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Short communication

Whole-exome sequencing revealed two novel mutations in Usher syndrome

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ABSTRACT

Usher syndrome is a clinically and genetically heterogeneous autosomal recessive inherited disorder accompanied by hearing loss and retinitis pigmentosa (RP). Since the associated genes are various and quite large, we utilized whole-exome sequencing (WES) as a diagnostic tool to identify the molecular basis of Usher syndrome. DNA from a 12-year-old male diagnosed with Usher syndrome was analyzed by WES. Mutations detected were confirmed by Sanger sequencing. The pathogenicity of these mutations was determined by in silico analysis.
A maternally inherited deleterious frameshift mutation, c.14439_14454del in exon 66 and a paternally inherited non-sense c.10830G>A stop-gain SNV in exon 55 of *USH2A* were found as two novel compound heterozygous mutations. Both of these mutations disrupt the C terminal of USH2A protein.
As a result, WES revealed two novel compound heterozygous mutations in a Turkish USH2A patient. This approach gave us an opportunity to have an appropriate diagnosis and provide genetic counseling to the family within a reasonable time.

1. Introduction

Usher syndrome is an autosomal recessive inherited disorder, accompanied by bilateral sensorineural hearing loss and retinitis pigmentosa (RP) with/without vestibular dysfunction (Sadeghi et al., 2004). This is the most frequent cause of concomitant deafness and blindness with an estimated prevalence of 3–6 per 100,000 live births (Steele-Stallard et al., 2013). Usher syndrome is a clinically and genetically heterogeneous syndrome and depending on the severity of the hearing loss with normal–abnormal vestibular response, three types of Usher syndrome were described; Usher type 1 (USH1), Usher type 2 (USH2) and Usher type 3 (USH3) (Steele-Stallard et al., 2013). USH1 is the most severe form of Usher syndrome characterized by profound congenital deafness, lack of vestibular function and prepubertal onset of RP. USH2 is characterized with mild to severe sensorineural hearing loss with normal vestibular response and progressive retinitis pigmentosa (RP), whereas, patients with USH3 suffers from progressive post-lingual hearing loss, post-pubertal onset of RP, and variable vestibular dysfunction (Huang et al., 2013; Steele-Stallard et al., 2013). USH2 is the most common type of Usher syndrome, accounting for more than half of the Usher syndrome cases. USH2 is classified into four subtypes, 2A–2B–2C–2D, based on their molecular etiology (USH Type II, Genereviews).

In the current study, following the clinical diagnosis of our patient with Usher syndrome, we performed WES, which has been commonly used as a diagnostic tool to identify the molecular basis of genetically heterogeneous disorders (Timal et al., 2012; Sawyer et al., 2013; Besnard et al., 2014), to screen all Usher-related genes and to look for novel candidate genes. WES results revealed two novel compound heterozygous mutations: one deleterious frameshift mutation at c.14439_14454del in exon 66 of *USH2A* which is inherited maternally and a nonsense c.10830G>A stop-gain SNV in exon 55 of *USH2A* which is inherited paternally. Then, we confirmed the detected mutations with Sanger sequencing. Here, we report these two novel compound

Abbreviations: RP, retinitis pigmentosa; WES, whole-exome sequencing; USH1, Usher type 1; USH2, Usher type 2; USH3, Usher type 3; USH2A, Usher type 2A; VEP, visual evoked potential; ERG, electroretinogram; DNA, deoxyribonucleic acid; GATK, Genome Analysis Toolkit; SNSs, single nucleotide substitutions; IGV, Integrative Genomics Viewer; SIFT, sorting intolerant from tolerant; SNV, single nucleotide variation; ARRP, autosomal recessive retinitis pigmentosa.

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heterozygous mutations in *USH2A* and discuss the benefits of performing WES in genetically heterogeneous syndromes.

2. Clinical findings and results

The patient was a single child born to a 20-year-old gravida 1, para 1 mother and the father was 24-year-old at that time. Patient's mother and father were healthy and had non-consanguineous marriage. Pregnancy was uncomplicated, with spontaneous vaginal delivery at term. The baby was male and had normal physical measurements at birth. He achieved unaided sitting at 7 months, and unaided walking at 12 months. At the age of 5, he complained about blurred vision in both eyes. Ophthalmological examination revealed myopia. He was recognized to have hearing loss when he was 6-year-old. Audiogram was compatible with bilateral mild-moderate sensorineural hearing loss. He continued his routine examinations for his progressive vision and hearing impairment. At 11 year-old, fundus examination showed bilateral peripapillary atrophy with tilted optic disk and attenuated vessels, prominent choroidal vessels were seen around optic disk in both eyes. Ishihara test was 10/24 plate in both eyes. P100 latency in Pattern VEP was 108 msn in the right eye and 109 msn in the left eye. Flash VEP was normal and symmetrical in both eyes. The response of Pattern ERG was minimal. Gansfeld ERG results were low under both photopic and scotopic conditions. P1 amplitude was decreased in all rings in multifocal ERG. MP1 microperimetry showed bilateral concentrical scotomas with good fixation properties in both eyes. Mean sensitivity in macula 20-degree tests was 5.1 dB in the right eye and 5.7 dB in the left eye. These findings were consistent with "Retinitis pigmentosa-cone rod dystrophy" (Fig. 1A). Recurrent otorhinolaryngological examinations showed bilateral moderate to severe sensorineural hearing loss with intact vestibular response (Fig. 1B). He was referred to us for retinitis pigmentosa and hearing loss. The clinical picture was compatible with Usher syndrome type 2. Normal vestibular response and unaided walking achieved before 18 month-old excluded the diagnosis of USH1. Moreover, the appearance time of retinitis pigmentosa helped excluding USH3.

