International Journal of Pediatric Otorhinolaryngology 81 (2016) 92–95

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

A novel recessive truncating mutation in MYO15A causing prelingual sensorineural hearing loss

Wei Li [a,c,1], Luo Guo [b,1], Yu Li [a,c,1], Qianru Wu [a,c], Qingzhong Li [a,c], Huawei Li [a,c], Chunfu Dai [a,c,*]

[a] Department of Otorhinolaryngology, Eye Ear Nose & Throat Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, China
[b] Research Center, Eye Ear Nose & Throat Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, China
[c] Hearing Medicine Key Laboratory of Ministry of Health, Eye Ear Nose & Throat Hospital, Fudan University, 83 Fenyang Road, Shanghai 200031, China

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form 18 December 2015
Accepted 19 December 2015
Available online 31 December 2015

Keywords:
MYO15A
Exon 2
Hearing loss

ABSTRACT

Hearing loss (HL) is one of the most common human defects which affects millions of people globally. The identification of deafness-related genes or loci may facilitate basic and clinical translational research on this disorder. Here, we investigated a Chinese family with autosomal recessive non-syndromic hearing impairment. Using targeted massively parallel sequencing, we identified a novel homozygous mutation, c.3525_3526insA and p.Q1175fsX1188 (NM_016239), in exon 2 of *MYO15A*. Sangersequencing confirmed that affected siblings were homozygous for the mutation, whereas both normal hearing parents were heterozygous. The mutation was absent in 96 healthy controls and public databases. The insertion leads to a frameshift and a truncated form of the protein, resulting in the pathogenic effect of hearing loss for the patients. Mutations in exon 2 of *MYO15A* may cause a less severe phenotype, facilitating the rapid identification of mutations in exon 2 among the 66 exons when linkage of less severe hearing loss to Deafness, Autosomal Recessive 3 (DFNB3) is detected. Our data provide additional molecular information for establishing a better genotype–phenotype understanding of DFNB3.

1. Introduction

Hearing loss (HL) is one of the most common human defects [1]. It is estimated that more than 50% of congenital HL is caused by genetic factors. Non-syndromic hearing loss (NSHL), in which hearing loss is the only clinical sign, accounts for 70% of genetic cases [2]. Autosomal recessive NSHL (ARNSHL), which accounts for up to 80% of NSHL, is a genetically heterogeneous disorder [3]. To date, more than 100 genetic loci and 55 genes have been identified for ARNSHL (Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org). The genes most commonly implicated in ARNSHL are, in order of frequency, *GJB2*, *SLC26A4*, *MYO15A*, *OTOF*, and *CDH23* [3].

Targeted massively parallel sequencing (MPS) has been employed to investigate inherited HL in several recent studies [4–10]. This technique, which takes advantage of target genome enrichment (TGE) and MPS, can simultaneously screen hundreds of deafness genes in a few days and generate high-quality candidate variants for further analysis. Because targeted MPS of deafness genes typically identifies thousands of exonic variants, a valid filtering strategy is critical to identify pathogenic mutations associated with the disease. Targeted MPS of affected and healthy members from one family followed by screening homozygous or compound heterozygous variants may be a powerful tool for quickly identifying pathogenic mutations in ARSNHL.

MYO15A(OMIM 602666) is composed of 66 exons distributed across 71 kb of DNA on chromosomal locus 17p11.2 [11]. Mutations in *MYO15A* cause congenital severe-to-profound hearing loss, Autosomal Recessive 3 (DFNB3). The protein encoded by *MYO15A*, myosin XVa, is an unconventional myosin critical for the differentiation and elongation of the stereocilia and has important roles in actin organisation in hair cells. Myosin XVa is localised at the tip of the stereocilia of cochlear and vestibular hair cells and is an actin-dependent molecular motor that uses adenosine triphosphate (ATP) to generate mechanical force with which myosin XVa in hair cells delivers whirlin to the stereociliary tips [12,13]. The hair cell stereocilia in *MYO15A* mutant mice are properly

* Corresponding author at: Eye Ear Nose & Throat Hospital, Fudan University, Department of Otorhinolaryngology, 83 Fenyang Road, Shanghai 200031, China. Tel.: +86 02164377134.
E-mail address: cfdai66@163.com (C. Dai).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.ijporl.2015.12.013
0165-5876/

positioned on the apical surface of hair cells without any links between the stereocilia, and the length of the stereocilia is shorter than the stereocilia of wild-type mice. Mutations in *MYO15A* result in congenital HL in both humans (DFNB3) and mice shaker 2 (sh2) [14–22]. Diagnostic testing for this gene is not routinely offered due to its large size. Thus, the frequency and spectrum of *MYO15A* mutations in most ethnic populations are largely unknown.

In this study, we present the genetic and molecular characteristics of a Chinese family with prelingual sensorineural HL. The affected individuals had moderate residual hearing at low frequencies and profound hearing loss at high frequencies. We used targeted genome enrichment and MPS to identify a novel homozygous insertion mutation in exon 2 of *MYO15A*. The insertion mutation results in a frame shift followed by an early stop codon, which leads to the loss of the majority of the functional protein and results in the pathogenic effect of hearing loss. This is the first report of an insertion mutation in *MYO15A* in the East Asian population. Our study has enriched the mutation spectrum of *MYO15A*, and proved that MPS is a reliable tool for genetic testing of hereditary hearing loss for large genes such as *MYO15A*.

