A, et al : Reversal of age-related neural timing delays with training. Proc Natl Acad Sci USA **110** : 4357-4362, 2013

(2013年12月27日受稿 2014年1月5日受理)

別冊請求先 : 〒113-8655
文京区本郷7-3-1
東京大学医学部耳鼻咽喉科学教室
山岨達也
Tatsuya Yamasoba
Department of Otolaryngology and Head and Neck Surgery, University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

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PLOS | ONE

Massively Parallel DNA Sequencing Facilitates Diagnosis of Patients with Usher Syndrome Type 1

Hidekane Yoshimura[1], Satoshi Iwasaki[2,3], Shin-ya Nishio[1], Kozo Kumakawa[4], Tetsuya Tono[5], Yumiko Kobayashi[6], Hiroaki Sato[6], Kyoko Nagai[7], Kotaro Ishikawa[8], Tetsuo Ikezono[9], Yasushi Naito[10], Kunihiro Fukushima[11], Chie Oshikawa[12], Takashi Kimitsuki[13], Hiroshi Nakanishi[14], Shin-ichi Usami[1]*

1 Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Nagano, Japan, **2** Department of Hearing Implant Sciences, Shinshu University School of Medicine, Matsumoto, Nagano, Japan, **3** Department of Otorhinolaryngology, International University of Health and Welfare, Mita Hospital, Minatoku, Tokyo, Japan, **4** Department of Otolaryngology, Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Minatoku, Tokyo, Japan, **5** Department of Otorhinolaryngology-Head and Neck Surgery, Miyazaki University School of Medicine, Miyazaki, Miyazaki, Japan, **6** Department of Otolaryngology-Head and Neck Surgery, Iwate Medical University, Morioka, Iwate, Japan, **7** Department of Otolaryngology-Head and Neck Surgery, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan, **8** Department of Otolaryngology, National Rehabilitation Center for Persons with Disabilities, Tokorozawa, Saitama, Japan, **9** Department of Otolaryngology, Saitama Medical University, Moroyama, Saitama, Japan, **10** Department of Otolaryngology, Kobe City Medical Center General Hospital, Kobe, Hyougo, Japan, **11** Department of Otorhinolaryngology, Head and Neck Surgery, Okayama University Postgraduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Okayama, Japan, **12** Department of Otorhinolaryngology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka, Japan, **13** Department of Otorhinolaryngology, Kyushu Central Hospital, Fukuoka, Fukuoka, Japan, **14** Department of Otorhinolaryngology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan

Abstract

Usher syndrome is an autosomal recessive disorder manifesting hearing loss, retinitis pigmentosa and vestibular dysfunction, and having three clinical subtypes. Usher syndrome type 1 is the most severe subtype due to its profound hearing loss, lack of vestibular responses, and retinitis pigmentosa that appears in prepuberty. Six of the corresponding genes have been identified, making early diagnosis through DNA testing possible, with many immediate and several long-term advantages for patients and their families. However, the conventional genetic techniques, such as direct sequence analysis, are both time-consuming and expensive. Targeted exon sequencing of selected genes using the massively parallel DNA sequencing technology will potentially enable us to systematically tackle previously intractable monogenic disorders and improve molecular diagnosis. Using this technique combined with direct sequence analysis, we screened 17 unrelated Usher syndrome type 1 patients and detected probable pathogenic variants in the 16 of them (94.1%) who carried at least one mutation. Seven patients had the *MYO7A* mutation (41.2%), which is the most common type in Japanese. Most of the mutations were detected by only the massively parallel DNA sequencing. We report here four patients, who had probable pathogenic mutations in two different Usher syndrome type 1 genes, and one case of *MYO7A/PCDH15* digenic inheritance. This is the first report of Usher syndrome mutation analysis using massively parallel DNA sequencing and the frequency of Usher syndrome type 1 genes in Japanese. Mutation screening using this technique has the power to quickly identify mutations of many causative genes while maintaining cost-benefit performance. In addition, the simultaneous mutation analysis of large numbers of genes is useful for detecting mutations in different genes that are possibly disease modifiers or of digenic inheritance.

Citation: Yoshimura H, Iwasaki S, Nishio S-y, Kumakawa K, Tono T, et al. (2014) Massively Parallel DNA Sequencing Facilitates Diagnosis of Patients with Usher Syndrome Type 1. PLoS ONE 9(3): e90688. doi:10.1371/journal.pone.0090688

Editor: Klaus Brusgaard, Odense University hospital, Denmark

Received September 6, 2013; **Accepted** February 3, 2014; **Published** March 11, 2014

Funding: This study was supported by a Health and Labour Sciences Research Grant for Comprehensive Research on Disability Health and Welfare from the Ministry of Health, Labour and Welfare of Japan (S.U.), and by 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 declared that no competing interests exist.

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

Introduction

Usher syndrome (USH) is an autosomal recessive disorder characterized by hearing loss (HL), retinitis pigmentosa (RP) and vestibular dysfunction. Three clinical subtypes can be distinguished. USH type 1 (USH1) is the most severe among them because of profound HL, absent vestibular responses, and prepubertal onset RP. USH type 2 (USH2) is characterized by congenital moderate to severe HL, with a high-frequency sloping configuration. The vestibular function is normal and onset of RP is in the first or second decade. The onset of the visual symptoms such as night blindness in USH usually occurs several years later than in USH1. USH type 3 (USH3) is characterized by variable onset of progressive HL, variable onset of RP, and variable impairment of vestibular function (normal to absent) [1,2].

To date, nine genetic loci for USH1(*USH1B-H*, *J*, and *K*) have been mapped to chromosomes 11q13.5, 11p15.1, 10q22.1, 21q21, 10q21-q22, 17q24-q25, 15q22-q23 (*USH1H* and *J*), and 10p11.21–q21.1 [2,3,4]. Six of the corresponding genes have been identified: the actin-based motor protein myosin VIIa

(*MYO7A*, USH1B) [5]; two cadherin-related proteins, cadherin 23 (*CDH23*, USH1D) [6] and protocadherin 15 (*PCDH15*, USH1F) [7]; and two scaffold proteins, harmonin (*USH1C*) [8] and sans (*USH1G*) [9]; the Ca^{2+}- and integrin-binding protein (*CIB2*, USH1J) [4]. In Caucasian USH1 patients, previous studies showed that mutations in *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, and *USH1G*, were found in 39–55%, 7–14%, 7–35%, 7–11%, and 0–7%, respectively (the frequency of *CIB2* is still unknown) [10,11,12]. In Japanese, Nakanishi et al. showed that *MYO7A* and *CDH23* mutations are present in USH1 patients [13], however, the frequency is not yet known. In addition, mutations in three corresponding genes (usherin *USH2A* [14], G protein-coupled receptor 98; *GPR98* [15], and deafness, autosomal recessive 31; *DFNB31* [16]) have been reported so far in USH2, and USH3 is caused by mutations in the clarin 1 (*CLRN1*) [17] gene.

Comprehensive molecular diagnosis of USH has been hampered both by genetic heterogeneity and the large number of exons for most of the USH genes. The six USH1 genes collectively contain 180 coding exons [4,9,10] the three USH2 genes comprise 175 coding exons [15,16,18], and the USH3 gene has five coding exons [17]. In addition some of these genes are alternatively spliced ([4,7,8,16,17] and NCBI database: http://www.ncbi.nlm.nih.gov/nuccore/). Thus far, large-scale mutation screening has been performed using direct sequence analysis, but that is both time-consuming and expensive. We thought that targeted exon sequencing of selected genes using the Massively Parallel DNA Sequencing (MPS) technology would enable us to systematically tackle previously intractable monogenic disorders and improve molecular diagnosis.

Therefore, in this study, we have conducted genetic analysis using MPS-based genetic screening to find mutations in nine causative USH genes (except *CIB2*) in Japanese USH1 patients.

Results

Mutation analysis of the nine USH genes in 17 unrelated USH1 patients revealed 19 different probable pathogenic variants, of which 14 were novel (Table 1).

All mutations were detected in only one patient each and sixteen of the 17 patients (94.1%) carried at least one mutation, while one patient had no mutations. Thirteen of the 16 mutation carriers each had two pathogenic mutations (Table 2).

Nonsense, frame shift, and splice site mutations are all classified as pathogenic, whereas missense mutations are presumed to be probable pathogenic variants based on results of prediction software for evaluation of the pathogenicity of missense variants (Table 1).

Of the 19 probable pathogenic mutations that we found, 17 were detected by MPS. The remaining two (p.Lys542GlnfsX5 in *MYO7A* and c.5821-2A>G in *CDH23*) were sequenced by direct sequence analysis.

Of our 17 USH patients, seven had *MYO7A* mutations (41.2%), three had *CDH23* mutations (17.6%), and two had *PCDH15* mutations (11.8%). We did not find any probable pathogenic mutations in *USH1C*, *USH1G*, and USH2/3 genes.

Four USH1 patients (Cases #3, 5, 8, 15) had probable pathogenic mutations in two different USH genes, with one being a biallelic mutation (Table 3). The other heterozygous/homozygous mutations were missense variants. Three of these patients (Cases #3, 5, 8) presented with earlier RP onset (night blindness) than in the other patients with two pathogenic mutations (Cases #1, 6, 7, 9, 11, 16) ($p = 0.007$) (Fig. 1).

One patient (Case #4) had heterozygote mutations in two USH1 genes (p.Ala771Ser in *MYO7A* and c.158-1G>A in *PCDH15*). His parents and one brother were found to also be carriers for these mutations. Another brother had no variants (Fig. 2).

