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Case Report

Identification of two novel pathogenic compound heterozygous MYO7A mutations in Usher syndrome by whole exome sequencing

Ying Jia[a,1], Xiaoge Li[b,1], Dong Yang[c,*], Yi Xu[c], Ying Guo[c], Xin Li[d,e]

[a] Department of Neurosurgery, Tianjin Medical University General Hospital, Tianjin, China
[b] Department of Pediatrics, Tianjin Jinnan Small Station Hospital, Tianjin, China
[c] Department of Otorhinolaryngology, Tianjin Medical University General Hospital, Tianjin, China
[d] Department of Otorhinolaryngology, Beijing Tsinghua Changgung Hospital, Beijing, China
[e] School of Clinical Medicine, Tsinghua University, Beijing, China

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ABSTRACT

The current study aims to identify the pathogenic sites in a core pedigree of Usher syndrome (USH). A core pedigree of USH was analyzed by whole exome sequencing (WES). Mutations were verified by polymerase chain reaction (PCR) amplification and Sanger sequencing. Two pathogenic variations (c.849 + 2T > C and c.5994G > A) in MYO7A were successfully identified and individually separated from parents. One variant (c.849 + 2T > C) was nonsense mutation, causing the protein terminated in advance, and the other one (c.5994G > A) located near the boundary of exon could cause aberrant splicing. This study provides a meaningful exploration for identification of clinical core genetic pedigrees.

1. Introduction

Deafness, also known as hearing loss, refers that lesions occurred in the auditory system of acoustic sound, acoustic nerve in the auditory pathway and all levels of the central nervous, resulting in hearing dysfunction or varied degrees of hearing loss. According to the latest statistics from World Health Organization (WHO), approximately 5% of population is suffering from hearing loss in the world [1]. Over 60% patients are caused by genetic mutations, and others are mainly caused by different genetic and environmental factors. About 30% of genetic deafness is syndromic accompanied by other symptoms, and other 70% is non-syndromic [2]. To date, over 400 genetic syndromes include hearing loss have been reported [3]. As a genetic syndrome form of hearing loss, Usher syndrome (USH) is an autosomal recessive disorder characterized by sensorineural hearing loss, accompanied by visual loss due to retinitis pigmentosa (RP) and sometimes vestibular dysfunction. The prevalence of USH is estimated at approximately 3–6/100000 [4]. According to the severity of symptoms, USHs can be classified into three subtypes, including USH1, USH2 and USH3. USH1 is characterized by congenital profound hearing impairment, a pre-pubertal onset of RP and constant absence of vestibular function [5]. USH2 is moderate to profound congenital hearing loss and later onset of RP with normal vestibular function [6]. USH3 is characterized by a congenital or early onset of progressive hearing loss, while the onset and severity of vestibular function as well as the RP are highly variable [7].

Many inherited diseases such as USH have complex mutations or obviously genetic and clinical heterogeneity. Due to the limitation of traditional gene detection technology (such as sanger sequencing) in throughput, cost and time, it is hardly to meet the requirements for the development of molecular genetics. Next-generation sequencing (NGS), as a major technological innovation in life sciences, has the advantages of high throughput and cost-efficient [8]. It is a powerful tool of genetic screening and diagnosis to discover the disease-related mutations at genetic level. Compared with whole genome sequencing (WGS), whole exome sequencing (WES) has lower cost and higher efficiency in selectively capturing exons from the coding regions of genome. Currently, WES is widely used in clinical genetic diagnosis of Mendelian inheritance Disease caused by mutations in coding regions, especially hereditary rare disease. It contributes to identify the mutations by analyzing allele frequency in population, sequencing conversation and hazard prediction though all kinds of gene mutation database. However, it is difficult to collect multi-generation pedigrees with genetic disease in clinical practice.

In the current study, we performed WES to screen USH-related causative mutations in two siblings from a core pedigree of two generations. Two novel compound heterozygous disease-segregating

* Corresponding author. Department of Otorhinlaryngology, Tianjin Medical University General Hospital, No.154 An Shan Dao, HePing District, Tianjin, 300052, China.
E-mail address: yangdong_dr@163.com (D. Yang).
[1] Contributed equally to this work.

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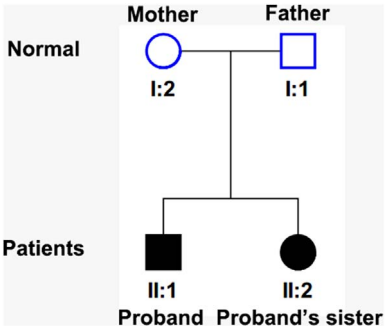

Fig. 1. A family pedigree of two generations I:1 the proband's father, I:2 the proband's mother, II:1 the proband, II:2 the proband's sister.

