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Genetic screening revealed usher syndrome in a paediatric Chinese patient

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Abstract

Introduction: Usher syndrome is the most common cause of hereditary deaf-blindness. Three clinical subtypes have been classified. Usher syndrome type I is the most severe subtype characterized by congenital severe-to-profound hearing loss, retinitis pigmentosa and vestibular dysfunction.

Methods: One family was analyzed and the analysis included the combination of a custom capture/next-generation sequencing panel of 180 known deafness gene, Sanger sequencing and bioinformatics approaches.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Results: Compound heterozygous mutations in the *MYO7A* gene: a known missense mutation c.494C>T (p.Thr165Met) and a novel missense mutation c.6113G>A (p.Gly2038Glu) were identified in a proband. This Chinese hearing-impaired child was misdiagnosed as non-syndromic hearing loss which was later changed to the diagnosis of Usher syndrome type I after comprehensive audiometric, vestibular and ophthalmological examinations at 9 years old.

Conclusions: Due to the features of genetic heterogeneity and variation in clinical manifestation, molecular diagnosis and ophthalmological examinations by skilled ophthalmologists with knowledge of Usher syndrome should be suggested as a routine assessment which may improve the accuracy and reliability of etiological diagnosis for hearing loss.

Keywords

Usher syndrome; Hearing loss; MYO7A; Mutation; Retinitis pigmentosa

INTRODUCTION

Usher syndrome (USH) is an autosomal recessive syndromic disorder associated with sensorineural hearing impairment, progressive night blindness attributable to retinitis pigmentosa (RP) and variable vestibular dysfunction. USH is the most common cause of hereditary deaf-blindness and responsible for 3–6% of childhood deafness and 50% of individuals who are deaf-blindness, with a prevalence of 3.2–6.2 per 100,000 depending on different population [1–7].

Based on clinical characterization, USH is classified into three subtypes. USH type I (USH1) is the most severe form, characterized by profound congenital hearing loss, vestibular dysfunction and early onset of retinitis pigmentosa in childhood [8,9]. USH type II (USH2) is the most common form of USH and has a less severe manifestation of hearing loss (moderate to severe), normal vestibular function and later onset of retinitis pigmentosa (usually begins in adolescence/adulthood). USH type III (USH3) is a relatively rare form of USH with progressive hearing and vision loss beginning in the first few decades of life.

USH is genetically heterogeneous. To date, six genes have been associated with USH1, namely *MYO7A* [10], *USH1C* [11,12], *CDH23* [13, 14], *PCDH15* [15,16], *USH1G* [17] and *CIB2* [18]. *MYO7A* is the most prevalent responsible gene, causing 24–59% of USH1 cases [9,19–24]. Over 300 mutations have been identified in *MYO7A* (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=MYO7A>).

Mutations in *MYO7A* also have been reported to cause two forms of nonsyndromic hearing loss (*DFNA11* and *DFNB2*) and USH type II [25–28]. *MYO7A* gene, located on chromosome 11q13.5, consists of 48 coding exons and encodes myosin VIIA, which is part of a group of proteins called unconventional myosins. As a membrane protein, myosin VIIA has been localized in a variety of cells in human, including the hair cells in the inner ear, pigment epithelium and photoreceptor cells of the retina [29]. The expression in the inner ear is restricted to the cell bodies and stereocilia of the sensory inner and outer hair cells in the organ of Corti and in the vestibule, but does not label other cells in the inner ear [29], suggesting their participation in mechano-transduction activity and morphogenesis of

stereocilia of the hair cells [30–32]. In the retina, myosin VIIA localizes in cilium of photoreceptors, apical region of retinal pigment epithelial (RPE) cells and melanosomes within RPE cells and may play a role in the migration of RPE cells, photoreceptor cells, and opsin transport [33–35].

In this study, we performed targeted gene capture and next-generation sequencing of 180 known deafness genes and identified compound heterozygous mutations, a known missense mutation c.494C>T(p.Thr165Met) and a novel missense mutation c.6113G>A(p.Gly2038Glu), in *MYO7A* gene as the cause of USH1 in a Chinese child.

MATERIALS AND METHODS

1. Subjects

A nine year old boy (III-2) with hearing-impairment from the Henan province of China was referred to the China Rehabilitation and Research Center for genetic diagnosis. He is the second child of healthy normal hearing and vision parents. His older brother (III-1) who is 14-years-old is healthy with normal hearing and vision (Fig. 1A). The patient did not undergo neonatal hearing screening. His parents suspected his hearing problem at age one and he was subsequently diagnosed with bilateral profound sensorineural hearing loss based on the absent auditory evoked potentials of the auditory brainstem response (ABR). Hearing level of both ears before cochlear implantation (CI) was evaluated by pure-tone air conduction audiometry. The patient underwent the examinations of videonystagmography (VNG), cervical vestibular evoked myogenic potential (cVEMP), and rotational vestibular testing (RVT) at 9 years of age to evaluate the vestibular function.