Discussion

For USH1, early diagnosis has many immediate and several long-term advantages for patients and their families [1]. However, diagnosis in childhood, based on a clinical phenotype, has been difficult because patients appear to have only non-syndromic HL in childhood and RP develops in later years. Although early diagnosis is now possible through DNA testing, performing large-scale mutation screening for USH genes in all non-syndromic HL children has been both time-consuming and expensive. Therefore, the availability of MPS, which facilitates comprehensive large-scale mutation screening [19] is a very welcome advance.

MPS technology enabled us to detect pathogenic mutations in USH1 patients efficiently, identifying one or two pathogenic/likely pathogenic mutations in 16 of 17 (94.1%) cases. This was comparable to previous direct sequence analysis results such as Bonnet et al. who detected one or two mutations in 24 out of 27 (89%) USH1 patients [11] and Le Quesne Stabej et al. who detected one or two mutations in 41 out of 47 (87.2%) USH1 patients [12].

In addition, MPS assists in the analysis of disease modifiers and digenic inheritance because it simultaneously investigates many causative genes for a specific disease, such as in our case, USH. Previous reports have described several USH cases with pathogenic mutations in two or three different USH genes [11,12,20]. In our study, four patients had two pathogenic mutations in one gene and missense variants in a different gene (Table 3). We considered the latter to possibly be a disease modifier. For example, *USH1C*:p.Tyr813Asp, which occurred in 0/384 control chromosomes and was predicted to be "probably damaging" by the Polyphen program, was found with a homozygous *CDH23* nonsense mutation (p.Arg2107X) (Case #15). As for what the variant "modifies", we speculate that for USH1 patients with a disease modifier, RP symptoms such as night blindness show an earlier onset. However, we think that profound HL and the absence of vestibular function in USH1 patients are not affected by modifiers as they are congenital and therefore not progressive.

Ebermann et al. described a USH2 patient with "digenic inheritance." a heterozygous truncating mutation in *GPR98*, and a truncating heterozygous mutation in PDZ domain-containing 7 (*PDZD7*), which is reported to be a cause of USH [20]. Our USH1 patient (Case #4) had segregated *MYO7A*:p.Ala771Ser and *PCDH15*:c.158-1G>A. Molecular analyses in mouse models have shown many interactions among the USH1 proteins [2]. In particular, *MYO7A* directly binds to *PCDH15* and both proteins are expressed in an overlapping pattern in hair bundles in a mouse model [21]. *PCDH15*:c.158-1G>A, predicted to alter the splice donor site of intron 3, has been classified as pathogenic. *MYO7A*:p.Ala771Ser is a non-truncating mutation, but was previously reported as disease-causing [13]. So, we consider the patient to be the first reported case of *MYO7A*/*PCDH15* digenic inheritance.

However, we should be aware of two limitations of MPS technology. First, the target region of MPS cannot cover all coding exons of USH genes. Actually, the coverage of the target exons was 97.0% in our study. So, it is impossible to detect a mutation in a region which is not covered using this system (Case #9: c.5821-2A>G). Secondarily, the MPS system used in this study, is not effective for detecting homo-polymer regions, for example poly C stretch [22] (Case #8: p.Lys542GlnfsX5). In addition, concerning

Table 1. Possible pathogenic variants found in this study.

Gene	Mutation type	Nucleotide change	Amino acid change	exon/intron number	Domain	control (in 384 alleles)	SIFT Score	PolyPhen Score	Reference
MYO7A	Frameshift	c.1623dup	p.Lys542GlnfsX5	Exon 14	-	N/A	-	-	Le Quesne Stabej et al. (2012)
		c.4482_4483insTG	p.Trp1495CysfsX55	Exon 34	-	N/A	-	-	This study
		c.6205_6206delAT	p.Ile2069ProfsX6	Exon 45	-	N/A	-	-	This study
	Nonsense	c.1477C>T	p.Gln493X	Exon 13	-	N/A	-	-	This study
		c.1708C>T	p.Arg570X	Exon 15	-	N/A	-	-	This study
		c.2115C>A	p.Cys705X	Exon 18	-	N/A	-	-	This study
		c.6321G>A	p.Trp2107X	Exon 46	-	N/A	-	-	This study
	Missense	c.2074G>A	p.Val692Met	Exon 17	Motor domain	0	0.09	0.982	This study
		c.2311G>T	p.Ala771Ser	Exon 20	IQ 2	0.0026	0.01	0.825	Nakanishi et al. (2010)
		c.6028G>A	p.Asp2010Asn	Exon 44	FERM 2	0	0	0.925	Jacobson et al. (2009)
CDH23	Frameshift	c.3567delG	p.Arg1189ArgfsX5	Exon 30	-	N/A	-	-	This study
		c.5780_5781delCT	p.Ser1927Cysfs16	Exon 44	-	N/A	-	-	This study
	Splicing	c.5821-2A>G	?	Intron 44	-	N/A	-	-	This study
	Nonsense	c.6319C>T	p.Arg2107X	Exon 48	-	N/A	-	-	Nakanishi et al. (2010)
PCDH15	Splicing	c.158-1G>A	?	Intron 3	-	N/A	-	-	This study
	Nonsense	c.1006C>T	p.Arg336X	Exon 10	-	N/A	-	-	This study
		c.2971C>T	p.Arg991X	Exon 22	-	N/A	-	-	Roux et al. (2006)
		c.3337G>T	p.Glu1113X	Exon 25	-	N/A	-	-	This study
	Missense	c.3724G>A	p.Val1242Met	Exon 28	Cadherin 11	0	0	1	This study

Computer analysis to predict the effect of missense variants on MYO7A protein function was performed with sorting intolerant from tolerant (SIFT; http://sift.jcvi.org/), and polymorphism phenotyping (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/).
N/A: not applicable.
doi:10.1371/journal.pone.0090688.t001

Table 2. Details of phenotype and genotype of 17 USH1 patients.

Sample No.	Age	Sex	Allele1	Allele2	Hereditary form	Onset of night blindness	Cataract	Hearing Aid	Cochlear Implant
MYO7A									
1	37	M	p.Gln493X	p.Trp1495CysfsX55	sporadic	13	no	unilateral	unilateral
2	41	W	p.I2069fsX6	p.I2069fsX6	AR	unknown	both eyes	bilateral	no
5	54	M	p.Val692Met	p.Val692Met	AR	5	both eyes	no	no
6	54	W	p.Arg570X	p.Arg570X	sporadic	6	no	no	no
8	14	M	p.Lys542GlnfsX5	p.Lys542GlnfsX5	sporadic	6	no	unilateral	unilateral
11	54	M	p.Asp2010Asn	p.Trp2107X	sporadic	13	no	no	no
17	56	W	p.Cys705X	p.Cys705X	sporadic	unknown	no	no	no
CDH23									
7	12	W	p.Arg1189ArgIfsX5	p.Arg1189ArgIfsX5	sporadic	12	both eyes	no	bilateral
9	9	M	p.Ser1927Cysfs16	c.5821-2A>G	sporadic	8	no	unilateral	unilateral
15	16	W	p.Arg2107X	p.Arg2107X	sporadic	unknown	no	no	no
PCDH15									
3	47	W	p.Glu1113X	p.Glu1113X	sporadic	5	both eyes	no	no
16	28	W	p.Arg991X	p.Arg991X	AR	10	no	no	no
10	62	M	p.Arg962Cys	unknown	sporadic	9	both eyes	no	no
12	52	M	p.Arg336X	unknown	sporadic	3	no	no	no
13	51	M	p.Val1242Met	unknown	sporadic	10	no	no	no
MYO7A[*1]/*PCDH15*[*2]									
4	21	M	p.Ala771Ser[*1]	c.158-1G>A[*2]	sporadic	10	no	unilateral	unilateral
unknown									
14	64	W	unknown	unknown	sporadic	15	both eyes	unilateral	no

*All subjects have congenital deafness and RP.

doi:10.1371/journal.pone.0090688.t002

pathogenecity of mutations identified, functional analysis will be necessary to draw the final conclusion in the future.

In UK and US Caucasian USH1 patients, USH1B (*MYO7A*) has been reported as the most common USH1 genetic subtype [11,12], while USH1F (*PCDH15*) has been reported as the most common USH1 genetic subtype in North American Ashkenazi Jews [23]. In Japanese, our study revealed that the most common type was *MYO7A* (41.7%), which was similar to the frequency in the above Caucasian patients (46.8~55%) [11,12]. However, the small number of USH1 patients in our study might have biased the frequency and further large cohort study will be needed in the future.

In addition, most of our detected mutations were novel. We have previously reported genes responsible for deafness in Japanese patients and observed differences in mutation spectrum between Japanese (who are probably representative of other Asian populations) and populations with European ancestry [24].

In conclusion, our study was the first report of USH mutation analysis using MPS and the frequency of USH1 genes in Japanese. Mutation screening using MPS has the potential power to quickly identify mutations of many causative genes such as USH while maintaining cost-benefit performance. In addition, the simultaneous mutation analysis of large numbers of genes was useful for detecting mutations in different genes that are possibly disease modifiers or of digenic inheritance.

Materials and Methods

Subjects

We screened 17 Japanese USH1 patients (aged 9 to 64 years): three from autosomal recessive families (non-affected parents and two or more affected siblings), and 14 from sporadic families. There were 9 males and 8 females. None of the subjects had any other noteworthy symptoms. All subjects or next of kin on the behalf of the minors/children gave prior written informed consent for participation in the project, and the Ethical Committee of Shinshu University approved the study and the consent procedure.

