ORL

ORL 2020;82:181–187
DOI: 10.1159/000506208
Received: November 14, 2019
Accepted: January 28, 2020
Published online: May 19, 2020

Research Article

The p.R206C Mutation in *MYO7A* Leads to Autosomal Dominant Nonsyndromic Hearing Loss

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Keywords
DFNA11 · *MYO7A* · Mutation · Targeted next-generation sequencing · Chinese Hans

Abstract
Background: Dominant mutations in *MYO7A* may lead to nonsyndromic deafness DFNA11. A p.R206C variant in *MYO7A* has previously been reported in a small deaf family from Taiwan but with ambiguous pathogenicity and inheritance pattern. ***Aims/Objectives:*** Our study aims to clarify the pathogenicity of this variant by clinical characterization and genetic analysis of a separate autosomal dominant deaf family harboring this variant in mainland China. ***Materials and Methods:*** Auditory features of hearing loss were characterized in representative affected family members. Mutation screening was performed by targeted next-generation sequencing of 138 known deafness genes in the proband. Candidate pathogenic mutations were confirmed by Sanger sequencing in family members and ethnically matched controls. ***Results:*** Consistent with typical DFNA11 phenotype, the affected family members in this study showed delayed-onset, progressive hearing loss affecting mostly high frequencies. Targeted next-generation sequencing identified a p.R206C mutation in *MYO7A* as the only candidate pathogenic mutation cosegregating with the hearing phenotype. This mutation is not seen in 200 Chinese Han normal-hearing controls. ***Conclusions and Significance:*** The

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ORL 2020;82:181–187
DOI: 10.1159/000506208

recurrent p.R206C variant in *MYO7A* is pathogenic and is likely in a mutation hot spot or due to a founder effect. Reports of such rare variants in multiple patients or families may facilitate exploitation of its pathogenicity.

Introduction

Hearing loss is one of the most common sensory disorders in humans [1]. The incidence of congenital hearing loss in mainland China was estimated to be approximately 1 in 1,000 [2]. Both hereditary and environmental factors can cause hearing loss, and the former accounts for about 60% of hearing loss [2, 3]. Depending on the absence or presence of other symptoms, hearing loss can be further divided into nonsyndromic (70%) and syndromic hearing loss, respectively [4]. Hereditary hearing loss is a highly heterogeneous disease. To date, over 100 nonsyndromic deafness genes have been identified (http://hereditary hearingloss.org).

MYO7A is located on chromosome 11q13.5 and encodes the actin-binding dynamic protein myosin VIIa, a member of nontraditional myosins [5]. Myosin VIIa consists of 2,215 amino acids. It contains a conserved N-terminal actin binding domain (motor head domain), an IQ5 neck domain, a coiled coil domain, and a C-terminal MyTH4-FERM tandem domain (tail) [6, 7]. Myosin VIIa is mainly responsible for intracapsular transport and plays an important role in the function of cochlear hair cells and eye development. It has been found that shaker1, a *Myo7a* mutant mouse, has cilium defects in cochlear hair cells, suggesting that *MYO7A* plays a crucial role in maintaining cilium hardness and structure [8, 9].

A variety of *MYO7A* mutations leads to both syndromic (Usher syndrome type 1B, USH1B) and nonsyndromic hearing loss (DNFB2 and DFNA11) [5, 10, 11]. USH1B is an autosomal recessive disorder characterized by severe congenital deafness, vestibular dysfunction, and retinitis pigmentosa [11]. DFNB2 is autosomal recessive, characterized by congenital, severe-to-profound hearing loss without vestibular dysfunction [10]. In contrast, DFNA11 is autosomal dominant, characterized by postlingual, progressive hearing loss affecting mostly high frequencies [5].

In this study, we identified a recurrent p.R206C variant in *MYO7A* in a Chinese Han DFNA11 family (family J-1). Though this variant has previously been reported in a small family from Taiwan, its pathogenicity and inheritance pattern remained unclear due to incomplete genetic and clinical information [12]. We, therefore, aimed to clarify the pathogenicity of this variant by clinical characterization and genetic analysis of family J-1.

