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SHORT COMMUNICATION

Hair roots as an mRNA source for mutation analysis of Usher syndrome-causing genes

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mRNA is an important tool to study the effects of particular mutations on the mode of splicing and transcripts. However, it is often difficult to isolate mRNA because the organ or tissue in which the gene is expressed cannot be sampled. We previously identified two probable splicing mutations (c.6485+5G>A and c.8559-2A>G) during the mutation analysis of *USH2A* in Japanese Usher syndrome (USH) type 2 patients, but we could not observe their effects on splicing because the gene is expressed in only a few tissues/organs, and is not expressed in peripheral lymphocytes. In this study, we used hair roots as a source of mRNA of USH-causing genes, and successfully detected the expression of seven, except *USH1C* and *CLRN1*, of the nine USH-causing genes. We used RNA extracted from the hair roots of a patient who has both c.6485+5G>A and c.8559-2A>G mutations in *USH2A* in a compound heterozygous state to observe the effects of these mutations on transcripts. Reverse-transcription PCR analysis revealed that c.6485+5G>A and c.8559-2A>G inactivated splice donor and splice acceptor sites, respectively, and caused skipping of exons. Thus, RNA extracted from hair roots is a potential powerful and convenient tool for the mutation analysis of USH-causing genes.
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Keywords: hair root; mRNA; Usher syndrome; *USH2A*

INTRODUCTION

To perform mutation analysis in the study of hereditary diseases, we generally used genomic DNA for the detection of mutations in exons and adjacent intronic regions of the gene of interest. Further, mRNA, if available, can also be used for the detection of mutations and for determining their effects on transcripts. However, except in the cases when the gene of interest is expressed in blood cells, it is difficult to isolate mRNA because the organ or tissue in which the gene of interest is expressed cannot be sampled.

We recently performed mutation analysis of *USH2A* gene for Japanese patients of Usher syndrome (USH) type 2, and identified 14 mutations, including 11 novel ones.[1] Of these mutations, two were splicing mutations, c.6485+5G>A and c.8559-2A>G in introns 33 and 42, respectively. We determined the pathogenicity of these mutations using supportive data, but could not examine their effect on pre-mRNA splicing because of the difficulty in obtaining *USH2A* mRNA. The expression of *USH2A* mRNA is restricted to a few tissues, including the retina and the cochlear, and is absent in peripheral lymphocytes.[2] Similarly, peripheral lymphocytes do not express mRNA of any other USH-causing genes, except *DFNB31*.[3]

Here, we attempted to use hair roots as a source of USH-causing gene mRNA. We successfully detected the mRNA expression of most USH-causing genes and analyzed the effect of the above-mentioned *USH2A* mutations on pre-mRNA splicing. This is the first report on the mRNA expression of USH-causing genes in hair roots.

MATERIALS AND METHODS

Collection of hair roots

At least 30 hair root samples were collected from the scalp of normal Japanese individuals and a USH type 2 patient. The patient had c.6485+5G>A and c.8559-2A>G mutations in *USH2A* in a compound heterozygous state (see the patient C152 in a previous report[1]). The institutional review board of Hamamatsu University School of Medicine approved this study, and written informed consent was obtained from all participants before enrollment.

Reverse-transcription PCR of USH-causing genes

Total RNA was extracted from the hair roots using the SV Total RNA Isolation System (Promega, Madison, WI, USA). Next, 2 μg of total RNA was reverse transcribed with oligo(dT) primers by using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) for all nine known USH-causing genes was amplified using specially designed PCR primers (Table 1). The PCR mixtures (total volume, 20 μl) contained 2 μg cDNA, 1.0 M betaine (Wako, Osaka, Japan), 1.5 mM $MgSO_4$, 0.3 μM each primer and 0.4 U KOD Plus DNA polymerase (Toyobo, Osaka, Japan). The amplification conditions were as follows: denaturation at 94 °C for

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Table 1 Nucleotide primers designed for PCR and sequencing of Usher syndrome-causing genes

Template	*Primer sequences (5′ to 3′)*	*Exon*	*Annealing temperature (°C)[a]*	*Product length (bp)*
MYO7A cDNA	F: TGAGATTGGGGCAGGAGTTCGACG	2	68	428
	R: GATGATGCAGCACTGGTCTCGGCT	4		
USH1C cDNA	F: AGTGGCCCGAGAATTCCGGCATAA	1	64	359
	R: CTGCCTGACCGCCTTTGATGAGGT	4		
CDH23 cDNA	F: GGTCGGCTTTGCCCTTCCACTCTT	11	64	449
	R: GTCCCGTGTCCTTGTCCAGCGAGA	14		
PCDH15 cDNA	F: TGCCAAACACTCGTGATTGCCGTC	8	64	330
	R: GACCGGCAAAGGCAGGAAGAGGAT	11		
USH1G cDNA	F: CCCACTCTCTGGGCTGCCTACCAT	1	68	443
	R: GTGAGGCTGGAGAAGCTGAGGGTGT	2		
USH2A cDNA	F: TAACTGCTTGCACTTTGGCTGGCT	31	64	613
	R: GTTAGGGCCTCACTGGCCTCACTC	35		
USH2A cDNA	F: GTGGTGACAGTGCTGGAACCCGAT	41	64	563
	R: ACAGTCACTTCTCGGCTCGGTGTAA	44		
GPR98 cDNA	F: ACTCACCTTTTGGCTTGGTGGGCT	53	64	533
	R: AAAGCTTCCAGCCAGCCGGACTAC	56		
DFNB31 cDNA	F: CTGCGCGTCAACGACAAATCCCTG	1	64	371
	R: CCTGGGTCCACGCCAGTGATGTAA	3		
CLRN1 cDNA	F: GCAATCCCAGTGAGCATCCACGTC	2	64	368
	R: GGGAACTGAAATCCAGCAAGTCGT	3		

Abbreviations: F, forward; R, reverse.
[a]The amplification conditions were as follows: denaturation at 94 °C for 2 min; followed by 40 cycles of treatment at 98 °C for 10 s, 64 or 68 °C for 30 s (see this column), and 68 °C for 1 min; and final extension at 68 °C for 5 min.

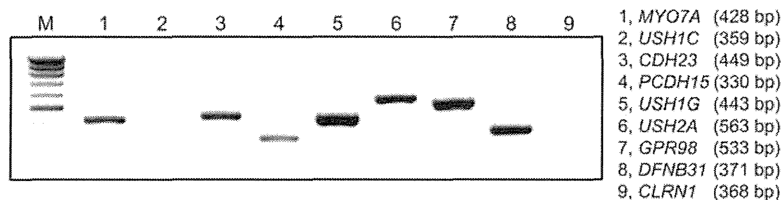

Figure 1 RT-PCR analysis of USH-causing genes. mRNA expression of all USH-causing genes, except *USH1C* and *CLRN1*, was detected in normal control hair roots. PCR was performed using 2 µg cDNA (total volume, 20 µl) with 40 cycles.

2 min; followed by 40 cycles of treatment at 98 °C for 10 s, 64 or 68 °C for 30 s (described in Table 1) and 68 °C for 2 min; and final extension at 68 °C for 5 min.

RESULTS

Detection of mRNA of USH-causing genes in hair roots

Total RNA was prepared from the scalp hair root samples obtained from normal individuals. Reverse-transcription PCR (RT-PCR) analysis revealed the mRNA expression of all USH-causing genes, except *USH1C* and *CLRN1*, in hair roots (Figure 1).

Detection of the splicing abnormality caused by *USH2A* splicing mutations

We next attempted to detect the splicing abnormality caused by the compound heterozygous mutations c.6485+5G>A and c.8559-2A>G in *USH2A*. Total RNA was prepared from the hair root samples obtained from the patient, and RT-PCR was performed using primers to amplify the cDNA between exons 31 and 35. Agarose gel electrophoresis of the RT-PCR products revealed two bands—a larger band corresponding to the normal sequence and a smaller band corresponding to the mutant sequence (Figure 2a). Sequence analysis of the mutants revealed that c.6485+5G>A causes skipping of exon 33 (160 bp) and presumably creates a premature stop codon in exon 34 through a frameshift. Similarly, RT-PCR performed using primers to amplify the cDNA between exons 41 and 44 revealed that c.8559-2A>G causes skipping of exon 43 (123 bp) (Figure 2b) and presumably induces a 41-amino-acid deletion. These results revealed that c.6485+5G>A and c.8559-2A>G inactivated splice donor and splice acceptor sites, respectively, and this finding confirmed the pathogenicity of these mutations.