2. Materials and methods

2.1. Subjects

Family D3125 is a two-generation Chinese family with autosomal recessive prelingual non-syndromic HL recruited from Zhejiang province, China (Fig. 1). All family members were evaluated by audiological tests. Pure tone audiometry was performed at frequencies of 125, 250, 500, 1000, 2000, 4000, and 8000 Hz. Immittance testing was applied to evaluate ear canal volumes, middle-ear pressure, and tympanic membrane mobility. Vestibular function was evaluated by Romberg and tandem gait tests. Physical examinations of the affected siblings were performed to detect syndromic features. High-resolution computed tomography (HRCT) scans of the temporal bone were obtained to exclude an enlarged vestibular aqueduct or other inner ear malformations. Written informed consent was obtained from all individuals, and this study was approved by the ethics committee of the Institutional Review Board of the Eye, Ear, Nose and Throat Hospital affiliated with Fudan University (Shanghai, China). To rule out the common deafness genes *GJB2*, *SLC26A4*, and *MT-RNR1*, the family members were prescreened by PCR amplification and Sanger sequencing.

2.2. Targeted exome sequencing

Genomic DNA was extracted from peripheral blood of all family members using a pure gene genomic DNA isolation kit (Qiagen, Hilden, Germany). A sequencing library was prepared using a DNA library preparation kit (New England Biolabs, Ipswich, MA, catalogue# E6040) and Human Deafness Panel oto-DA3 (Otogenetics Corporation). A total of two million paired-end sequencing reads were generated.

2.3. Bioinformatics and validation of the variants

Sequencing reads were produced by the Illumina CASAVA v1.8 pipeline and aligned to the human reference genome (hg19) using the Burrows–Wheeler Aligner (BWA) program [15]. Variants were called using the GATK software package. All variants were annotated and characterised using ANNOVAR [16]. To validate the variants, Sanger sequencing of the *MYO15A* exon 2 was performed on genomic DNA from all family members and 96 normal hearing controls. Primers were designed by Primer3. PCR products were sequenced on a 3730XL sequencer (Applied Biosystems) according to the manufacturer's instructions.

3. Results

3.1. Family and clinical presentations

Family D3125 is a non-consanguineous Chinese family that includes two affected siblings (25 and 20 years old) and two unaffected parents (Fig. 1A). The first diagnosed subject and her younger brother had congenital HL. Audiograms showed bilateral severe to profound sensorineural HL mainly affecting high frequencies, with moderate residual hearing at low frequencies (Fig. 1B). They recalled that their HL was stable over the years, and progressive hearing loss was not observed during the 2 years of follow-up. A vestibular functional test of the two patients revealed no abnormalities. An HRCT scan of the temporal bone revealed no inner ear malformations. We also performed pure tone audiometry on the parents and detected no HL features. Otoscopy and full physical examination with special attention to electrocardiac, renal, and ophthalmological features revealed no additional abnormalities.

3.2. Prescreening for mutations in common deafness genes

We prescreened for mutations in three common deafness genes, *GJB2*, *SLC26A4*, and *MT-RNR1*, in the family members by Sanger sequencing. No mutations were identified. Therefore, we proceeded to sequence the exons of known deafness genes in the samples.

3.3. Targeted massively parallel sequencing

We performed targeted MPS of all exons and exon–intron boundaries for 131 deafness genes in the proband. MPS yielded

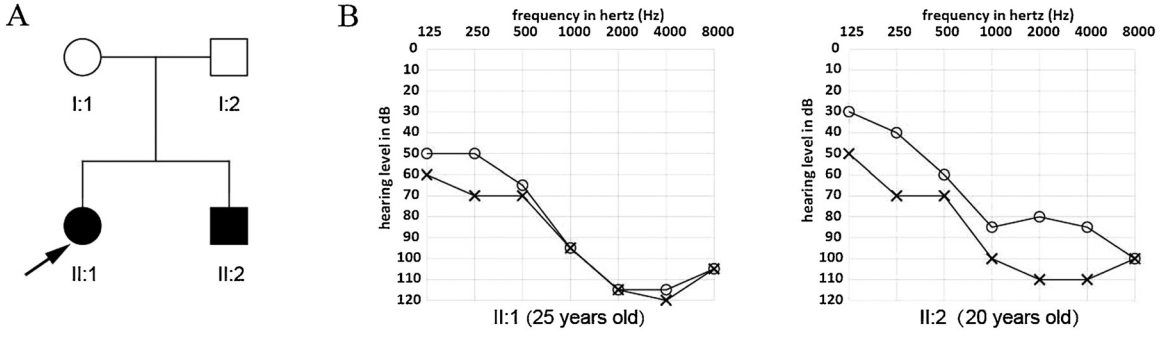

Fig. 1. (A) Pedigree of the family with non-syndromic autosomal recessive hearing loss. Darkened symbols denote affected individuals. (B) Audiograms of the two affected siblings in the family. Right ear indicated by 'O', left ear indicated by 'X'.