A homozygous *MYO7A* mutation associated to Usher syndrome and unilateral auditory neuropathy spectrum disorder

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Abstract. Usher syndrome (USH) is an autosomal recessive disorder characterized by sensorineural hearing loss, progressive visual loss and night blindness due to retinitis pigmentosa (RP), with or without vestibular dysfunction. The purpose of this study was to detect the causative gene in a consanguineous Chinese family with USH. A c.3696_3706del (p.R1232Sfs*72) variant in the myosin VIIa gene (*MYO7A*) was identified in the homozygous state by exome sequencing. The co-segregation of the *MYO7A* c.3696_3706del variant with the phenotype of deafness and progressive visual loss in the USH family was confirmed by Sanger sequencing. The variant was absent in 200 healthy controls. Therefore, the c.3696_3706del variant may disrupt the interaction between myosin VIIa and other USH1 proteins, and impair melanosome transport in retinal pigment epithelial cells. Notably, bilateral auditory brainstem responses were absent in two patients of the USH family, while distortion product otoacoustic emissions were elicited in the right ears of the two patients, consistent with clinical diagnosis of unilateral auditory neuropathy spectrum disorder. These data suggested that the homozygous c.3696_3706del variant in the *MYO7A* gene may be the disease-causing mutation for the disorder in this family. These findings broaden the phenotype spectrum of the *MYO7A* gene, and may facilitate understanding of the molecular pathogenesis of the disease, and genetic counseling for the family.

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Introduction

Usher syndrome (USH) is an autosomal recessive, clinically and genetically heterogeneous disorder. It is characterized by sensorineural hearing loss, progressive visual loss and night blindness due to retinitis pigmentosa (RP), with or without vestibular dysfunction (1-4). USH occurs in ~4.2-6.2 out of 100,000 individuals (5,6) and is responsible for >50% of deaf-blindness patients (1). Hearing aids and cochlear implantation may assist hearing and oral language acquisition, while few strategies offer help to visual impairments (7).

USH is divided into three clinical subtypes based on involvement of vestibular function and the level of deafness: USH type I (USH1), type II (USH2) and type III (USH3) (1,5). USH1, the most serious type, is responsible for ~33-41.6% of USH patients (5,6) and manifests as congenital bilateral profound sensorineural deafness with unintelligible speech, constant vestibular dysfunction since birth, and childhood onset progressive RP (1,4). USH2 accounts for 46.1-47% of USH cases (5,6), and patients with USH2 display non-progressive moderate to severe hearing impairments modified by hearing aids, good speech development, pubertal onset of RP and without vestibular dysfunction (1,3). USH3 is characterized by post-lingual progressive moderate to severe deafness, inconstant vestibular function and the onset of RP symptoms (1).

To date, 15 loci and 11 USH genes, including myosin VIIa (*MYO7A*), harmonin, cadherin 23, protocadherin 15, the scaffold protein containing ankyrin repeats and SAM domain (*SANS*), calcium- and integrin-binding protein 2, USH type 2A, G protein-coupled receptor 98, whirlin, clarin 1 and histidyl-tRNA synthetase have been associated with USH (http://hereditaryhearingloss.org). Additionally, the PDZ domain-containing 7 gene was reported as a contributor to digenic USH (8).

It is time-consuming to identify the pathogenic gene mutations in USH using regular Sanger sequencing, due to the genetic heterogeneity and large size of these USH genes (9). Exome sequencing, a cost-effectiveness strategy, was introduced to detect the causative gene mutations in USH (10). In the present study, a c.3696_3706del mutation in the *MYO7A*

gene was identified in the homozygous state in a consanguineous Chinese family with USH by exome sequencing.

Materials and methods

Ethics. The present study was approved by the Institutional Review Board of the Third Xiangya Hospital, Central South University (Changsha, China) and in accordance with the Declaration of Helsinki. Written informed consent was received from all the participants or guardians.

Subjects. A four-generation Chinese Han family with USH was enlisted, and five family members took part in this study (Fig. 1A). Congenital bilateral hearing impairments, a delay in sitting and walking without assistance were observed in the two brothers (IV:3 and IV:4), and night blindness was discovered in the first decade of the proband (IV:4) and the third decade of the patient (IV:3). Neither hearing aids nor cochlear implantation was offered to the two patients during their childhood. However, their parents (III:1 and III:2) and two unaffected siblings (IV:1 and IV:2) had normal hearing, vision, speech and motor development. A total of 200 ethnically matched unrelated subjects (100 males and 100 females, aged 49.0±7.5 years) without hearing, visual or vestibular impairments were recruited as healthy controls.

