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USH2 caused by *GPR98* mutation diagnosed by massively parallel sequencing in advance of the occurrence of visual symptoms

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Abstract

Objective—We present two patients who were identified with mutations in the *GPR98* gene that causes Usher syndrome type 2 (USH2).

Methods—One hundred ninety-four (194) Japanese subjects from unrelated and families were enrolled in the study. Targeted genomic enrichment and massively parallel sequencing of all known non-syndromic hearing loss genes were used to identify the genetic causes of hearing loss.

Results—We identified causative mutations in the *GPR98* gene in one family (two siblings). The patients had moderate sloping hearing loss, and no progression was observed over a period of 10 years. Fundus examinations were normal. However, electroretinogram revealed impaired responses in both patients.

Conclusion—Early diagnosis of Usher syndrome has many advantages for patients and their families. This study supports the use of comprehensive genetic diagnosis for Usher syndrome, especially prior to the onset of visual symptoms, to provide the highest chance of diagnostic success in early life stages.

Keywords

Hearing loss; genetics; *GPR98*; Usher syndrome; massively parallel sequencing

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DECLARATION OF CONFLICTING INTERESTS

All authors have declared no competing financial interests.

INTRODUCTION

Usher syndrome (USH) is an autosomal recessive disorder characterized by hearing loss (HL), retinitis pigmentosa (RP) and vestibular dysfunction. There are three clinical subtypes classified by the severity and onset of HL, onset of RP and vestibular symptoms. However, it can be difficult to recognize these clinical manifestations due to its extremely heterogeneous trait. USH type 1 (USH1) has the most severe forms that are characterized by congenital profound HL, prepubertal onset RP and absent vestibular responses. USH type 2 (USH2) shows congenital moderate to severe with a high-frequency sloping HL and normal vestibular functions. The onset of RP is later than in USH1, and visual symptoms such as night blindness in USH2 usually occur in the second decade. USH type 3 (USH3) has variable onset and severity in these three manifestations[1, 2]. Due to its complexity, diagnosis of USH in childhood, based on a clinical phenotype, can be difficult because patients appear to have only non-syndromic HL in childhood and RP develops in later years. Based solely on frequency, if a patient has only HL, it is hard to diagnose accurately syndromic HL.

Early diagnosis has many immediate and several long-term advantages for patients and their families, and is now possible through genetic testing[1]. The advantage of genetic testing for the diagnostic approach to USH has been fully established. We also previously reported a case in which *MYO7A* mutation analysis diagnosed USH1 in advance of the appearance of the visual symptoms, and genetic testing allowed us to give appropriate genetic counseling[3]. To date, ten of the corresponding genes have been identified as a cause of USH: *MYO7A* (USH1B), *USH1C* (USH1C), *CDH23* (USH1D), *PCDH15* (USH1F), *USH1G* (USH1G) and *CIB2* (USH1J); and *USH2A* (USH2A), *GPR98* (USH2C) and *DFNB31* (USH2D); and *CLRN1* for USH3 (Hereditary Hearing loss Homepage; http://hereditaryhearingloss.org). However, these targeted genes are significant in size and number of exons, and much labor and expense are necessary for analyzing whole genes corresponding to USH. Recent advances in targeted genomic enrichment with massively parallel sequencing (TGE+MPS) have made possible the sequencing of all known causative genes simultaneously[4, 5].

In this study, we performed genetic testing on 194 Japanese hearing loss patients. Here, we describe two patients with hearing loss in whom we identified novel mutations in the *GPR98* gene. Based on the result of genetic testing, we performed ophthalmological tests for the patients and diagnosed USH2 even before they suffered from any visual symptoms. This is the first report of a diagnosis of USH2 caused by *GPR98* mutations in advance of visual defects in the cohort of non-syndromic HL patients, and highlights the importance of comprehensive genetic testing for the clinical usage of diagnosis for hearing loss patients.

SUBJECTS and METHODS

Subjects

One hundred ninety-four (194) Japanese subjects (114 females) from unrelated and non-consanguineous 194 families were ascertained through 33 otolaryngology clinics in 28 prefectures across Japan. All subjects had presumed non-syndromic HL. For each proband, informed consent was obtained to participate in this study, which was approved by the human subjects ethical committee associated with each clinic.