Amplicon Library Preparation

An Amplicon library of the target exons was prepared with an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies, Carlsbad, CA) designed with Ion AmpliSeq Designer (https://www.ampliseq.com/browse.action) for nine USH genes by using Ion AmpliSeq Library Kit 2.0 (Applied Biosystems, Life Technologies) and Ion Xpress Barcode Adapter 1–16 Kit (Applied Biosystems, Life Technologies) according to the manufacturers' procedures.

In brief, DNA concentration was measured with Quant-iT dsDNA HS Assay (Invitrogen, Life Technologies) and Qubit Fluorometer (Invitrogen, Life Technologies) and DNA quality was confirmed by agarose gel electrophoresis. 10 ng of each genomic DNA sample was amplified, using Ion AmpliSeq HiFi Master Mix (Applied Biosystems, Life Technologies) and AmpliSeq Custom primer pools, for 2 min at 99°C, followed by 15 two-step cycles of

Table 3. The patients with mutations in two different genes.

Sample	Genes with two pathogenic mutations	Gene with one heterozygous mutation	Nucleotide change	Amino acid change	control	SIFT score	PolyPhen score	Referense
5	*MYO7A*	*CDH23*	c.C719T	p.P240L*	0.26	0.06	0.999	Wagatsuma et al. (2007)
8	*MYO7A*	*CDH23*	c.2568C>G	p.Ile856Met	0	0.08	1	This study
15	*CDH15*	*USH1C*	c.2437T>G	p.Tyr813Asp	0	0.19	0.932	This study
3	*PCDH15*	*USH1G*	c.28C>T	p.Arg10Trp	0	0.19	1	This study

*homozygotes.

doi:10.1371/journal.pone.0090688.t003

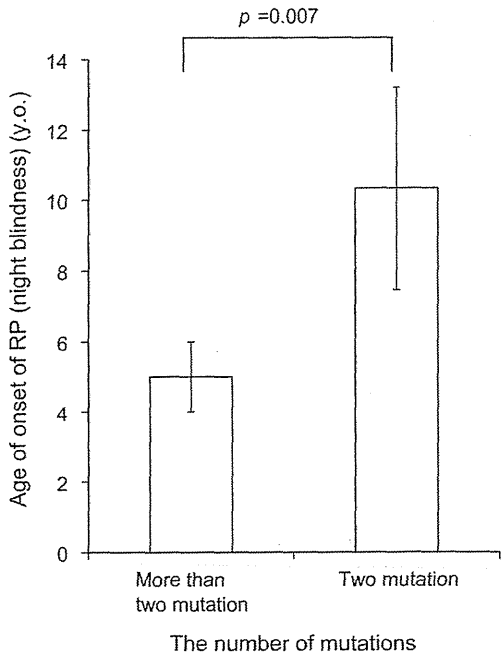

Figure 1. The number of mutations and the age of RP onset in Usher syndrome type 1 patients. The age of RP onset is earlier in the patients with more than two pathogenic mutations. RP: retinitis pigmentosa.

doi:10.1371/journal.pone.0090688.g001

99°C for 15 sec and 60°C for 4 min, ending with a holding period at 10°C in a PCR thermal cycler (Takara, Shiga, Japan). After the Multiplex PCR amplification, amplified DNA samples were digested with FuPa enzyme at 50°C for 10 min and 55°C for 10 min and the enzyme was successively inactivated for 60°C for 20 min incubation. After digestion, diluted barcode adapter mix including Ion Xpress Barcode Adapter and Ion P1 adaptor were ligated to the end of the digested amplicons with ligase in the kit for 30 min at 22°C and the ligase was successively inactivated at 60°C for 20 min incubation. Adaptor ligated amplicon libraries were purified with the Agencourt AMPure XP system (Beckman Coulter Genomics, Danvers, MA). The amplicon libraries were quantified by using Ion Library Quantitation Kit (Applied Biosystems, Life Technologies) and the StepOne plus realtime PCR system (Applied Biosystems, Life Technologies) according to the manufacturers' procedures. After quantification, each amplicon library was diluted to 20 pM and the same amount of the 12 libraries for 12 patients were pooled for one sequence reaction.

Emulsion PCR and Sequencing

The emulsion PCR was carried out with the Ion OneTouch System and Ion OneTouch 200 Template Kit v2 (Life Technologies) according to the manufacturer's procedure (Publication Part Number 4478371 Rev. B Revision Date 13 June 2012). After the emulsion PCR, template-positive Ion Sphere Particles were enriched with the Dynabeads MyOne Streptavidin C1 Beads (Life Technologies) and washed with Ion OneTouch Wash Solution in the kit. This process were performed using an Ion OneTouch ES system (Life Technologies).

After the Ion Sphere Particle preparation, MPS was performed with an Ion Torrent Personal Genome Machine (PGM) system

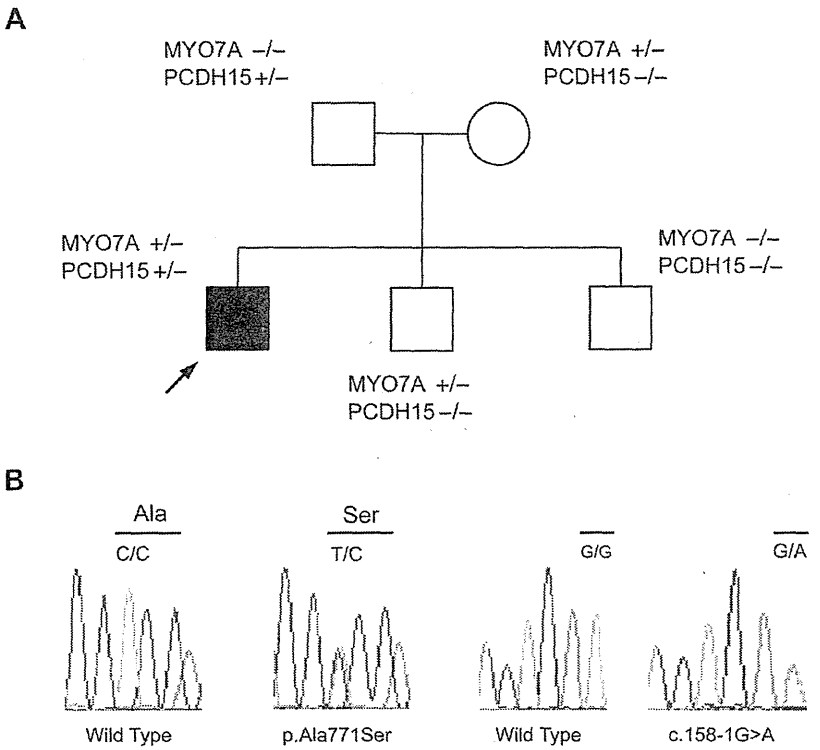

Figure 2. Pedigree and sequence chromatograms of the patient with the p.Ala771Ser in *MYO7A* and c.158-1G>A in *PCDH15* mutations. (A) The pedigree and sequence results of the proband and family. (B) Sequence chromatograms from wild-type and mutations. The proband, his mothor and one brother carried a heterozygous 2311G>T transition in exon 20, which results in an alanine to a serine (Ala771Ser) in *MYO7A*. Another variation, 158-1G>A in intron 3 of *PCDH15*, was derived from the proband and his father. Another brother had no variants.
doi:10.1371/journal.pone.0090688.g002

using the Ion PGM 200 Sequencing Kit and Ion 318 Chip (Life Technologies) according to the manufacturer's procedures.

Base Call and Data Analysis

The sequence data were processed with standard Ion Torrent Suite Software and Torrent Server successively mapped to human genome sequence (build GRCh37/hg19) with Torrent Mapping Alignment Program optimized to Ion Torrent data. The average of 562.33 Mb sequences with about 4,300,000 reads was obtained by one Ion 318 chip. The 98.0% sequences were mapped to the human genome and 94% of them were on the target region. Average coverage of depth in the target region was 314.2 and 93.8% of them were over 20 coverage.

After the sequence mapping, the DNA variant regions were piled up with Torrent Variant Caller plug-in software. Selected variant candidates were filtered with the average base QV (minimum average base quality 25), variant frequency (40–60% for heterozygous mutations and 80–100% for homozygous mutations) and coverage of depth (minimum coverage of depth 10). After the filtrations, variant effects were analyzed with the wANNOVAR web site [25,26] (http://wannovar.usc.edu) including the functional prediction software for missense variants: Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/), and Polymorphism Phenotyping (PolyPhen2; http://genetics.bwh.harvard.edu/pph2/). The sequencing data was available in the DNA databank of Japan (Accession number: DRA001273).

Algorithm

Missense, nonsense, and splicing variants were selected among the identified variants. Variants were further selected as less than 1% of: 1) the 1000 genome database (http://www.1000genomes.org/), 2) the 5400 exome variants (http://evs.gs.washington.edu/EVS/), and 3) the in-house control. Candidate mutations were confirmed by Sanger sequencing and the responsible mutations were identified by segregation analysis using samples from family members of the patients. In addition, the cases with heterozygous or no causative mutation were fully sequenced by Sanger sequencing for USH1 genes in order to verify the MPS results.