Exome sequencing and variant analysis. Genomic DNA was obtained from peripheral blood samples of all the subjects by standard method of phenol-chloroform extraction. No less than 1.5 µg genomic DNA from the proband was used for exome sequencing at the Novogene Bioinformatics Institute (Beijing, China). According to the manufacturers' protocol, the genomic DNA was first sheared, and then enriched, hybridized, and captured by the Agilent SureSelect Human All Exon V5 (Agilent Technologies, Inc., Santa Clara, CA, USA), and the enriched library was sequenced using Illumina HiSeq 2000 sequencing instruments (Illumina, Inc., San Diego, CA, USA). The mean sequencing depth on the target exome was 70.80x, which covered 98.0% of the targeted exome (11).

The Burrows-Wheeler Alignment tool was applied to map the clean reads to the human reference genome (UCSC hg19, http://genome.ucsc.edu/). The Sequence Alignment/Map tools (version 1.0, http://samtools.sourceforge.net/) were used for identifying single nucleotide polymorphisms (SNPs) and insertions/deletions, and Picard (version 1.111, https://broadinstitute.github.io/picard/) was applied to mark duplicate reads. Previously reported variants detected in the SNP database version 137 (dbSNP137) with minor allele frequency >1% and 1000 Genomes Project with a frequency of >0.5% were screened out. Following this, variants in the exonic and splicing junction regions were retained, and synonymous variants were screened out. Sorting Intolerant from Tolerant (http://sift.jcvi. org/) and Polymorphism Phenotyping version 2 (http://genetics. bwh.harvard.edu/pph2/) were used for the functional prediction of nonsynonymous SNPs. The ANNOVAR (Annotate Variation, version 2013, August 23) software was applied to the annotation of candidate variants.

Sanger sequencing and functional prediction. The potential pathogenic variant was confirmed by Sanger sequencing using an ABI3500 sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and segregation analysis was performed (12). Two primer sequences for variants in the *MYO7A* gene (NM_000260.3) were designed as follows: Forward, 5'-GCTGCCCTCAAAATCCACAT-3' and reverse, 5'-CACACACGCATTTACACACG-3'. The functional prediction of the *MYO7A* variant was conducted using the MutationTaster online tool (http://www.mutationtaster.org/).

Clinical, audiometric and ophthalmologic evaluations. Subjects in the family had clinical, audiometric and fundus examinations conducted in the Third Xiangya Hospital. These auditory and ophthalmologic evaluations included pure tone audiometry (PTA), auditory brainstem responses (ABR), tympanometry, acoustic reflex (AR) thresholds, transient evoked otoacoustic emissions (TEOAE), distortion product otoacoustic emissions (DPOAE), magnetic resonance imaging (MRI) of the inner ear and optic nerve, the naked visual acuity, and images of the fundus with a dilated pupil. Hearing degree was distributed into healthy (<20 dBHL), mild deafness (20-40 dBHL), moderate deafness (41-70 dBHL), severe deafness (71-95 dBHL), and profound deafness (>95 dBHL) (13).

Results

Exome sequencing. Exome sequencing of the proband generated 19,306,510 paired reads, with a mean read length of 150 bp, and 98.66% (19,047,947) of paired reads passed the quality evaluation. Approximately 99.88% clean reads were mapped to the human reference genome (14). After filtering out common variants and nonpathogenic variants, a homozygous *MYO7A* c.3696_3706del variant was observed in the proband, and other probable variants in the known causative genes for hearing loss or RP were excluded.

Identification of the pathogenic mutation. The *MYO7A* c.3696_3706del variant was confirmed in a homozygous state in the proband by Sanger sequencing (Fig. 1B). The *MYO7A* c.3696_3706del variant co-segregated with the phenotype of USH in the family; the homozygous c.3696_3706del variant was identified in the two affected brothers, and the heterozygous variant was detected in their unaffected father (Fig. 1C) and siblings. The *MYO7A* c.3696_3706del variant was predicted to result in a shift in the reading frame and a premature stop codon (p.R1232Sfs*72), the nomenclature was checked by the Mutalyzer online tool (http://www.lovd.nl/mutalyzer/). The variant was absent in two hundred healthy controls (Fig. 1D).