Clinical information and blood samples were obtained for each proband and for all consenting affected and unaffected relatives.

Targeted Genomic Enrichment and Massively Parallel Sequencing

Genomic DNA was assessed for quality by gel electrophoresis and spectrophotometry (Nanodrop 1000; Thermo Fisher Scientific, Waltham, MA; 260/280 ratio of 1.8–2.2) and quantity by fluorometry (Qubit 2.0 Fluorometer; Life Technologies, Carlsbad, CA). TGE of all exons of all genes implicated in non-syndromic HL, including non-syndromic HL mimics, was completed as described, targeting 89 genes as part of the OtoSCOPE® v5 platform. Libraries were prepared using a modification of the solution-based Agilent SureSelect target enrichment system (Agilent Technologies, Santa Clara, CA),[6]. Of the 198 samples, 58 samples were processed manually; the remainder was prepared robotically using the Sciclone NGS Workstation.

In brief, 3μg gDNA was randomly fragmented to an average size of 250 bp (Covaris Acoustic Solubilizer; Covaris Inc., Woburn, MA), fragment ends were repaired, A-tails were added, and sequencing adaptors were ligated before the first amplification. Solid phase reverse immobilization purifications were performed between each enzymatic reaction. Hybridization and capture with RNA baits was followed by a second amplification before pooling for sequencing. Minimal amplification was used – typically 8 cycles for the pre-hybridization PCR (range 8–10 cycles) using NEB Phusion HF Master Mix (New England BioLabs Inc, Ipswich, MA) and 14 cycles for the post-hybridization PCR (range 12–16 cycles) using Agilent Herculase II Fusion DNA Polymerase. All samples were barcoded and multiplexed before sequencing on either an Illumina MiSeq or HiSeq (Illumina Inc, San Diego, CA) in pools of 4–6 or 48, respectively, using 100-bp paired-end reads.

Bioinformatics Analysis

Data were analyzed as described using a local installation of the open-source Galaxy software (http://galaxyproject.org) and the following open-source tools: BWA[7] for read mapping, Picard for duplicate removal, GATK[8] for local re-alignment and variant calling and NGSRich[9] for enrichment statistics[5]. We reported and annotated variants with custom software.

Variant Confirmation

All pathogenic variants were confirmed by Sanger sequencing and segregation analysis using exon-specific custom primers.

RESULTS

Mutation analysis

We indentified novel causative mutations that were one frame-shift mutation and one missense mutation in *GPR98* in the cohort of this study (194 hearing loss patients). The former mutation corresponded to c.16604_16611delGTACCCAG (NM_032119) and led to frame-shift mutation and truncate (p.Ser5535ArgfsX6). The second mutation was c. 9464C>A (p.Ala3155Asp). We also performed Sanger sequencing for the family

segregation study and a confirmation of the variant MPS outputted result. As shown in Figure 1, Sanger sequencing results revealed that the parents had one of either mutation in the heterozygote, and the proband's brother had biallelic mutations.

Case Details

The proband is a 16-year-old female (II-2; SNS 3356). She had no complications in the perinatal period. She had not undergone newborn hearing screening. At the age of 6, HL was suspected at an elementary school wellness check-up, and she was referred to Shinshu University Hospital, Department of Otolaryngology for audiological examinations. An older brother (II-1; SNS 3357) (8 years old) visited the hospital at the same time as she received her consultation as he had not undergone newborn hearing screening. They were diagnosed with moderate HL, with a high-frequency sloping configuration (Figure 2A, 3A). Subsequently, they began to wear hearing aids. A deterioration of their HL was not observed over the period of ten years (Figure 2A, 3A).

They participated in this study in 2004; however, we could not find the responsible genes within the common genes, such as GJB2 and mitochondrial 1555AG mutations. Genetic testing using MPS was carried out in 2013. Mutations in *GPR98* gene as mentioned above were detected, thus we considered that they might have USH2C rather than non-syndromic HL. At the time of testing, he (II-1) was aware of night blindness at the age of 18. However, she (II-2) had no apparent nyctalopia or dark adaptation problems at the age of 16. Ophthalmological testing showed that the fundus examinations of both patients were normal (Figure 2C, 3C). However, his electroretinogram (ERG) revealed a complete bilateral absence (Figure 3D) and her ERG revealed a weaker response than that of normal levels (Figure 2D).

