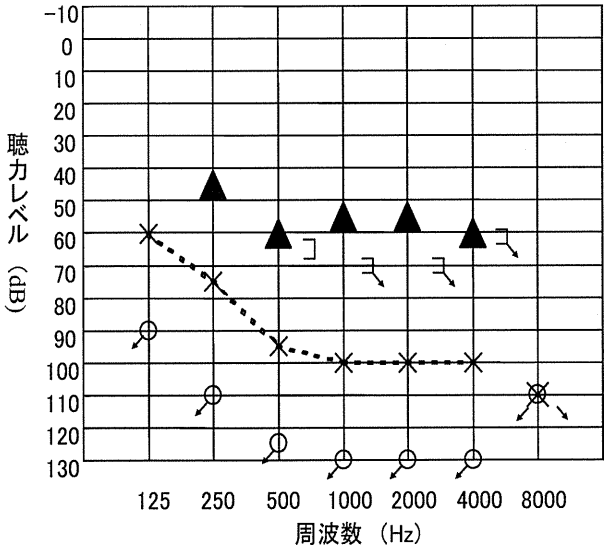

図１ａ　初診時オージオグラム（遊戯聴力検査）▲は補聴器装用時閾値を示す。

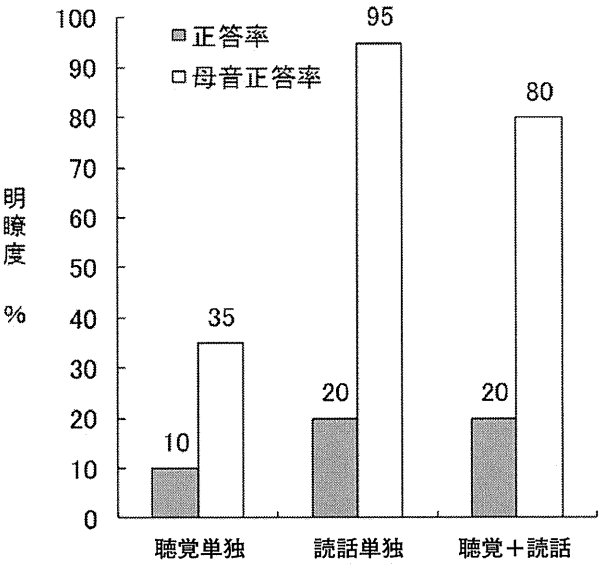

図１ｂ　音節明瞭度（67-S語表：肉声）

される。就学期あるいは入学してから言語の遅れにより発見されることもNHS導入以前には多く，その場合言語発達の遅れは小学校高学年あるいは中学まで続き，高校入試での国語力の低下にまでつながることも少なくなかった。NHSによって軽度から中等度難聴児も早期に発見されるようになり，早期教育を始めることで言語発達の遅れの予防が可能になりつつある。しかし裸耳で音に反応があり，HAは自己負担で購入せねばならず，外観上抵抗があることなどの理由から，高度難聴児以上にHA装用指導に困難を伴いやすいことも事実である。

杉内ら[5]はNHS導入前にHA外来にて聴覚管理を行ってきた軽度・中等度難聴児30名を対象に，難聴診断時期，HA装用開始時期，HA使用状況などを調査し，24名にWISC-III知能検査を行った。その結果難聴を疑った時期は平均2歳10カ月と遅く，診断は平均4歳2カ月，HA装用開始が平均5歳3カ月と，難聴を疑いながらも診断・補聴がさらに遅れる傾向にあること，HAを有効に活用できていない児のいることを示した。また知能検査を施行した24例中14例では言語性IQが動作性IQより15以上も低く，言語発達に遅れが見られたと報告している。田中[6]は通常小学校での学業ないしは就学にあたって苦慮した5歳以上の難聴児22名（感音性19名，伝音性3名）を調べたところ，良聴耳は30～97.5dBに分布し（18名は80dB以下），難聴発見は3歳代5例，4歳代3例，5歳以上5例であったとしている。このうち言語発達の遅れは19例にみられ，学校教育においては発見の遅れる中等度難聴児ほど深刻な問題を抱える傾向にあると啓蒙している。