Direct Sequence Analysis

Primers were designed with the Primer 3 plus web server (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Each genomic DNA sample (40 ng) was amplified using Ampli Taq Gold (Life Technologies) for 5 min at 94°C, followed by 30 three-step cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 5 min, ending with a holding period at 4°C in a PCR thermal cycler (Takara, Shiga, Japan). The PCR products were treated with ExoSAP-IT (GE Healthcare Bio, Buckinghamshire, UK) and by incubation at 37°C for 60 min, and inactivation at 80°C for 15 min. After the products were purified, we performed standard cycle sequencing reaction with ABI Big Dye terminators in an ABI 3130xl sequencer (Life Technologies).

Accession numbers

MYO7A, [NM_000260.3]; *USH1C*, [NM_ 153676.3]; *CDH23*, [NM_ 022124.5]; *PCDH15*, [NM_ 033056.3]; *USH1G*, [NM_ 173477.2]; *USH2A*, [NM_206933.2]; *GPR98*, [NM_ 032119.3]; *DFNB31*, [NM_ 015404.3]; *CLRN1*, [NM_ 174878.2]; *PDZD7*, [NM_ 001195263.1].

Acknowledgments

We thank A. C. Apple-Mathews for help in preparing the manuscript.

Author Contributions

Conceived and designed the experiments: HY SI SN SU. Performed the experiments: HY SN. Analyzed the data: HY SN SU. Contributed reagents/materials/analysis tools: HY SI SN KK TT YK HS KN KI TI YN KF CO TK HN SU. Wrote the paper: HY SN SU.

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Acta Oto-Laryngologica. 2014; 134: 58–65

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ORIGINAL ARTICLE

Clinical features of rapidly progressive bilateral sensorineural hearing loss

IPPEI KISHIMOTO[1†], HIROSHI YAMAZAKI[1,2*,†], YASUSHI NAITO[1,2], SHOGO SHINOHARA[1], KEIZO FUJIWARA[1], MASAHIRO KIKUCHI[1], YUJI KANAZAWA[1], RISA TONA[2] & HIROYUKI HARADA[1]

[1]*Department of Otolaryngology, Kobe City Medical Center General Hospital, Kobe and* [2]*Institute of Biomedical Research and Innovation, Kobe, Japan*

Abstract

Conclusion: Rapidly progressive bilateral sensorineural hearing loss (SNHL) often develops as a symptom of intracranial diseases or systemic vasculitis. For early diagnosis and treatment of these potentially fatal diseases, a history of hearing deterioration within 2 months and associated symptoms may be important. *Objectives:* To reveal clinical features and causative diseases for rapidly progressive bilateral SNHL. *Methods:* The inclusion criterion was patients with bilateral progressive SNHL, who had experienced difficulty in daily conversation within 4 days to 1 year after the onset of hearing loss awareness. This study was a retrospective evaluation of 12 patients with rapidly progressive bilateral SNHL who visited our hospital between 2007 and 2011. *Results:* The causative disease for hearing loss was identified in 11 of 12 patients; intracranial lesions including nonbacterial meningitis, meningeal metastasis of lymphoma, and superficial siderosis in 4 patients, systemic vasculitis in 2, auditory neuropathy spectrum disorder in 1, and an isolated inner ear disorder in 4. Relatively rapid hearing deterioration within 2 months showed a significant association in six patients with an intracranial lesion or systemic vasculitis. Moreover, all these six patients complained of dizziness and/or non-cochleovestibular symptoms such as fever, headache, and/or altered mental state in addition to hearing loss.

Keywords: *Auditory perception, intracranial disease, systemic vasculitis, magnetic resonance imaging, hearing threshold*

Introduction

Sensorineural hearing loss (SNHL) is caused by various disorders, including sudden deafness, presbycusis, hereditary hearing loss, drug-induced hearing loss, and Meniere's disease. Various clinical data are used to diagnose the cause of SNHL, of which the time course of hearing deterioration may be particularly important for estimating the nature of the disorder. For example, sudden deafness has an onset period of < 72 h [1], while presbycusis deteriorates by 1–2.5 dB per year over a long period of time. We also encounter patients with bilateral SNHL whose hearing deteriorates more slowly than that in sudden deafness but more quickly than that in presbycusis. Such patients often have serious complicating diseases, although only a few studies have examined this type of hearing loss. In this study, we report 12 cases of rapidly progressive bilateral SNHL and analyze the clinical features and causative diseases for hearing loss.

Material and methods

The study was a retrospective review of medical records. Of the 908 patients diagnosed with bilateral SNHL who visited the Department of Otolaryngology at Kobe City Medical Center General Hospital from

Correspondence: Yasushi Naito, Department of Otolaryngology, Kobe City Medical Center General Hospital, 2-2-1 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan. Tel: +81 78 302 4321. Fax: +81 78 302 7537. E-mail: naito@kcho.jp
*Present address: Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Japan.
†Ippei Kishimoto and Hiroshi Yamazaki contributed equally to this work.

(Received 17 June 2013; accepted 30 July 2013)

ISSN 0001-6489 print/ISSN 1651-2251 online
DOI: 10.3109/00016489.2013.831993

Table I. Characteristics of 12 patients with rapidly progressive bilateral sensorineural hearing loss.

Case no.	Onset (age in years)	Time from onset to difficulty in daily life (days)	Gender	Causative disorder	Category of causative disorder	Worst hearing (dB)		Hearing after treatment (dB)		Clinical symptoms
						R	L	R	L	
1	33	4	M	Cryptococcal meningitis	Intracranial lesion	115	115	68.3	25	Fever, headache, altered mentation, dizziness
2	45	60	M	Chronic herpes meningitis + labyrinthitis		115	108.3	No improvement		Fever, tinnitus
3	60	6	M	Meningial metastasis of lymphoma		75	50	45	48.3	Fever, dizziness
4	79	30	F	Superficial siderosis		65	61.7	No improvement		Dizziness, tinnitus
5	73	45	F	Cogan's syndrome	Systemic vasculitis	115	115	No improvement		Fever, headache, dizziness
6	44	4	F	Vasculitis syndrome		93.3	81.7	51.7	38.3	Fever, headache, altered mentation
7	26	7	F	Auditory neuropathy	ANSD	115	113.3	No improvement		Tinnitus
8	63	120	F	Isolated inner ear disorders	Isolated inner ear disorder	65	56.7	No improvement		Tinnitus
9	67	90	M	Isolated inner ear disorders		103.3	103.3	No improvement		Tinnitus
10	69	360	M	Isolated inner ear disorders		95	115	No improvement		Tinnitus
11	69	360	F	Isolated inner ear disorders		80	73.3	No improvement		Tinnitus
12	61	14	F	Undefined disorder	Undefined	53.3	55	41.7	41.7	Fever, backache

January 2007 to December 2011, 12 (1.3%, 5 males and 7 females; Table I) who met the following criteria for rapidly progressive bilateral SNHL were selected: (1) pure-tone audiometry data showing bilateral SNHL and average hearing thresholds at 500, 1000, and 2000 Hz of ≥ 50 dB; (2) difficulty in daily conversation without lip-reading or sign language within 4 days to 1 year after the onset of hearing loss awareness; and (3) exclusion of cases with bilateral Meniere's disease or functional hearing loss. Wegener's granulomatosis [2], Churg-Strauss syndrome [3], and eosinophilic otitis media [4], are also known to induce progressive hearing loss, but were excluded from this study because these diseases lead to mixed hearing loss rather than SNHL. The median age at onset of hearing loss was 62 years (range 26–79 years). The precise deterioration speed of the patients' pure-tone audiometric thresholds could not be calculated because most of them came to our hospital after having moderate or severe SNHL and their initial pure-tone audiometry thresholds before the onset of hearing loss had not been tested. Therefore, we defined progressive bilateral SNHL on the basis of subjective time course of deterioration in auditory perception.

The diagnoses of causative diseases of rapidly progressive bilateral SNHL were based on medical interviews, physical findings, and examinations by otologists, internal medicine specialists, and radiologists. The examinations included blood autoantibody tests, microbiological culture tests, radiographic examinations (CT and MRI), and cerebrospinal fluid (CSF) tests, as well as conventional otological examinations including pure-tone audiometry, distortion product otoacoustic emissions (DPOAEs), and auditory brainstem response (ABR). The causative diseases were categorized into five groups: (1) an intracranial lesion for which CT, MRI, and/or CSF tests revealed an abnormality in the central nervous system; (2) systemic vasculitis, diagnosed by positive blood tests for autoantibodies and systemic inflammation and vasculitis-specific skin lesion, retinal vasculitis, or non-syphilitic interstitial keratitis; (3) auditory neuropathy spectrum disorder (ANSD), diagnosed on the basis of good responses in DPOAE and a lack of obvious responses in ABR; (4) isolated inner ear disorder, with no abnormality on CT or MRI scans and no symptoms other than cochleovestibular symptoms; and (5) an undefined disorder with symptoms other than cochleovestibular symptoms.

The time course of hearing deterioration was evaluated using subjective manifestations. The time course was defined as the time period from the onset of hearing loss awareness to the onset of difficulty in understanding speech in daily life, and it was classified as follows: (1) 4 days to 1 week, (2) 1 week to 1 month, (3) 1–6 months, and (4) 6 months to 1 year. We also focused on clinical manifestations other than hearing loss, which were divided into cochleovestibular symptoms including tinnitus and dizziness and noncochleovestibular symptoms including fever, headache, and altered mental state.

