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Identification of novel *OTOF* compound heterozygous mutations by targeted next-generation sequencing in a Chinese patient with auditory neuropathy spectrum disorder

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ABSTRACT

Objectives: The molecular causes of auditory neuropathy spectrum disorder (ANSD) are not well known. Identification of the pathogenic mutations underlying nonsyndromic ANSD is difficult because of its extremely heterogeneous trait. The aim of the present study was to identify the genetic etiology of a single Chinese patient diagnosed with congenital ANSD by targeted next-generation sequencing.
Methods: Targeted next-generation sequencing of 79 known deafness genes was performed in a child that was clinically diagnosed with ANSD and received cochlear implantation. Candidate pathogenic variants were confirmed by Sanger sequencing. Post-implantation outcome were evaluated in a 40 months span.
Results: Novel compound heterozygous mutations p.R1583H/p.Q1883X in *OTOF* were identified as the pathogenic cause of the patient, correlated with a good post-implantation outcome in terms of sound detection and communication skills.
Conclusion: Targeted next-generation sequencing is effective for molecular diagnosis of ANSD and may provide important information for clinical management of this disease.

1. Introduction

Auditory neuropathy spectrum disorder (ANSD) is a hearing disorder characterized by normal outer cochlear hair cell function (preservation of otoacoustic emissions and cochlear microphonics) and abnormal neural conduction of the auditory pathway (absent or severely abnormal auditory brainstem potentials) [1]. Though initially deemed rare, recent studies indicated that the prevalence of ANSD may be as high as 8–15% of newly diagnosed cases of pediatric hearing loss [2]. For infants and young children, the deleterious effect of ANSD on language development and academic achievement can be significant. It represents a serious disability for normal communication and social integration.

ANSD can be either congenital or acquired. It was estimated that most of pediatric ANSD cases are attributable to genetic causes and the majority are non-syndromic with an autosomal recessive mode of transmission [3,4]. Due to lack of conventional auditory measures to evaluate the site and type of lesion in ANSD children [5], audiological management and speech and language intervention for infants and young children with this disorder is at times uncertain [6,7]. On the other side, identification of specific mutations of causative genes may have important implications for rehabilitation, which sees cochlear implantation (CI), to date, as the only tool for restoration of speech perception by bypassing the site of the lesion [8]. CI does not benefit all children with this disorder and genetic analysis may help determine who may benefit. For this reason, genetic and molecular diagnosis of ANSD is invaluable to ensure the appropriate therapeutic management [3,9–11].

The genetic basis of ANSD is highly heterogeneous. Mutations in many genes have been found associated with ANSD, including *OTOF*, *PJVK*, *DIAPH3*, *GJB2* and *MTRNR1* [12]. In addition, the mutation spectrums were broad and diverse. In Chinese, for example, it was found that a large proportion of non-syndromic ANSD was not caused by recurrent mutations or mutational hot spots [13,14]. To answer the challenge of efficient molecular diagnosis of ANSD in individual patients, new technologies such as targeted DNA capturing and next-generation sequencing (NGS) may make it possible to analyze most – if not all – deafness genes, as opposed to screening of each individual gene by conventional

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Sanger sequencing. In this study we report a case of ANSD patient who received CI at 20 months of age. By targeted NGS of 79 known deafness genes, we identified two novel compound heterozygous *OTOF* mutations as the pathogenic cause, which was correlated with his good post-CI outcome.

2. Subjects and methods

2.1. Family description

In this paper, we reported a small Chinese family associated with ANSD, named Family 1 (Fig. 1A). According to the criteria proposed by Guidelines Development Conference at Newborn Hearing Screening (NHS) 2008, Como, Italy [1], the affected member II-2 was diagnosed with ANSD. The other family members had normal hearing. No systemic diseases were present in any of the family members. High-resolution computed tomography (HRCT) and magnetic resonance imaging showed that II-2 had normal inner ear structure and cochleovestibular nerves. He underwent CI of the left ear with a Nucleus HiRes 90K device (Cochlear Corporation, AB, CA, USA) at the age of 20 months.

2.2. Clinical evaluation

Clinical and audiometric evaluations were performed on all family members in Xinhua Hospital, Shanghai. Medical and family history was obtained. Audiometric evaluations are consisted of otoscopy, pure-tone audiometry (PTA), tympanometry, acoustics stapedial reflex, auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE) and auditory steady-state response (ASSR) test. Adequate collaboration and coordination between specialists in different fields were involved in the management of the proband, who had completed a series of detailed assessments process before surgery, as previously described [15]. Post-CI Behavioral sound field results and speech and sentence recognition scores were reviewed.

