Association of a novel missense mutation in MYO15A with nonsyndromic hearing loss: a case report

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Case Report

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Abstract

Background

Hearing loss is a common disease globally, and more than 50% of the cases are genetic. Autosomal recessive nonsyndromic hearing loss (ARNSHL) is one of the most common types of hereditary hearing loss. Here, a novel *MYO15A* missense mutation was identified in a Chinese family with ARNSHL, using targeted genetic sequencing and Sanger sequencing.

Case presentation:

A 6-year-old girl with congenital nonsyndromic sensorineural deafness was presented from the First Affiliated hospital of Chongqing Medical University, China. We used targeted region sequencing, Sanger sequencing, functional prediction, and three-dimensional protein structure modeling to identify and verify the genes responsible for deafness in the family.

Conclusions

We found pathogenic compound heterozygous mutations in *MYO15A*, including a novel missense mutation, c.6353T > C (p.Leu2118Pro). It could provide help not only for genetic counseling but also for further understanding of the functional role of *MYO15A* mutations.

Background

Hearing loss (HL) is a common disease, with one to two in every 1,000 newborns suffering from severe congenital deafness [1]. The etiology of HL is diverse and includes genetic, environmental, or multifactorial factors. However, genetic factors predominate; more than 50% of HL is genetic [2]. Approximately 70% of deafness with no other symptoms can be categorized as nonsyndromic HL (NSHL). Autosomal dominant, autosomal recessive, mitochondrial, and x chromosome inherited are the four genetic modes of hereditary deafness. Autosomal recessive inherited nonsyndromic HL (ARNSHL) is the most common genetic type [3]. *GJB2* was the first gene shown to be responsible for hereditary HL, and to date, more than 80 genes and 100 genetic loci have been shown to cause ARNSHL (<https://hereditaryhearingloss.org>).

MYO15A (OMIM 602666), as one of the most common pathogenic genes involved in nonsyndromic HL, is located on chromosome 17p11.2 (total length of 71 KB) and comprises 66 encoded exons [4]. The *MYO15A* gene encodes myosin XVA protein, which is comprised of 3530 amino acids and includes two isoforms. Both isoforms are expressed in the human inner ear [5].

In this study, we reported a novel *MYO15A* missense mutation using targeted region sequencing, Sanger sequencing, functional prediction, and three-dimensional protein structure modeling to identify and verify the responsible genes for deafness in this Chinese family with ARNSHL. The results identified compound heterozygous mutations in *MYO15A*, including the novel missense mutation c.6353T > C (p.Leu2118Pro) and

the previously reported pathogenic mutation c.1185dupC(p.Glu396ArgfsTer36). To the best of our knowledge, this missense mutation has not been reported in any international database. Our findings could provide help not only for genetic counseling for the family but also for further understanding of the functional role of MYO15A mutations.

Case Presentation

Ethics statement

This study followed the guidelines of the Helsinki declaration and was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). All participants signed the relevant informed consent forms before the beginning of the study.

Participants and findings

This study recruited a Chinese family with ARNSHL from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). Three family members, including the parents (normal hearing) and a deaf daughter, spanning two generations, participated in this study.

Table 1 summarizes the clinical data of the ARNSHL family. The proband was a 6-year-old girl with deafness, whose parents had normal hearing (Fig. 1a). Pure tone audiometry (PTA), auditory brainstem responses (ABRs), tympanometry (TP), distortion product otoacoustic emission (DPOAE), and auditory steady state response (ASSR) were evaluated. Congenital inner ear malformation was excluded using temporal bone computed tomography (CT) and magnetic resonance imaging (MRI). PTA showed bilateral profound sensorineural HL with a threshold of more than 100 dBHL (Fig. 1b). Her ASSR suggested the same result (Fig. 1c). She failed the ABR and DPOAE. Type A tympanometric curves were obtained through acoustic immittance measurement, and the temporal bone CT and MRI of the patient showed no abnormalities in the inner ear.