中等度難聴の影響は感音難聴だけでなく伝音難聴においても認められる。千原ら[7]は両側中等度伝音難聴が6歳，7歳，12歳まで補聴されなかった3症例について言語性IQと動作性IQの聴力改善手術前後の推移を調べたが，治療前も後も言語性IQは低いままであり，言語発達の遅れもみられたと報告している。Psarromatisら[8]は726例の言語発達遅滞児を調べ，72例の症候性を除く654例のうち87例（13.3%）に難聴が認められ，55例は感音難聴，32例は伝音難聴であったと報告している。

このような事実から軽度から中等度難聴児においても早期発見・早期聴覚支援を考慮するべきである。林ら[9]は「平均聴力が30dB後半から40dB台と聴力が比較的良好でも言語性IQが80台の症例には装用装用や言語指導を勧める」としている。我々は40dB程度以上の難聴がある場合は積極的にHA装用を勧め，30-40dBの場合は定期的な聴力検査と言語発達の評価により，方針を決めることにしている。

3）高度難聴の影響

高度難聴児においては早期教育の効果は明らかである。難聴児の療育開始が6歳からの義務教育であった時代には，多くの高度難聴児の言語力・学力は健常児の9歳レベル以上には向上せず，言語力にいわゆる“9歳の壁”があることが知られていた。その後Yoshinaga-Itanoら[4]が6カ月以前に難聴を診断し療育を開始することの重要性を示し，本邦でも早期介入の効果を示す追試で証明された。例えば内山と徳光[10]は6歳時点での良耳聴力平均が80dB以上，動作性IQが正常範囲，他障害がない難聴児を対象とし，療育開始時期が0歳代（6名），1歳代（19名），2歳代（14名）の三つの群における言語性IQを比較した結果，6歳時点の聴力およびPIQに群間差はなかったが，言語性IQは0歳群では平均98，1歳群では平均88，2歳群では平均77と療育開始年齢が遅れるとともに低くなる傾向があり，0歳群と2歳群では有意差が見られたと報告した。さらに小学校就学後も追跡ができた難聴児の言語性IQについても，0歳台群（6名）は2歳代群（10名）より有意に高かった。これらの結果は高度難聴児において0歳代からの早期療育が効果的であることを示している。

しかしながら聴力レベルが100dB以上の難聴児ではHAを装用しても装用閾値は50dBに達しないことも多く，言語音の聴き取りが容易ではないため，聴覚を活用しつつも読話を併用する必要があった。難聴児が読話併用を会話理解の手段として利用する限り，日常生活の中で健常児と同様の言語習得は困難であった。このようなより高度の難聴児に対してもCIを装用すると装用閾値約35dB程度で聴くことが多くの症例で可能になった。この結果CI装用児は相手の発話を聴覚だけで聴き取り，正確な構音を聴覚活用により習得することが可能となって

いる。残念なことにこのCIの利点が広く認知されておらず，CI装用児に対しても従来のHA装用重度難聴児に対する療育法がいまだ多くの施設でなされている現状がある。小児CI症例を多数経験している海外では，十分な聴取能が得られなかった症例や重複障害を伴う症例などを除き，すでにauditory verbal communicationが療育の主流となっている。本邦ではCI症例に対しても依然としてtotal communicationを主に用いている施設が多く，CIの効果が十分活かされていないことは大きな問題である。

難聴進行の可能性

NHSでpassとなった症例でもその後に難聴が出現することがあり，また先天性難聴症例で経過中にさらに難聴が進行することもある。前者の代表的なものには先天性横隔膜ヘルニア（横隔膜の裂肛閉鎖障害により，胃，腸管などの腹腔内臓器が胸郭内に侵入して呼吸障害を来たす先天性疾患）があり，難聴は約3割に生じるが遅発性のことが多い。後者としては，先天性CMV感染症，遺伝性難聴，Auditory Neuropathyなどがある。遺伝性難聴で進行性を示す遺伝子には，常染色体優性では*COCH*，*KCNQ4*，*WFS1*，*TECTA*など，常染色体劣性では*SLC26A4*，*CDH23*など，ミトコンドリア遺伝子異常では3243位点変異，1555位点変異などがある。*SLC26A4*遺伝子異常による前庭水管拡大症では聴力が変動や進行しやすく，特に頭部打撲などをきっかけに難聴が進行しやすい。先天性難聴のなかで遺伝子異常として最も頻度の高い*GJB2*遺伝子変異例で両側性に進行を示す症例は数%程度と考えられている。

