

ORIGINAL ARTICLE

Frequency of Usher syndrome type 1 in deaf children by massively parallel DNA sequencing

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Usher syndrome type 1 (USH1) is the most severe of the three USH subtypes due to its profound hearing loss, absent vestibular response and retinitis pigmentosa appearing at a prepubescent age. Six causative genes have been identified for USH1, making early diagnosis and therapy possible through DNA testing. Targeted exon sequencing of selected genes using massively parallel DNA sequencing (MPS) technology enables clinicians to systematically tackle previously intractable monogenic disorders and improve molecular diagnosis. Using MPS along with direct sequence analysis, we screened 227 unrelated non-syndromic deaf children and detected recessive mutations in USH1 causative genes in five patients (2.2%): three patients harbored *MYO7A* mutations and one each carried *CDH23* or *PCDH15* mutations. As indicated by an earlier genotype–phenotype correlation study of the *CDH23* and *PCDH15* genes, we considered the latter two patients to have USH1. Based on clinical findings, it was also highly likely that one patient with *MYO7A* mutations possessed USH1 due to a late onset age of walking. This first report describing the frequency (1.3–2.2%) of USH1 among non-syndromic deaf children highlights the importance of comprehensive genetic testing for early disease diagnosis.

Journal of Human Genetics (2016) 61, 419–422; doi:10.1038/jhg.2015.168; published online 21 January 2016

INTRODUCTION

Usher syndrome (USH) is a collection of three autosomal recessive disorder subtypes that results in hearing loss (HL), retinitis pigmentosa (RP) and/or vestibular dysfunction. Among these, USH type 1 (USH1) is the most severe due to its profound hearing loss, absent vestibular response and RP appearing at a prepubescent age. USH type 2 (USH2) shows congenital moderate-to-severe with a high-frequency sloping HL and normal vestibular functions. RP of USH2 appears in the first or second decades of life. USH type 3 (USH3) is typified by the variable onset of progressive HL and RP and a range of vestibular function impairment, from normal to absent.¹

To date, 10 causal genes have been identified for USH: *MYO7A* (USH1B), *USH1C* (USH1C), *CDH23* (USH1D), *PCDH15* (USH1F), *USH1G* (USH1G) and *CIB2* (USH1J) for USH1; *USH2A* (USH2A), *GPR98* (USH2C) and *DFNB31* (USH2D) for USH2; and *CLRN1* (USH3A) for USH3 (Hereditary Hearing Loss Homepage; <http://hereditaryhearingloss.org>). As these target genes are large with many exons, considerable labor and cost are required for their analysis by using conventional Sanger sequencing. However, recent advances in targeted re-sequencing by massively parallel DNA sequencing (MPS) have made it possible to analyze all known causative genes simultaneously;^{2,3} we recently employed MPS to identify the frequency of USH-related gene mutations in Japanese USH1 patients⁴ and characterize USH2 and USH3 patients.^{5,6}

The diagnosis of USH in childhood based on clinical phenotypes can be challenging since patients often appear to have non-syndromic

HL only in their youth until RP develops in later years. However, early diagnosis through genetic testing provides many immediate and long-term advantages for patients and their families.⁷ We previously described a case in which *MYO7A* and *GPR98* mutation analysis allowed the diagnosis of USH prior to the appearance of visual symptoms, and subsequent DNA testing enabled appropriate genetic counseling.^{5,8}

In the present study, we performed genetic analysis using MPS technology to simultaneously screen for four USH1 causative genes (*MYO7A*, *USH1C*, *CDH23* and *PCDH15*) in unrelated, non-syndromic, severe-to-profound HL children.

MATERIALS AND METHODS

Subjects

Among the 1373 Japanese HL patients registered in our DNA sample bank from 53 otorhinolaryngology departments across Japan, we selected 227 patients who met the criteria of: (i) congenital HL (i.e., HL onset was prelingual/early at <6 years of age), (ii) severe-to-profound HL (above 71 dB on average over 500, 1000, 2000 and 4000 Hz in the better hearing ear) and (iii) DNA sampling prior to 10 years of age due to the prepubertal nature of USH1.

Of the 227 non-syndromic deaf children screened, 21 were from autosomal recessive families, 22 from autosomal dominant families and 184 from sporadic onset families. There were 127 boys and 100 girls. All subjects (or guardians) gave prior written informed consent for participation in the study. This study was approved by the Ethics Committee of Shinshu University School of Medicine.