The patient also received comprehensive ophthalmological examinations including best-corrected visual acuities (BCVA) test, slit lamp biomicroscopy, fundus photography, visual field test (VF), electroretinogram (ERG), fundus autofluorescence imaging (FAF), and optical coherence tomography (OCT) at 9 years of age.

This study was approved by the local Institutional Review Board at the University of Miami in USA and the ethnicity committee of China Rehabilitation and Research Center for Deaf Children. A signed informed consent form was obtained from each subject and their parents before blood sampling and genetic testing.

2. DNA sequence analysis

2.1 Targeted gene capture and Next-generation sequencing—Genomic DNA was extracted from peripheral blood of the patient and his family members and from a control group of 100 Chinese healthy unrelated individuals with normal hearing and vision, using a TIANamp Blood DNA Kit (TIANGEN Biotech CO., Beijing, China). DNA samples of the patient were initially screened using Sanger sequencing to detect the most prevalent deafness-related mutations of the three genes. They are the coding sequence of *GJB2* and *SLC26A4*, and the sequence flanking the mutations C1494T and A1555G of *12s rRNA*, but no mutation was found. Then, based our first vision of a custom designed next-generation sequencing gene panel with 146 targeted gene [36], a recently refined version of panel with 180 targeted gene was used to capture the targeted genes [37]. Thus far, ten genes known to

cause USH were included in this panel: six genes *MYO7A*, *USH1C*, *CDH23*, *PCDH15*, *USH1G*, and *CIB2* associated with USH1; three genes *USH2A*, *GPR98*, and *DFNB31* associated with USH2; only one gene, *CLRN1*, was included in for USH3. The other two USH3-related genes, *HARS* and *ABHD12*, were not included [38, 39]. The next-generation sequencing (NGS) was processed at the Hussman Institute for Human Genomics (HIHG) Sequencing core facilities, University of Miami. The Agilent's SureSelect Target Enrichment (Agilent, Santa Clara, CA) of in-solution hybridization capture system was used following the manufacturer's instruction. The variant calls were determined by the GENomes Management Application [40].

2.2 Mutation analysis by Sanger sequencing on genomic DNA—The mutations identified by the panel were subsequently confirmed by direct Sanger sequencing. Exon 6+7 and Exon 45 of the *MYO7A* gene has been amplified for the detection of variants c.494C>T (p.Thr165Met) and c.6113G>A (p.Gly2038Glu), respectively, in 6 members (I1, I2, II1, II2, III1, III2) (Fig. 1A) of the family. The following primer pairs: *MYO7A*-6+7F (5'-CTGGGCTGAGTTCCAGTTGGTGG-3') and *MYO7A*-6+7R (5'-GGAGCAATACGGGCAGCAATACG-3'); *MYO7A*-45F (5'-TCTGCCAGGTCCCTGCACGCCTGT-3') and *MYO7A*-45R (5'-CCTGGGCTGCTGGCCAGTGTCTG-3') were used for PCR as well as for bidirectional sequencing. The PCR reaction condition was follows: the specimens were preheated at 95°C for 3 min followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s. Sequence data were aligned and compared to *MYO7A* reference sequence (GenBank Accession Number: [NG_009086.1](#)).

2.3 Bioinformatics analysis—The Illumina CASAVA v1.8 pipeline was used to assemble the sequence read. The processes for the alignment of sequence reads, variants calls, variant filtering and annotation were described previously [37]. An overall quality check of uploaded read data was performed, including the coverage and average read depth of targeted regions, and quality scores. A total of 1577 variants are left after filtering by gene features. Genesis 2.0 (<https://www.genesis-app.com/>) was then used for variant filtering based on quality/score read depth and minor allele frequency (MAF thresholds of 0.005 for ARNSHL) as reported in dbSNP141, the National Heart, Lung, ND Blood Institute Exome Sequencing Project Exome Variant Server, Seattle, WA Project (Exome Variant Server, 2012), ExAC server (<http://exac.broadinstitute.org/>), the 1000 Genome Project Database and our internal database of > 3,000 samples from European, Asian, and American ancestries. Using these data analysis criteria, we classified 13 nonsynonymous variants as potential true variants; of these, 7 variants (in 5 genes) are predicted to be disease-causal, including the 2 variants (1 known and one novel) in the *MYO7A* gene.

The functional impacts of the mutations were analyzed and indicated by two web-based bioinformatics data analysis tools, including PolyPhen-2 (Polymorphism Phenotyping-2; <http://genetics.bwh.harvard.edu/pph2/>) and SIFT Human Protein (http://sift.jcvi.org/www/SIFT_enst_submit.html) and two databases, ExAC and NHLBI ESP.