In addition, their vestibular functions (determined by means of caloric tests and cervical vestibular evoked myogenic potential (cVEMP)) were both normal (Figure 2B, 3B). With these findings, they were diagnosed with USH2C caused by *GPR98* mutations.

DISCUSSION

In this report, we identified novel heterogygous mutations in the *GPR98* gene among autosomal recessive inherited and presumably non-syndromic HL, and finally diagnosed USH2C. One (p.Ser5535ArgfsX6) was considered pathogenic due to a truncating mutation. The second (p.Ala3155Asp) was strongly suspected as pathogenic, and this mutation was not described in any mutation databases. PhyloP and GERP showed this residue is well conserved among various species. We also employed functional prediction software (Polyphen2, SIFT, Mutation Taster and LRT) that indicated the second mutation as damaging (1.00, 0.00, 0.99 and 1.00, respectively).

GPR98 gene (also previously known as *VLGR1* gene) is localized on chromosome 5q13 and contains 90 exons and has a range of 600kb. The *GPR98* was first described as implicated in USH2 in 2004[10]. Weston et al. identified mutations in *GPR98* among patients who had deaf-blindness, and also showed that the expression of *GPR98* was observed in human fetal retina and cochlea by RT-PCR [10]. Subsequently, there have been several cases identified, thus, it

was considered as a USH2 causative gene[11–13]. Mutations of some USH causative genes (*MYO7A, USH1C, CDH23, PCDH15, CIB2, DFNB31*) can result in both non-syndromic hearing loss and USH (Hereditary Hearing loss Homepage), while mutations of the *GPR98* gene are only responsible for USH. The *GPR98* protein (VLGR1) is one of the major components of the ankle link of the hair bundle in the cochlea[14] and plays an important role in the normal development of cochlea hair bundles[15]. McGee et al. reported that *Vlgr1* mutated mice exhibited early hair bundle defects resulting in hearing loss at high frequencies, whereas normal vestibular function was observed. Normal transduction currents were measured in vestibular hair cells[15]. These findings are also consistent with our present cases that have normal vestibular functions.

In USH2 patients, USH2A (*USH2A*) has been reported as the most common USH2 genetic subtype (57~95.8%)[16–18], while USH2C (*GPR98*) and USH2D (*DFNB31*) accounted for 5.2~19% and 0~9.5%[13, 16–18]. All published mutations in USH genes have been recorded in public database, USHbases (https://grenada.lumc.nl/LOVD2/Usher_montpellier/USHbases.html). Nakanishi et al. reported that the frequency of USH2 genetic subtypes in Japanese USH patients, and that *USH2A* gene mutations were found in 8 of 10 patients (80%)[19]. Our study is the first to identify the *GPR98* gene mutations. However, we recruited a cohort of suspected non-syndromic HL patients, so that the frequency of USH2C in a Japanese population is still unclear.

Abadie et al. reported that in USH2C, Moderate HL was predominant (76%) and a gently down-sloping configuration characterized most audiograms in 66%[20]. This is consistent with our cases. It has been shown that USH2C patients had severer HL than USH2A, however, it is impossible to predict the candidate gene based on audiograms due to the heterogeneity of USH[20].

This is the first report of the mutations in *GPR98* identified by genetic testing using MPS, in which we were able to diagnose USH2C before the patients suffered from obvious visual symptoms. With regard to USH2C patients, the median age of HL diagnosis was 5 years, although the median age at USH2 diagnosed was 34.5 years[20]. That is because visual symptoms with RP appear later in life, mostly in the second decade[21]. In general, diagnosis of RP can be possible before the appearance of visual symptoms by ERG[22], however, there is usually no indication to carry out an ERG before RP symptoms appear. Based on the present cases, we suggest that if mutations are found in genes concerned with USH, ophthalmological testing should be provided to the patients for differential diagnosis. We previously recommended ophthalmologic tests for young non-syndromic HL patients, in whom candidate mutations in USH gene had been found[3].

Further evolution of genetic testing, such as MPS will make more accurate diagnosis of hearing loss possible, but we should also be more rigorous in confirmation of the phenotypes, including hearing loss and other manifestations. Regarding hearing loss caused by mutations in *GPR98*, all clinicians should provide optimal management of hearing abilities in order to improve patient's quality of life. We should provide genetic counseling to patients about the risk of future vision loss, and also provide applicable educational support.

