Novel frameshift Variant c.3056delA of the DHX38 Gene in a Chinese Family With Retinitis Pigmentosa

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Case Report

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Abstract

Background

Retinitis pigmentosa (RP) is the most common inherited retinal degeneration. Our purpose was to describe disease-causing variants in a Chinese patient with RP. We described the clinical features and identify a novel (p.Lys1019fs) variant in *DHX38*.

Case presentation

A 47-year-old Chinese man complained of persistent visual impairment. To clarify the diagnosis, the clinical symptoms were observed and analysed in combination with comprehensive ophthalmologic examinations and genetic analysis. Abnormal fundus manifestations were found, including thinning of retinal arteriovenous vessels, obscure reflection in macular fovea, and scattered osteocyte-like pigment in the retina. We identified two mutations of *USH2A* gene (c.2802T > G and c.8559-2A > G ) and one novel mutation of *DHX38* gene (c.3056delA) in the proband. The parents with visual loss were heterozygous carriers. The compound heterozygous mutations in *USH2A* are the underlying cause of this case. And the novel variant results in the mutation of amino acid 1019 from lysine to arginine and bring a new reading frame, the 37th codon followed by the mutation site turn to be a stop codon, resulting in a premature protein truncation.

Conclusions

The study identified two compound heterozygous *USH2A* variants (c.2802T > G and c.8559-2A > G) and one novel *DHX38* variants (c.3056delA) in an RP patient. It is conducive to a clearer understanding of genotype-phenotype correlation in the non-syndromic RP patients. Our study expands the spectrum of *DHX38* variants in RP as well.

Background

Retinitis pigmentosa (RP) is the most common inherited retinal degeneration, with a global incidence of 4000 and affecting approximately 2.5 million people worldwide [1, 2]. Typical RP is characterized by night blindness and tunnel vision development due to the defect of photosensitive rod, while the loss of cone function results in the damage of central vision[3]. The typical clinical signs of RP include retinal arteriolar attenuation, generalized and diffuse pattern of mottled and moth-eaten retinal pigment epithelium (RPE). Autosomal dominant (adRP, 15%-25%), autosomal recessive(arRP, 5%-20%), x-linked(xlRP, 5%-15%) or unknown(40%-50%) are the common modes of inheritance of RP[3, 4]. To date, more than 100 genes have been shown to cause non-syndromic or syndromic RP (https://sph.uth.edu/Retnet/), and the rate of disease progression is highly variable between different types of RP. Non-syndromic RP and syndromic RP are closely related to the tissue or cell in which the gene is expressed. In general, non-syndromic RP is where the mutation mainly occurs in genes which is expressed in retinal cells or tissues[5, 6].
Mutations in *USH2A* (MIM:608400) gene account for almost 7–23% of arRP cases and are key causes of RP, leading to non-syndromic RP or Usher syndrome type IIa (USH2a). The highest frequencies of *USH2A* mutations are c.2802T > G (11.88%) and c.8559-2A > G (9.28%), which are autosomal recessive mutations and were supposed to be hot spot mutations in Chinese population[7]. *DHX38* was supposed as a potential candidate gene for RP, which encodes pre-mRNA processing factor 16 (PRP16) located on chromosome 16q22.2. PRP16 is also known as DEAH (Asp-Glu-Ala-Asp)-box helicase 38 and is suggested to be involved in catalyzing pre-mRNA splicing thus it is critical in regulating the fidelity of splicing[8]. Mutations of *DHX38* may disrupt the balance of splicing regulatory network and resulting in deficiency of pre-mRNA. A missense *DHX38* mutation c.971G > A, p. (Arg324Gln) was reported recently, which was confirmed playing role in RP phenotypes[9].

In this study, whole exon sequencing (WES) was applied to detect the RP-related mutations in patients with a clinical diagnosis of RP and family history of visual impairment. Two reported *USH2A* mutations and a novel heterozygous mutation of *DHX38* were observed in the proband and his family members. We find that the proband’s parents have different degrees of vision loss, with computational and functional predictions, we highly suspect that it may be caused by carrying a single mutation.

**Case presentation**

A 47-year-old Chinese man was admitted to Fengdu women and children hospital Chongqing, China because of persistent visual impairment. The proband, II:2, developed visual disorders at the age of 38 and his parents showed visual impairment after 65 years old. The proband was without vestibular abnormalities and had an initial bilateral cataract with poor night vision for 8 years. Then, peripheral venous blood samples were collected for Sanger sequencing. We performed a comprehensive ophthalmic examination, including best-corrected visual acuity (BCVA) determination, visual field examination, optical coherence tomography (OCT), visual evoked potentials (FVEP), and fluorescein fundus angiography (FFA) for clinical diagnosis. The clinical features of the proband are showed in Fig. 1. Figure 2A shows the pedigree chart, and the Sanger sequencs are illustrated in Fig. 2B.2C.2D.