Clinical, audiometric and ophthalmologic results. Both patients displayed bilateral hearing loss, visual impairment and vestibular dysfunction. PTA revealed bilateral profound sensorineural deafness with thresholds >100 dBHL (Fig. 2A). AR, ABR (Fig. 2B) and TEOAE were absent in the two patients. A type A tympanometric curve was present in bilateral ears of the patient (IV:3) and the left ear of the proband, while a type C tympanometric curve was present in the right ear of the proband. No inner ear or optic nerve anomaly was detected by MRI in the two patients. DPOAE was absent in the left ears of the two patients. However, DPOAE was elicited in the right ears of the two patients (Fig. 3A). The
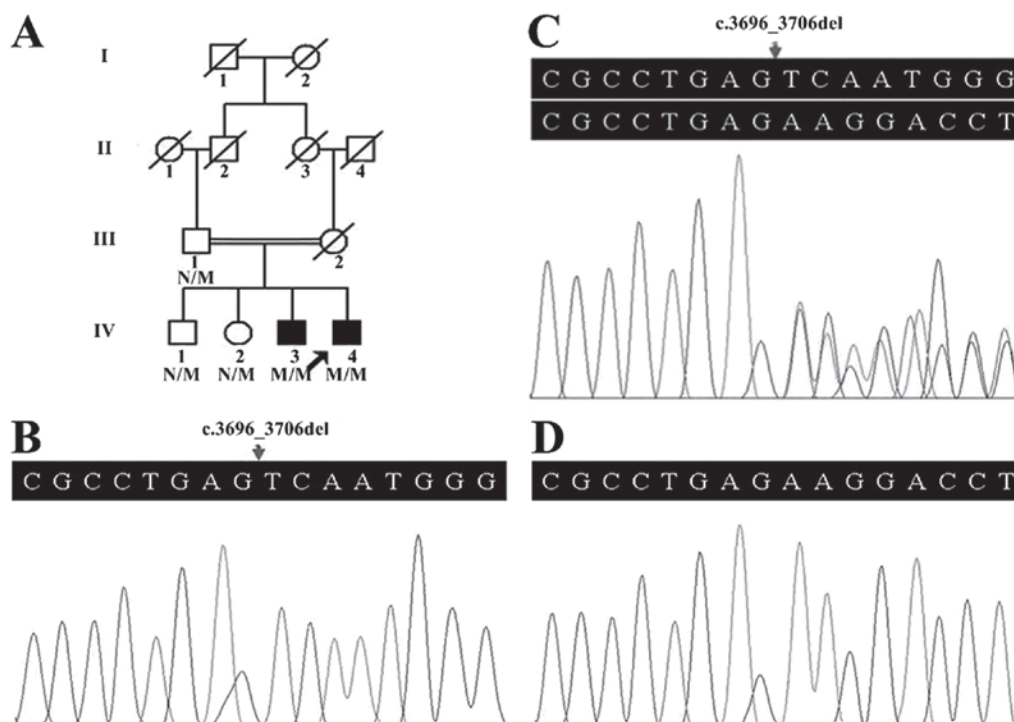
Figure 1. Pedigree and mutation analysis of a Chinese family with Usher syndrome. (A) Pedigree of the USH family. N, normal, M, the *MYO7A* c.3696_3706del variant. (B) The homozygous *MYO7A* c.3696_3706del variant in the affected individual (IV:4). (C) The heterozygous *MYO7A* c.3696_3706del variant in the unaffected individual (III:1). (D) The *MYO7A* gene sequence in a healthy control. *MYO7A*, the myosin VIIa gene.
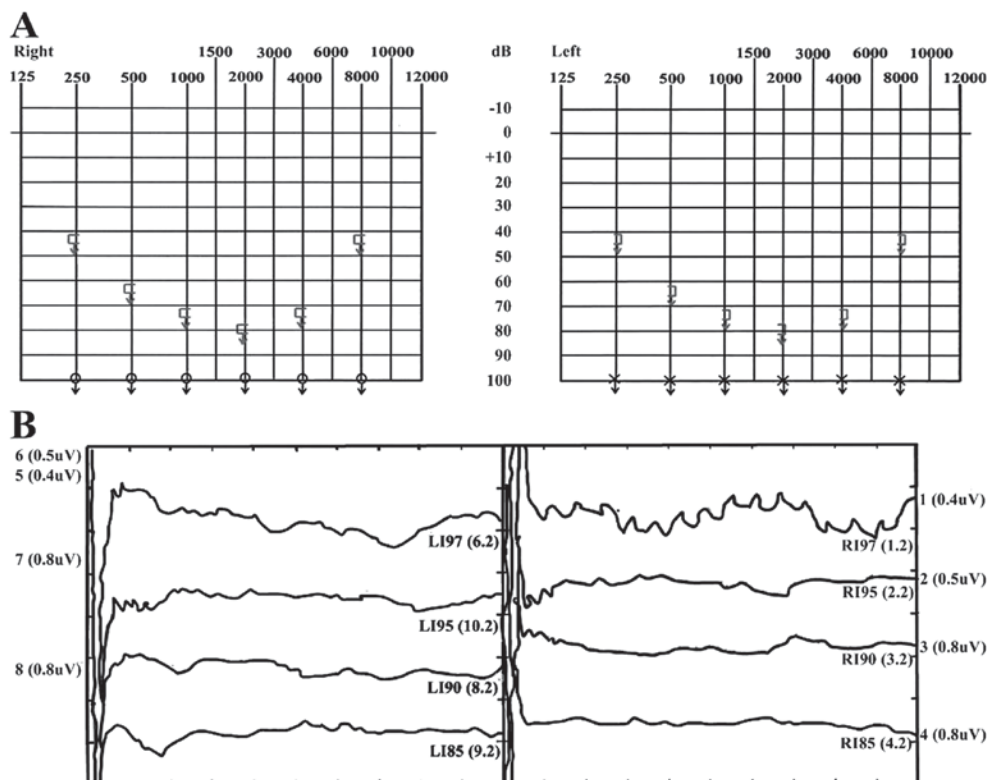