DISCUSSION

RT-PCR analysis revealed the mRNA expression of seven of the nine USH-causing genes in hair roots. It has been reported that the mRNA of one USH-causing gene, *MYO7A* (causes USH type 1B), can be detected in the nasal epithelium;[4] however, obtaining *MYO7A* mRNA would necessitate invasive and painful tissue sampling methods. In contrast, collecting hair roots from the scalp is not an invasive procedure. Further, analysis of total RNA obtained from the hair roots of the patient with USH type 2 revealed that the two intronic mutations c.6485+5G>A and c.8559-2A>G inactivated a splice donor and splice acceptor sites, respectively, and both these mutations resulted in exon skipping. This is the first report to describe the RT-PCR analysis of *USH2A* mutations and show that the mutations close to the splice donor/acceptor sites cause splicing errors.

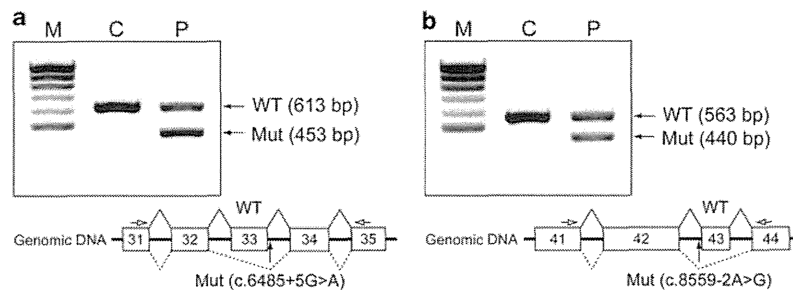

Figure 2 (**a**) Products of RT-PCR performed using primers to amplify *USH2A* cDNA between exons 31 and 35. The c.6485+5G>A mutation caused skipping of exon 33 (160 bp) and was presumed to create a premature stop codon in exon 34 through a frameshift. (**b**) Products of RT-PCR performed using primers to amplify *USH2A* cDNA between exons 41 and 44. The c.8559-2A>G mutation caused skipping of exon 43 (123 bp) and was presumed to create a 41-amino-acid deletion. Boxes with a number represent the exons. The solid and dotted lines that connect exons show the manner of splicing in the wild type and mutant, respectively. The distance between exons does not indicate the actual intron sizes. The open arrows indicate the PCR primers, and the closed arrows indicate mutations in introns. M, molecular marker (100 bp ladder); C, control; P, patient; WT, wild type; Mut, mutant.

Generally, mRNA is very useful for mutation analysis, especially in the case of coding-sequence mutations in large multi-exon genes, splicing mutations and regulatory-region mutations that affect the expression levels. Of these, the use of mRNA to determine the effect of a mutation on splicing as we revealed in this report is the most important advantage because we still cannot accurately predict splicing changes from DNA sequence alterations, especially if the alterations occur at a distance from splicing donor/acceptor sites[5] or within exonic splicing enhancers.[6]

Thus, mRNA extracted from hair roots is a potentially powerful and convenient tool for mutation analysis in USH-causing genes. Further, it is also reasonable to hypothesize that the mRNA of genes that cause deafness can be detected in hair roots, and this may facilitate easier and more accurate mutation analysis.

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ORIGINAL ARTICLE

Mutation analysis of the *MYO7A* and *CDH23* genes in Japanese patients with Usher syndrome type 1

Hiroshi Nakanishi[1,2], Masafumi Ohtsubo[2], Satoshi Iwasaki[3], Yoshihiro Hotta[4], Yoshinori Takizawa[1], Katsuhiro Hosono[2,4], Kunihiro Mizuta[1], Hiroyuki Mineta[1] and Shinsei Minoshima[2]

Usher syndrome (USH) is an autosomal recessive disorder characterized by retinitis pigmentosa and hearing loss. USH type 1 (USH1), the second common type of USH, is frequently caused by *MYO7A* and *CDH23* mutations, accounting for 70–80% of the cases among various ethnicities, including Caucasians, Africans and Asians. However, there have been no reports of mutation analysis for any responsible genes for USH1 in Japanese patients. This study describes the first mutation analysis of *MYO7A* and *CDH23* in Japanese USH1 patients. Five mutations (three in *MYO7A* and two in *CDH23*) were identified in four of five unrelated patients. Of these mutations, two were novel. One of them, p.Tyr1942SerfsX23 in *CDH23*, was a large deletion causing the loss of 3 exons. This is the first large deletion to be found in *CDH23*. The incidence of the *MYO7A* and *CDH23* mutations in the study population was 80%, which is consistent with previous findings. Therefore, mutation screening for these genes is expected to be a highly sensitive method for diagnosing USH1 among the Japanese.
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Keywords: *CDH23*; hearing loss; *MYO7A*; retinitis pigmentosa; Usher syndrome

INTRODUCTION

Usher syndrome (USH) is an autosomal recessive disorder characterized by retinitis pigmentosa (RP) and hearing loss (HL), with or without vestibular dysfunction.[1] It is the most common cause of combined deafness and blindness in industrialized countries, with a general prevalence of 3.5–6.2 per 100 000 live births.[2–7] The syndrome is clinically and genetically heterogeneous and can be classified into three clinical subtypes on the basis of the severity and progression of HL and the presence or absence of vestibular dysfunction.[8–10]

USH type 1 (USH1) is characterized by congenital severe-to-profound HL and vestibular dysfunction; it is the second common type after USH type 2 and accounts for 25–44% of the USH cases.[7,11] Five causative genes have been identified: myosin VIIA (HUGO gene symbol *MYO7A*); Usher syndrome 1C, harmonin (*USH1C*); cadherin-related 23 (*CDH23*); protocadherin-related 15 (*PCDH15*); and Usher syndrome 1G, Sans (*USH1G*).[12–18] Mutations in these genes have been observed in patients with USH1 from various ethnic origins, including Caucasian, African and Asian.[19] However, there have been no reports of mutation analysis for any responsible genes for USH1 in Japanese patients.

Of the five causative genes, the mutation frequency of *MYO7A* is the highest (39–55% of the total cases), followed by that of *CDH23* (19–35% of the total cases).[20,21] These two genes account for approximately 70–80% of the USH1 cases that have been analyzed.[20,21]

The aim of this study was to analyze mutations in the *MYO7A* and *CDH23* genes in Japanese patients with USH1.

MATERIALS AND METHODS

Subjects and diagnosis

Five unrelated Japanese patients (C103, C224, C312, C517 and C720) from various regions of Japan were referred to Hamamatsu University School of Medicine for genetic diagnosis of USH. All patients met the following criteria for USH1: RP, congenital severe-to-profound HL and vestibular dysfunction.[8] The clinical evaluation of the affected patients consisted of elicitation of the medical history, and ophthalmological and audiovestibular examinations. The medical history included the age at onset of walking, age at diagnosis of HL, nature of HL and age at diagnosis of RP.

The ophthalmological evaluation consisted of best-corrected visual acuity measurement, slit-lamp microscopy, ophthalmoscopy, Goldmann perimetry and electroretinography. Visual fields were evaluated by Goldmann perimetry of both eyes, and the isopters for the V/4e, III/4e and I/4e test targets were measured. Electroretinography was performed according to the International Society for Clinical Electrophysiology of Vision protocol.[22]

The auditory examination consisted of otoscopy, pure-tone audiometry (125–8000 Hz) and tympanometry. The severity of HL was classified using the pure-tone average over 500, 1000, 2000 and 4000 Hz in the better hearing ear as follows: normal hearing, <20 dB; mild HL, 21–40 dB; moderate HL, 41–70 dB; severe HL, 71–90 dB; and profound HL, >91 dB.

Vestibular function was evaluated on the basis of the medical history concerning childhood motor development and the results of caloric tests.

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Caloric stimulation of each ear was performed with cold water (20 °C, 5 ml) and the results were classified according to the peak slow-phase velocity as follows: normal, ⩾20°/s; canal paresis, <20°/s.[23] For the patient diagnosed with canal paresis, stronger stimulation with iced water (4 °C, 5 ml) was used to determine the presence of a residual response.

For all patients, parent samples were obtained for segregation analysis. A set of 135 control subjects, selected from Japanese individuals with no visual or hearing impairment, was used to assess the frequency of nucleotide sequence variations. The institutional review board of Hamamatsu University School of Medicine approved this study, and written informed consent was obtained from all subjects before enrollment.