In conclusion, this study supports the use of comprehensive genetic diagnosis for USH, particularly in advance of visual symptoms, to provide the highest chance of diagnostic success in the early life stages. The benefit of early identification lies in the potential to provide future treatment to prevent RP[1]. Genetic diagnosis using MPS will contribute to early intervention, and may provide an opportunity for the development of novel therapeutic possibilities.

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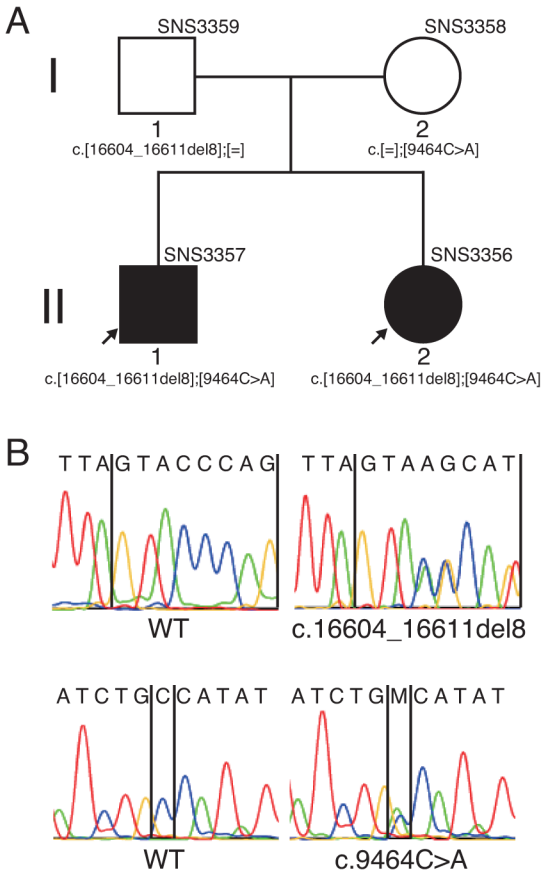

Figure 1.

Pedigree of the patients ID: SNS 3356 and 3357, and Sanger sequence results. The affected patients carried c.[16604-16611delGTACCCAG] (p.Ser5535ArgfsX6) and c.[9464C>A] (p.Ala3155Asp) mutations. These mutations were segregated in this family.

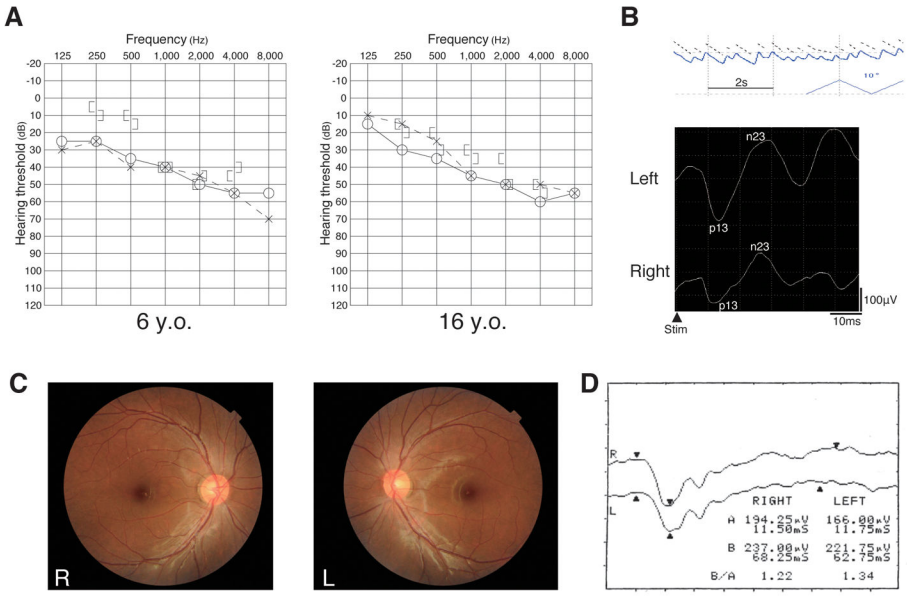

Figure 2.