西澤[11]は両側中等度もしくは高度難聴児を5年以上または10歳以上まで経過観察できた63例について平均11.6年経過を追跡し，初診時と最終診察時の聴力検査の比較で連続する2周波数において15dB以上の閾値上昇を認めたものは25例（39.7%）であり，うち半数では10歳以下に進行が見られたと報告した。また家族または血縁に難聴者のみられる水平型聴力のものに難聴進行が多く見られたとしている。杉内ら[12]は小児感音難聴児45例を9–22年間経過観察し，1周波数で15dB以上または2周波数で10dB以上閾値上昇した場合を聴力悪化と判定し，聴力の急性増悪時には副腎皮質ステロイドを用いた積極的治療と補聴指導を行った。その結果，36例（80%）が聴力変動を示し，うち29例は急性増悪であったと報告している。また経過観察最終時まで初期の聴力が維持されたのは23例（51.1%）であったが，うち14例は急性増悪が治療で回復したこと，初期聴力が維持されなかった22例中7例では徐々に聴力が低下し，15例は急性増悪であったがその多くは治療に反応したことも示している。

難聴が進行した場合，本人の訴えや家族により気付かれることも多いが見過ごされる場合もある。上述したように小児の難聴増悪は稀ではなく，療育における影響も大きい。従って難聴増悪のリスクが高い場合は1カ月に1度，その他の場合でも最低3カ月に1度は聴力検査で評価することが望まれる。難聴増悪時には副腎皮質ステロイドなどの積極的治療が必要である。先天性CMV感染症では保険適応はないが抗ウイルス薬投与が有効な場合もある。難聴が増悪して固定した場合はHAの調整が必要である。

重複障害の影響

難聴が知的障害や脳性麻痺などの他障害と合併することは少なくない。その内容は多様であり，身体的疾患（眼疾患，心臓疾患，腎臓疾患，外表奇形，口蓋裂，小耳症など），運動機能障害（脳性まひなど），発達障害（精神運動遅滞，広汎性発達障害など）などが，単一または複合した障害として合併しうる。重複障害の頻度は概ね25–35%程度と推定される。英国で1980–1995年に誕生した両側40dB以上の難聴児17,160名の疫学調査では約30%に他の障害が見られたと報告されている[13]。内山は平成5年から15年に在籍した難聴児100名を調べ，身体的疾患・奇形・障害を合併するものは32%，発達遅滞・自閉的発達障害・学習障害などの発達障害を合併するものは25%，両者を伴うものは16%であったとしている[14]。合併する多様な障害の中で療育上特に問題となるのは，自閉的傾向，学習障害，多動傾向などの軽度発達障害を合併している難聴児である。

一般に重複障害児では聴力レベルの確定に時間のかかることが少なくない。また発達の評価も困難なことが多い。特に発達障害が軽度な場合，難聴が原

因であるための発達の偏りなのか，認知機能・行動の偏りなのか，判断が困難な場合もある。重複障害の発見には発達評価は必須であり，さらに日常生活，療育場面での行動観察も重要である。比較的軽度の発達障害であっても早期療育を進めるうえで支障となりやすいが，HAを装用して障害・発達程度に応じて働きかけることで聴覚活用は可能である。

CIに関しても一定の効果があるとの報告が多い。Pymanら[15]は20例の重複障害例（認知発達遅滞17例を含む）と障害の無い55例の術後聴取成績の変化を調べ，重複障害を持つ場合，特に認知発達が緩慢な場合は障害のないCI装用児に比べ聴取能の向上は遅れるが，装用経験年数に伴って発達は見られると報告している。同様にWaltzmanら[16]も31例のCIを装用した重複障害児の経過を観察し，聴取能の向上は遅れるがCIの効果は明らかであり，17例（59%）はoral communicationを行うようになったと報告している。