Mutation analysis

Genomic DNA was extracted from peripheral lymphocytes by using standard procedures. In brief, the DNA samples were first screened for mutations in *MYO7A*, and the negative cases were screened for *CDH23* mutations. All exons (*MYO7A*, 49 exons; *CDH23*, 69 exons) and their flanking sequences were amplified by PCR. The PCR products were purified with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) or treated with Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, MA, USA). Direct sequencing was performed using the BigDye Terminator version 3.1 Cycle Sequencing Kit on an ABI 3100 Autosequencer (Applied Biosystems, Foster City, CA, USA). PCR amplification of *MYO7A* was performed using the primers described by Kumar *et al.*[24] with a slight modification. The PCR primers for *CDH23* amplification were newly designed. Information of the nucleotide sequence and appropriate annealing condition of all primers for PCR and sequencing is available on request. Using direct sequencing or restriction enzyme-based assay, we tested the Japanese control chromosomes for all the novel mutations identified during the mutation analysis.

Reverse-transcription PCR of *CDH23*

Reverse-transcription PCR (RT–PCR) of *CDH23* was preformed using total RNA extracted from hair roots as described previously.[25] The PCR primers were newly designed: forward primer, GCTTTTGGTGCTGATCTCTGGATGC located in exon 1; reverse primer, TGGTCGCTGACAGAGAACTCCACG in exon 4. The amplification condition was as follows: denaturation at 94 °C for 2 min; 40 cycles of treatment at 98 °C for 10 s, 64 °C for 30 s and 68 °C for 1 min; and final extension at 68 °C for 5 min.

RESULTS

Mutation analysis

Mutation analysis of *MYO7A* and *CDH23* in the five unrelated Japanese patients revealed five probable pathogenic mutations in four patients (Tables 1 and 2; Figure 1). Of these, two mutations (p.Tyr1942SerfsX23 in *CDH23* and p.Ala771Ser in *MYO7A*) were novel (Table 2). The former was a large deletion affecting 3 exons (Figure 2). The mutation was found in a homozygous state, which is probably accounted by consanguinity (Supplementary Figure 1). As the deletion caused the loss of 3 exons, resulted in a frameshift generating a premature stop codon at 23-codon downstream and was not identified in 64 control chromosomes, it was considered

Table 1 Clinical information of patients with probable pathogenic mutations

Patient	Age	Sex	Responsible gene	Mutations: Allele 1	Mutations: Allele 2	Age[a]: Walking	Age[a]: HL	Age[a]: RP	Visual acuity: Right	Visual acuity: Left	Visual field	ERG	Fundus of the eye	Cataract	Severity of HL	Caloric test
Homozygotes[b]																
C517	26	M	*CDH23*	p.Tyr1942 SerfsX23	p.Tyr1942 SerfsX23	22	2	3	0.1	0.1	5–10° with residual temporal field (V/4e)	Extinguished	Typical RP	No	Profound	CP
C720	13	F	*CDH23*	p.Arg2107X	p.Arg2107X	24	2	12	0.7	0.6	10–15° (V/4e)	Extinguished	Typical RP	No	Profound	CP
Compound heterozygotes																
C312	36	F	*MYO7A*	p.Arg150X	p.Arg1883Gln	24	2	10	0.5	0.7	5° (V/4e)	Extinguished	Typical RP	Both eyes	Profound	CP
Heterozygote																
C103	39	M	*MYO7A*	p.Ala771Ser	Unknown[c]	18	3	27	0.4	0.3	10–15° with residual temporal field (III/4e)	Extinguished	Typical RP	Both eyes	Profound	CP

Abbreviations: CP, canal paresis; ERG, electroretinography; HL, hearing loss; RP, retinitis pigmentosa.
[a]Age at onset of walking (months) and at diagnosis of HL and RP (years) are shown.
[b]The family of patient C517 has consanguinity (see Supplementary Figure 1), whereas that of patient C720 does not.
[c]The pathogenic allele remained undetected.

Table 2 Probable pathogenic mutations identified in the Japanese patients with USH1 examined in this study

Responsible gene	Nucleotide change	Predicted translation effect	Mutation type	Exon number	Domain[a]	Conservation in h/d/r/m/c/z species[b]	Number of alleles	Alleles in control chromosomes	Reference
CDH23	c.5821-?_6253+?del5078	p.Tyr1942SerfsX23	Deletion	44–46	EC18		2	0/64	This report
	c.6319C>T	p.Arg2107X	Nonsense	47	EC20		2	0/64	26
MYO7A	c.448C>T	p.Arg150X	Nonsense	5	Motor		1	0/64	12
	c.2311G>T	p.Ala771Ser	Missense	20	IQ	A/A/A/A/V/A	1	0/270	This report
	c.5648G>A	p.Arg1883Gln	Missense	41	MyTH4	R/R/R/R/R/R	1	0/200	21

[a]Detailed locations of the mutations are shown in Figure 1.
[b]h/d/r/m/c/z denote human/dog/rat/mouse/chicken/zebrafish myosin IIVa orthologs, respectively.

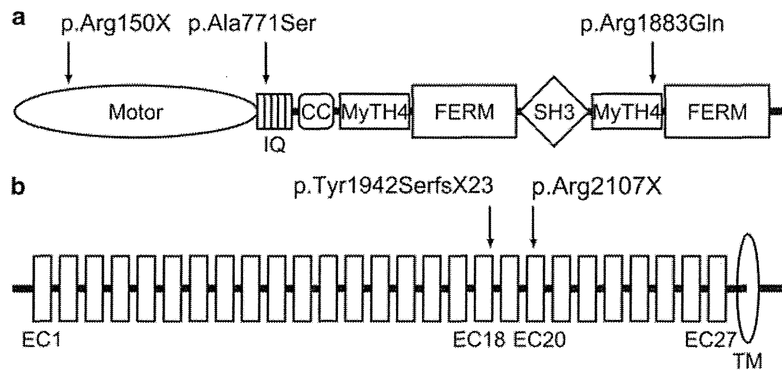

Figure 1 (**a**) Schema of myosin VIIa domains with mutations identified in *MYO7A*. The p.Arg150X, p.Ala771Ser and p.Arg1883Gln mutations were located in the Motor domain, IQ motif and MyTH4 domain, respectively. IQ, isoleucine-glutamine motif; CC, coiled-coil domain; MyTH4, myosin tail homology 4 domain; FERM, 4.1, ezrin, radixin, moesin domain; SH3, Src homology 3 domain. (**b**) Schema of cadherin 23 domains with mutations identified in *CDH23*. The p.Tyr1942SerfsX23 mutation changed Tyr1942 located in EC18 to Ser and created a premature stop codon at 23-codon downstream. The p.Arg2107X mutation was located in EC20. EC, extracellular domain; TM, transmembrane domain.

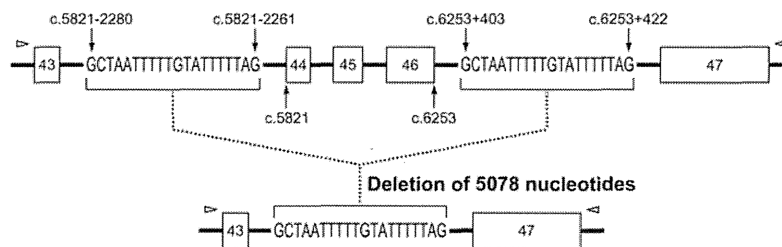

Figure 2 Schema of mutation p.Tyr1942SerfsX23 in the *CDH23* gene. The deletion occurred between introns 43 and 46, and both boundaries had 20-nucleotide sequence string GCTAATTTTTGTATTTTTAG. Upstream and downstream strings were located between c.5821-2280 and c.5821-2261 and between c.6253+403 and c.6253+422, respectively. Although the precise breakpoints could not be determined, the deletion size was elucidated to be 5078 nucleotides. The deletion was notated as c.5821-?_6253+?del5078. The boxes with a number represent exons. The distance between exons does not indicate the actual intronic sizes. The open arrowheads indicate the primer pairs used for PCR to amplify exons 43–47.

pathogenic. The other novel mutation (p.Ala771Ser in *MYO7A*) was considered pathogenic because it was not detected in 270 control chromosomes and Ala771 has been found to be almost conserved in various vertebrates (Table 2). Another mutation in patient C103 remained unclear. The remaining mutations (p.Arg150X and p.Arg1883Gln in *MYO7A*, and p.Arg2107X in *CDH23*) were previously reported and none of them was detected in the Japanese control chromosomes (Table 2).

In addition to the probable pathogenic mutations listed in Table 2, various sequence alterations were identified in *MYO7A* and *CDH23* (Table 3; Supplementary Tables 1 and 2). These alterations were predicted to be nonpathogenic for various reasons. Some of them have been reported as polymorphism in previous reports (Supplementary Tables 1 and 2). The newly identified alteration in exon 30 of *MYO7A* (p.Pro1261Pro) was also found in the control chromosomes. The newly found alterations in introns, except for c.68-3C>T in *CDH23* of patient C224, were distant from splicing donor or acceptor sites. The exception was not detected in any of the 270 control chromosomes but was considered benign because the RT–PCR analysis revealed that the alteration had no influence on splicing (Figure 3).