Figure 2. Audiograms and auditory brainstem responses in the proband with Usher syndrome. (A) Pure tone audiometry revealed bilateral profound sensorineural deafness. (B) Auditory brainstem responses were absent in the proband.

proband had more severe vision loss (complete blindness) and vestibular dysfunction than the patient (IV:3), whose bilateral naked visual acuity was measured as 0.1 by the E Standard Logarithm Visual Acuity Chart at 5 m. Pale optic nerve, vascular attenuation and retinal bone spicule pigmentations were detected in the bilateral eyes of the two patients
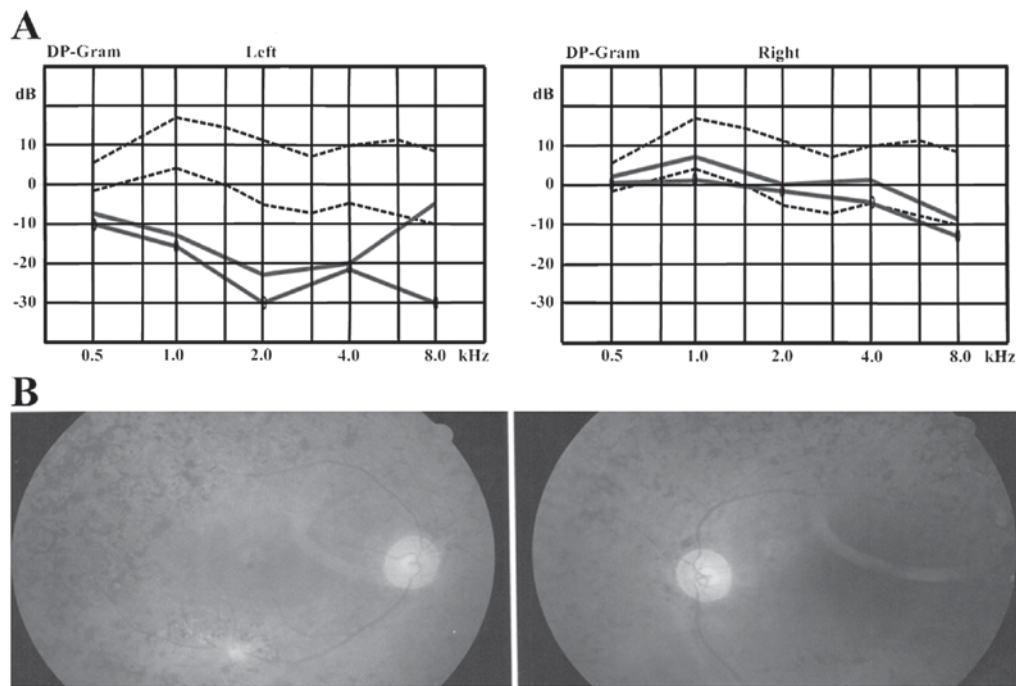
Figure 3. DPOAE and fundus images in two patients with Usher syndrome. (A) DPOAE was absent in the left ear of the proband, but was elicited in the right ear. (B) Fundus images of the patient IV:3 displayed a pale optic nerve, vascular attenuation and retinal bone spicule pigmentations. DPOAE, distortion product otoacoustic emission.

by fundus imaging (Fig. 3B). The two patients presented with bilateral profound hearing impairments, constant vestibular dysfunction and progressive retinitis pigmentosa, and were diagnosed with USH1.

