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Targeted next generation sequencing identified a novel mutation in *MYO7A* causing Usher syndrome type 1 in an Iranian consanguineous pedigree

Daniz Kooshavar[a,1], Masoumeh Razipour[a,1], Morteza Movasat[b], Mohammad Keramatipour[a,*]

[a] Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Usher syndrome (USH) is characterized by congenital hearing loss and retinitis pigmentosa (RP) with a later onset. It is an autosomal recessive trait with clinical and genetic heterogeneity which makes the molecular diagnosis much difficult. In this study, we introduce a pedigree with two affected members with USH type 1 and represent a cost and time effective approach for genetic diagnosis of USH as a genetically heterogeneous disorder.

Methods: Target region capture in the genes of interest, followed by next generation sequencing (NGS) was used to determine the causative mutations in one of the probands. Then segregation analysis in the pedigree was conducted using PCR-Sanger sequencing.

Results: Targeted NGS detected a novel homozygous nonsense variant c.4513G > T (p.Glu1505Ter) in *MYO7A*. The variant is segregating in the pedigree with an autosomal recessive pattern.

Conclusion: In this study, a novel stop gained variant c.4513G > T (p.Glu1505Ter) in *MYO7A* was found in an Iranian pedigree with two affected members with USH type 1. Bioinformatic as well as pedigree segregation analyses were in line with pathogenic nature of this variant. Targeted NGS panel was showed to be an efficient method for mutation detection in hereditary disorders with locus heterogeneity.

1. Introduction

Usher syndrome (USH) is a genetically and clinically heterogeneous disorder with an autosomal recessive inheritance pattern that is characterized by congenital, bilateral deafness and a later onset of vision impairment, caused by retinitis pigmentosa (RP) [1–3]. USH accounts for about 50% of all deafblindness cases [1,4] and its prevalence ranges from 1/6000 to 1/10,000 [2]. According to the severity of the sensorineural hearing loss (SNHL), the age of onset of RP, and the presence or absence of vestibular dysfunction, USH is divided into three clinical subtypes as USH type 1, USH type 2 and USH type 3. USH type 1 is the most severe form of USH and is defined by congenital bilateral profound sensorineural hearing loss, severe vestibular dysfunction, and prepubertal onset of RP. USH type 2 patients suffer from mild to severe congenital hearing impairment and RP with prepubertal onset. Their vestibular function is normal. USH type 3 is characterized by mild and progressive hearing impairment and variability in age of onset of RP and vestibular function [1,2].

To date, 14 genes have been identified to be associated with Usher syndrome. The USH type 1 genes include *MYO7A* encoding the motor protein myosin VIIa, *USH1C* encoding harmonin, *USH1G* encoding SANS, *CDH23* and *PCDH15* encoding cadherin 23 and protocadherin 15 respectively, and *CIB2* which encodes calcium and integrin binding protein 2. The USH type 2 genes are *USH2A* encoding usherin and *ADGRV1* (*GPR98*) encoding adhesion G protein-coupled receptor VI. Another gene associated with USH type 2 is *WHRN* (*DFNB31*) encoding whirlin. The USH type 3 gene is *CLRN1* encoding Clarin1 [2,5]. Furthermore, *HARS* encoding histidyl tRNA synthetase and *ABHD12* encoding Lysophosphatidylserine (LPS) lipase have been identified in atypical USH [6,7]. In addition, two modifier genes have been recently identified, *PDZD7* and *CEP250* encoding PDZ domain containing protein 7 and centrosome associated protein 250, respectively [8,9]. The USH proteins are organized in a mutual “interactome” in retinal photoreceptors and inner ear hair cells. Dysfunction or absence of any of these molecules can cause the clinical symptoms of USH [10,11].

Because there are several genes that can cause USH with a large number of coding exons, identification of causative mutations is costly and time-consuming by traditional methods like direct Sanger sequencing. NGS has provided a high-throughput and cost-effective approach for detection of mutations in genetic diseases with high locus

* Corresponding author. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Poursina Ave., Keshavarz Blvd., Tehran 1417613151, Iran.
E-mail address: keramatipour@sina.tums.ac.ir (M. Keramatipour).
[1] These authors contributed equally to this work.

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heterogeneity such as USH. In this study, a targeted NGS method was used for genetic investigation of an Iranian pedigree with two affected members suspected of USH type 1. Here we represent the detailed data about the identification of a novel variant in this pedigree.

2. Materials and methods

2.1. Subjects

A pedigree with two affected members was investigated. A 16 years old male with profound hearing loss, bilateral rod-cone degeneration, and clinical diagnosis of Usher syndrome type 1 who was a result of a consanguineous marriage was referred to Farabi Eye Hospital, Tehran, Iran for genetic counseling/analysis. His hearing loss was documented at about the age of 1 year. According to the parents' reports, he had a delay in walking and language development. He started to walk at the age of 2 years. This history supports vestibular dysfunction in the proband. His ophthalmologic problems were initiated with night blindness and amblyopia at the age of 3–4 years. Eye examination including electroretinography (ERG) at the age of 14 reported bilateral rod-cone degeneration.

The proband had a one-year-old cousin, also result of a consanguineous marriage, with profound hearing loss. Fig. 1 demonstrates the pedigree of the family. Written informed consent was taken from the parents of the patients and peripheral blood samples were collected from the patients and their family.