Uniprot software (<http://www.uniprot.org/>) was applied to evaluate the evolutionary conservation of the mutated amino acids through multiple sequence alignment of the myosin VIIA orthologous proteins of the following eight species: *Homo sapiens* (UniProtKB-

Q13402), *Mus musculus* (UniProtKB-P97479), *Rattus norvegicus* (UniProtKB-Q8CJE3), *Danio rerio* (UniProtKB-Q9DGG8), *Bos taurus* (UniProtKB-F1MHG0), *Canis lupus familiaris* (UniProtKB-F1PB31), *Pan troglodytes* (UniProtKB-H2Q4G1), and *Sus scrofa* (UniProtKB-Q28970).

RESULTS

Clinical evaluation

A nine year old Chinese hearing-impaired boy was initially diagnosed with congenital non-syndromic sensorineural hearing loss (SNHL) at age one. His hearing loss was stable, bilateral and profound (Fig. 1B), while his old brother has normal hearing (Fig. 1C) by audiometry examination. Motor milestones were delayed in the patient with the ability to walk occurring at age of 24 months. Both high-resolution computed tomography (CT) scan of temple bone and magnetic resonance imaging (MRI) scan of brain of the patient were normal, excluding malformation of inner ear and brain (images not shown). Due to the poor language development despite the use of bilateral hearing aids, right ear cochlear implantation (CI) was performed in the patient at the age of 6. He is currently enrolled in the third grade of a regular elementary school.

After the present genetic diagnostic result suggesting the mutations in gene *MYO7A* as the cause of the hearing loss, the clinical history of the patient was gathered carefully. Difficulty with night vision and visual field narrowing were initially noticed at age of 5. The patient had several occasions of falling during regular walking at age 4–5. However, the fine motor abilities were not delayed. Both mental and physical development of the patient was normal. Vestibular function evaluation was normal at age of 9 assessed by videonystagmography (VNG), cervical vestibular evoked myogenic potential (cVEMP), and rotational vestibular testing (RVT) (data not shown).

The orthoptic and ophthalmologic examination at age of 9 revealed the characteristic features of retinitis pigmentosa. The current BCVA of the patient was 20/20 in the right eye and 20/25 in the left eye. The anterior segment and intraocular pressure (IOP) were normal in both eyes. The fundus photography revealed that the patients had typical RP features including waxy pallor of the optic discs, attenuated retinal arterioles (Fig. 2A and C), the diffuse mottling of retinal pigmented epithelium and scattered peripheral bone spicule-type pigment deposits (Fig. 2B and D). His vision field (VF) was significantly constricted to tubular vision (Fig. 2E and F). The ERG wave amplitudes of the patient were undetectable from the baseline (date not shown). Fundus autofluorescence imaging (FAF) imaging showed perifoveal hyperautofluorescent rings and widespread hypoautofluorescence in both eyes (Fig. 2G and H). The optical coherence tomography (OCT) images showed attenuated outer nuclear layer (ONL) and retinal pigmented epithelium (RPE), loss of outer/inner segments (IS/OS) junction layer at extrafoveal region and preserved outer/inner segments (IS/OS) junction layer at foveal region (Fig. 2I and J).

Targeted gene capture and mutation analysis

Patient III-2 was screened first for mutations of the coding sequence of genes *GJB2* and *SLC26A4*, common mutation C1494T and A1555G of *12s rRNA*, but no mutation was found. Subsequently, the DNA sample was subjected to the 180 gene NGS panel analysis. Two missense variants were detected. These include a known mutation c.494C>T (p.Thr165Met) [Ouyang et al., 2005; Roux et al., 2006; Rong et al., 2014] and a novel sequence change c.6113G>A (p.Gly2038Glu), located within exon 6 and exon 45 of *MYO7A* gene, respectively. Using Sanger sequencing, the compound heterozygous variants were confirmed in the patient (Fig. 3B). Six family members were screened for the segregation analysis of these two variants. The maternal grandfather (II1), the mother (II2) and the older brother (III1) were found to be carriers of heterozygous c.494C>T (p.Thr165Met) mutation, while heterozygous c.6113G>A (p.Gly2038Glu) was identified in the father (III1) (Fig. 1A). The maternal grandmother (I2) was homozygous wild-type genotype for both sequence variants. Therefore, the mutations were segregated within the family members.