Materials and Methods

Subjects and Clinical Evaluation

All subjects were recruited at the Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

Members of family J-1 were recruited from the Jiangxi Province in mainland China (Fig. 1). A detailed family history was obtained from the affected family members I:1 and II:3, who were also physically examined. Focal points of the clinical evaluation included ophthalmological abnormalities such as visual field changes, visual impairment, strabismus, diplopia, and amblyopia. A Madsen 502 portable audiometer (Denmark) was used to detect the pure-tone air conduction threshold (0.250, 0.500, 1, 2, 4, and 8 kHz) of the family members. Hearing curves were drawn to determine the degree and type of hearing loss.

Targeted Next-Generation Sequencing

Genomic DNA was extracted from peripheral blood samples of I:2, II:1, II:3, and II:4 using a blood DNA extraction kit (Tiangen Biotech Inc., Beijing, China). For DNA library preparation of the targeted next-generation sequencing, genomic DNA was fragmented to an average size of 180 bp. DNA repair, adapter ligation,

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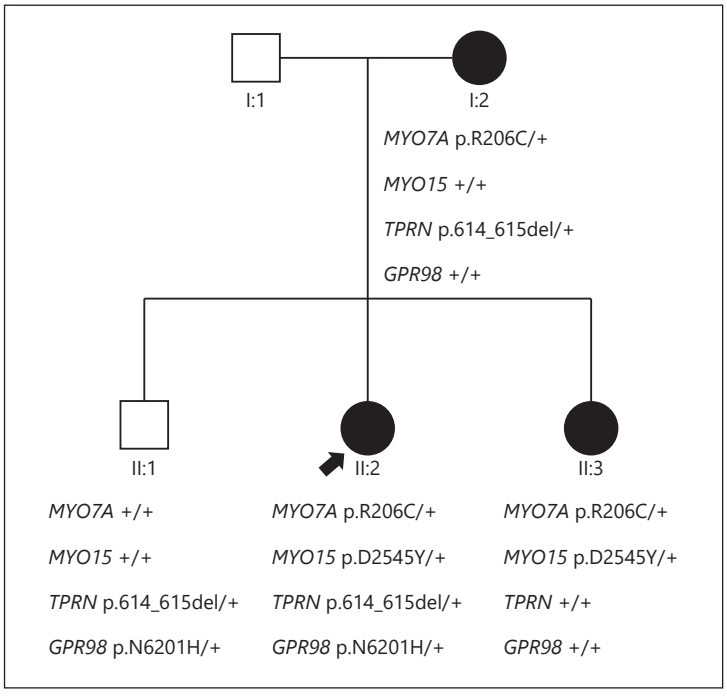

Fig. 1. Pedigree and the genotype of family J-1. Arrow indicates the proband.

and PCR enrichment were performed as recommended by the Illumina protocols. The amplified DNA was captured using the GenCap deafness capture kit (MyGenostics, Beijing, China). The DNA probes were designed to tile along the exon and partial intron regions of the 144 deafness genes (listed in online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000506208). The enrichment libraries were sequenced on Illumina HiSeq X ten sequencer (Illumina, San Diego, CA, USA) with paired reads of approximately 150 bp. After quality control, the clean reads were mapped to the UCSC hg19 human reference genome using the BWA software. Variants were annotated by ANNOVAR, compared with multiple databases, including 1000 Genomes, ESP6500, dbSNP, and EXAC, and predicted by computational programs SIFT, PolyPhen-2, and Mutation Taster. Potential pathogenic variants were filtered using the minor allele frequency threshold of ≤0.0005 for dominant inheritance. Cosegregation of the disease phenotype and the candidate variants were confirmed by Sanger sequencing of the family members. The primers are listed in online supplementary Table S2.

Results

Clinical Characterization

The family J-1 lived in Jiangxi Province for 3 generations and has at least 13 family members in record (Fig. 1). Among them, 3 were diagnosed with sensorineural hearing loss. The oldest and youngest patients with hearing loss were 67 years (I:2) and 38 (II:3) years old, respectively. The 4 members in the third generation (III:1–III:4) are less than 20 years old, and uncertainty exists regarding their future hearing phenotype. The family pedigree is consistent with autosomal dominant inheritance (Fig. 1). All 3 affected family members had bilateral, delayed-onset, progressive hearing loss starting in their third to fourth decades. The hearing loss first affected higher frequencies (from 1 to 8 kHz) and gradually developed to all frequencies (Fig. 2). All affected members denied vestibular and ophthalmological dysfunction. Funduscopic examination showed that none had retinitis pigmentosa. The hearing phenotype of family J-1 is consistent with previous reports for DFNA11.