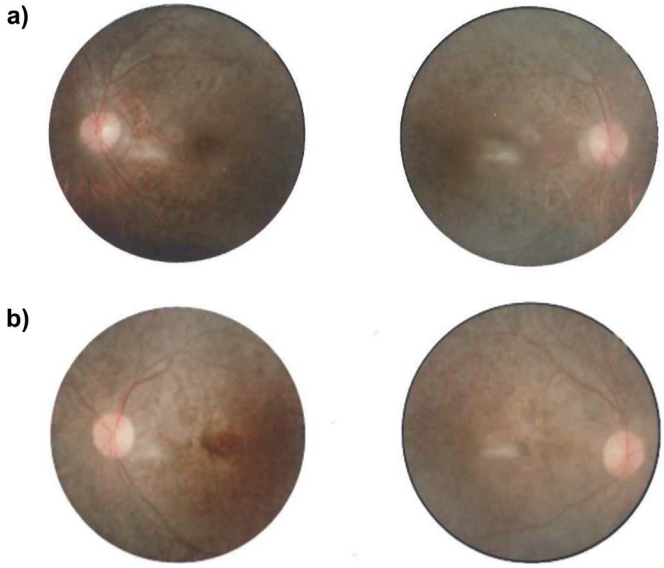

Fig. 2. Image of the fundus photographs of two affected individuals from the core family. a) The proband, b) The proband's sister.

Table 1
Coverage and depth statistics of four samples.

Sample name	Total bases of target region	Average depth	Coverage over 1X	Coverage over 10X	Coverage over 20X
II:1	6734793499	133.65	99.8	99.2	98
II:2	5966777557	118.41	99.9	99.2	97.6
I:1	7129530413	141.49	99.8	99.3	98.2
I:2	6618615369	131.35	99.9	99.4	98.1

mutations in MYO7A were successfully identified. The study provides a meaningful exploration for the identification of the pathogenic sites in core genetic pedigrees.

2. Materials and methods

2.1. Subjects

A Chinese family with two siblings was recruited in this study (Fig. 1). The proband is a 12 years old boy, and his sister is 16 years old. Patients were speechless and had congenital hearing loss, which never been treated, while their parents showed the normal hearing and vision. Series of listening test and eye examination were performed. Pure tone audiometry (PTA) examination revealed binaural sensorineural deafness. Auditory brainstem response (ABR) examination revealed no wave of dichotic test observed in the proband and average binaural hearing threshold of 95dB in the proband's sister. The proband had uncorrected visual acuity (UCVA, 0.2/0.08) and best-corrected visual acuity (BCVA, 0.2/0.15). The proband's sister had UCVA (0.15/0.1) and BCVA (0.15/0.1), respectively. Optic coherent topography (OCT) examination showed the normal macular area in the proband and the blurred fundus macular due to eye fibrillation in the proband's sister. Fundus oculi and intraocular pressure (IOP) revealed 14/17 mmHg in the proband and 16/17 mmHg in the proband's sister. The proband showed normal optic disc color, retinal blood vessels converge, a lot of peripheral retinal osteoblast like cells and foveal reaction. And the proband's sister had pink optic disc, retinal blood vessels converge, a lot of osteoblast like cells, foveal reaction (Fig. 2). Vestibular function was not assessed due to no related symptoms observed in the patients. Both the proband and his sister were diagnosed as binocular retinitis pigmentosa (RP) and total binaural deafness, and suspected USH. Written informed consent was obtained from patients' guardians, and the study protocol was approved by the Ethical Committee of Tianjin Medical University General Hospital.

Table 2
Ranking of candidate gene deleteriousness by pVAAST.

Rank	Gene name	Deleterious score	Linkage disequilibrium score
1	MYO7A	41.29	0.6
2	ATXN3	36.46	0
3	PCDH15	34.01	0
4	APOBEC3A	32.12	0.6
5	MLL2	31.202	0.6
6	RP1L1	28.88	0.6
7	RGL3	24.972	0.6
8	KRTAP10-4	22.72	0
9	ANKRD28	21.978	0
10	LOC646862	21.32	0
11	FAM166A	19.6	0
12	INPP5K	19.59	0
13	PIK3R6	19.328	0.6
14	LCN10	18.87	0
15	CCDC153	18.31	0
16	SLC25A5	18.31	0.6
17	IL1RN	18.2	0
18	TRMT2A	17.86	0
19	HOXD3	16.95	0
20	UTP11L	16.95	0

2.2. Whole exome sequencing (WES)

Genomic DNA was extracted from 400 μL of patients anticoagulant whole blood samples using a QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) and diluted to 50 ng/uL (Qubit, Invitrogen). Each of 1.5 μg genomic DNA was randomly sheared to 300–500 bp fragments by high adaptive focused-ultrasonicator (Covaris). DNA fragments were repaired by adding 3′ adenine (A), followed by adapter ligation. DNA library was prepared by PCR amplification and followed magnetic beads purification using the AMPure DNA Purification kit (Beckman Agencourt, Danvers, MA). In addition, the qualified DNA library was hybridized by the whole exon capture kit (Agilent Technologies, Santa Clara, CA, US). The enriched exon region DNA was eluted and amplified by PCR. Libraries from samples were finally developed. WES was performed on Illumina Hiseq 2500, mainly through hybridizing the fragment containing target genome DNA with probe containing adapter joint. After DNA enrichment by PCR extending and bridge amplification, “sequencing by synthesis” were performed by adding four kinds of end-blocked and fluorescent labeling bases.

2.3. Bioinformatics data analysis

FastQC software was used for quality control after sequencing and Burrows-Wheeler Aligner (BWA) software was used for alignment with