Discussion

In 1992, a disease gene locus for USH type 1B (USH1B) was localized to 11q13.5 by linkage analysis of 27 families with USH (15). In 1995, mutations in the *MYO7A* gene were identified in five unrelated families with USH1B (4). Mutations in the *MYO7A* gene are responsible for 6.25% of Pakistani families with pre-lingual hearing loss (16). According to the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk), to date >402 mutations in the *MYO7A* gene have been reported. The *MYO7A* p.R212C, p.R212H, p.R302H, p.R666* and p.Q821* mutations were reported to be common USH1B mutations (17-19), and p.C31* and p.R241G mutations were identified as founder mutations in the Danish and Italian populations, respectively (20,21).

Of note, a few mutations in the *MYO7A* gene were also reported in patients with other hearing and/or visual disorders, such as autosomal recessive nonsyndromic deafness 2 (22), autosomal dominant nonsyndromic deafness 11 (23), USH2 (24) and Leber congenital amaurosis (25).

The *MYO7A* gene contains 49 exons, and encodes an unconventional myosin VIIa with several alternatively spliced transcripts. The long transcript is composed of 2215 amino acids, which consist of the motor domain containing a typical adenosine triphosphate binding site and an actin binding site, the regulatory domain harboring five light chain binding IQ motifs, and the tail region consisting of a coiled-coil domain, two myosin tail homology 4 (MyTH4) domains, a Src-homology-3 (SH3) domain and two band 4.1, ezrin, radixin and moesin (FERM) domains (26-28).

The *MYO7A* gene is expressed in the cochlear and vestibular sensory hair cells, particularly in the actin-rich hair bundle, and in the pigment epithelium and the photoreceptor cells of the retina (27,29). Myosin VIIa is implicated in sensory hair cell bundle integrity and may be essential for endocytosis at the apical end of the sensory hair cell (30). The protein also contributes to the phagocytosis of photoreceptor disk membranes by the retinal pigment epithelium, which is vital to photoreceptor cell viability (31). Defects in the morphogenesis of the inner ear sensory cell stereocilia may cause deafness and vestibular dysfunction, and pigment epithelium and photoreceptor cells may be implicated in the retinal degenerative process (27).

Both *Shaker-1* mice and *polka* mice with homozygous *Myo7a* mutations displayed the phenotype of deafness and vestibular dysfunction, disorganization of the inner ear hair bundles, and defective melanosome localization in the apical retinal pigment epithelium (32-35). Defective hair bundles were also observed in zebrafish *mariner* mutants due to *Myo7a* mutations (30).

In the present study, the c.3696_3706del variant in the *MYO7A* gene co-segregated with the USH1 phenotype in the family, and therefore is likely to be the causative mutation. The c.3696_3706del variant (rs397516303) was previously reported in a compound heterozygous state with the *MYO7A* c.4398G>A or c.4251delC variant in two Chinese patients with USH1 (36). The c.3696_3706del variant in the *MYO7A* gene, located in the first MyTH4 domain, was predicted to result in a shift in the reading frame and a premature stop codon (p.R1232Sfs*72), and lead to a truncated protein missing part of the first and second MyTH4 domain, the SH3 domain, and the two FERM domains in the tail region of myosin VIIa.

Myosin VIIa may interact with other USH1 proteins, harmonin and *SANS*, by the C-terminal MyTH4 and FERM domains, and contribute to the shaping of hair bundles (28,37). The C-terminal FERM domain of Myosin VIIa is essential for melanosome transport in the retinal pigment epithelial cells (35). The c.3696_3706del variant in the *MYO7A* gene may disrupt the interaction between Myosin VIIa and other USH1 proteins, and impair melanosome transport in the retinal pigment epithelial cells. Therefore, the variant may be the pathogenic mutation in the USH family, and was reported for the first time in a homozygous state.

The proband had early onset of RP and more severe phenotype than the affected brother (IV:3), potentially due to genetic modifiers or environmental factors (25). Notably, bilateral ABR was absent in both patients, while DPOAE was elicited in the right ears of the two patients, suggesting preservation of healthy cochlear outer hair cell function in their right ears. The deafness phenotypes of the two patients were consistent with clinical diagnosis of unilateral auditory neuropathy spectrum disorder (ANSD) (38). It is the first report of the association between the phenotype of ANSD and a mutation in the *MYO7A* gene, which may contribute to further elucidation of the molecular pathogenesis.

In conclusion, the homozygous c.3696_3706del variant in the *MYO7A* gene was the disease-causing mutation in this USH family. The present study indicated that exome sequencing is an effective and systematic molecular diagnostic strategy for USH, a disorder with clinical and genetic heterogeneity. These findings expand the phenotype spectrum of the *MYO7A* gene, and may facilitate understanding on the molecular pathogenesis and genetic counseling.

Acknowledgements

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