Clinical findings

All four patients in whom at least one mutant allele was detected had been diagnosed with RP by ophthalmologists at ages 3–27 years (mean ± s.d., 13.0 ± 10.1 years; Table 1). In all the patients, the visual fields were symmetrically constricted, pigmentary degeneration was typical of RP with peripheral bone-spicule pigmentation and standard combined electroretinography was extinguished. The best-corrected visual acuity ranged from 0.7 to 0.1. Two patients (C312 and C103) reported having cataracts, but none underwent cataract surgery.

Table 3 Presumed nonpathogenic alterations that have never been reported

Nucleotide change	*Predicted translation effect*	*Exon/Intron number*	*Number of alleles*	*Alleles in control chromosomes*
Alterations in MYO7A among 5 patients (C103, C224, C312, C517 and C720)				
c.1691-125_126insT		Intron 14	5	
c.1797+55A>G		Intron 15	3	
c.3783C>T	p.Pro1261Pro	Exon 30	1	1/270
c.5944+57G>A		Intron 43	5	
c.5944+67C>T		Intron 43	5	
Alterations in CDH23 among 4 patients (C103, C224, C517 and C720)				
c.68-3C>T		Intron 1	1	0/270
c.3370-46T>C		Intron 27	4	
c.4206+61T>A		Intron 32	8	
c.4207-90G>A		Intron 32	4	
c.4489-98delA		Intron 35	3	

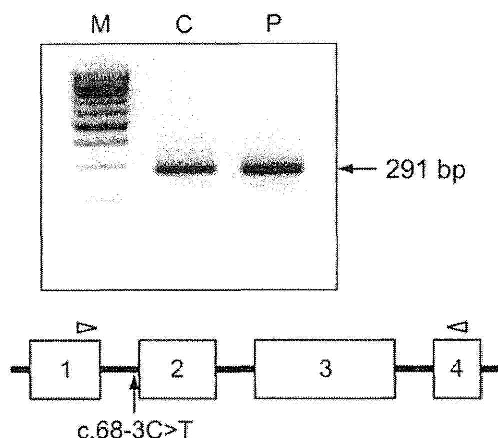

Figure 3 Products of reverse-transcription PCR (RT–PCR) performed using primers to amplify *CDH23* complementary DNA (cDNA) between exons 1 and 4. Agarose gel electrophoresis of the RT–PCR products revealed a single band with the proper size predicted from the normal sequence, indicating that the nucleotide change (c.68-3C>T) had no effect on splicing and was presumably nonpathogenic. PCR was performed using 2 μg cDNA (total volume, 20 μl) with 40 cycles. The boxes with a number represent exons. The distance between exons does not indicate the actual intronic sizes. The open arrowheads indicate the PCR primers, and the arrow indicates the nucleotide change. M, molecular marker (100-bp ladder); C, control; P, patient.

The patients were diagnosed with hearing impairment by otorhinolaryngologists at ages 2–3 years (2.3 ± 0.5 years; Table 1). Despite using hearing aids immediately after the diagnosis, all the patients did not develop speech ability and used sign language to communicate. Tympanometry yielded normal results, consistent with the clinical findings of a normal tympanic membrane and middle ear cavity. Audiograms showed bilateral profound sensorineural HL in all the patients. None of the patients complained of progressive HL.

All the patients reported delayed walking, with starting ages ranging from 18 to 24 months (22 ± 2.8 months; Table 1). The caloric test with cold water revealed canal paresis in all the patients, and no response was induced with the iced water. These results indicated that all the patients had congenital vestibular dysfunction.

DISCUSSION

This is the first report on mutation analysis of *MYO7A* and *CDH23* in Japanese patients with USH1. We found at least one mutant allele in four of the five patients in either of the genes. Although the number of patients examined was small, this frequency (80%) is similar to that among Caucasians, indicating that mutation screening for these genes is a highly sensitive method for diagnosing USH1 among the Japanese.

Of the five mutations identified in this study, three mutations (p.Arg150X and p.Arg1883Gln in *MYO7A*, and p.Arg2107X in *CDH23*) were previously identified in European-Caucasians.[12,21,26] All of these mutations occurred by transition (C/G→T/A) at CpG sites and were considered to be recurrent, which meets the fact that they are not specific to a particular ethnic group. This finding is consistent with a result of an analysis by Baux *et al.*,[27] who reported that a high proportion of *MYO7A* and *CDH23* mutations are represented by single base-pair substitutions and that 51.5 and 48.5% of them in *MYO7A* and *CDH23*, respectively, involve a CpG dinucleotide. Interestingly, neither of the two novel mutations found in the present study is of the transition type.

Mutation p.Tyr1942SerfsX23 (in *CDH23*) was found by PCR using a specially designed primer pair far distant from each other. After failing to amplify each of exons 44–46 in patient C517, we hypothesized the homozygous deletion of a long genomic region including at least exons 44–46. We successfully obtained an amplified product using a primer pair, one (forward) in intron 42 and the other (reverse) in intron 47 (Figure 2). Sequence analysis showed that the amplified DNA contains intact exon 43, truncated intron 43, truncated intron 46 and intact exon 47, indicating a deletion from introns 43 to 46. The boundary between truncated introns 43 and 46 had 20-nucleotide sequence string GCTAATTTTTGTATTTTTAG. Interestingly, the same 20-nucleotide sequences exist in normal introns 43 and 46, and lie within AluSx repetitive sequences. It is speculated that the deletion occurred with Alu-mediated recombination. We could not determine the precise breakpoints in both introns because of the exact sequence identity around possible breakpoints, but the deletion size was elucidated to be 5078 nucleotides regardless of the position of break. We notated the deletion as c.5821-?_6253+?del5078 according to a nomenclature guideline recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).[28]

The deleted sequence in p.Tyr1942SerfsX23 included exons 44, 45 and 46 (103, 126 and 204 nucleotides long, respectively) and the total deletion size in mRNA was 433 nucleotides. Therefore, the mutation was presumed to create a premature stop codon at 23-codon downstream in exon 47 by a frameshift. This is the first large deletion to be found in *CDH23*. We could detect the mutation because of the loss of the same exons in both alleles by consanguinity. However, a large deletion of this type in only one allele cannot be easily detected by PCR because of the existence of the normal allele. In addition, we found a mutation p.Arg2107X in *CDH23* of patient C720. Both of these mutations are of a truncated type (nonsense, deletion/insertion with frameshift, or splicing). This finding is consistent with the previously reported genotype/phenotype relationship for *CDH23*: at least one of the two mutations is of a truncated type in USH1 cases, and both mutations are of a missense type in nonsyndromic HL cases.[29]

In conclusion, the mutation analysis of *MYO7A* and *CDH23* led to the identification of five mutations in four patients. This frequency (80%) indicates that mutation screening for these genes is a highly sensitive method for diagnosing USH1 among the Japanese. One novel mutation, p.Tyr1942SerfsX23 of *CDH23*, was a large deletion causing the loss of 3 exons: the homozygosity resulting from consanguinity probably led to the relatively easy identification. It is possible that similar exonal deletions latently exist in a compound heterozygous state in some USH1 cases in which only one mutation has been found.

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臨　　床

非典型的臨床症状を示した*USH2A*遺伝子変異例

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An Atypical Usher Syndrome Type 2 Patient with *USH2A* Mutations

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Usher syndrome (USH) is an autosomal recessive disorder characterized by hearing loss and retinitis pigmentosa. USH can be classified into 3 clinical subtypes (USH type 1-3: USH1-3) on the basis of the severity and progression of hearing loss and the presence or absence of vestibular dysfunction.

We conducted a mutation analysis of *USH2A*, one of the disease-causing genes of USH2, and identified c.8559-2A>G and p.Trp3150X in a heterozygous state in a USH patient. Though USH2 is characterized by non-progressive moderate-to-severe hearing loss and normal vestibular dysfunction, the patient showed atypical USH2 phenotype–rapidly progressive hearing loss. In atypical patients, environmental factors or modifier genes are presumed to influence the clinical findings. Because the patient had no history of noise exposure, ototoxic medication, or ultraviolet exposure, modifier genes were likely to have influenced the atypical phenotype with *USH2A* mutations.

Considering *MYO7A*, *CDH23*, and *USH3A* as modifier genes, we conducted a mutation analysis of these genes. We identified 16, 44, and 2 sequence alterations in *MYO7A*, *CDH23*, and *USH3A*, respectively, none of which was presumed to be a mutation.

Though we could not identify the causes of the atypical phenotype, we considered it very important in the expansion of the genetic analysis of USH that the causes of atypical USH patients should be identified.