Clinical findings of the patient, II-2; SNS 3356. (A) Left: Audiogram of at the age of 6 shows moderate hearing loss. Right: Audiogram at the age of 16 shows no progression of hearing loss. (B) Upper: The caloric testing for the left ear shows normal response. Lower: There were no obvious differences between both ears in the cervical vestibular evoked myogenic potential (cVEMP). (C) Fundus examination at the age of 18 was normal. (D) Full-field electroretiongram result exhibits weaker response.

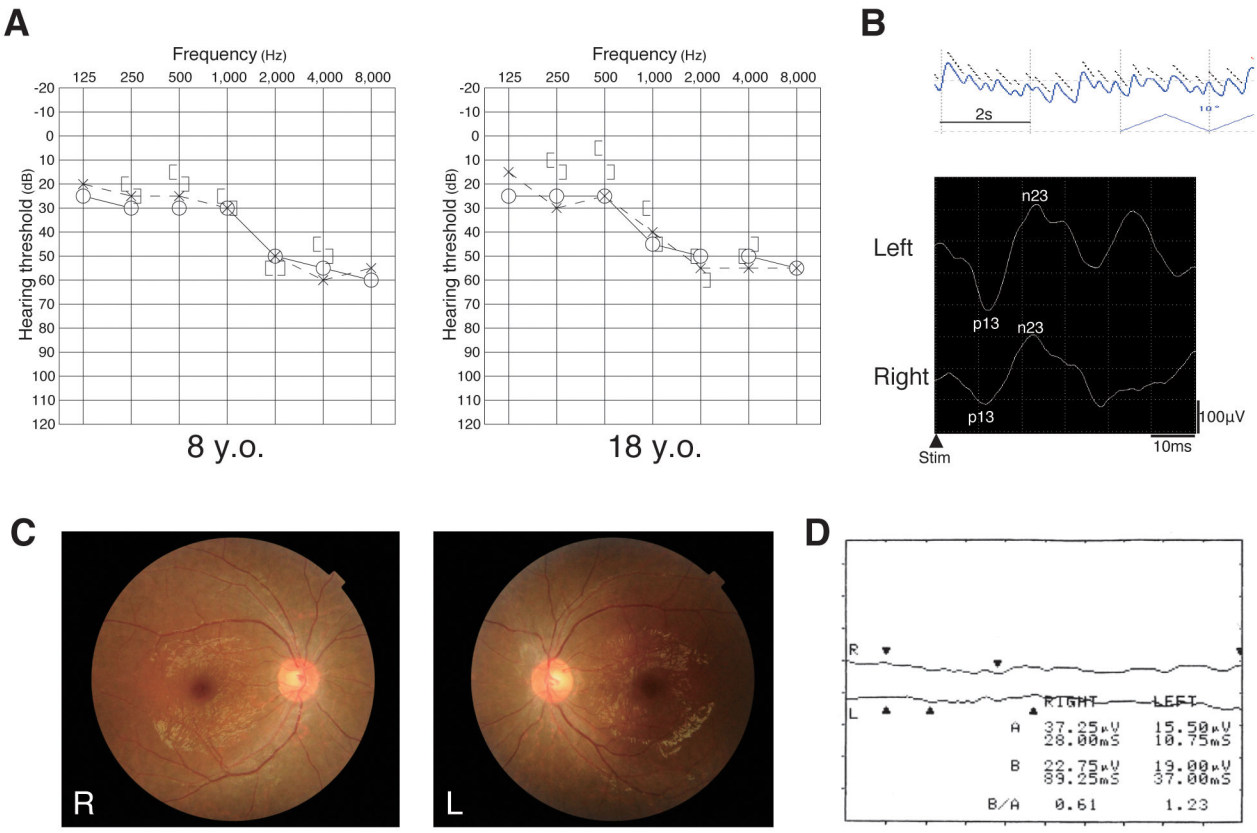

Figure 3.

Clinical findings of the patient, II-1; SNS 3357. (A) Left: Audiogram of at the age of 8 shows moderate hearing loss. Right: Audiogram at the age of 18 shows no progression of hearing loss. (B) Upper: The caloric testing for the left ear shows normal response. Lower: There were no obvious differences between both ears in the cervical vestibular evoked myogenic potential (cVEMP). (C) Fundus examination at the age of 18 was normal. (D) Full-field electroretiongram result exhibits complete bilateral absence.