Clinical evaluations

All the imaging and audiological assessments were completed at the First Affiliated Hospital of Chongqing Medical University (Chongqing, China), including PTA, ABRs, DPOAE, TP, ASSR, CT, and MRI. The classification of hearing impairment was based on the pure tone average at frequencies of 0.5, 1, 2, and 4kHz. It was classified as normal hearing (≤ 25 dBHL), mild deafness (26 ~ 40 dBHL), moderate deafness (41 ~ 60 dBHL), severe deafness (61 ~ 80 dBHL), and profound deafness (> 80 dBHL) [6].

Preparation of genomic DNA

Genomic DNA was extracted from the peripheral venous blood of the participants, using a blood DNA extraction kit (TIANGEN, Beijing, China), and was stored in a refrigerator at -20 °C.

Target region sequencing

Targeted sequencing was used to analyze the known or suspected association of specific mutations with phenotypes in a set of genes or regions. We could identify the candidate genes or mutations associated with

the disease by using this approach. All exon, splicing site, and lateral intron sequences of five HL-related genes, including *GJB2*, *MYO7A*, *MYO15A*, *MT-RNR1*, and *SLC26A4*, were captured by multiplex polymerase chain reaction (PCR). Targeted deafness gene sequencing was conducted by Mingma Inc. (Shanghai, China), and data collection and bioinformatics analysis were conducted using an in-house pipeline.

Direct Sanger sequencing

We identified two candidate mutations using targeted region sequencing. PCR and Sanger sequencing were used to determine whether these mutations co-segregated with HL in the family. PCR primers were designed using the PrimerZ online software (<http://grch37.genepipe.ncgm.sinica.edu.tw/primerz/beginDesign.do>). Primers that were used for amplification are as follows: human *MYO15A* c.1185dupC mutation, forward 5'-CCCTACTTTGATCCCTACGGAGT-3' and reverse 5'-GAGGTCCCCTGACGCTCTA-3'; human *MYO15A* c.6353T > C mutation, forward 5'-CAACTTTCTGAGATGCCCAGGA-3' and reverse 5'-GAAGGTGCAAAGCCACTGAG-3'. The PCR products were sequenced directly using ABI 3730XL gene analyzer (Applied Biosystems, Foster City, CA, USA).

Results of sequencing analyses

Targeted deafness gene capture sequencing

Five HL-related genes were sequenced in the proband. We detected two mutations in the *MYO15A* gene, c.1185dupC (p.Glu396ArgfsTer36), and c.6353T > C (p.L2118P); and the co-segregation of mutations and deafness suggested that these mutations may be responsible for deafness in this ARNSHL family.

Confirmation of the variants

The compound heterozygous mutations in *MYO15A*, including c.1185dup (p.Glu396ArgfsTer36) and c.6353T > C (p.L2118P) were confirmed by direct sequencing in the proband. Parents with normal hearing carried only one heterozygous mutation (Fig. 2a). To the best of our knowledge, this missense mutation of c.6353T > C (p.L2118P) has not been reported in any international database. The mutation c.6353T > C in *MYO15A*, a missense mutation, leads to an alternation in a leucine with a proline at amino acid position 2118 (p.L2118P).

Amino acid sequences of *MYO15A* of different species: *H. sapiens*, *R. norvegicus*, *G. gallus*, *Pongo abelii*, *Pan troglodytes*, *Macaca mulatta*, *B. taurus*, and *Macaca fascicularis* were analyzed to look for conservation of amino acid residues (Fig. 2b). It was revealed that this residue was important for the normal function of proteins, because p.L2118 was conserved in many species.

Functional prediction

ClustalX1.83 was used to compare the amino acid sequences of different species. The following species were included: NP_057323.3 (*Homo sapiens*), XP_001077498.1 (*Rattus norvegicus*), XP_015149902.1 (*Gallus gallus*), XP_024090661.1 (*Pongo abelii*), XP_009430930.1 (*Pan troglodytes*), XP_014974242.1 (*Macaca mulatta*), XP_015323568.1 (*Bos taurus*), and XP_015293218.1 (*Macaca fascicularis*).