2.3. Mutation analysis

Informed consent and blood samples were obtained from all subjects according to a protocol approved by the ethics committee of the Xinhua Hospital, Shanghai Jiao Tong University School of Medicine. For targeted NGS, genomic DNA were extracted from the blood samples using the Blood DNA kit (Tiangen Biotech, Beijing, China) and fragmented to 200–300 base pairs using an ultrasonoscope (Covaris S2, Massachusetts, USA). A customized capture array (NimbleGen, Roche) was designed to capture all exons, splicing sites and immediate flanking intron sequences of 79 deafness genes as previously described [16]. End-repair, adenylation and adapter ligation were performed for library preparation following standard Illumina protocols. Targeted DNA fragments were captured by hybridization to the capture array and sequenced on Illumina HiSeq2000 Analyzers for 90 cycles per read. Image analysis, error estimation and base calling were performed using the Illumina Pipeline (version 1.3.4). Reads were aligned to the National Center for Biotechnology Information (NCBI) 37/hg19 assembly using the BWA Multi-Vision software package. SNPs and indels were identified using the SOAPsnp software and the GATK IndelGenotyper, respectively. Previously identified SNPs and their allele frequencies were determined using the NCBI dbSNP, 1000 Genomes and the in-house sequencing data of 200 Chinese Han normal hearing controls as previously described [16].

Candidate pathogenic variants identified by targeted NGS were further confirmed by PCR amplification and Sanger sequencing.

3. Results

3.1. Clinical characteristics

There was one hearing impaired subject (no. II-2) in this family. His physical examination and otoscopy results were normal. No predisposing factors were revealed for his medical and family history. No subject in his family complained of vertigo or dizziness. II-2 failed the automated ABR testing during the neonatal hearing screening and was referred and diagnosed with profound sensorineural hearing loss (SNHL). Characteristic of ANSD, his ABRs showed that both ears were not elicited reproducible wave at 100 dBnHL while DPOAE were present bilaterally. Behaviorally, he had no response to stimulation in the sound field. Speech perception ability was severely impaired. No spontaneous improvement in hearing was seen when monitored over time by repeat ABR and/or behavioral audiological testing. In addition, the child had used a powerful hearing aid since the diagnosis, but 6-month trial of conventional amplification failed to show benefit.

At age 20 months, II-2 underwent left-side CI. Intraoperative electrode impedance and waveform testing was within normal limits. No complications related to surgery were observed. The child commenced daily training and daily sessions with the speech therapist after switching on one month later. His hearing was followed up until age five years. Sound field testing revealed hearing thresholds of 25 dB at 500, 1000, 2000, and 4000-Hz tones consistently. He shows excellent speech and language recognition score and is enrolled in regular school. The results of CI were considered successful in terms of sound detection and communication skills.

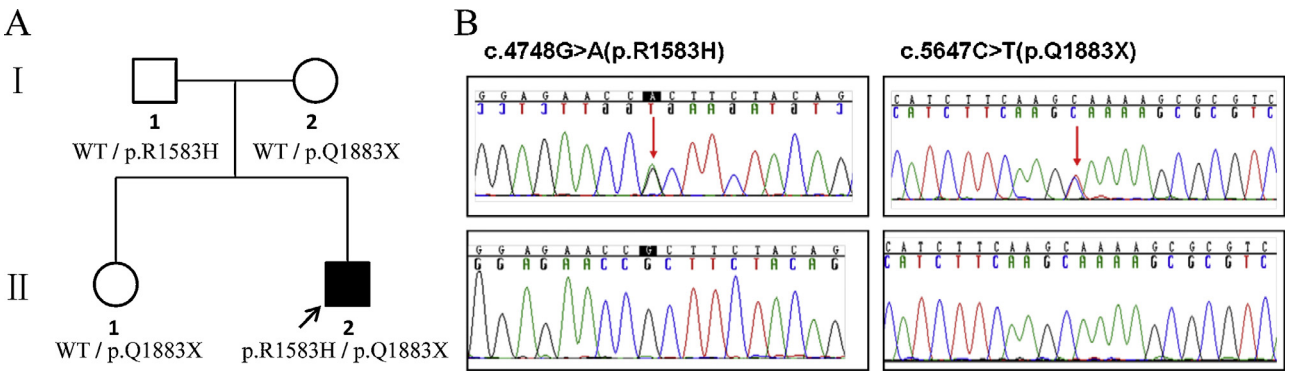

Fig. 1. Segregation of compound heterozygous mutations of *OTOF* in Family 1. The affected family member with ANSD is pointed by an arrow in the pedigree (A). Sanger sequencing results of the p.R1583H and p.Q1883X mutations were shown in (B).