2.2. DNA extraction

Genomic DNA was extracted from whole peripheral blood using the GeneAll®Exgene™ kit (GeneAll Biotechnology Co., LTD, Seoul, Korea), according to the manufacturer's instructions.

2.3. Targeted NGS

Target region capturing of 13 Usher syndrome-related genes (*CDH23*, *DFNB31*, *GPR98*, *MYO7A*, *PCDH15*, *USH1C*, *USH1G*, *USH2A*, *CLRN1*, *HARS*, *PDZD7*, *CIB2*, and *ABHD12*) was performed with Nimblegen chip. NGS was performed on an Illumina HiSeq NGS System (Illumina Inc., San Diego, CA, USA) provided by Beijing Genomics Institute (BGI).

2.4. Validation of the detected variant

Polymerase Chain Reaction (PCR) followed by Sanger sequencing was used to validate the detected variant and analyze the segregation in the pedigree. The sequences of designed primers are as follows; Forward primer: TGGCACGTAGCGAGTTTGT and Reverse primer: CCCCACCACTGTTATGCGAG. PCR reaction was performed in 30 µl total volume containing 15 µl the *Taq* DNA Polymerase 2× Master Mix (Ampliqon A/S, Odense, Denmark), 10.5 µl DH2O, 1.5 µl of each 5 pM primer and 1.5 µl of 50 ng/µl DNA. The PCR condition was based on the touchdown PCR protocol for efficient amplification of fragments. Initial denaturation was performed at 95 °C for 5 min, followed by 37 cycles of 95 °C for 30 s, annealing step for 30 s, 72 °C for 40 s, and a final extension of 72 °C for 7 min. The annealing temperature was decreased 1 °C in every cycle from 72 to a touchdown temperature at 60 °C; this final annealing temperature was maintained for the remaining 25 cycles.

In addition, bioinformatic investigation was conducted by multiple *in-silico* predictive tools including Mutation Taster (http://www.mutationtaster.org) and CADD (Combined Annotation Dependent Depletion, http://cadd.gs.washington.edu/) to determine the pathogenicity of the novel variant which was not previously reported in any of the population/disease databases such as ExAC, 1000G, dbSNP, ClinVar, and HGMD®. Furthermore, the phylogenetic comparison was performed to show the conservation of the residue using the consurf server (http://consurf.tau.ac.il/).

3. Results

Targeted NGS of 13 Usher syndrome-related genes revealed a novel homozygous stop gained variant in the proband V:3.

The variant was a G-to-T transition at the first base of codon 1505 in exon 34 of the *MYO7A* that causes the substitution of Glu (GAG) by a stop codon (TAG). As demonstrated in Fig. 1, segregation of the variant was investigated in the pedigree. The variant was well-cosegregated with the disease in the pedigree. Both affected pedigree members were TT homozygotes, their parents and some other pedigree members were G/T heterozygote carriers, and some other pedigree members were normal GG homozygotes. Fig. 2 demonstrates sequence chromatograms of the variant in two affected members and their parents.

According to our survey, the variant c.4513G > T (p.Glu1505Ter) in *MYO7A*, has not been previously reported in any of the population/

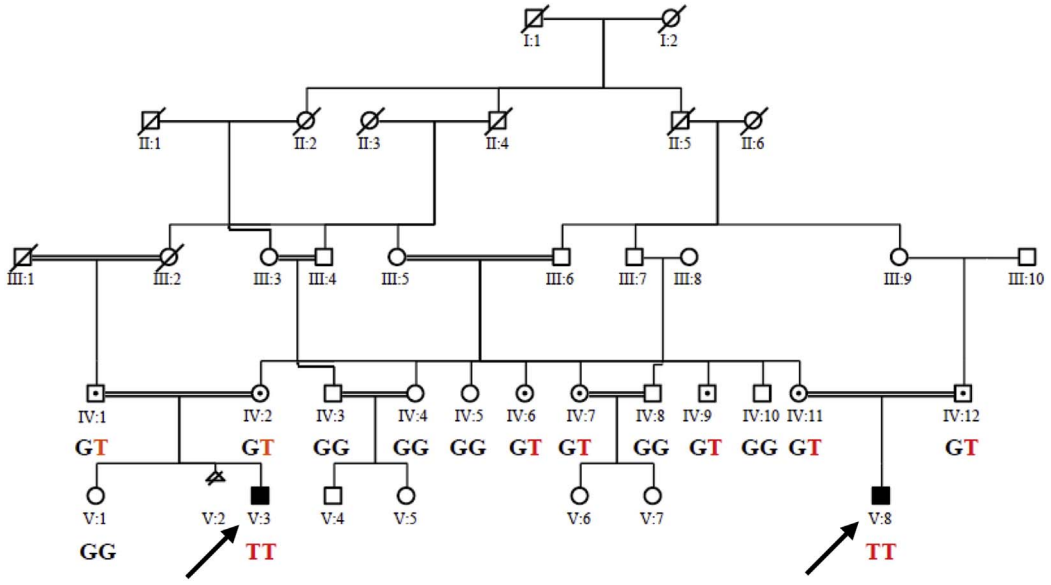

Fig. 1. The pedigree of the Usher syndrome family. Genotype of the variant c.4513G > T in studied members is shown in the pedigree. Arrows indicate the probands.