Results

Clinical manifestations

The time course of hearing deterioration was from 4 days to 1 week in four patients, from 1 week to 1 month in two patients, from 1 to 6 months in four patients, and from 6 months to 1 year in two patients. The median hearing level (i.e. the worst value for each patient) of the 12 patients was 94 dB for the right ear and 93 dB for the left ear (Table I). With respect to manifestations related to noncochleovestibular disorders, fever was the leading symptom and was observed in six patients (50%). Among these patients with fever, three also complained of severe headache and two of these further suffered from altered mental state. Tinnitus was observed in seven patients including all six patients without noncochleovestibular symptoms. Dizziness was reported in four patients and three of these were also associated with a noncochleovestibular symptom, but the other complained of only tinnitus and dizziness (Table I).

MRI findings

Brain MRI was performed in nine patients including all six with a noncochleovestibular symptom, one with both tinnitus and dizziness, and two with tinnitus. Association of noncochleovestibular symptoms and dizziness with bilateral SNHL suggests the presence of systemic or intracranial lesions in the former and a retrocochlear or unusual inner ear disease in the latter. In fact, the diagnosis of an intracranial lesion or systemic vasculitis was confirmed or supported by MRI in five of seven patients with a noncochleovestibular symptom or dizziness (Figure 1). In case 4, T2-weighted MRI revealed superficial hypointensity on the surface of the brainstem and cerebellum, which was diagnosed as superficial siderosis. In the other four patients, gadolinium-enhanced T1-weighted MRI showed abnormal enhancement in the inner ear or internal auditory canal. In five cases complaining solely of tinnitus in addition to hearing loss, only two underwent brain MRI. In the other three cases, results of neurological examinations implied that the lesion was restricted in the cochleae and, therefore, careful follow-up of pure-tone audiometry, ABR,

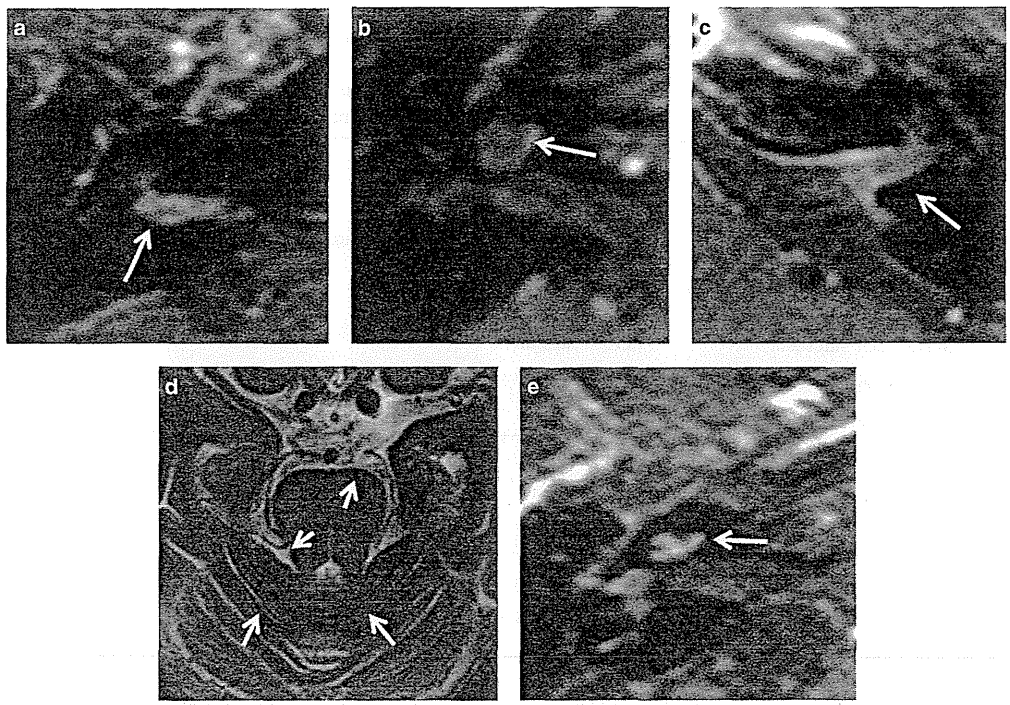

Figure 1. (a) Case no. 1. Cryptococcus meningitis with enhancement of bilateral internal auditory canal (IAC) on gadolinium-enhanced MRI. The enhanced right IAC is shown. (b) Case no. 2. Chronic viral meningitis plus labyrinthitis with enhancement of bilateral cochlea on gadolinium-enhanced MRI. The enhanced basal turn of the right cochlea is shown. (c) Case no. 3. Meningeal metastasis of lymphoma with enhancement of bilateral IAC on gadolinium-enhanced MRI. Enhanced left IAC is shown. (d) Case no. 4. Superficial siderosis with hypointensity along the brainstem and cerebellum on T2-weighted MRI. (e) Case no. 5. Cogan's syndrome with enhancement of bilateral cochlea on gadolinium-enhanced MRI. The right whole cochlea is enhanced.

DPOAE, and/or blood tests for autoimmune antibodies rather than brain MRI were conducted to evaluate cochlear disorders.

Categories of causative diseases

The causative diseases for hearing loss are shown in Table I. Systemic evaluation showed abnormalities restricted to the inner ear in four patients (isolated inner ear disorder). Intracranial lesions were detected in four patients and systemic vasculitis in two, with these disorders diagnosed as the causes of bilateral SNHL. The intracranial lesions included Cryptococcus meningitis, chronic meningitis due to herpes simplex virus, meningeal metastasis of lymphoma, and superficial siderosis. The two patients with systemic vasculitis were diagnosed with Cogan's syndrome and Sjögren syndrome with aseptic meningitis, retinal vasculitis, and skin lesions.

Relationship between category of causative diseases and clinical manifestations

The time course for deterioration in auditory perception was ≤ 60 days in the six patients with an

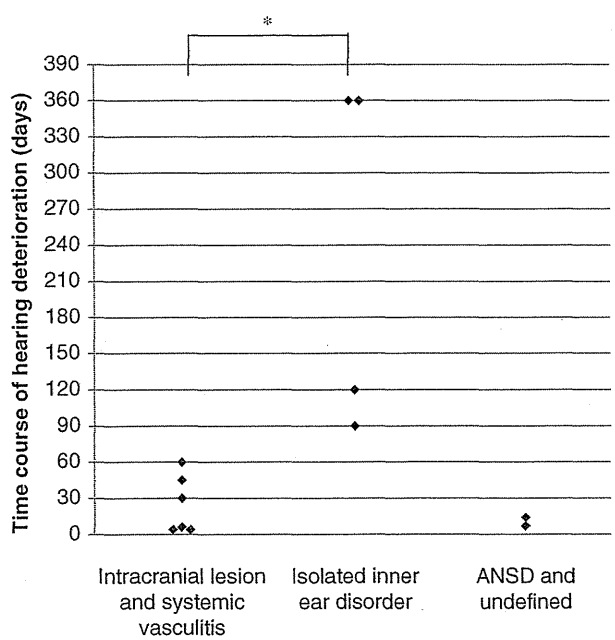

Figure 2. Time course of hearing deterioration in different categories of causative disorders. There was a significant difference between patients with intracranial lesion and systemic vasculitis, and those with an isolated inner ear disorder.
*: $p < 0.05$

Table II. Characteristics of six patients with an intracranial lesion or systemic vasculitis.

Case no.	Diagnosis	Treatment	Time before treatment (days)	Hearing improvement
1	Cryptococcal meningitis	Antifungal drug	3	Improved
2	Chronic herpes meningitis + labyrinthitis	Steroid and anti-HSV agents	Unknown	Not improved
3	Meningial metastasis of lymphoma	Steroid and anticancer drug	6	Improved
4	Superficial siderosis	No treatment		Not improved
5	Cogan's syndrome	Steroid	90	Not improved
6	Sjögren syndrome	Steroid	4	Improved

intracranial lesion or systemic vasculitis and ≥ 90 days in the four patients with an isolated inner ear disorder. The Mann–Whitney U test showed a significant difference ($p < 0.05$) between these groups (Figure 2). As shown in Table I, all patients with an intracranial lesion or systemic vasculitis complained of dizziness and/or noncochleovestibular symptoms in addition to hearing loss. Four of these six patients had dizziness and five of them had fever, headache, or altered mental state. These symptoms were not observed in patients with ANSD or an isolated inner ear disorder, who had only tinnitus as an associated symptom.

Hearing improvement after treatment for the causative diseases

The causative disease was treated in five patients with an intracranial lesion or systemic vasculitis, except in case 4 who had superficial siderosis (Table II). Hearing improved in three patients, who did not require hearing aids in daily life. The delay from the onset of hearing loss awareness to the beginning of treatment was within 1 week in cases 1, 3, and 6, who showed an improvement in hearing. However, it took as long as 90 days in case 5, who showed no change in hearing threshold after treatments. In case 4, the origin of bleeding that caused hemosiderosis was not determined despite radiographic evaluations, including brain and spinal MRI, and the patient showed no improvement in hearing at follow-up. Improvement in hearing loss did not occur in any of the patients with ANSD or an isolated inner ear disorder, despite systemic administration of steroids and/or circulation activators.