Structure modeling

SWISS-MODEL Workplace (<http://swissmodel.expasy.org/>) was used for modeling the three-dimensional (3D) molecular structure of the first myosin-tail homology 4 (MyTH4) domain, including the templates of the wild-type and c.6353T > C mutant MYO15A proteins. Furthermore, PYMOL software ([http:// www.pymol.org/](http://www.pymol.org/)) captured and presented our analytical images.

Results of structural modeling and functional evaluation

SWISS-MODEL (<http://swissmodel.expasy.org/>) was used to construct the templates of the wild-type and the p.L2118P mutant MYO15A proteins (pdb ID: 3pvl.1.A). Compared with the template proteins, both the p.L2118P mutant MYO15A proteins and the wild-type proteins showed a consistency of more than 30% in the protein sequence; thus, these met the basic requirements of the homologous modeling program. The 3D protein structures simulated 1751–2380 residues of myosin XVA protein, and p.L2118P is in the first MyTH4 domain of myosin XVA (Fig. 3a). The simulated structures of the wild type and mutant in the MYO15A MyTH4 region were visualized using PYMOL software (Fig. 3b). The results indicated that the mutant p.L2118P showed disruption of the amino acid side chain, which further affected the stability of the protein (Fig. 3c).

Discussion And Conclusions

MYO15A is one of the most common pathogenic genes for NSHL. Indeed, after *GJB2* and *MYO7A*, *MYO15A* is the third most commonly identified gene associated with ARNSHL (<https://hereditaryhearingloss.org>). In this report, we found pathogenic compound heterozygous mutations in *MYO15A*, including a novel missense mutation c.6353T > C (p.Leu2118Pro) that has not been reported previously. PTA and ASSR of the proband showed profound deafness. In addition, the patient/s ABR and DPOAE were still negative under strong stimulation, suggesting that the patient was severely deaf. Moreover, Sanger sequencing confirmed that the new mutation p.L2118P co-segregated with the disease phenotypes of the family, and strong genetic evidence was obtained using sequence conservation analysis and prediction of the protein structure changes caused by mutations at those specific points.

Myosin XVA is an unconventional myosin encoded by the human *MYO15A* gene and consists of 3530 amino acids. It is expressed at the tips of hair cell stereocilia in the inner cochlear region [7–9]. Three evolutionally conserved regions (head, neck, and tail) are present in this protein. The head region includes the N-terminal domain and the motor domain. The motor region plays a key role in ATP activity and contains two binding sites for adenosine triphosphate and actin. The neck contains two IQ motifs associated with calmodulin light chain binding. The longest tail region includes two MyTH4 domains, two band F/ezrin/radixin/moesin (FERM) domains, a Src-homology-3 (SH3) domain, and the C-terminal isoform I and PDZ-binding ligand domain [10–13]. The novel missense mutation, L2118P, found in this study was in exon 29 of the first MyTH4 domain. In studies performed in different countries, several mutations, including c.V2114M, p.R2124Q, p.R2146Q, and p.A2153fs, have been reported in the first MyTH4 domain [14–17] and these mutations may lead to deafness and play an important role in the functions of myosin XVA.

Mutations that cause HL were first identified at the deafness, autosomal recessive 3 (DFNB3) site in the Bengkala kindred and two unrelated consanguineous Indian families [18]. Since then, many countries in

South Asia, such as Pakistan, India, and Turkey, have reported various mutations [5, 19–23]. In East Asians, however, the first mutations in *MYO15A* were not reported until 2013 [16]. A study had shown that the p.The R2146Q mutation broke the surface links between MYTH4 and FERM and caused deafness. The p.The R2146Q protein contains a hydrophobic sac similar in structure to that of R1190 of MYO7A myth4-ferm reported previously. Other mutations reported in the MyTH4 region of myosin XVA have been shown to interfere with the interaction between myosin XVA and whirlin, thus inhibiting the formation of the complex needed for normal hearing. [15]. The MyTH4 region is closely associated with the microtubule binding and acting binding on the plasma membrane [24]. It has been shown that the MyTH4/FERM domains in myosin XVA are necessary for myosin XVA to locate to the tip of the stereocilia, which is the key to the formation of the transmembrane actin microfilament assembly complex [24–26]. Homozygous shaker-2 (sh2) mice show profound deafness similar to that observed in humans with DFNB3, but vestibular defects not present in human patients. The stereocilia of hair cells in sh2 mice are located correctly during development, but they are much shorter and lack the typical stepped structure compared with wild-type mice [27]. The other mutation, c.1185dupC, was first reported in 2012 [28] and is a frameshift by the introduction of an eventual stop codon in the *MYO15A* open reading framework (p.E396fsX431).