NHSによる難聴の早期発見や他覚的検査精度の向上などに伴い，難聴の診断を受けた後に他障害が気付かれる事例も増えている。医療者，療育担当者は保護者に適切な情報提供を行い，コミュニケーション発達について目標を共有して療育に取り組む必要がある。HA装用，CI装用のいずれにおいても重複障害児では療育効果が明らかになるまで時間がかかることから，長期的で総合的な療育プログラムを組むことが求められる[17]。

人工内耳をめぐる問題

1）人工内耳と補聴器装用の差

90dB以上の高度難聴児をCIで療育した場合とHAで療育した場合，将来の言語能力に差が生じるのか，まだよくわかっていない。海外ではCIの効果は裸耳聴力が80–90dB程度の難聴者のHA装用レベルに相当するとみなされるようになっている[18]。例えばBlameyら[19]は聴覚口話法で教育している47例のCI装用児（平均聴力106dB）と40例のHA装用児（平均聴力78dB）の聴取能，言語力，言語獲得速度などを比較し，明らかな差がなかったことを報告している。日本において難聴以外に身体および知的・学習機能に差がない難聴の小児を対象として，CI装用児とHA装用児の間の言語能力について多数例を用いて統計的に比較検討した報告はほとんどない。

加我ら[20]は2005年3月まで難聴幼児通園施設で療育を受け，同年4月に普通小学校に就学した難聴児に対し，療育修了時点で行ったWPPSI知能検査をもとに療育効果を検討した。対象児は知的障害等の他障害が合併しない21例で，CI装用児7名，聴力80dB以上のHA装用児7名（平均91dB），聴力80dB未満のHA装用児7名（平均60dB）である。3群とも動作性IQに差はみられない。CI装用群の半数はNHSにより難聴の診断を受けている。療育開始月齢はCI群とHA群（80dB以上）は平均14ヵ月で統計学的に差がないが，HA群（80dB未満）は平均42.4ヵ月と有意に遅れていた。就学時の言語性IQはCI群が平均101（標準偏差14），HA群（80dB以上）が平均90（標準偏差16），HA群（80dB未満）が平均82（標準偏差15）であり，CI群は同年齢の正常児とほぼ同等の言語性IQを示した。CI群の言語性IQは，統計的に有意ではないがHA群（80dB以上）より良い傾向にあり，HA群（80dB未満）より有意に良好であった。CI群がHA群（80dB未満）より言語性IQが良かったのは療育が早期に開始されたためと考えられる。80dB以上の症例数を増加するとCI優位の傾向がより明らかとなるかどうかについては今後の検討課題である。

2）騒音下での聴取成績

CIにおける単音節明瞭度は症例により個人差が大きく，また個々の装用環境により効果は異なり，CI聴覚の実用性については多様と想定される。これらCIの日常生活に即した音声処理特徴を測定しようと，近年では雑音を負荷した評価が報告されているが，これまで日本語音声による小児例の報告はほとんど無い。そこで我々はCI埋込み術を施行した小児例における雑音負荷条件の聴取能について中途失聴成人例及び健聴児と比較した（赤松裕介他，音声言語医学会2011発表）。対象は当科にて聴覚管理を行っている小児CI例41名であり，対照群として成人CI装用者35名，健聴児童20名に同様の評価を行った。評価はCI2004語音聴取評価の文章課題を用い，CI装用下に2台のスピーカから検査語音と加重不規則雑音を提示した。検査語音レベルを一定にし，静寂条件，SN比20dB，10dB，5dB，0dB

の５条件で雑音を変化させた。その結果健聴児ではSN比0dB条件で減衰率が有意に低下したが個人差は少なかったのに対し，小児CI例ではSN比10dBで減衰率の有意な低下がみられ個人差が大であった。成人CI例でも小児CI例と同様の結果であった。

この結果はCI装用により静寂下で良好な聴取能を示す症例においても健聴者との雑音下での聴覚情報量の差が明らかであることを示している。従って，SN比改善のためのプログラム設定や補助機器などを積極的に活用することには大きな意義がある。