Key words : Usher syndrome, *USH2A*, hearing loss, retinitis pigmentosa

はじめに

アッシャー症候群（Usher syndrome：USH）は，難聴に網膜色素変性症を合併する常染色体劣性遺伝性疾患である．難聴に視覚障害を合併する疾患は，現在までに約40種類知られているが，その中で全患者数の約半数を占める最多の疾患である[1)2)]．

USHは，難聴の程度と前庭機能障害の有無などの臨床症状により，タイプ1～3（USH1～USH3）の3つのタイプに分類され，さらに原因遺伝子がマッピングまたはクローニングされたものは，サブタイプに分類されてい

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る．現在までに，タイプ 1B ～ 1H，2A，2C，2D，3A，3B の 12 のサブタイプが知られている[3)～5)]．

われわれは，臨床症状より USH と診断した患者を対象に，USH2 の原因遺伝子の一つである *USH2A* の遺伝子解析を行い，疾患原因と考えられる変異を同定した．USH2 は，非進行性の中等度～高度難聴を示し，前庭機能障害は合併しないことが特徴であるが[6)]，本症例は，難聴が進行する非典型的な臨床症状を示していた．

このような非典型的な症例では，*USH2A* 遺伝子変異のほかに，環境因子や修飾遺伝子が関与していると考えられている[7)]．本症例では，臨床症状に影響を与えるような環境因子は存在しなかったため，修飾遺伝子が関与している可能性が高いと思われた．

そこで，修飾遺伝子の候補として，*USH2A* 以外の USH の原因遺伝子である *MYO7A*（タイプ 1B の原因遺伝子）[8)]，*CDH23*（タイプ 1D）[9)]，*USH3A*（タイプ 3A）[10)] を考え遺伝子解析を行ったので，*USH2A* 遺伝子解析結果とともに報告する．

症例報告

症　例：21 歳，男性．

主　訴：難聴，網膜色素変性症．

既往歴：特記事項なし．

家族歴：兄に難聴，網膜色素変性症あり．

現病歴：正期産・正常分娩にて出生，周産期に特記すべきことはなかった．運動発達は正常で，1 歳時には自立歩行可能であった．3 歳時に，後方からの呼びかけに対して反応が鈍いことに両親が気付き，近医耳鼻咽喉科を受診し両難聴と診断された．

13 歳時から夜盲を自覚していたが放置，16 歳時に視野狭窄を合併してきたため，総合病院眼科を受診し網膜色素変性症と診断された．21 歳時に，USH の遺伝子検査目的にて当院を受診した．

難聴に影響を与える内耳毒性のある薬剤の服用歴や騒音曝露歴，網膜色素変性症に影響を与える紫外線曝露歴等は認めなかった．

臨床検査結果

耳鼻科的検査：外耳道・鼓膜には異常所見を認めず，ティンパノグラムは両耳 A 型であった．標準純音聴力検査で，両側感音難聴（3 分法にて右耳 75.0 dB，左耳 80.0 dB）を認めた（図 1 A）．DP グラム（MADSEN Capella）は両耳とも 1，2，4，8 kHz の全周波数帯でノイズレベルであり，ABR（日本光電 NeuropackΣ，クリック音）における V 波の反応閾値は両耳とも 90 dB（ダイヤル値）であった．

カロリックテスト（20°C，5 ml）における最大緩徐相速度は，両側とも 20 °/s 以上であった．

眼科的検査：前眼部に異常所見はなく，白内障も認めなかった．矯正視力は，右眼 0.6・左眼 0.9 であった．眼底検査にて，網膜血管の狭細化・骨小体様色素沈着を認め，網膜電図は両眼とも non-recordable であり，典型的な網膜色素変性症の所見を示していた．Goldmann 視野計による視野測定にて V/4e イソプターが 5° 以下（図 2）であり，年齢に比較して求心性視野狭窄は非常に高度であった（同年齢では，V/4e イソプターは 30° 以上のことが多い[11)12)]）．

聴力経過

本症例の聴覚症状をさらに詳しく評価するため，当院

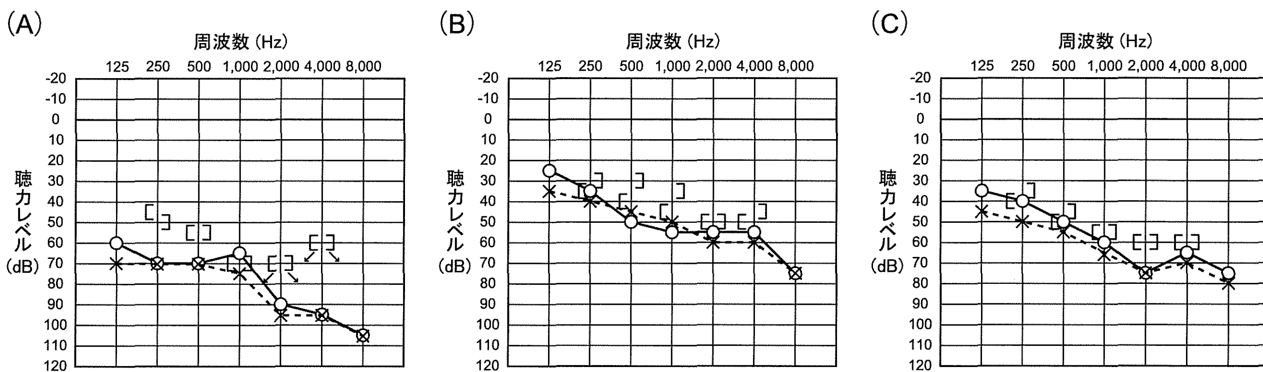

図 1　オージオグラム

A：21 歳時，B：12 歳時，C：17 歳時

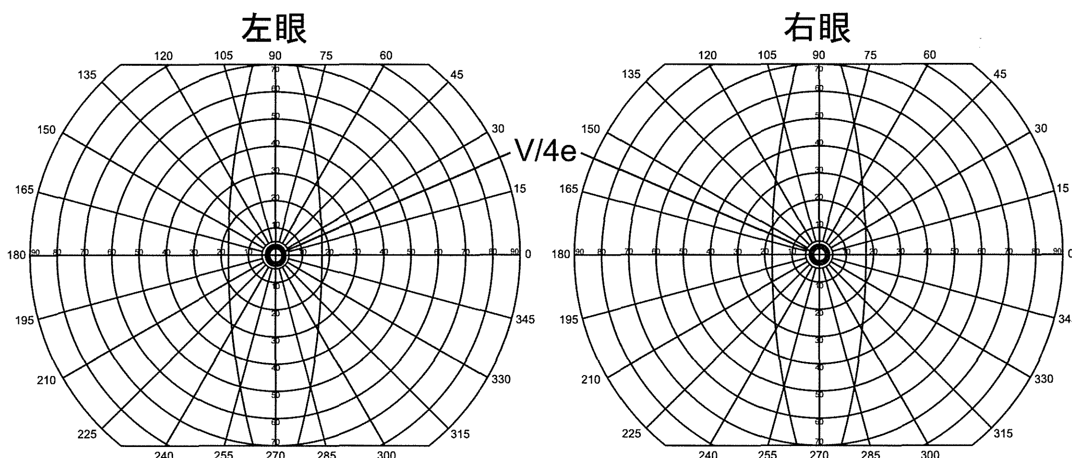

図 2　視野検査結果
高度な求心性視野狭窄を認めた．

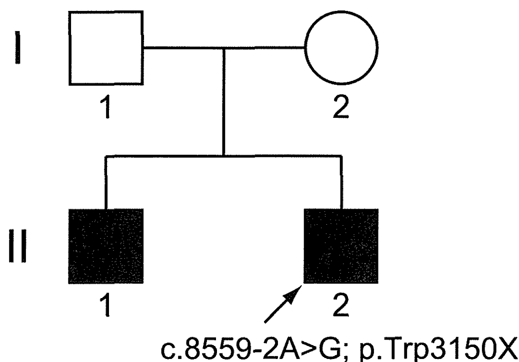

図 3　家系図および *USH2A* 遺伝子解析結果
c.8559-2A>G，p.Trp3150X 変異をヘテロ接合体で認めた．

と前医における聴力検査結果を比較した．12 歳時の聴力検査結果は，右耳 53. 3dB，左耳 51. 7dB（図 1 B），17 歳時は，右耳 61. 7 dB，左耳 65. 0 dB（図 1 C）であり，難聴が進行していることが明らかになった．