Multiple sequence alignment of the myosin VIIA orthologous proteins in eight species was performed using Uniprot software to evaluate the evolutionary conservation of the mutated amino acids. Both of the variants localize in the evolutionary highly conserved positions of myosin VIIA protein (Fig. 3C). Similar to the known mutation c.494C>T (p.Thr165Met), the novel c.6113G>A variant is also a missense mutation, resulting in a change of amino acid from glycine to glutamine. PolyPhen-2 and SIFT bioinformatics tools were used to predict the possible impact of novel coding sequence substitution p.Gly2038Glu on the structure and function of myosin VIIA protein. The p.Gly2038Glu mutant was predicted as deleterious and damaging. In addition, the novel mutation p.Gly2038Glu was not detected in 100 healthy normal hearing and vision controls and absent in the ExAC and NHLBI ESP databases, thus may be considered pathogenic.

DISCUSSION

Most cases of congenital hearing loss are now identified within 3–6 months after birth as a result of the universal neonatal hearing screen (UNHS) [41–44]. However, the clinical diagnosis of USH1 is often missed due to the later-presentation of retinal degeneration. Our case of USH1 presented with congenital sensorineural hearing loss but the diagnosis of USH1 was not made until later in life. The patient had difficulties with night vision and narrowed visual field since the age of 5. Since his central vision is not affected, his parents did not seek further ophthalmologic evaluation. Similar cases in which the diagnosis of USH delayed have been previously reported by Zina et al. and Astuto et al [45, 46]. In both reports, retinal degeneration was not discovered until detailed examination by funduscopy.

Although USH is the most common syndrome affecting both hearing and vision, vestibular impairment is also observed in Usher syndrome. From a retrospective view, the vestibular dysfunction of this patient was obvious at young age as shown by his late independent ambulation (24 months). Other congenital disorders, Pendred syndrome or enlarged vestibular aqueduct (EVA), are also associated with early-onset of bilateral sensorineural hearing loss and vestibular weakness in children [47]. Approximately 45% of patients with

EVA has vestibular signs and symptoms [48, 49]. Additionally, children with Pendred syndrome or EVA often present with delayed gross motor milestones, specifically walking [47]. For the hearing-impaired children with vestibular dysfunction such as delays in walking, imaging examination will assist in differentiation diagnosis. Normal CT result will definitively rule out Pendred syndrome or EVA. If there is no signs of inner ear malformation in CT, molecular diagnosis and thorough and repeated retinal examinations should be suggested which can provide more confident clues toward the USH. Characteristic ophthalmoscopic findings including the atrophy of the RPE, pigment deposition in the retina, attenuated retinal arterioles, tubular vision and undetectable ERG wave amplitudes of the patient supported the diagnosis of RP. The presence of RP is crucial for differentiating USH from non-syndromic hearing loss [50].

However, comprehensive genetic diagnosis of USH is not a simple task because of the genetic heterogeneity and numerous exons for the USH genes. Although *MYO7A* plays a major role in Usher syndrome and mutations in *MYO7A* are responsible for 24–59% of USH1 cases [5] and 12–14% of total USH patients [51], but there is an absence of hot spot mutations in the *MYO7A* gene. Gene *MYO7A* alone has 48 coding exons, not to mention USH1 has six disease-associated genes and 185 coding exons totally, e.g. *USH1C* (27 coding exons), *CDH23* (67 coding exons), *PCDH15* (34 coding exons), *USH1G* (3 coding exons), and *CIB2* (6 coding exons). Conventional Sanger sequencing of dozens of exons in the candidate genes sequentially is time and labor consuming and costly. NGS-based gene panel provides an efficient methods to improve the positive rate of molecular diagnosis [52]. After routine clinical screening of coding sequence of the genes *GJB2* and *SLC26A4* and common C1494T and A1555G mutations in *12S rRNA*, follow by the use of the gene panel is certainly a faster and straightforward practice for genetic hearing loss diagnosis. In this panel, both syndromic and non-syndromic hearing loss related genes are included as hearing loss is a genetically heterogeneous disorders with high phenotypic heterogeneity and variability. In addition, panel analysis is performed independently of the clinical manifestation of patients, thus facilitating the detection of mutations located in genes usually associated with other clinical diagnosis and of putative digenic inheritances. Although the genetic screening can increase the prevalence of suspected Usher syndrome with identified mutations in USH genes [6], the molecular results should be interpreted and reviewed carefully because some of these mutations only lead to nonsyndromic hearing loss [25,26].