Discussion

This study was performed as a retrospective review of 12 cases with progressive bilateral SNHL who complained of difficulty in daily conversation within 4 days to 1 year after the onset of hearing loss awareness. The patients with bilateral SNHL presenting this time course of deterioration were relatively rare and accounted for only 1.3% of those with bilateral SNHL in this study. However, retrospectively, distinguishing this type of SNHL from others was meaningful because 6 of these 12 patients (50%) developed SNHL from an intracranial lesion or systemic vasculitis, which can be fatal without appropriate treatment. It is also noteworthy that all three patients with an intracranial lesion or systemic vasculitis, who showed improvement in hearing, underwent early treatment of the causative diseases, suggesting that accurate diagnosis and appropriate treatments for the causative disease at its early stage may be important to restore hearing as well as to lower the mortality. In the present study, the rapidly progressive SNHL was also caused by ANSD or an isolated inner ear disorder, but clinical manifestations of intracranial lesions and systemic vasculitis were different from those observed in other categories of causative diseases. Our study showed that in patients with intracranial lesions and systemic vasculitis, the time from onset of hearing loss to difficulty in daily life was within 2 months and significantly shorter than that in patients with an isolated inner ear disorder. In addition to the rapidly progressing hearing loss, noncochleovestibular symptoms and/or dizziness were always associated with intracranial lesions and systemic vasculitis, while all five patients with an isolated inner ear disorder or ANSD complained of only tinnitus. Among noncochleovestibular symptoms, fever was the leading symptom (6 of 12 patients), followed by headache and an altered mental state. In all cases with fever, the origin of fever was difficult to identify at first and systemic inflammation or intracranial infection was identified later based on systemic evaluations by otologists, internal medicine specialists, and radiologists. The presence of headache and an altered mental state also suggests that lesions may

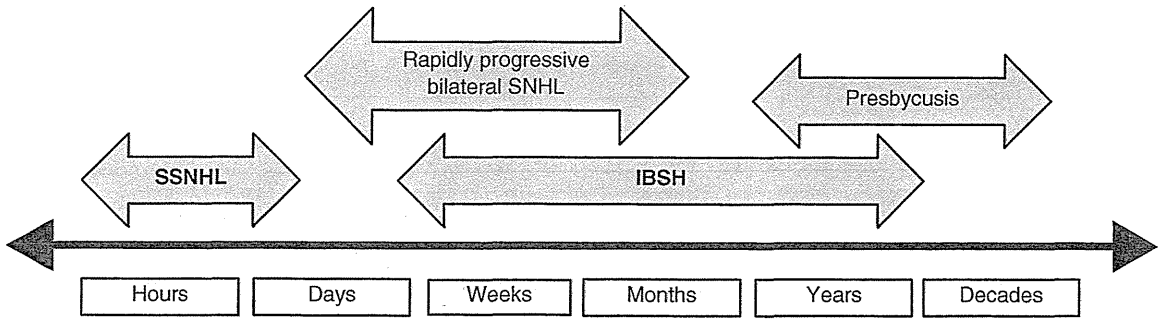

Figure 3. The time course in various types of bilateral sensorineural hearing loss (SNHL). IBSH, idiopathic bilateral SNHL; SSNHL, sudden SNHL.

involve other areas of the central nervous system in addition to the auditory neural pathway. Interestingly, obvious vestibular dysfunction was not observed in patients with an isolated inner ear disease, although four of the six patients with an intracranial lesion or systemic vasculitis had dizziness. The inner ear lesions in the present series may have been limited to the cochlea, with central compensation possibly making the vestibular symptoms less prominent despite the presence of some vestibular involvement.

We performed brain MRI in nine patients including all seven with a noncochleovestibular symptom or dizziness. Headache, altered mental state or other abnormal neurological findings in addition to the eighth cranial nerve dysfunction suggests the presence of an intracranial lesion. In this situation, brain MRI is necessary to evaluate intracranial diseases. Even though the neurological disorders were limited to the eighth cranial nerve, association of dizziness with SNHL might be caused by labyrinthitis or lesions in internal auditory canals and brain MRI may be recommended. Prolonged unknown origin of fever associated with bilateral SNHL is also an indication for brain MRI to evaluate labyrinthitis and nonbacterial meningitis.

In the present study, pure-tone hearing thresholds were improved in case 1 with Cryptococcus meningitis and case 3 with meningeal metastasis of lymphoma after the intracranial administration of antifungal and anticancer drugs, respectively. Hearing recovery is usually difficult in patients with Cryptococcus meningitis [5], although a patient with this disease was reported to show partial recovery of hearing after treatment [5]. Hearing improvement after treatment has also been reported in patients with bacterial and viral meningitis [6,7]. Vasculitis causes SNHL in patients with connective tissue diseases such as systemic lupus erythematosus and polyarteritis nodosa [8], with this type of hearing loss reported to improve following plasmapheresis or immunosuppressive therapy using steroids or cyclophosphamide [2,9]. In our study, case 6, who had Sjögren syndrome, showed hearing improvement after steroid treatment. In contrast, hearing loss in case 5, who had Cogan's syndrome, was not improved by steroids. Although hearing improvement has been described in a patient with Cogan's syndrome [10], it is often difficult to improve hearing loss in such patients.

Previous case reports indicate that the etiology of bilateral SNHL, which deteriorates more slowly than sudden deafness and more quickly than presbycusis, also includes meningeal carcinomatosis [11], metastasis of carcinoma in the bilateral internal auditory canal [12], mitochondrial neurogastrointestinal encephalopathy (MINGIE) [13], and polyarteritis nodosa [14]. These diseases were not found in the present study due to the small size of the study. The rapidly progressive bilateral SNHL can be induced by various types of diseases with different etiologies described above and, moreover, within each type of a disease, severity of symptoms may vary widely between patients. Therefore, further study investigating more patients with rapidly progressive bilateral SNHL is needed to lead to definite conclusions about the importance of clinical manifestations and indications for MRI for diagnosis of the causative diseases.

The definition of rapidly progressive SNHL in previous reports varies, including SNHL deteriorating in days [15] or in weeks to months [14,16–18]. However, the disease entity described in these reports is almost identical, which is the SNHL that progresses more slowly than sudden deafness and more rapidly than presbycusis. Thus, in line with those previous reports, we defined rapidly progressive SNHL as the one that deteriorates in days to months. The time course of rapidly progressive bilateral SNHL compared with that of other types of common bilateral SNHL is illustrated in Figure 3. Idiopathic bilateral SNHL (IBSH) is a progressive bilateral SNHL of unknown etiology and

was proposed as a clinical entity in 1976. In IBSH, hearing loss usually progresses over several years; therefore, deterioration in hearing loss is slower than that observed in the current patients [19], suggesting different etiologies. In the current study, the four patients with isolated inner ear disorders showed a significantly slower deterioration in hearing loss compared with the other patients. IBSH sometimes shows rapid progression of hearing loss within several days or weeks; therefore, patients with similar pathology to that observed in IBSH could meet our criteria for rapidly progressive bilateral SNHL if they visit a hospital in the rapid phase of the disease.

A noteworthy aspect of the patients reported in this study was that early treatment of intracranial lesions and systemic vasculitis improved hearing loss, suggesting the importance of early diagnosis of the causative disease, although further investigation of large numbers of patients is necessary to prove the effectiveness of early treatment. Early diagnosis is also important because the causative diseases for rapidly progressive bilateral SNHL include fatal conditions such as meningitis or malignant diseases, or diseases that may result in irreversible neurological deficits such as superficial siderosis. In patients with superficial siderosis, decreasing the risk for a poor outcome requires early diagnosis of the disease and identification and ablation of the bleeding source [20].

Conclusion

Rapidly progressive bilateral SNHL is rare, but it often develops as a symptom of intracranial disease or systemic vasculitis, both of which are potentially fatal. Hearing may recover in patients who undergo treatment at an early stage of the causative disease. This indicates that early diagnosis followed by appropriate treatment of the causative disease is critical for the management of these patients.

Acknowledgments

We would like to thank Dr Michi Kawamoto and Dr Nobuo Kohara in our institute for advice about diagnosis and treatment of patients. This study was supported by a Grant-in-Aid for Scientific Research (C) (22591894) and a Grant-in-Aid for Young Scientists (B) (22791642) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

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

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Notice of correction

The Early Online version of this article published online ahead of print on 21 Nov 2013 was missing information about the authors.
The corrected version is shown here.

International Journal of Pediatric Otorhinolaryngology 78 (2014) 285–289

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Evaluation of cortical processing of language by use of positron emission tomography in hearing loss children with congenital cytomegalovirus infection☆

Hideaki Moteki [a,1], Mika Suzuki [a,1], Yasushi Naito [b], Keizo Fujiwara [b], Kazuhiro Oguchi [c], Shin-ya Nishio [a], Satoshi Iwasaki [d], Shin-ichi Usami [a,*]

[a] Department of Otorhinolaryngology, Shinshu University School of Medicine, Matsumoto, Japan
[b] Department of Otorhinolaryngology, Kobe City Medical Center General Hospital, Kobe, Japan
[c] Positron Imaging Center, Aizawa Hospital, Matsumoto, Japan
[d] Department of Hearing Implant Sciences, Shinshu University School of Medicine, Matsumoto, Japan

ARTICLE INFO

Article history:
Received 24 May 2013
Received in revised form 22 November 2013
Accepted 24 November 2013
Available online 2 December 2013

Keywords:
FDG-PET
CMV
Hearing loss
Visual language task

ABSTRACT

Objective: To predict cochlear implant efficacy and investigate the cortical processing of the visual component of language in profoundly deafened patients with asymptomatic congenital cytomegalovirus (CMV) infection.

Methods and cases: The cortical activity of two children with CMV-related hearing loss was evaluated with fluorodeoxyglucose-positron emission tomography (FDG-PET) with a visual language task before cochlear implantation. Total development and auditory perception ability were assessed one year after implantation.