遺伝子解析

MYO7A，*CDH23*，*USH2A*，*USH3A* の遺伝子解析を行った．遺伝子解析を行う前に，患者に十分な説明を行い，インフォームド・コンセントを得た後，末梢血を採血した．末梢血よりゲノム DNA を抽出し，*MYO7A*（全エキソン 1 〜 49），*CDH23*（全エキソン 1 〜 69），*USH2A*（全エキソン 1 〜 73），*USH3A*（全エキソン 1 〜 3）について，PCR ダイレクトシーケンス法にて遺伝子解析を行った．*MYO7A*，*USH2A*，*USH3A* の遺伝子解析に用いるプライマーは，Kumar ら[13]，van Wijk ら[14]，Adato ら[15]の報告と同様のものを使用した．*CDH23* の遺伝子解析に用いるプライマーは mPrimer3（http://bioinfo.ebc.ee/mprimer3/）を用いて設計した．本遺伝子解析研究は，当院のヒトゲノム・遺伝子解析研究倫理審査委員会にて承認されている．

遺伝子解析結果

USH2A の遺伝子解析結果：USH2A のイントロン 42 に c.8559-2A>G（IVS42-2A>G），エキソン 48 に p.Trp3150X（c.9449G>A）変異をヘテロ接合体で認めた（図 3）．本症例では，家族の検体が得られず，変異と発症の対応解析を行うことができなかった．しかし，両変異とも疾患原因変異としてすでに報告[16]されている変異であるため，c.8559-2A>G，p.Trp3150X 変異により発症した USH2 と診断した．

MYO7A，*CDH23*，*USH3A* の遺伝子解析結果：*MYO7A* に 16 種，*CDH23* に 44 種，*USH3A* に 2 種の塩基変化を同定した（表 1 〜 3）．*MYO7A* の 12 種，*CDH23* の 27 種，*USH3A* の 2 種の塩基変化は，多型としてすでに報告されている塩基変化であった．*MYO7A* の 4 種，*CDH23* の17種の塩基変化は現在までに報告されていない新規の塩基変化であったが，これらは，エキソンから離れたイントロン内の塩基変化，または 3' 非翻訳領域の塩基変化であり，臨床症状に影響を与える可能性は低いと考えられた．

考　察

USH は，難聴の程度と前庭機能障害の有無などの臨床

表1 *MYO7A* の遺伝子解析結果

ヌクレオチドの変化	産物蛋白質への影響	エキソン／イントロン番号	文献
c.47C>T	p.Ser16Leu	エキソン3	22
c.783T>C	p.Gly261Gly	エキソン8	22
c.1004-35C>G		イントロン9	22
c.1343+8G>A		イントロン12	22
c.1691-125_126insT		イントロン14	未報告
c.1797+55A>G		イントロン15	未報告
c.1936-23G>A		イントロン16	23
c.3375+33G>C		イントロン26	24
c.3505+11_32del		イントロン27	25
c.4755T>C	p.Ser1585Ser	エキソン35	26
c.4996T>A	p.Cys1666Ser	エキソン36	26
c.5715G>A	p.Lys1905Lys	エキソン41	26
c.5857-7A>T		イントロン42	26
c.5944+57G>A		イントロン43	未報告
c.5944+67C>T		イントロン43	未報告
c.6051+17T>A		イントロン44	24

症状により，USH1～3の3つのタイプに分類されている．USH1は重度難聴に前庭機能障害を合併するもの，USH2は中等度～高度難聴を認めるが前庭機能は正常なもの，USH3は難聴が進行するもの（前庭機能障害の有無はさまざま）と定義されている[6]．さらに原因遺伝子がマッピングまたはクローニングされたものは，サブタイプに分類されており，現在までに9種の原因遺伝子が同定されている（表4）[3)～5)]．

本症例では，*USH2A* の遺伝子解析にて，c.8559-2A>G，p.Trp3150X変異がヘテロ接合体で同定された．一般的に *USH2A* 遺伝子変異例はUSH2の臨床症状を呈することが多い．聴力検査にて高度感音難聴を示していたこと，歩行開始年齢やカロリックテストから高度前庭機能障害は否定的であったことは，USH2の臨床症状に合致していた．しかし，難聴が進行したことは，USH2の臨床症状とは異なっていた．このように，*USH2A* 遺伝子変異例の中にも，難聴が進行する非典型的な臨床症状を示す症例が存在することは，遺伝子検査を臨床応用する際に大変重要であると思われる．つまり，現時点では，遺伝子検査のみから臨床症状を予測することには限界があり，遺伝子解析結果を患者に説明する際には，この点を必ず伝えなければならない．

このような非典型的な臨床症状を示す症例では，*USH2A* 遺伝子変異に加えて修飾遺伝子の関与が示唆されている[7]．修飾遺伝子の候補として，難聴・網膜色素変性症の原因遺伝子，遺伝子発現に関与する遺伝子などさまざまな遺伝子が考えられる．本症例では，①難聴（進行性難聴）と網膜色素変性症（高度視野狭窄）の両症状に影響が出ていること，② *USH2A* の産物蛋白質は有毛細胞の不動毛に発現し，他のUSH原因遺伝子の産物蛋白質と相互作用していること[3]（一般的に，原因遺伝子と修飾遺伝子の産物蛋白質は相互作用することが多い[17]）から，*USH2A* 以外のUSH原因遺伝子が修飾遺伝子として有力であると考えた．また，本症例では難聴が進行することが特徴であり，難聴の進行と関連している遺伝子が修飾遺伝子である可能性が高い．USH原因遺伝子の中で，*USH3A* は難聴が進行することを特徴とするUSH3の原因遺伝子である．さらに，USH1の原因遺伝子である *MYO7A*，*CDH23* は，中等度～高度感音難聴を示し難聴が進行する非典型的な症例の原因遺伝子としても報告[18)19)]されている．そこで，修飾遺伝子の候補として，*MYO7A*，*CDH23*，*USH3A* を考えた．

MYO7A，*CDH23*，*USH3A* の遺伝子解析を行い，*MYO7A* に16種，*CDH23* に44種，*USH3A* に2種の塩基変化を同定したが，臨床症状に影響を与えるような変異を同定することはできなかった．PCRダイレクトシーケンス法では，大きな欠失・挿入変異を同定することは困難であるため，本方法による限界もあると思われる．また，

表 2　*CDH23* の遺伝子解析結果

ヌクレオチドの変化	産物蛋白質への影響	エキソン／イントロン番号	文献
c.145+135C>T		イントロン 3	27
c.146-125T>C		イントロン 3	未報告
c.366T>C	p.Val122Val	エキソン 6	19
c.429+13G>A		イントロン 6	19
c.429+26A>G		イントロン 6	19
c.1449+225G>A		イントロン 13	未報告
c.1515-193A>G		イントロン 14	未報告
c.1753-78A>T		イントロン 15	27
c.2060-176C>T		イントロン 18	未報告
c.2289+135C>T		イントロン 20	19
c.2289+240T>C		イントロン 20	未報告
c.2290-268G>A		イントロン 20	未報告
c.2290-267C>T		イントロン 20	未報告
c.2290-240C>G		イントロン 20	未報告
c.2316T>C	p.Asn772Asn	エキソン 21	19
c.2388T>C	p.Asp796Asp	エキソン 21	19
c.2397+26T>C		イントロン 21	19
c.2953+146G>A		イントロン 24	未報告
c.3369+123G>A		イントロン 27	未報告
c.4051A>G	p.Asn1351Asp	エキソン 31	19
c.4206+61T>A		イントロン 32	未報告
c.4206+131T>C		イントロン 32	未報告
c.4207-254T>A		イントロン 32	未報告
c.4207-184T>C		イントロン 32	未報告
c.4360-333T>C		イントロン 34	未報告
c.4488+32C>G		イントロン 35	19
c.4617+154C>T		イントロン 36	未報告
c.4618-77C>T		イントロン 36	27
c.4723G>A	p.Ala1575Thr	エキソン 37	19
c.5187+44C>G		エキソン 39	19
c.5187+99C>T		イントロン 39	27
c.5503-10A>G		イントロン 41	19
c.6130G>A	p.Glu2044Lys	エキソン 46	19
c.6830-81G>A		イントロン 48	19
c.7055-16A>G		イントロン 49	27
c.7073G>A	p.Arg2358Gln	エキソン 50	19
c.7139C>T	p.Pro2380Leu	エキソン 50	19
c.7225-22C>T		イントロン 50	27
c.9319+72_73delTC		イントロン 64	19
c.9380+109G>A		イントロン 65	27
c.9380+111C>T		イントロン 65	27
c.7572G>A	p.Ala2524Ala	エキソン 53	27
c.9873G>A	p.Thr3291Thr	エキソン 69	19
c.*66C>T		エキソン 69	未報告

表3 *USH3A* の遺伝子解析結果

ヌクレオチドの変化	産物蛋白質への影響	エキソン／イントロン番号	文献
c.-71A>G		エキソン1	15
c.57A>T	p.Ala19Ala	エキソン1	15