Myosin VIIA protein contains 2215 amino acid sequence with a typical unconventional myosin polypeptide structure, i.e. a motor head domain with the highly conserved ATP-binding and actin-binding sites, a neck with 5 isoleucine glutamine (IQ) motifs and a long tail domain which includes two myosin tail homology 4 (MyTH4) domains, two FERM (F for the band 4.1 protein, E for ezrin, R for radixin and M for moesin) domains and one SRC Homology 3 (SH3) domain [9,53] (Fig. 3A). The motor domain enables movement of myosin VIIA on actin filaments. The mutation p.Thr165Met identified in this study had been previously reported in both Caucasian and Chinese population, leading to a substitution of amino acid threonine at position of 165 in the ATP-binding site (amino acid 158–165) with amino acid methionine [20,21,24,27,54]. The mutation p.Gly2038Glu caused by a c. 6113G>A transition in exon 45 is a novel change and locates in the second FERM domain (amino acid 1902–2205) of the protein. The FERM domain is a widespread protein module

involved in localizing proteins to the plasma membrane and cytoskeleton-membrane crosslinking [55,56]. It has been reported that C-terminal FERM domain of myosin VIIA is critical for melanosome transport in retinal pigment epithelial (RPE) cells [57]. The p.Gly2038Glu substitution may interfere with the binding of its second FERM domain to the putative membrane ligand. This substitution was absent in 100 normal hearing and vision controls and predicted to have damaging effects on protein structures and functions by software PolyPhen2 and SIFT. A possible polymorphic nature for this variant was discarded because they were not detected in control samples and co-segregated with the disease in the family. This change is likely to be pathogenic and responsible for the disease.

In summary, we reported the clinical features and genetic diagnosis of a Chinese patient with USH1. Compound heterozygous mutations, a known mutation c.494C>T (p.Thr165Met) and a novel mutation c.6113G>A (p.Gly2038Glu), were identified using the next-generation sequencing gene panel and confirmed by Sanger sequencing. In order to provide an earlier and more precise diagnosis of Usher syndrome, a clinical history including signs of vestibular and vision dysfunctions should be evaluated carefully. Molecular diagnosis can uncover the cause even before the onset of vision symptoms, which will provide the time windows for therapeutic interventions to prevent or retard the progressive loss of vision due to the RP of USH.

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REFERENCES

1. Boughman JA, Vernon M, Shaver KA. Usher syndrome: definition and estimate of prevalence from two high-risk populations. *J Chronic Dis.* 1983;36:595–603. [PubMed: 6885960]
2. Hope CI, Bunday S, Proops D, Fielder AR. Usher syndrome in the city of Birmingham--prevalence and clinical classification. *Br J Ophthalmol.* 1997;81:46–53. [PubMed: 9135408]
3. Rosenberg T, Haim M, Hauch AM, Parving A. The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. *Clin Genet.* 1997;51:314–321. [PubMed: 9212179]
4. Espinós C, Nájera C, Millán JM, Ayuso C, Baiget M, Pérez-Garrigues H, et al. Linkage analysis in Usher syndrome type I (USH1) families from Spain. *J Med Genet.* 1998;35:391–398. [PubMed: 9610802]
5. Jaijo T, Aller E, Oltra S, Beneyto M, Nájera C, Ayuso C, et al. Mutation profile of the MYO7A gene in Spanish patients with Usher syndrome type I. *Hum Mutat.* 2006;27:290–291.
6. Kimberling WJ, Hildebrand MS, Shearer AE, Jensen ML, Halder JA, Trzupek K, et al. Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. *Genet Med.* 2010;12:512–516. [PubMed: 20613545]
7. Yan D, Liu XZ. Genetics and pathological mechanisms of Usher syndrome. *J Hum Genet.* 2010;55:327–335. [PubMed: 20379205]
8. Fishman GA, Kumar A, Joseph ME, Torok N, Anderson RJ. Usher's syndrome. *Arch Ophthalmol.* 1983;101:1367–1374.
9. Lévy G, Levi-Acobas F, Blanchard S, Gerber S, Larget-Piet D, Chenal V, et al. Myosin VIIA gene: heterogeneity of the mutations responsible for Usher syndrome type IB. *Hum Mol Genet.* 1997;6:111–116. [PubMed: 9002678]
10. Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, et al. Defective myosin VIIA gene responsible for Usher syndrome type IB. *Nature.* 1995;374:60–61. [PubMed: 7870171]