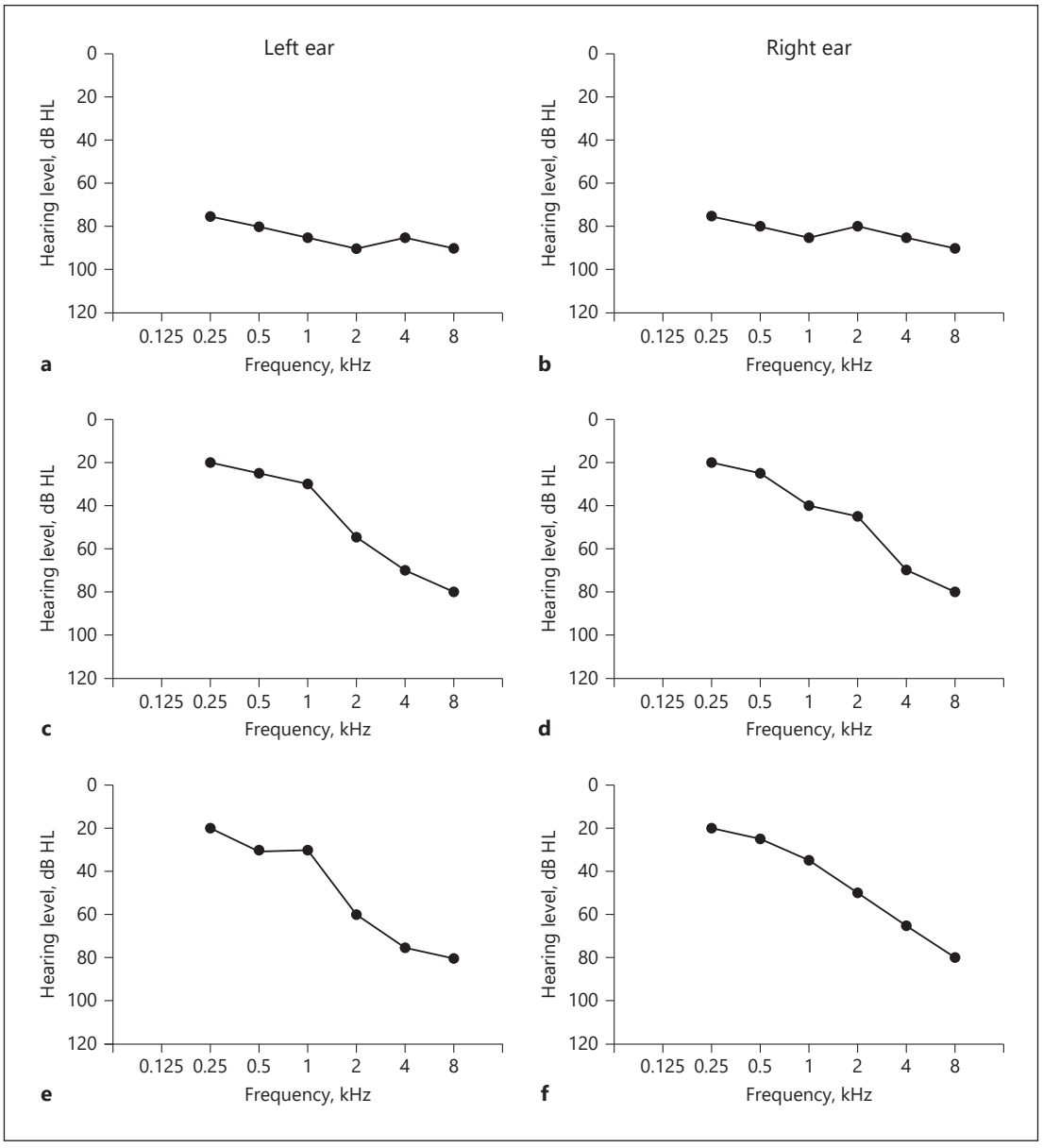

Fig. 2. Audiograms of pure-tone audiometry for affected members I:2 (**a**, **b**; 67 years of age) and II:3 (**e**, **f**; 40 years of age) in family J-1. **c**, **d** II:2 (38 years of age).