表4 USH の原因遺伝子と産物蛋白質

タイプ	サブタイプ	遺伝子座	原因遺伝子	産物蛋白質
1	1B	11q13. 5	*MYO7A*	Myosin VIIa
	1C	11q15. 1	*USH1C*	Harmonin
	1D	10q22. 1	*CDH23*	Cadherin 23
	1E	21q21	未同定	
	1F	10q21. 1	*PCDH23*	Protocadherin 15
	1G	17q25. 1	*USH1G*	Usher syndrome type 1G protein
	1H	15q22-q23	未同定	
2	2A	1q41	*USH2A*	Usherin
	2C	5q14. 3	*GPR98*	G-protein coupled receptor 98
	2D	9q32	*DFNB31*	Whirlin
3	3A	3q25. 1	*USH3A*	Clarin 1
	3B	20q	未同定	

MYO7A, *CDH23*, *USH3A* 以外のUSHの原因遺伝子や，USH 以外の原因遺伝子の関与も考えられる．現在までに，*USH2A* 遺伝子変異症例において，難聴が進行する症例や，年齢に比較して視野狭窄が高度である症例などの非典型的な臨床症状を示す患者に関する報告[7)20)]はあるが，そのような患者において修飾遺伝子の遺伝子解析を行った報告はなく，われわれの報告が初めてである．

Cremersら[21)]は，DNAマイクロアレイを用いてUSHの原因遺伝子を網羅的に解析し，*USH2A* にp.Lys182fs, p.Glu767fs変異をヘテロ接合体で認め，さらに*MYO7A*にp.Arg1343Ser変異をヘテロ接合体で認めた症例を報告している．この症例の臨床症状は記載されていなかったため，*MYO7A* のp.Arg1343Ser変異が修飾遺伝子として臨床症状に影響を与えていたのか不明であるが，複数の遺伝子に変異を認める症例が存在することは大変興味深い．今後，このような網羅的解析により，非典型的な臨床症状を示すUSH症例の発症機序が明らかになることが期待される．

USHでは，難聴が出現してから数年後に網膜色素変性症が発症するため，臨床症状からでは診断が遅れることが多く，遺伝子検査が早期診断のために有効であると考えられている．遺伝子検査により，早期診断とともに臨床症状の予後を予測することが可能となれば，遺伝子検査の結果をもとに早期治療を行うことができると期待される．そのためにも，本症例のような非典型的な臨床症状を呈する症例の発症機序の解明が必要と思われる．

まとめ

臨床症状よりUSHと診断した患者を対象に*USH2A*の遺伝子解析を行い，c.8559-2A>G, p.Trp3150X 変異をヘテロ接合体で同定した．本患者は，進行性難聴を認め，USH2と異なる臨床症状を示す非典型的*USH2A*遺伝子変異例と考えられた．このような症例では，*USH2A* 遺伝子変異に加えて修飾遺伝子の関与が示唆されているため，*MYO7A*, *CDH23*, *USH3A* を修飾遺伝子の候補と考え遺伝子解析を行った．遺伝子解析にて，同遺伝子に変異を同定することはできなかったが，*USH2A* 遺伝子変異例の中にも非典型的な臨床症状を示す症例が存在することは，遺伝子検査を臨床応用する際に大変重要であり，今後，このような患者の発症機序を解明することが必要であると思われた．

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Usher症候群の全国アンケート調査結果の検討

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Evaluation of a National Survey Study for Usher Syndrome

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Usher syndrome is a major cause of genetic deafness and blindness. The standard classification of Usher syndrome recognizes three clinical types. The present state of Usher syndrome in Japan was evaluated by questionnaire including information on the number and the clinical types of patients diagnosed with the syndrome. We targeted 697 hospitals in which the Oto-Rhino-Laryngological Society of Japan approved as training facilities.

As a result, there were 111 cases reports. There were two possible reasons for this. The one was that the patients were not examined by any otolaryngologist, and the other was that they were not followed-up even if the patients presented themselves in otolaryngology department. In addition, the responders classified them into 15 patients with type 1, 27 patients with type 2, and 17 patients with type 3, but 52 patients (46.8%) were unclassified. We speculated that basis for the classification of Usher syndrome was ambiguous.

In order to achieve accurate classification, we thought that it is necessary to establish the datebase of Usher syndrome patients in cooperation with ophthalmologists.

Key words：hearing loss, retinitis pigmentosa, epidemiology
和文キーワード：難聴，網膜色素変性症，疫学調査

論文要旨

Usher症候群は視聴覚障害を生じる疾患の代表であり、臨床症状により3つにタイプ分類されている。平成22年度より難治性疾患克服研究事業として「Usher症候群に関する調査研究」が全国13施設の共同研究で開始された。そこで我々は本症候群の実態把握のため全国アンケート調査を行った。

対象は日本耳鼻咽喉科学会の定める認可研修施設（697施設）とし、患者数、ならびにタイプ判定を質問項目とした。

61.1%の施設より回答が得られたが、報告された患者数は111名に留まり、半数近くの52例（46.8%）がタイプ判定困難な症例であった。患者数が極端に少ない理由として、本症候群患者であっても耳鼻咽喉科を受診していない場合や、受診してもその後経過観察されていないことが考えられ、タイプ判定が困難な理由は分類基準の曖昧さと思われた。

より正確な実態把握をしていくためには眼科医との連

携とタイプ分類をどの施設でも容易にする統一された分類方法の工夫が必要と考えられた。

はじめに

Usher症候群は「感音難聴に網膜色素変性症を伴う疾患」の代表であり、1858年にvon Graefeにより初めて報告[1]され、1914年にUsherが遺伝的側面についても言及し、「Usher症候群」として報告した[2]。その後、1945年にLindenovによりUsher症候群は常染色体劣性遺伝形式であることが報告され[3]、1959年にはHallgrenがUsher症候群には少なくとも２つのタイプがあり、タイプの違いは原因遺伝子によるものと仮説を立てた[4]。1977年にDavenportらにより難聴の程度や前庭機能障害の有無などの臨床症状により４つのタイプに分類された[5]。しかし、Ｘ連鎖劣性遺伝形式をとるとされたタイプ４症例は連鎖解析にて常染色体劣性遺伝形式であることが判明し[6]、以後Usher症候群はタイプ１～３の３つにタイプ分類されている。「Usher症候群に関する調査研究班」による試案（平成22年度、表１）によれば、タイプ１は「幼少期より高度難聴を呈す。めまいを伴う例が多く、視覚症状は10歳前後より生じる。」、タイプ２は「若年期より高音障害型の難聴を呈する。視覚症状は思春期以降に生じる。めまいを伴わない例が多い。」、タイプ３は「難聴、視覚症状とも思春期以降に生じ、難聴は徐々に進行する。」とされている。Usher症候群の頻度は海外では国により多少の差はあるが、人口10万人あたり3.0～6.2人と報告されている（表２）[4),7)～12)]。本邦におけるUsher症候群の頻度はタイプ１の小児（19歳以下）を対象にした1978年の大鳥らの報告[13]では10万人あたり0.6人と極端に少ない結果であったが、2006年の岩崎らの網膜色素変性症患者を対象とした自覚症状に基づいたアンケート調査[14]では10万人あたり6.7人であり諸外国と類似した頻度が報告されている。しかし、本邦においては未だUsher症候群の頻度を含めた実態は

表１　Usher症候群診断基準（試案）（Usher症候群に関する調査研究班による）

１．疾患の定義

遺伝子変異が原因で、両側性感音難聴と網膜色素変性症を併発する症候群。難聴の程度には軽度難聴～最重度難聴まで幅が大きく自覚症状を伴わない例もある。網膜色素変性に関しては10歳～思春期ごろより夜盲など自覚症状が現れ、徐々に視野狭窄が進行して社会的失明となる例が多い。

２．自覚症状

・夜盲、視野狭窄、視力低下などの視覚障害（網膜色素変性症）
・両側性感音難聴、めまいなどの耳症状（蝸牛、前庭症状）

３．臨床検査所見

・網膜色素変性症に関する所見
（1）眼底所見：網膜血管狭小、粗糙胡麻塩状網膜、骨小体様色素沈着、白点状
（2）網膜電図の振幅低下、又は消失
（3）蛍光眼底造影所見で網膜色素上皮萎縮による過蛍光
・感音難聴に関する所見
（1）純音聴力閾値検査（気導・骨導）の閾値上昇
（2）中枢性疾患、Auditory Neuropathy、伝音難聴が否定できる