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Received 31 July 2015; revised 7 December 2015; accepted 8 December 2015; published online 21 January 2016

Table 1 Possible pathogenic variants found in this study

Gene	Nucleotide change	Amino acid change	Exon/ intron number	Domain	Allele frequency in HL		Polyphen2 HDIV	SIFT	Mutation		Reference
					patients based on MPS (in 3864 alleles)	controls based on MPS (in 538 alleles)			Taster	LRT	
MYO7A	c.2115C>A	p.C705X	Exon 18	Motor	—	—	—	—	—	—	4
	c.3508G>A	p.E1170K	Exon 28	MyTH4	0.00026	0	1.0	1.0	1.0	1.0	22
	c.4501C>T	p.Q1504X	Exon 34	FERM1	—	—	—	—	—	—	This study
	c.5636+1G>T	?	Intron 40	—	—	—	—	—	—	—	This study
	c.6542T>C	p.L2181P	Exon 48	FERM2	0.00026	0	1.0	1.0	1.0	1.0	This study
CDH23	c.6551C>T	p.T2184M	Exon 48	FERM2	0.00052	0	1.0	1.0	1.0	1.0	This study
	c.(2090-2093) insG	p.L697fs	Exon 14	EC7	—	—	—	—	—	—	This study
PCDH15	c.289C>T	p.Q97X	Exon 4	EC1	—	—	—	—	—	—	This study
	c.334C>T	p.R112X	Exon 4	EC1	—	—	—	—	—	—	This study

Table 2 Genotypic and phenotypic characteristics of five patients

Sample no.	Present age (years)	Age at DNA sampling (months) ^a	Sex	Allele 1	Allele 2	Hereditary form	Age (months)	
							Walking	C ^b
<i>MYO7A</i>								
#3840	10	12	M	p.C705X	p.L2181P	Sporadic	17	12 (unilateral)
#4627	6	8	M	p.E1170K	p.T2184M	AR ^c	12	29, 42 (bilateral)
JHLB1637	2	23	M	p.Q1504X	c.5636+1G>T	Sporadic	24	23 (unilateral)
<i>CDH23</i>								
JHLB624	2	5	F	p.L697fs	p.L697fs	Sporadic	24	22 (unilateral)
<i>PCDH15</i>								
#4859	4	7	M	p.Q97X	p.R112X	Sporadic	31	21, 56 (bilateral)

^aAge at participation in this study.^bAge at receiving cochlear implant(s) (CI).^cAutosomal recessive.

Massively Parallel Sequencing

Targeted genes. We screened for mutations in *MYO7A* [NM_000260], *USH1C* [NM_153676], *CDH23* [NM_022124] and *PCDH15* [NM_033056].

Amplicon library preparation. Amplicon libraries for MPS analysis were prepared according to the manufacturer's instructions with an Ion AmpliSeq Custom Panel (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) for 63 standard genes that reportedly cause non-syndromic HL (including *MYO7A*, *USH1C*, *CDH23* and *PCDH15*) as described elsewhere.⁹ The amplicon libraries were diluted to 20 μ M, and equal amounts of six libraries from six patients were pooled for one sequence reaction.

Emulsion polymerase chain reaction and sequencing. Emulsion polymerase chain reaction and sequencing were performed according to the manufacturer's instructions and the protocol of an earlier report.⁹ MPS analysis was performed with an Ion Torrent PGM using an Ion PGM 200 Sequencing Kit and Ion 318 Chip (Life Technologies).

Base call and data analysis. Sequence results were mapped against the human genome sequence (build GRCh37/hg19) with the Torrent Mapping Alignment Program. After sequence mapping, variant regions were compiled with Torrent Variant Caller plug-in software. Each variant effect was then analyzed using ANNOVAR software.^{10,11} Identified missense, nonsense, insertion/deletion and splicing variants were further selected if their incidence was less than 1% of the 1000 Genome database, the 6500 exome variants in the Exome Variant Server, the data set of 1208 Japanese exome variants in the Human Genetic Variation Database and 269 in-house Japanese normally hearing controls. We excluded all

pathogenic mutations of *CDH23*-caused HL (DFNB12), on which we have previously reported.¹²

To predict the pathogenicity of missense variants, the following functional prediction software included in ANNOVAR was used: Sorting Intolerant from Tolerant (SIFT; <http://sift.jcvi.org/>), Polymorphism Phenotyping (PolyPhen2; <http://genetics.bwh.harvard.edu/pph2/>), LRT (http://www.genetics.wustl.edu/jflab/lrt_query.html) and MutationTaster (<http://www.mutationtaster.org/>). Candidate mutations were confirmed using Sanger sequencing, and segregation analysis was also performed using samples from the patients' family members. The sequencing data are available in the DDBJ databank of Japan (Accession number: DRA003791).