Mutation Analysis

Mutation screening of 144 known deafness-associated genes was performed in proband II:3 by targeted next-generation sequencing. The averaged sequencing depth on the targeted region was 456.28-fold, with 94.56% of the region covered by at least 20×, demonstrating high quality of the sequencing data. Four candidate variants were identified (Table 1). Sanger sequencing in the family members I:2, II:1, II:3, and II:4 showed that only variant c.616C>T in *MYO7A* (NM_001127180) cosegregated with hearing loss phenotype in family J-1 (Fig. 1, 3a). This nucleotide change is located in exon 7 of *MYO7A*, resulting in amino acid substitution at position 206 from arginine to cysteine (p.R206C).

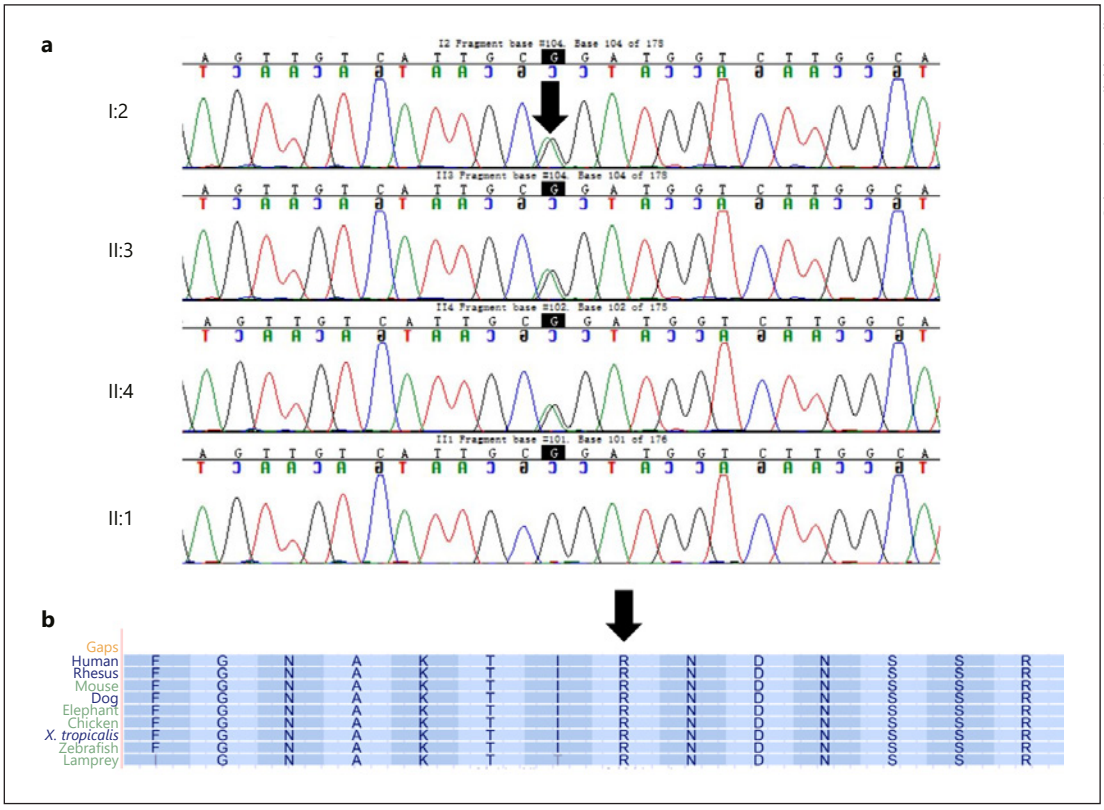

Fig. 3. Sequencing chromatographs (**a**) and evolutionary conservation (**b**) of the p.R206C mutation in *MYO7A* (cf. arrows).