In order to identify the genetic etiology, WES was performed. Informed consents were obtained from the family. Exonic DNA library was prepared by using Illumina TruSeq Sample Preparation kit and Illumina TruSeq Exome Enrichment kit. Illumina TruSeq PE Cluster Kit v3-cBot-HS was used for paired-end cluster generation and TruSeq SBS Kit v3-HS reagent kit used for sequencing the post-capture libraries. Paired-end sequencing was done on an Illumina HiSeq 2000 system with read length of 101. The paired-end sequence reads were aligned and analyzed the human genome (hg19) using BWA, SAMtools, BED tools, The Genome Analysis Toolkit v1.6 (GATK) IndelRealigner, GATK UnifiedGenotyper, dbSNP (build 135) tools. The variations on suspected genes were visually inspected by using Integrative Genomics Viewer (IGV).

WES revealed two novel compound heterozygous mutations, one of which is an exonic frameshift deleterious mutation c.14439_14454del in exon 66 and the other one is an exonic stop-gain SNV c.10830G>A in exon 55. Sanger sequencing was used as described previously (Seven et al., 2013), which confirmed the WES findings in the patient, and revealed that parents were heterozygous carriers for these mutations (Fig. 1C, D). To our knowledge, these mutations have not been previously reported in the literature and they are not reported as variations in the databases like HGMD professional, "1000 genomes" and Exome Variant Server. Furthermore, these mutations are not found in IGBAM in-house exome database, which contains 728 samples.

c.10830G>A substitution results in an early stop codon instead of tryptophan residue. SIFT analysis (Kumar et al., 2009), whose prediction is made using the degree of conservation of amino acid residues in the sequence alignments derived from closely related sequences, predicted that mutant form of the transcript is presumably exposed to nonsense-mediated decay and cannot be expressed in protein level.

The second mutation, c.14439_14454del is a frameshift mutation, which results in a truncated 4812-amino acid-long protein lacking the C terminal region. Since there is no tool for evaluation of novel deletions

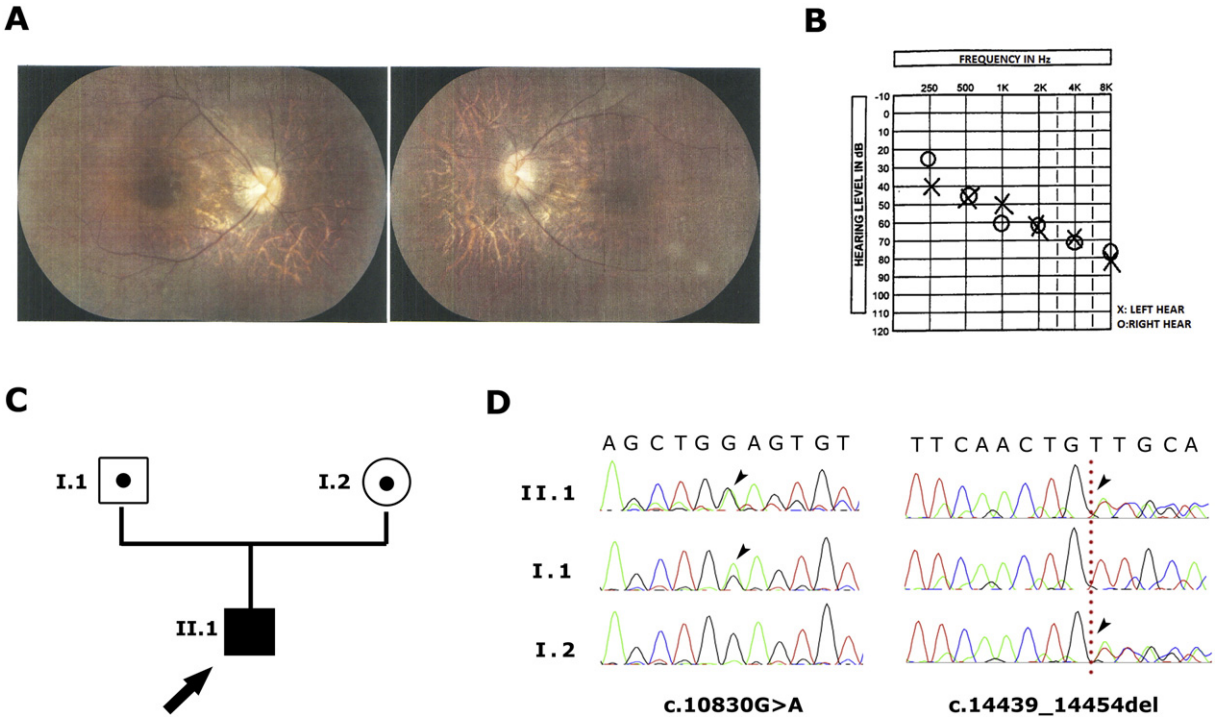

Fig. 1. (A) Fundus images of the patient demonstrating retinitis pigmentosa changes including bilateral peripapillary atrophy with tilted optic disk and attenuated vessels, prominent choroidal vessels around optic disk. (B) The audiogram of the patient was documented as moderate-severe sensorineural hearing loss. (C) Pedigree of the family and (D) chromatographs of sequence analysis of *USH2A* in proband (II.1) and parents (I.1, I.2).