RESULTS

Identified mutations

Mutation analysis of 4 selected USH1-associated genes in 227 non-syndromic deaf children revealed 9 different probable pathogenic variants, among which 7 were novel. We observed one frameshift mutation, four nonsense mutations, one splice site mutation and three missense mutations (Table 1).

Whereas the nonsense, frameshift and splice site mutations were all considered pathogenic, the missense mutations were presumed to be probable pathogenic variants based on the results of prediction software evaluation of pathogenicity (Table 1). These residues were well conserved among several species. Functional prediction software (Polyphen2, SIFT, MutationTaster and LRT) indicated mutations to be damaging at scores of 1.0, 1.0, 1.0 and 1.0, respectively.

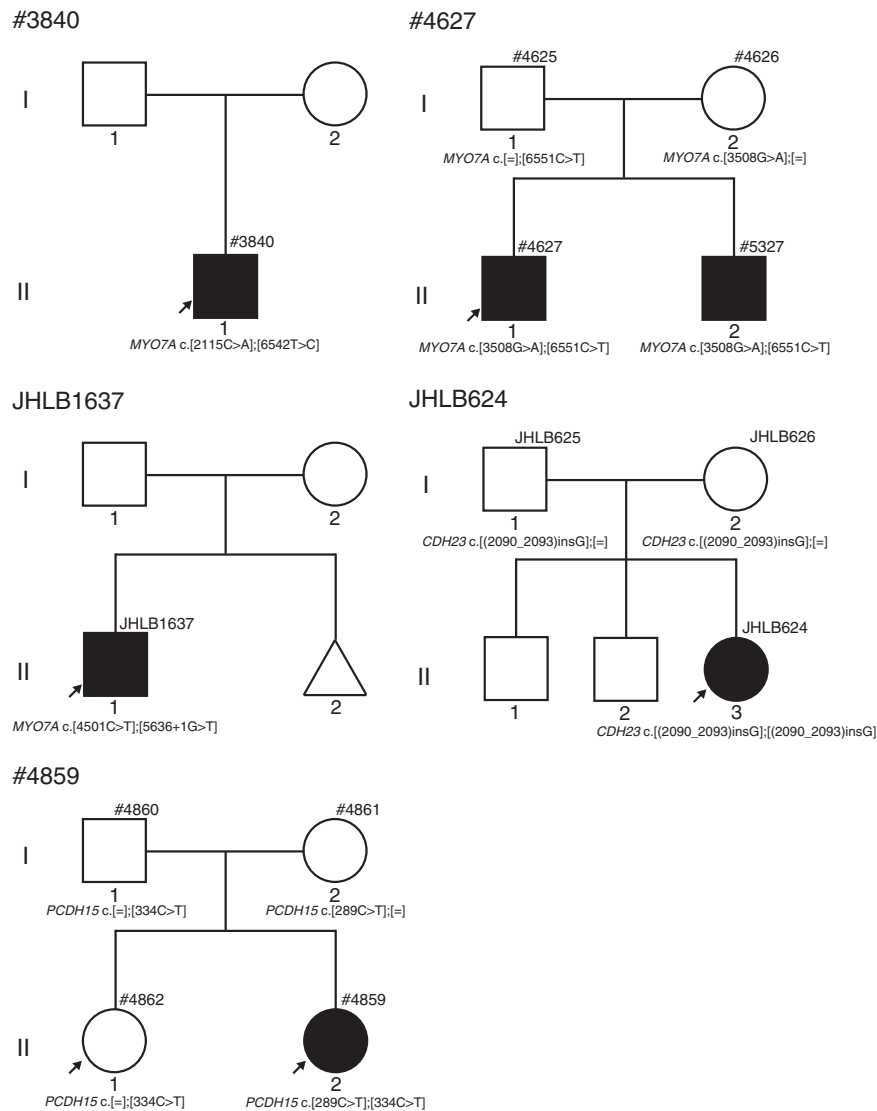


Figure 1 Pedigree and sequencing results for the probands and their families. Three patients (#3840, #4627 and JHLB1637) harbored *MYO7A* mutations and one each carried *CDH23* (JHLB624) or *PCDH15* (#4859) mutations.

In the cohort, five patients had recessive mutations in a USH1 causative gene (2.2%). Of them, three were in *MYO7A*, one was in *CDH23* and one was in *PCDH15* (Table 2).

Clinical findings

The family histories of the five patients identified in this study were compatible with autosomal recessive inheritance (Figure 1). Although all patients entered this study before 12 months of age, no common responsible genes, such as *GJB2* or mitochondrial 1555AG mutations, were found at the time. Genetic testing using MPS was later carried out in 2013–2014.