With the widespread application of next-generation sequencing technology, reports on *MYO15A* mutations are no longer limited to the Middle East and other countries, where inbreeding is common. Reports of *MYO15A* mutation sites are increasing worldwide, particularly for complex heterozygous mutation sites, resulting in continuous enrichment of the *MYO15A* gene mutation database.

In summary, a novel mutation of the *MYO15A* gene was identified in this study. The mutation was in the first MyTH4 domain of myosin XVA and affected its protein function. The results of this study indicated that second-generation target region sequencing was feasible for the identification of rare variants in HL. Our findings expanded our knowledge of pathogenic variants in the *MYO15A* gene in patients with ARNSHL and could have implications in genetic counseling for families with HL.

Abbreviations

HL: Hearing loss ; NSHL: Nonsyndromic hearing loss; ARNSHL: Autosomal recessive nonsyndromic hearing loss; PTA: Pure tone audiometry; ABRs: Auditory brainstem responses; TP: Tympanometry; DPOAE: Distortion product otoacoustic emission; ASSR: Auditory steady state response; CT: Computed tomography; MRI: Magnetic resonance imaging; PCR: polymerase chain reaction; 3D: three-dimensional; MyTH4: Myosin-tail homology 4; FERM: F/ezrin/radixin/moesin; SH3: Src-homology-3; DFNB3: Autosomal recessive forms of deafness 3;

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). All participants or their guardians signed the relevant informed consent forms

before the beginning of the study.

Consent for publication

All participants or guardians provided written informed consent to publish the results of the study using their clinical case data and sequencing results.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

There is no conflict of interest.

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Authors' contributions

HK and SW initiated and designed the study. ZC and XO recruited suitable family members and completed relevant audiology and clinical examination. JD and SW collected blood samples and extracted DNA. The bioinformatics analysis was done by HK. LZ and CX assisted with follow-up. FY and SW studied molecular genetics and mutation filtration. HK and SW prepared the manuscript. All authors read and approved the final manuscript.

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Table

Table 1: Clinical features of the family									
Subjects	Age	Age onset	PTA	ABR	TP	DPOAE	ASSR	CT	MRI
Proband	6 years	6 years	Profound	—	A curve	—	Profound	Normal	Normal
Father	41 years	/	Normal	/	/	/	/	/	/
Mother	39 years	/	Normal	/	/	/	/	/	/

PTA, pure tone audiometry; ABR, auditory brainstem response; TP, tympanometry; DPOAE, distortion product otoacoustic emission; ASSR, auditory steady state response; CT, computed tomography; MRI, magnetic resonance imaging; —, failed inspection; /, the test was not performed.

Figures

Figure1

Figure 1

a. Family pedigree diagram. b. PTA of the left and right ears of the affected proband, showing profound hearing loss. c. ASSR of the left and right ears of the affected proband, showing profound hearing loss.

b.

Figure 2

a. Family pedigree diagram. b. PTA of the left and right ears of the affected proband, showing profound hearing loss. c. ASSR of the left and right ears of the affected proband, showing profound hearing loss.

Figure3

Figure 3

a. Schematic structure of myosin XVA. Black arrows indicate the location of the mutation p.L2118P. b. Three-dimensional molecular modeling of the first MyTH4 domain of myosin XVA. c. Simulated structures of wild-type and mutant p.L2118P residues in this model.