４．疾患のタイプ分類

タイプ１：幼少期より高度難聴を呈す。めまいを伴う例が多く、視覚症状は10歳前後より生じる。
タイプ２：若年期より高音障害型の難聴を呈する。視覚症状は思春期以降に生じる。めまいを伴わない例が多い。
タイプ３：難聴、視覚症状とも思春期以降に生じ、難聴は徐々に進行。

５．治療

網膜色素変性に対する有効な治療法は確立されていない。

聴覚障害に対しては、先天性重度難聴を呈するタイプ１症例に対しては人工内耳が有効であり、早期からの人工内耳装用により大幅な聴取能の向上が可能である。中等度～重度の難聴となるタイプ２、３症例に関しては、難聴の自覚を伴わない場合があるため、難聴の自覚の有無にかかわらず網膜色素変性症に罹患した患者の聴力検査を行う事が望ましい。その後、聴力に応じて補聴器・人工内耳を装用する事で、聴取能の向上が可能である。

表2　Usher症候群の頻度とタイプ別割合に関する報告

著者（報告年）	国	頻度	タイプ1	タイプ2	タイプ3	非典型例
Hallgren（1959）[4]	スウェーデン	3.0	—	—	—	—
Nuutila（1970）[7]	フィンランド	3.0	—	—	—	—
Boughman（1983）[8]	アメリカ	4.4	—	—	—	—
Grøndahl（1987）[9]	ノルウェー	3.6	50.0%	36.0%	14.0%	—
Pakarinen（1995）[15]	フィンランド	—	34.5%	12.2%	13.0%	40.2%
Rosenberg（1997）[10]	デンマーク	3.8	39.3%	57.3%	3.4%	—
Hope（1997）[11]	イギリス	6.2	33%	47%	20%	—
Spandau（2002）[12]	ドイツ	6.2	17.7%	82.3%	0%	—
大島ら（1978）[13]	日本	0.6	—	—	—	—
岩崎ら（2006）[14]	日本	6.7	—	—	—	—

頻度は人口10万人あたりの人数を示す。

分かっていない。

平成22年度より難治性疾患克服研究事業として「Usher症候群に関する調査研究」が全国13施設の共同研究で開始され、Usher症候群患者の疫学、臨床像および治療法の調査と、診断基準および治療指針の策定がすすめられている。そこでまず我々は平成22年度に、Usher症候群の実態把握として、日本耳鼻咽喉科学会の定める認可研修施設を受診した本症候群患者の概数把握を試みた。本論文では調査結果、および調査により判明した今後の課題につき検討する。

対象と方法

調査の対象は、日本耳鼻咽喉科学会の定める認可研修施設一覧（平成22年版）をもとに、全国の大学病院（分院含む）114施設、ならびに地域の基幹病院583施設の、合計697施設とした。対象施設に調査の趣旨を説明し協力を求める文章とアンケートを記載した葉書を郵送し、添付した返信用葉書により回収した。調査期間は2006年1月1日～2010年12月31日とした。

本調査の質問事項はUsher症候群に対する診療実態を把握することを目的に、①各施設を受診したUsher症候群の患者数（新患、再来患者のすべて）、ならびに②患者それぞれの「Usher症候群に関する調査研究班」の試案（表1）に基づいたタイプ判定の2項目とした。該当患者がいない場合は「0例」と記載、またタイプ判定が困難な場合は「分類不明」としてもらうようにした。回答施設や回答者を同定するための施設名、回答者名、連絡先の記載は任意とした。

結　果

郵送した697通（当施設含む）のうち最終的に426通の回答が寄せられた（回収率：61.1%）。調査用紙において回答施設名や、回答者自身に関する情報は匿名でも可としたが、426通すべてで記載されていた。

回答施設は、大学病院（分院含む）が85施設（送付施設中74.6%）、地域基幹病院が341施設（同58.5%）であった（表3）。患者報告施設は、大学病院（分院含む）が26施設（回答施設中22.8%）、地域の基幹病院が8施設（同1.4%）であった（表3）。また返信のなかった都道府県はなかったが、患者報告施設は23都道府県にわたり、地方別にみると表4の結果であった。

患者報告は111名（34施設）であった。大学病院（分院含む）は94名（26施設）であるのに対し、地域基幹病院は17名（8施設）の報告があった（図1）。内訳はタイプ1が15名、タイプ2が27名、タイプ3が17名、分類不明が52名であった。またタイプ判定できた症例の中で、タイプ1は25.4%、タイプ2は45.8%、タイプ3は28.8%であった（図2）。

表3　回答施設属性によるアンケート調査結果

	送付施設数	回答施設数	患者報告施設数
大学（分院含む）	114	85（74.6%）	26（22.8%）
地域基幹病院	583	341（58.5%）	8（1.4%）
合計	697	426（61.1%）	34（4.9%）

表4　回答施設数、患者報告施設数、および症例報告数の地方別分布

地方名	回答施設数	患者報告施設数	症例報告数
北海道地方	20	0	0
東北地方	35	2	5
関東地方	115	13	37
中部地方	78	5	28
近畿地方	87	5	26
中国地方	33	5	6
四国地方	18	1	1
九州地方	40	3	8
合計	426	34	111

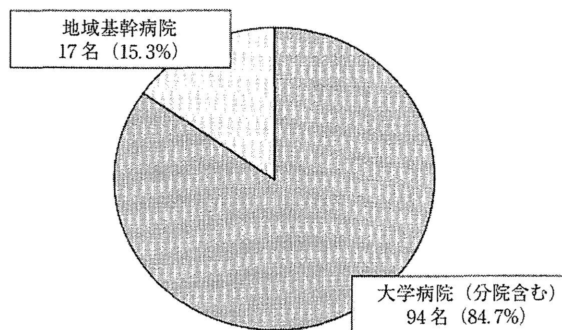

図1　患者報告施設の属性

属性は大学病院（分院含む）、地域基幹病院に分類した。

考　察

Usher症候群は聴覚・視覚の重複障害を呈する代表的疾患であり、日常生活には特別な支援が必要である。しかし本邦でUsher症候群の研究を進める上で、その有病率や発生動向は未だ明らかとは言えないのが現状である。本研究では、本邦におけるUsher症候群の診断の現状を明らかにし、患者数を可能な限り実数に近い形で把握したいという目的で、全国の日本耳鼻咽喉科学会の定める認可研修施設に対して調査を行った。調査にあたっては、Usher症候群に関する調査研究班による診断基準（試案）を参考にした。なお、今回の調査はUsher症候群の疾患概念を「感音難聴に網膜色素変性症を伴う疾患」としており、頻度は少ないが他の視聴覚障害を呈する疾患（Refsum症候群、Alstrom症候群、Cockayne症候群など）が鑑別されていない可能性もある。しかし詳細な調査は二次調査で行うこととし、今回は広く患者数を把握することに努めた。

結果として、回答総数は配布数の半数を超え、郵送調査としては高い回収率（61.1%）であった。回答施設は大学病院（分院含む）と地域基幹病院と比較して回収率に著明な差はみられなかった（表3）。患者報告は111名であったが、施設に関しては大学病院（分院含む）が94名（84.7%）、地域基幹病院が17名（15.3%）であり（図1）、現在のところUsher症候群患者は大学病院（分院含む）でより多く経過観察されているようである。地方別のデータ（表4）に関しては地域によっては対象人数が少ないため、地域ごとの比較は困難であると思われたが、広く全国から患者報告があったことは今後の二次調査を行うにあたって大変有意義な調査であったと考え

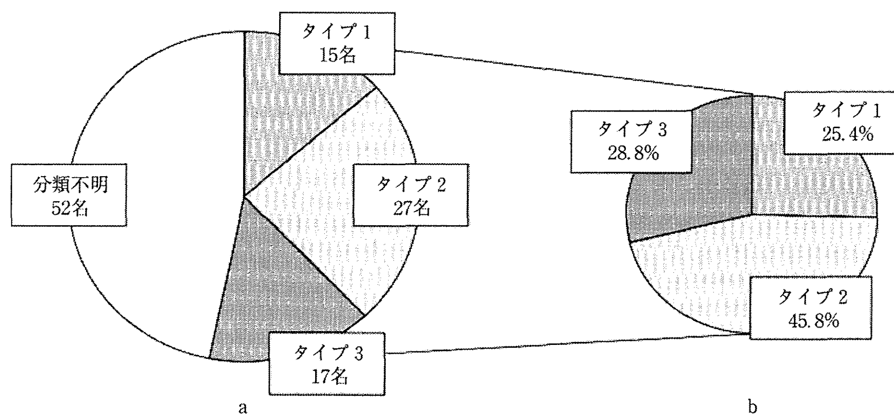

図2　タイプ別報告症例数・頻度

a：タイプ別報告症例数
b：タイプ分類できた症例でのタイプ別頻度