The onset of walking in two patients (#3840 and #4627) was normal (12 and 17 months, respectively), while that in three patients (JHLB1637, JHLB624 and #4859) was delayed (24, 24 and 31, respectively; Table 2).

At the time of MPS testing, the identified patients were between 2 and 10 years old. Three had received a unilateral cochlear implant (CI) and two had received bilateral CIs (Table 2). One patient (#3840) had not experienced night blindness by the age of 10 years and ophthalmologic data were therefore not available.

DISCUSSION

In this report, we identified nine mutations among three USH1 causative genes (*MYO7A*, *CDH23* and *PCDH15*) in five patients. However, since mutations among these genes could have resulted in non-syndromic HL as well as USH1 (Hereditary Hearing Loss Homepage; <http://hereditaryhearingloss.org>), a careful differential diagnosis was crucial. As suggested by an earlier genotype–phenotype correlation study, USH1D (*CDH23*) and USH1F (*PCDH15*) were typically associated with truncating mutations, while DFNB12 (*CDH23*) and DFNB23 (*PCDH15*), which had a milder phenotype, were associated with non-truncating mutations.^{13,14} Accordingly, we considered the diagnosis in 2 patients (JHLB624 and #4859) to be USH1 based on genetic findings.

No obvious correlations have been reported between mutations in the *MYO7A* gene and the resulting phenotype.¹ However, clinical confirmation of hallmark symptoms may enable the differential diagnosis of non-syndromic HL and USH1. The most frequent clinical sign of USH1 in a cohort of prelinguistically deaf children was a delayed onset of walking (>20 months) due to bilateral vestibular

dysfunction.¹⁵ Therefore, it was highly likely that the clinical subtype in one patient with *MYO7A* mutations (JHLB1637) was USH1B because he began walking at 24 months of age. Astuto *et al.* evaluated the published clinical data for non-syndromic HL patients with *MYO7A* mutations (DFNB2). They concluded that there was no convincing evidence supporting a DFNB2 phenotype in patients with recessive *MYO7A* mutations and that such deaf individuals most likely had USH. In the present study, we considered the remaining two patients with recessive mutations in *MYO7A* (#3840 and #4627) to possess non-syndromic HL (DFNB2) because their onset time of walking was normal. However, careful monitoring for ophthalmic symptoms is needed.

Based on the above findings, we calculated that the frequency of USH1 patients in 227 deaf children was 1.3–2.2% (3–5/227) on the basis of MPS. We have performed mutation screening of four major USH1-causing genes. It is known that the majority of cases of USH1 are caused by four genes (*MYO7A*, *USH1C*, *CDH23* and *PCDH15*). We have not included *USH1G* (*USH1G*) and *USH1J* (*CIB2*) in this study, because these USH1-causing genes have been reported to be very rare.^{16–19} Based on microarray analysis, Kimberling *et al.* showed that in 155 deaf children receiving CIs, 1.9% (3/155) carried recessive USH1 mutations. Of them, however, two patients had non-truncating recessive mutations in *CDH23*. We considered these to be cases of non-syndromic HL, resulting in an adjusted frequency of USH1 in deaf children of 0.6% (1/155). This difference (1.3–2.2% vs 0.6%) may be attributed to the method of genetic testing (MPS vs microarray analysis) and/or the mutation spectrum between Japanese (and by association other Asian populations) and populations with European ancestry.

According to a conservative estimate of the frequency of childhood deafness of approximately 1/1000,²⁰ we can calculate the incidence of USH1 in the Japanese population to be 1.3–2.2 per 100 000 individuals. Previous studies have reported the prevalence of USH1 based on clinical data as 1.1–2.0 per 100 000,^{21–24} which is compatible with our findings.

With regard to treatment, deaf children identified as harboring USH1 causative mutations should be offered unilateral or bilateral CIs. There is a strong need to provide USH children with the best hearing amplification available, with a preference for CIs, accompanied by intensive training and habilitation before the onset of RP.²⁵ In fact, all patients highlighted in this study have received a CI, two bilaterally, with another (JHLB1637) about to receive a second implant. Careful ongoing surveillance for visual symptoms is also needed for deaf children with identified USH1 causative mutations.

In conclusion, based on MPS, this study showed the frequency of USH1 among deaf children to be 1.3–2.2% and underscored the importance of comprehensive genetic testing for diagnosing USH among non-syndromic deaf children. Otolaryngologists and audiologists should bear USH in mind when dealing with deaf children with the aims of prompt therapy and habilitation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a Health and Labour Sciences Research Grant for Research on Rare and Intractable Diseases and 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 Ministry of Education, Science, and Culture of Japan (S.U.). The authors thank Mr Trevor Ralph for his help in preparing the manuscript.

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