３）人工内耳の術後成績に影響する因子

中途失聴成人がCIを装用した場合，言語の認知に関与する聴皮質・聴覚連合野を含む高次中枢は形成されているため，その術後聴取能は末梢から入力される情報量，すなわち挿入電極の種類・数，蝸牛の状態（残存神経線維数など）に主に依存する。例えば失聴期間が長くなるほど聴取成績が悪化する事はよく知られているが，これは蝸牛神経・ラセン神経節の変性が進行するためと考えられる。従って適応を適切に決定すれば，もちろんリハビリテーションは重要であるが，成人例の大半はCI装用後に良好な聴取能を再獲得することができる。一方先天性高度難聴の小児例では新たに聴覚活用を介して言語を獲得するという行程を要するため，多くの因子がその術後成績に関与する。すなわち，難聴の原因，内耳奇形の有無など末梢からの情報入力量に影響する因子だけでなく，聴覚補償の開始時期，療育状況，重複障害の有無なども重要な因子と考えられる。例えばNiparkoら[21]は米国の６つの医療施設で５歳までにCIを施行した188例を３年間追跡する前向き，縦断的，多次元的解析を施行し，言語理解も発話行動も術前より著明に改善したが若年で手術を受けた方がより速やかであり，術前の残存聴力が良いほど，両親と子供の交流関係が密なほど，また経済的に恵まれた環境にいるほど，言語の理解と表出が良好であったと報告している。

海外からの報告の多くは日本語を話す我々においてもかなりあてはまると考えられるが，言語の違いや療育環境の差なども考慮する必要がある。日本では小児例に関するまとまった報告が少なく，比較的明らかな傾向，例えば早期CI装用ほど術後成績が良いこと[22]や内耳奇形の種類により成績に差が出ること[23]などについてはいくつかの報告があるが，海外のように多数例によるエビデンスは確立されていない状況にあった。これは一施設当たりのCI症例数が一部の施設を除き限られているためである。日本ではこれまで六千数百例にCI手術が施行されたが，小児例はその４割に過ぎない。最近は年間四百数十例のCI手術のうち小児例が約６割を占めるようになってきているが，３歳以下は全体の約３割にとどまっている。一方CI手術を施行する施設数は100施設近くに及ぶため，まだ多くの施設で小児例を少数しか経験していない状態にある。

このような背景から，我々は厚生労働科学研究費の支援を得て，「人工内耳を装用した先天性高度感音難聴小児例の聴覚・言語能力の発達に関するエビデンスの確立」に関する多施設共同研究（研究代表者：山岨達也，研究分担者：土井勝美，熊川孝三，伊藤健，坂田英明，安達のどか）を2008年から2010年に施行した。本研究の結果はまだ論文化されていないため詳細は割愛するが，概略は以下の通りである。方法は後方視的，縦断的，多次元的解析研究であり，６歳までに大阪大学・東京大学・虎の門病院でCI手術を受け就学年齢に達した324例のうち，言語習得後症例を除く316例を対象とした。NHS導入以前の症例も多く含まれているため診断年齢は遅く平均1.3歳（標準偏差1.5歳）であり，HA装用開始年齢は1.6歳（標準偏差1.7歳），CI手術時年齢は4.4歳（標準偏差2.7歳），評価時年齢は9.9歳（標準偏差5.0歳）である。患者基本情報として，現在年齢，性別，難聴の原因，重複障害の有無，診断年齢，HA装用年齢，CI装用年齢，療育先，主たるコミュニケーションモード，機種・音声処理法，術中NRT情報を登録した。評価項目には施設間に差があるため，Meaningful Auditory Integration Scale（MAIS），Meaningful Use of Speech Scale（MUSS），語音聴取能（67-S語表，CI2004），絵画語彙発達検査（Picture Vocabulary Test：PVT），言語能力（言語性IQ）を解析した。そして，CI装用開始年齢，難聴の原因，重複障害の有無，コミュニケーションモードなどの影響について調べた。難聴の原因は側頭骨CT，MRIにより内耳奇形の有無を調べ，*GJB2*