Results: The two children with CMV-related hearing loss showed activation in the auditory association area where no activation was found in the controls, and exhibited nearly identical cortical activation patterns to those seen in patients with profound congenital hearing loss. In contrast, differences in total development in verbal ability and discrimination of sentences between the two cases were revealed one year after implantation.

Conclusion: These results might indicate that the differences of cortical activities according to hearing abilities could have been influenced by CMV infection that involves higher function of the brain directly and/or affects the cochlea peripherally. Additionally, if CMV infection might have affected only the cochlea, these cortical activation patterns were influenced secondary by the time course of hearing loss characterized by CMV infection, which had varied manifestations.

Accurate diagnosis and cochlear implantation at the appropriate time are important for successful speech development, and each patient needs a personalized habilitation program based on their etiology and brain function.

1. Introduction

Functional brain imaging provides important evidence of the plasticity of the central auditory pathway following a profound loss of hearing, and is one of the effective methods for investigating the cortical processing of language [1,2]. Previous studies have shown low levels of auditory cortical activity in subjects with profound deafness, i.e. lower levels of activity are observed with longer durations of deafness [3,4]. The importance of early hearing inputs by hearing aids or cochlear implantation (CI) has also been shown. Children with prelingual deafness can acquire spoken language by CI, but this approach is less effective in older children who have not acquired language during the critical language acquisition periods [5,6]. The development of the auditory cortex is believed to depend on the patient's auditory experience within 'critical periods' in the early lifetime. Positron emission tomography (PET) activation study by visual language task has shown that low glucose metabolism in the temporal auditory cortex predicts a good CI outcome in prelingually deafened children, which suggests that low metabolism in the

* Corresponding author at: 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 address: usami@shinshu-u.ac.jp (S.-i. Usami).

[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.ijporl.2013.11.025

auditory cortex may indicate its potential of plasticity for spoken language acquisition [7].

Congenital cytomegalovirus (CMV) infection is the most common environmental cause of developmental disability and sensorineural hearing loss (SNHL) in children [8]. Approximately 90% of infected infants are born with no clinical symptoms of congenital CMV infection, such as microcephaly, growth retardation, hepatomegaly, jaundice, or abnormal neurologic findings. SNHL is found in 6–23% of these asymptomatic infection cases and is often late-onset, fluctuating and progressive in nature within the first 6 years of childhood [9,10]. Hence, newborn hearing screening often does not detect problems in children with asymptomatic congenital CMV infection, and at the time of eventual SNHL diagnosis, the exact time course and manifestations cannot be determined [11]. The development of auditory skills and experiences of children with congenital CMV infection with associated hearing loss are unclear due to various clinical histories. Hearing impairment resulting from (even asymptomatic) congenital CMV infection might be not only of cochlear origin but also have central nerve involvement and entail possible risk of CMV-associated disorders later in life. Brain function and CI outcomes have not been examined in asymptomatic congenital CMV-associated hearing loss. In this study, we used ^{18}F-fluorodeoxyglucose (FDG)-PET to measure cortical glucose metabolism with a visual language task before CI in two profoundly deaf children with asymptomatic congenital CMV infection in order to assess the activities of the auditory cortex and predict the CI outcomes.

2. Methods and cases

2.1. Diagnosis of congenital CMV infection

To analyze congenital CMV infection, we used CMV DNA quantitative PCR (qPCR) analysis. Before qPCR analysis, total DNA including genomic DNA and CMV DNA was extracted from preserved dried umbilical cords. Each 10 pg total DNA was analyzed by a Step One Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using a TaqMan Universal Master Mix II (Applied Biosystems). The detailed methods of qPCR have been described previously (Furutate et al.) [12].

2.2. FDG-PET scanning and image analysis

FDG-PET scanning and image analysis were performed using the methods described by Fujiwara et al. [7]. 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). The patients were then sedated by an anesthesiologist, and their heads were immobilized with a bandage during the scan. 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 by a t test in the basic model of SPM2. The control group consisted of six normal-hearing (pure tone average hearing levels within 20 dB HL) right-handed adult (27.5 ± 3.8 years) subjects. The statistical significance level was set at $p < 0.001$ (uncorrected).

2.3. Measurement of language and total development

Before CI, we evaluated the patients' mental development by the Kyoto scale of psychological development (*K*-test) in which Cognitive-Adaptive development [13] that consists of non-verbal reasoning or visuospatial perceptions is measured. This test is used commonly to assess developmental status for Japanese language users and the results are described as a developmental quotient (DQ) in comparison to normal controls. In the *K*-test, developmental delay is defined by DQ below 80.

One year after CI, auditory perception ability was assessed by word and sentence discrimination tests, which are components of the CI2004 test battery for children. Audible word discrimination tests were administered by a speech therapist with live voice stimuli presented at 70 dB in a soundproof room. We also evaluated intellectual development using the Japanese version of the WISC-III that corresponds to the Wechsler Intelligence Scale for Children (WISC) and contains non-verbal and verbal ability components. The Japanese WISC-III includes five subsets for performance IQ (PIQ) (picture completion, picture arrangement, block design, object assembly and coding) and five subsets for verbal IQ (VIQ) (information, comprehension, similarities, arithmetic and vocabulary).

This study was approved by the Ethics Committee of Shinshu University School of Medicine and prior written consent was obtained from the parents of both children after a full explanation of the study.

2.4. Details of cases

2.4.1. Case 1

This case was a 5-year-old girl. She had no particular events in the perinatal period and passed the newborn hearing screening. However at age 4 years 11 months, her mother suspected hearing loss because of poor response to sound. She only had mild expressive language impairment; her fine motor skills were unaffected. An auditory steady state response (ASSR) test showed bilateral hearing loss (approximately, right: 60 dB, left: 110 dB) (Fig. 1A). She was promptly fitted for bilateral hearing aids. After one month, a follow-up ASSR test indicated deterioration of hearing in her right ear to over 110 dB (Fig. 1C). At this point, DNA testing for hereditary hearing loss e.g. screening for mutations in the *GJB2* and *SLC26A4* genes, and checking for congenital CMV infection using preserved dried umbilical cord (above mentioned) was performed to diagnose the cause of hearing loss. These tests revealed that there were no pathological mutations causing hearing loss, but there were positive results for CMV infection. It was suspected that this late-onset, and rapidly progressive for one month, hearing loss was due to asymptomatic congenital CMV infection. Computed tomography (CT) findings of the middle and inner ear were normal. Hearing aids were not expected to be adequate to acquire spoken language, therefore CI was performed in the left ear at the age of 5 years 5 months.

2.4.2. Case 2

This 4-year-old girl had no particular events in the perinatal period and had not undergone newborn hearing screening. Her parents noticed that she did not respond to their voices when she had just turned 3 years old. She visited a hospital for a checkup where she was diagnosed by ASSR test at the age of 3 years 6 months with hearing loss that was approximately right: 60 dB, and left: 110 dB (Fig. 1B). She attended rehabilitation for hearing, using a combination of finger signing and gestures. In the following year, her hearing deteriorated further to right: 90 dB, left: 110 dB at the age of 4 years five months and her speech development was not

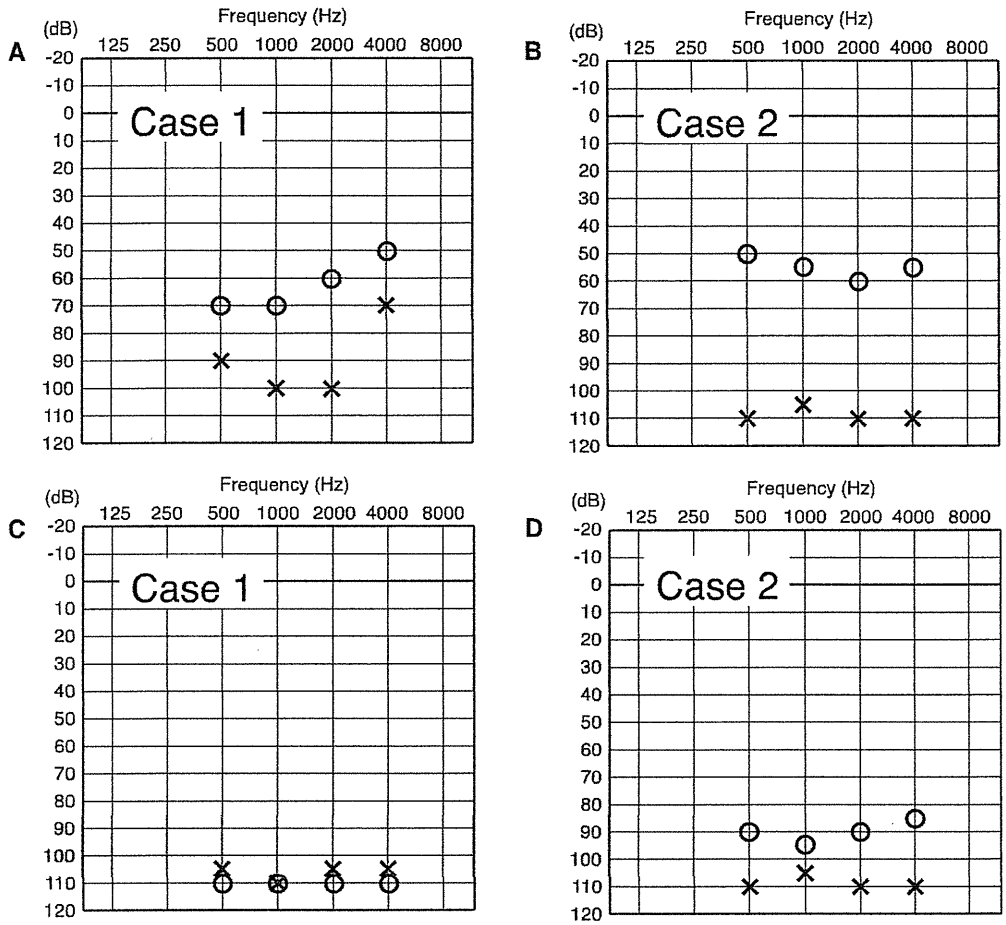

Fig. 1. (A) Case 1; a 5-year-old girl with asymptomatic congenital CMV infection (threshold using ASSR test). (B) Case 2; a 5-year-old girl with asymptomatic congenital CMV infection. These were results of first diagnosed with hearing loss. (C and D) Deterioration in hearing, for one month and for one year, respectively.

significant (Fig. 1D). She was referred to our hospital for further examinations, and her preserved umbilical cord demonstrated a positive result for congenital CMV infection. Late-onset and slowly progressive hearing loss for one year was suggested. There were no inner ear abnormalities seen in the CT findings. She underwent CI surgery in the left ear at the age of 4 years 9 months.