**Disease-targeted gene panel and Sanger sequence**

Genomic DNA (gDNA) samples were extracted from the blood of the patient and his lineal consanguinity using a Qiagen genomic DNA isolation kit (Qiagen, Hilden, Germany). All DNA samples were quantified by NanoDrop (Thermo). Libraries were prepared as described previously[10]. Captured libraries were sequenced on an Illumina NovaSeq. Image analysis and base calling was performed using Illumina Real Time Analysis with default parameters. At least 10G raw data were obtained, aimed exome depth of 100X. Then 10–12 GB of data volume were obtained, assessed with stringent bioinformatic filters. At least 100× average target coverage, 99.5% of nucleotides had at least 20× coverage. The BWA program and GATK software were employed to align clean reads to the human reference genome and recalibrate quality scores. Sequence Alignment/Map tools (SAM tools) 3 was used to remove duplicated reads, and only unique mapping reads were used to detect mutations. SNVs and indels were detected with the GATK...
Unified Genotyper and GATK Indel GenotyperV2. RefSeq (hg19, from UCSS) and UCSS annotation were used to annotate the variant by an in-house bioinformatics tool. Several databases (such as dbSNP138, 1000 Genomes, and an in-house Asian database) were used to filter the SNPs/indels that appeared with > 5% frequency in these databases. SIFT, PolyPhen, and MutationTaster were employed to predict the probable pathogenic mutations[11]. Sanger sequencing on an ABI 3130 DNA Analyzer (Applied Biosystems, USA) was performed to verify the candidate gene mutation sites and the sequencing data were analyzed using chromas, version 2.26.

Two mutations of USH2A gene (c.2802T > G in exon 13 and c.8559-2A > G in intron 42, NM_206933.2) and one mutation of DHX38 gene (c.3056delA in exon 22, NM_014003.3) were observed in the proband by extensive bioinformatics analysis. Then Sanger sequencing of the targeted mutation sites was performed on his family members. His father carries splicing mutation c.8559-2A > G, his mother and son are heterozygous carriers of USH2A c.2802T > G and DHX38 c.3056delA while his sister and daughter are heterozygous carriers of USH2A c.8559-2A > G. However, only three mutations in DHX38 related to RP have been reported [9, 16, 17].

**Harmfulness prediction**

DHX38 c.3056delA, a frameshift variant that results in a nucleotide deletion in exon 22 to form a premature stop-codon downstream of the variant c.3056delA, has not been reported in literatures or databases. This new shift variant c.3056delA causes mutation of amino acid 1019 from lysine to arginine and a new reading frame, followed by mutation of codon 37 to a stop codon. It leads to producing a truncated protein or being degraded. The results of several databases or softwares shows the USH2A c.2802T > G tend to be pathogenic. Table 1 shows the information of the mutation.

**Protein structure modeling and analysis**

Pymol (Version 4.6.0) software was applied to simulate the structures of wild-type and mutated proteins. DHX38 (c.3056delA, p.Lys1019fs), a frame shift mutation, causes lysine 1019 mutated to arginine and produces a new truncated protein since the subsequent 37th codon turns to be a termination codon. The mutation may cause the protein to be degraded and may have a serious impact on the PRP16’s structure and function. The 3D structures of the protein model were predicted as shown in Fig. 3.

**Discussion and conclusions**

As a incurable eye disease, Retinitis pigmentosa has clinically-genetic heterogenity. In our study, the patient of 47 years old carries two mutations on USH2A and a novel frameshift mutation on DHX38. USH2A variants display a wide phenotypic spectrum, therefore, most prevalent USH2A variants phenotype-genotype correlation may contribute to genetic counselling. It also improves the prognosis of affected individuals, and provides guideline for patient-specific treatment[12]. USH2A (c.8559-2 A > G) variant site located in the structural domain of protein FN3 and causes an exon 43 jump, affecting protein function. USH2A (c.2802T > G, p.Cys934Trp) is located in the 8th EGF-Lam. It interferes with the proper folding of the secondary structure of Usherin, through changing two β sheets “E”with two coil “C” at
amino acids 935 and 940 respectively[13]. It is a ‘retinal disease-specific’ USH2A allele, such as c.2802T > G, that maintains normal hearing in patients with USH2A-related disease[14]. The genotype results of previous studies are consistent with our findings, further supporting that the patient has non-syndromic RP.