11. Bitner-Grindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, et al. A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. *Nat Genet.* 2000;26:56–60. [PubMed: 10973248]
12. Verpy E, Leibovici M, Zwaenepoel I, Liu XZ, Gal A, Salem N, et al. A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nat Genet.* 2000;26:51–55. [PubMed: 10973247]
13. Bolz H, von Brederlow B, Ramírez A, Bryda EC, Kutsche K, Nothwang HG, et al. Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nat Genet.* 2001;27:108–112. [PubMed: 11138009]
14. Bork JM, Peters LM, Riazuddin S, Bernstein SL, Ahmed ZM, Ness SL, et al. Usher syndrome 1D and nonsyndromic autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. *Am J Hum Genet.* 2001;68:26–37. [PubMed: 11090341]
15. Ahmed ZM, Riazuddin S, Bernstein SL, Ahmed Z, Khan S, Griffith AJ, et al. Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. *Am J Hum Genet.* 2001;69:25–34. [PubMed: 11398101]
16. Alagramam KN, Yuan H, Kuehn MH, Murcia CL, Wayne S, Srisailpathy CR, et al. Mutations in the novel protocadherin PCDH15 cause Usher syndrome type 1F. *Hum Mol Genet.* 2001;10:1709–1718. [PubMed: 11487575]
17. Weil D, El-Amraoui A, Masmoudi S, Mustapha M, Kikkawa Y, Lainé S, et al. Usher syndrome type I G (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Hum Mol Genet.* 2003;12: 463–471. [PubMed: 12588794]
18. Riazuddin S, Belyantseva IA, Giese AP, Lee K, Indzhukulian AA, Nandamuri SP, et al. Alterations of the CIB2 calcium- and integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. *Nat Genet.* 2012;44:1265–1271. [PubMed: 23023331]
19. Bharadwaj AK, Kasztejna JP, Huq S, Berson EL, Dryja TP. Evaluation of the myosin VIIA gene and visual function in patients with Usher syndrome type I. *Exp Eye Res.* 2000;71:173–181. [PubMed: 10930322]
20. Ouyang XM, Yan D, Du LL, Hejtmancik JF, Jacobson SG, Nance WE, et al. Characterization of Usher syndrome type I gene mutations in an Usher syndrome patient population. *Hum Genet.* 2005;116:292–299. [PubMed: 15660226]
21. Roux AF, Faugère V, Le Guédard S, Pallares-Ruiz N, Vielle A, Chambert S, et al. French Usher Syndrome Collaboration, Survey of the frequency of USH1 gene mutations in a cohort of Usher patients shows the importance of cadherin 23 and protocadherin 15 genes and establishes a detection rate of above 90%. *J Med Genet.* 2006;43:763–768. [PubMed: 16679490]
22. Jaijo T, Aller E, Beneyto M, Najera C, Graziano C, Turchetti D, et al. MYO7A mutation screening in Usher syndrome type I patients from diverse origins. *J Med Genet.* 2007;44:e71. [PubMed: 17361009]
23. Nakanishi H, Ohtsubo M, Iwasaki S, Hotta Y, Takizawa Y, Hosono K, et al. Mutation analysis of the MYO7A and CDH23 genes in Japanese patients with Usher syndrome type 1. *J Hum Genet.* 2010;55:796–800. [PubMed: 20844544]
24. Bonnet C, Grati M, Marlin S, Levilliers J, Hardelin JP, Parodi M, et al. Complete exon sequencing of all known Usher syndrome genes greatly improves molecular diagnosis. *Orphanet J Rare Dis.* 2011 6:21. [PubMed: 21569298]
25. Liu XZ, Walsh J, Mburu P, Kendrick-Jones J, Cope MJ, Steel KP, et al. Mutations in the myosin VIIA gene cause non-syndromic recessive deafness. *Nat Genet.* 1997;16:188–190. [PubMed: 9171832]
26. Sun Y, Chen J, Sun H, Cheng J, Li J, Lu Y, et al. Novel missense mutations in MYO7A underlying postlingual high- or low-frequency non-syndromic hearing impairment in two large families from China. *J Hum Genet.* 2011;56:64–70. [PubMed: 21150918]
27. Rong W, Chen X, Zhao K, Liu Y, Liu X, Ha S, et al. Novel and recurrent MYO7A mutations in Usher syndrome type 1 and type 2. *PLoS One.* 2014;9:e97808. [PubMed: 24831256]
28. Zhai W, Jin X, Gong Y, Qu LH, Zhao C, Li ZH. Phenotype of Usher syndrome type II associated with compound missense mutations of c.721 C>T and c.1969 C>T in MYO7A in a Chinese Usher syndrome family. *Int J Ophthalmol.* 2015;8:670–674. [PubMed: 26309859]