Each child received the same rehabilitation according to auditory oral method by the same speech therapist after implantation.

Table 1
The activated areas of the brain in profoundly deaf individuals during speech-reading.

Case	Gender/age (years)	Activated areas	
		Right hemisphere	Left hemisphere
1	Female/5	Superior temporal gyrus [BA22] Cingulate gyrus [BA31] Middle frontal gyrus [BA9]	Middle temporal gyrus [BA21] Inferior parietal lobe [BA40] Occipital gyrus [BA19] Precueus [BA7]
2	Female/5	Middle temporal gyrus [BA21] Postcentral gyrus [BA3/1/2] Middle occipital gyrus [BA20] Middle frontal gyrus [BA9]	Precentral gyrus [BA4] Precuneus [BA31] Precuneus [BA19] Cingulate gyrus [BA24]

3. Results

3.1. Brain imaging with PET

Table 1 and Fig. 2 show the areas that were activated in each child during a speech-reading task. The following cortical areas showed significantly higher metabolism during speech-reading in the children compared to normal hearing control subjects. In Case 1, the activated areas were the bilateral auditory association area [BA21], the bilateral precuneus, somatosensory cortex [BA7], the left secondary visual area [BA19], and the left inferior parietal lobule [BA40].

The activated areas in Case 2 were similar to those in Case 1, but the activation of the visual association areas in the parietal lobe were lower and smaller than in Case 1.

3.2. Assessment before cochlear implant, and outcome

Table 2 shows the children's scores in the *K*-test before CI, in the word and sentence discrimination tests, and in the Japanese WISC-III at one year after implantation. *K*-test scores that assessed Cognitive-Adaptive development of each child were almost similar. Both cases showed 30–40 dB of aided hearing thresholds at all frequencies with CI. One year after CI, the results of the Japanese WISC-III showed a clearer difference in VIQ than PIQ, in which Case 1 had a better score compared with Case 2. Case 1 did

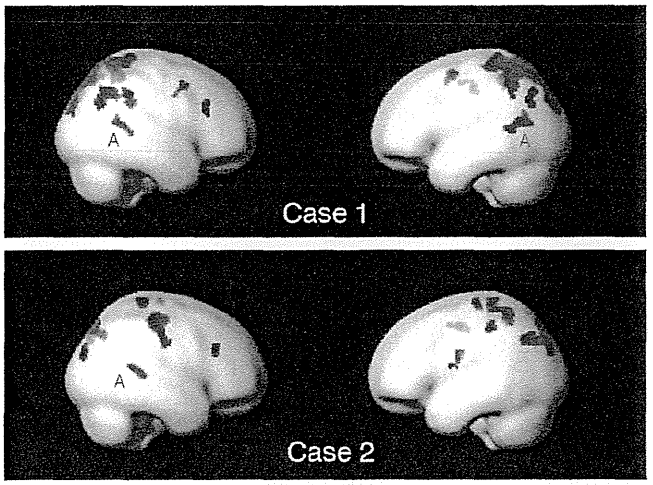

Fig. 2. Cortical activation by language-related visual stimuli in two profoundly deafened with congenital CMV infection cases. Case 1 and 2 showed significant activation in auditory association areas (A) (SPM2, $p < 0.001$, uncorrected).

Table 2
The results of total development before and after cochlear implant, and auditory assessment.

	Before CI	One year after CI	
	K-test (Cognitive-Adaptive)	WISC-III (Japanese version)	Infant word and sentence discrimination
Case 1	99	PIQ 101 VIQ 84	Word 98% Sentence 90%
Case 2	93	PIQ 93 VIQ 56	Word 100% Sentence 53%

CI, cochlear implant; *K-test*, Kyoto scale of psychological development; WISC, Wechsler Intelligence Scale for Children; PIQ, performance IQ; and VIQ, verbal IQ.

better as well in the sentence discrimination component of the auditory perception ability assessment while their results were similar regarding words in the word and sentence discrimination test for children.

4. Discussion

This is the first report on the evaluation of cortical processing of language in hearing loss children with congenital CMV infection. In infants with congenital CMV infections, as many as 20% will suffer from some degree of SNHL, either fluctuating or progressive [14]. This may present a late onset hearing loss, even if the results of newborn hearing screening were normal. The clinical courses of hearing loss in Cases 1 and 2 were typical for asymptomatic congenital CMV infection. Performance and outcome of children with CIs have a strong relation to hearing variables such as onset and course of hearing loss, age of hearing aids fitting, and social background variability, which depends on habilitation and education. According to Fukushima et al. and Kawasaki et al., children with *GJB2* mutations as the etiology for hearing loss have an advantage in their CI outcomes and speech acquisition with normal cognitive development compared with children with unknown etiologies, but this is because the hearing loss is of cochlear origin [15,16]. On the other hand, widely varying conclusions regarding CI outcomes with congenital CMV infection have been reported. Some studies reported the efficacy being not inferior to that of other CI recipients, while others reported it being much poorer [9,17–20]. Accordingly, prediction of CI outcomes for hearing loss with CMV infection is still difficult, unclear, and inconsistent because of various manifestations, progression and the possibility of involvement of higher brain function. Yamazaki et al. suggested that CI with CMV infection outcomes vary widely depending on the psycho-neurological disorders, with their differences in proportion and severity [19].

In this study, the auditory association area in the temporal lobe was activated bilaterally in Case 1 and unilaterally in Case 2. Fujiwara et al., in a FDG-PET study using the same methods and tasks as the present study, showed that subjects with better spoken language skills had less temporal lobe activation [7]. These cases exhibited nearly identical cortical activation patterns to those of congenitally deafened children, suggesting that they did not have enough hearing to develop the cortical network for audition. Previous studies have suggested that plastic changes in auditory cortices were strongly determined by the duration of auditory deprivation [21,22]. However, our two cases of children with CMV-related hearing loss were affected with severe bilateral hearing loss over a short period and were able to acquire spoken language with only a little delay for their age group. It is an interesting but unsolved question why they exhibited results that were the same as previous reports of pre-lingually deafened patients who did not receive sufficient auditory signals and therefore depended on visual cues. One possibility was that high speech-reading activation in the temporal auditory area might be linked to the condition of lacking auditory speech skills at that point, rather than reflecting a consequence of replacement by visual cross-modal plasticity due to a hearing loss of long duration. Besides, visual language activation in the auditory area may change even if affected by hearing loss of a short duration, or it might be influenced by the age-related metabolic changes during the critical period for spoken language acquisition. Another possibility was that these results might indicate that both cases had not received sufficient hearing stimulation as a foundation of language during their early years, which may be attributed to the central nervous system impairment of CMV infection.

Regarding the results of assessment after CI, there was a difference of cognitive ability with VIQ and hearing ability of sentence discrimination, with Case 1 having better CI performance than Case 2 (Table 2). In the assessment of auditory performance, Case 2 especially had difficulty in sentence discrimination despite having the same score in word discrimination as Case 1, who had better CI performance. Sentence discrimination tests require not only audible sound coded by CI, but also recognition of semantics and syntax that would be developed and established with hearing experiences during growth. Indeed, because of the differences between our two cases of the brain imaging, especially in the auditory cortex, we were uncertain whether it might be affected by CMV infection or the onset of their hearing loss itself. However, it raised the possibility that involvement of central nerve and high brain function relevant to CMV infection may lead to retardation of sentence discrimination and speech acquisition in Case 2. On the other hand, there was a difference of activation patterns in the parietal visual association areas. Case 2 showed lower and smaller than in Case 1. Fujiwara et al. predicted that the children with deafness were likely to depend more on vision than normal hearing children do. In Case 1, when someone talked to her, she might have been able to pay much more attention to their facial expression, gestures and visual cues for understanding better than Case 2. Lee et al. reported the comparison of brain metabolic activity between good and poor CI outcomes [23]. The activity patterns in the parietal regions of those with good CI outcomes in their study were similar to our result in Case 1.

We considered that these results might indicate that the differences of cortical activities according to hearing abilities could have been influenced by CMV infection that involves higher function of the brain directly and/or affects the cochlea peripherally. Additionally, if CMV infection might have affected only the