Table 1. Candidate variants identified by targeted next-generation sequencing

Gene	Transcription	Variant		Family cosegregation
MYO7A	NM_001127180	c.616C>T	p.R206C	Yes
MYO15A	NM_016239	c.7633G>T	p.D2545Y	No
TPRN	NM_001128228	c.1842_1844del	p.614_615del	No
GPR98	NM_032119	c.18601A>C	p.N6201H	No

The arginine206 residue is highly conserved among humans, rhesus monkeys, mice, elephants, chicken, frogs, zebrafish and lampreys (Fig. 3b). The p.R206C variant was not seen in over 200 Chinese Han normal-hearing controls. The pathogenicity prediction scores of SIFT, PolyPhen-2, and Mutation Taster are 0.912, 0.971, and 0.81, respectively, all suggesting a damaging effect.

Discussion

Our present study suggested that the p.R206C variant in *MYO7A* is likely a pathogenic mutation leading to dominant nonsyndromic hearing loss DFNA11 in family J-1. To date, more than 340 different mutations in *MYO7A* have been documented to be associated with

hearing loss (http://www.umd.be/MYO7A/). In contrast, including p.R206C, only 9 mutations have been confirmed to be associated with dominant, nonsyndromic hearing loss DFNA11 [13–20]. Notably, 7 of 9 DFNA11 mutations are located at the motor domain of MYO7A [13–20]. Most of the DFNA11 patients exhibited postlingual progressive hearing loss similar to that of family J-1 (Fig. 3a).

A previous study reported the p.R206C variant in *MYO7A* as a suspected pathogenic mutation in a small family with 2 deaf siblings in Taiwan [12]. The heterozygous p.R206C variant was detected in both affected siblings, but the inheritance pattern of this variant is dubious because neither of the parents had any hearing impairment, the father is wild type, and the mother's DNA sample is not available. The hearing phenotype was not described in detail in that family.

In our study, we provided further evidence to support that the p.R206C variant is likely a pathogenic mutation for DFNA11: (1) the p.R206C variant segregated with the hearing loss in family J-1 in an autosomal dominant pattern; (2) the phenotype, characterized by nonsyndromic, late-onset, progressive hearing loss with down-sloping audiogram, is consistent with previous reports of other DFNA11 families; (3) the p.R206C variant was not seen in 200 ethnically matched normal-hearing controls; (4) the p.R206C variant is predicted to be pathogenic by computational tools (SIFT, PolyPhen-2 and Mutation Taster) and is classified as pathogenic following criteria by the American College of Medical Genetics. In combination with the high degree of evolutionary conservation of the Arg206 position in the motor domain, we concluded that p.R206C is likely a pathogenic mutation for DFNA11.

Interestingly, 5 of the 11 dominant mutations in *MYO7A* reported so far were identified in Chinese families. The p.R206C variant reported in 2 separate families in both Taiwan and mainland China could either be inherited from a distantly related ancestor or re-occur separately. Our study demonstrated that reports of such rare variants in multiple patients or families may facilitate exploitation of its pathogenicity.

Acknowledgments

We thank all patients who participated in this study.

Statement of Ethics

This research was in compliance with the Declaration of Helsinki and approved by the Ethics Committee of the Ninth People's Hospital, Shanghai Jiaotong University School of Medicine. Informed consent of the patients or guardians was obtained before collecting clinical information and peripheral blood samples of the family members.

Disclosure Statement

The authors declare no conflicts of interest.

Funding Sources

This research was supported by grants from the National Key R&D Program of China (2017YFC1001804 to H.W.), National Science Foundation of China (81570930 to T.Y.), Shanghai Municipal Science and Technology Commission (14DZ2260300 to H.W.), and Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant (20152519 to T.Y.).

Author Contributions

Conceptualization: H.W. and T.Y.; formal analysis: J.L. and P.C.; funding acquisition: H.W. and T.Y.; investigation: J.L. and P.C.; methodology: T.C. and L.L.; project administration: H.W. and T.Y.; supervision, H.W. and T.Y.; writing/original draft: P.C. and J.L.; and writing/review and editing: T.Y.

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