29. Hasson T, Heintzelman MB, Santos-Sacchi J, Corey DP, Mooseker MS. Expression in cochlea and retina of myosin VIIa, the gene product defective in Usher syndrome type IB. *Proc Natl Acad Sci USA*. 1995;92:9815–9819. [PubMed: 7568224]
30. Self T, Mahony M, Fleming J, Walsh J, Brown SD, Steel KP. Shaker-1 mutations reveal roles for myosin VIIA in both development and function of cochlear hair cells. *Development*. 1998;125:557–566. [PubMed: 9435277]
31. Kros CJ, Marcotti W, van Netten SM, Self TJ, Libby RT, Brown SD, et al. Reduced climbing and increased slipping adaptation in cochlear hair cells of mice with Myo7a mutations. *Nat Neurosci*. 2002;5:41–47. [PubMed: 11753415]
32. Adato A, Michel V, Kikkawa Y, Reiniers J, Alagramam KN, Weil D, et al. Interactions in the network of Usher syndrome type 1 proteins. *Hum Mol Genet*. 2005;14:347–356. [PubMed: 15590703]
33. Wolfrum U, Liu X, Schmitt A, Udovichenko IP, Williams DS. Myosin VIIa as a common component of cilia and microvilli. *Cell Motil. Cytoskeleton*. 1998;40:261–271. [PubMed: 9678669]
34. Udovichenko IP, Gibbs D, Williams DS. Actin-based motor properties of native myosin VIIa. *J Cell Sci*. 2002;115:445–450. [PubMed: 11839794]
35. El-Amraoui A, Petit C. Usher I syndrome: unravelling the mechanisms that underlie the cohesion of the growing hair bundle in inner ear sensory cells. *J Cell Sci*. 2005;118:4593–4603. [PubMed: 16219682]
36. Tekin D, Yan D, Bademci G, Feng Y, Guo S, 2nd Foster J, et al. A next-generation sequencing gene panel (MiamiOtoGenes) for comprehensive analysis of deafness genes. *Hear Res*. 2016;333:179–184. [PubMed: 26850479]
37. Yan D, Tekin D, Bademci G, 2nd Foster J, Cengiz FB, Kannan-Sundhari A, et al. Spectrum of DNA variants for non-syndromic deafness in a large cohort from multiple continents. *Hum Genet*. 2016;135:953–961. [PubMed: 27344577]
38. Eisenberger T, Slim R, Mansour A, Nauck M, Nürnberg G, Nürnberg P, et al. Targeted next generation sequencing identifies a homozygous nonsense mutation in ABHD12, the gene underlying PHARC, in a family clinically diagnosed with Usher syndrome type 3. *Orphanet J Rare Dis*. 2012;7:59. [PubMed: 22938382]
39. Puffenberger EG, Jinks RN, Sougnez C, Cibulskis K, Willert RA, Achilly NP, et al. Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PLoS One*. 2012;7:e28936. [PubMed: 22279524]
40. Gonzalez MA, Lebrigio RF, Van Booven D, Ulloa RH, Powell E, Speziani F, et al. GENomes Management Application (GEM.app): a new software tool for large-scale collaborative genome analysis. *Hum Mutat*. 2013;34:842–846. [PubMed: 23463597]
41. Vohr BR, Carty LM, Moore PE, Letourneau K. The Rhode Island hearing assessment program: experience with statewide hearing screening. *J Pediatr*. 1998;133:353–357. [PubMed: 9738715]
42. Windmill IM. Universal screening of infants for hearing loss: Further justification. *J Pediatr*. 1998;133:318–319. [PubMed: 9738708]
43. Elden LM, Potsic WP. Screening and prevention of hearing loss in children. *Curr. Opin. Pediatr*. 2002;14:723–730. [PubMed: 12436045]
44. Nikolopoulos TP. Neonatal hearing screening: what we have achieved and what needs to be improved. *Int J Pediatr Otorhinolaryngol*. 2015;79:635–637. [PubMed: 25758196]
45. Zina ZB, Masmoudi S, Ayadi H, Chaker F, Ghorbel AM, Drira M, et al. From DFNB2 to Usher syndrome: variable expressivity of the same disease. *Am J Med Genet*. 2001;101:181–183. [PubMed: 11391666]
46. Astuto LM, Kelley PM, Askew JW, Weston MD, Smith RJ, Alswaid AF, et al. Searching for evidence of DFNB2. *Am J Med Genet*. 2002;109:291–297. [PubMed: 11992483]
47. National Institute on Deafness and Other Communication Disorders, Pendred syndrome. <https://www.nidcd.nih.gov/health/pendred-syndrome>, 2015 (accessed 16.09.16)
48. Jung J, Seo YW, Choi JY, Kim SH. Vestibular function is associated with residual low-frequency hearing loss in patients with bi-allelic mutations in the SLC26A4 gene. *Hear Res*. 2016;335:33–39. [PubMed: 26900070]

49. Zalewski CK, Chien WW, King KA, Muskett JA, Baron RE, Butman JA, et al. Vestibular Dysfunction in Patients with Enlarged Vestibular Aqueduct. *Otolaryngol Head Neck Surg.* 2015;153:257–262. [PubMed: 25968061]
50. Cremers CW, Delleman WJ. Usher's syndrome, temporal bone pathology. *Int J Pediatr Otorhinolaryngol.* 1988;16:23–30. [PubMed: 3203983]
51. Jiang L, Liang X, Li Y, Wang J, Zaneveld JE, Wang H, et al. Comprehensive molecular diagnosis of 67 Chinese Usher syndrome probands: high rate of ethnicity specific mutations in Chinese USH patients. *Orphanet J Rare Dis.* 2015;10:110. [PubMed: 26338283]
52. Yan D, Tekin M, Blanton SH, Liu XZ. Next-generation sequencing in genetic hearing loss. *Genet Test Mol Biomarkers.* 2013;17:581–587. [PubMed: 23738631]
53. Weil D, Levy G, Sahly I, Levi-Acobas F, Blanchard S, El-Amraoui A, et al. Human myosin VIIA responsible for the Usher 1B syndrome: a predicted membrane-associated motor protein expressed in developing sensory epithelia. *Proc Natl Acad Sci USA.* 1996;93:3232–3237. [PubMed: 8622919]
54. Heissler SM, Manstein DJ. Functional characterization of the human myosin-7a motor domain. *Cell Mol Life Sci.* 2012;69:299–311. [PubMed: 21687988]
55. Rees DJ, Ades SE, Singer SJ, Hynes RO. Sequence and domain structure of talin. *Nature.* 1990;347:685–689. [PubMed: 2120593]
56. Takeuchi K, Kawashima A, Nagafuchi A, Tsukita S. Structural diversity of band 4.1 superfamily members. *J Cell Sci.* 1994;107:1921–1928. [PubMed: 7983158]
57. Schwander M, Lopes V, Sczaniecka A, Gibbs D, Lillo C, Delano D, et al. A novel allele of myosin VIIa reveals a critical function for the C-terminal FERM domain for melanosometransport in retinal pigment epithelial cells. *J Neurosci.* 2009;29:15810–15818. [PubMed: 20016096]

Figure 1.

Family pedigree and pure tone audiometry results. (A) Pedigree of the Chinese Family with USH1 and segregation of the mutations in *MYO7A*. The proband is indicated by filled square. Empty symbols represent the unaffected individuals. Bilateral profound sensorineural hearing loss is revealed in patient III:2 (B). The unaffected subject III:1 has normal hearing (C). (red, right ear; blue, left ear). Mutations T165M and G2038E are the abbreviations for p.Thr165Met and p.Gly2038Glu, respectively.

Figure 2.

(A-D) Fundus photography of the patient III:2. Typical RP fundus are shown by waxy pallor of the optic discs (white arrow heads), attenuated retinal arterioles (black arrow heads), diffuse mottling of retinal pigmented epithelium and scattered peripheral bone spicule-type pigment deposits (white arrows), while the macular regions are relatively preserved in both eyes of the patient. A and C: right eye; B and D: left eye. (E-F) Visual field of the patient. Bilateral vision field got significantly constricted to tubular vision. E: right eye; F: left eye. (G-H) Fundus autofluorescence imaging showed the preserved central autofluorescence surrounded by a ring of high density (hyperautofluorescent ring) (white arrows) and widespread hypoautofluorescence consistent with RPE atrophy. G: right eye; H: left eye. (I-J) Optical coherence tomography (OCT) imaging test showed attenuated outer nuclear layer (double white arrows), retinal pigmented epithelium (double white arrow heads), and preserved outer/inner segments (IS/OS) junction layer at foveal region (double red arrows). I: right eye; J: left eye.

A

B

C

	p.T165M		p.G2038E
MYO7A_HUMAN	ESGAGKTESTKLILQFLAAISG	180	2037 LGALIYRVKFEEEDKSYF
MYO7A_MOUSE	ESGAGKTESTKLILQFLAAISG	180	2037 LGALIYRVKFEEEDKSYF
Q8CJE3_RAT	ESGAGKTESTKLILQFLAAISG	180	1999 LGALIYRVKFEEEDKSYF
Q9DGG8_DANRE	ESGAGKTESTKLILQFLAAISG	180	1999 LGALIYRVKFEEEDKSHF
F1MHG0_BOVIN	ESGAGKTESTKLILQFLAAISG	179	2032 LGALIYRVKFEEEDKSYF
F1PB31_CANLF	ESGAGKTESTKLILQFLAAISG	180	1999 LGALIYRVKFEEEDKSYF
H2Q4G1_PANTR	-----	97	1718 LGALIYRVKFEEEDKSYF
F1SUK5_PIG	ESGAGKTESTKLILQFLAAISG	180	1998 LGALIYRVKFEEEDKSYF
			*****:***:

Figure 3.

Protein domain structure, conservation and mutational analysis of the identified mutations of *MYO7A*. (A) Schematic diagram of domain structure with the relative linear locations of two mutations: c.494C>T (p.Thr165Met) and c.6113G>A (p.Gly2038Glu) identified in the present study in context of protein structure. (B) DNA sequencing trace for the two identified mutations of *MYO7A*. (C) Protein alignment showed the evolutionary conservation of residues T165 (p.Thr165) and G2038 (p.Gly2038) across eight species. Mutations p.T165M and p.G2038E are the abbreviations for p.Thr165Met and p.Gly2038Glu, respectively.