In our report, we discovered a novel frameshift variant c.3056delA in DHX38. This variant was not reported in gnomAD, and was classified as likely pathogenic, which suggesting that DHX38 is a candidate gene for severe early-onset RP. Here, we analyzed how an amino acid substitution leading to RP in the DEAH-box helicase of DHX38 affects DHX38 function. The frame-shift mutation resulted in the mutation of amino acid 1019 from lysine to arginine and bring a new reading frame. The 37th codon followed by the mutation site turn to be a stop codon. Thus this frame-shift mutation resulted in a premature protein truncation. Therefore, it seems that this heterozygous mutation, as a loss of function mutation, leads to elimination of DEAH box function. Mina Obuća proved that Meanwhile, DHX38 is well-connected with the splicing of RHO which could produce Rhodopsin[15]. Based on structure prediction results, the mutant protein is partially missing and its 3D structure is obviously different from wild-type protein. Therefore, we speculate that it will affect its function and the prediction of MutationTaster and PROVEAN confirmed our suspicions. However, given the proband is the only one that has visual abnormality in 3 observed DHX38 mutation carriers, the compound heterozygous mutations in USH2A are the underlying cause of this case.

Three mutations have been reported in PubMed, including c.2571C > T, c.995G > A, c.971G > A. The site of c.995G > A is considered “Likely pathogenic”. According to the reported mutations in DHX38, all mutations segregate with the arRP phenotype and only homozygous mutations can cause RP, so we can infer that if DHX38 (c.3056delA) mutation is homozygous which may be pathogenic. Besides, according to Tian Zhu, reporting that a Chinese patient with USH2A (c.9453T > A) and CDH23 (c.7145G > A) compound heterozygote have had symptoms of RP since the age of 17[18]. CDH23 is the classic gene of the USHER syndrome, and the compound mutations in the two genes severely impaired the protein function. And DHX38 is a candidate gene for RP, so we speculate that the vision loss of the impaired mother carries both USH2A (c.2802T > G) and DHX38 (c.3056delA) mutations. Meanwhile, the proband’s son carries the same pathogenic mutation as the mother, but his phenotype is normal, which may be due to the fact that the son has not reached the age of onset.

However, carrying a single pathogenic mutation is hard to cause retinitis pigmentosa independently, and there is no reliable experimental supportment at present. So we speculated that father’s vision loss may be due to aging. In addition, the remaining families with a single pathogenic mutation have not yet had vision problems, we can observe their future conditions of vision, especially the son of proband to verify our conjecture.

This case study shows a non-syndromic RP patient with three variants: USH2A (c.8559-2A > G), USH2A (c.2802T > G) and DHX38 (c.3056delA), which is conducive to a clearer understanding of genotype-phenotype correlation in the non-syndromic RP patients and increasing the number of pathogenic or pathogenic-like variations of DHX38. The present study has certain limitations. (i)There is a possibility of
prediction errors in the limited number of prediction software. (ii) The obtained clinical data is limited and there is no detailed examination of the patient’s family members. (iii) The limited duration of follow-up.

**Abbreviations**

RP
Retinitis pigmentosa
USH2A
Usher syndrome 2A
DHX38
DEAH-BOX HELICASE 38
PRE
Retinal Pigment Epithelium
PRP16
Pre-mRNA Processing Factor 16
USH2a
Usher Syndrome Type IIa
gDNA
Genomic DNA
WES
Whole Exon Sequencing
BCVA
Best corrected visual acuity
OCT
Optical coherence tomography
FVEP
Visual evoked potentials
FFA
Fluorescein fundus angiography
FN3
Fibronectin type-III
EGF-Lam
Laminin-type EGF-like modules
CDH23
Cadherin Related 23
FSCN2
Fascin Actin-BUNDLING PROTEIN 2
gnomAD
Genome Aggregation Database
HGMD
Human Gene Mutation Database

Declarations

Availability of data and materials

The data reported in the paper have been deposited in the ClinVar, under accession number SUB12330076 at https://submit.ncbi.nlm.nih.gov/subs/clinvar_wizard/SUB12330076/overview.

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Contributions

XJ and JL conceived the idea and take responsibility for the integrity of the data. SZ and YX collected the samples and wrote the manuscript. HZ, XL, XH and MQ acquired, analyzed, and interpreted the data. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

The present study was performed according to the guidelines approved by the ethics committee of Fengdu women and children hospital. Written informed consent for genetic testing and medical photography was obtained from all subjects prior to participation.

Consent for publication

The patient’s parents provided the written consent for the case report to be published. Informed consent from parents regarding data and clinical details for publication has been obtained.

Competing interests

The authors declare that they have no competing interests.

References


Tables
Table 1
Summary of the novel frameshift mutation in *DHX38*

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<thead>
<tr>
<th>Nucleotide change</th>
<th>c.3056delA</th>
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<tr>
<td>Amino acid change</td>
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</tr>
<tr>
<td>HGMD®</td>
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<td>PROVEAN</td>
<td>Deleterious</td>
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Figures
Clinical features of the proband

OCT demonstrated retinal tissue thickness thinned and the ellipsoidal zone reflections were extensively lost (a). FFA demonstrated attenuated retinal vessels and intraretinal pigment on periphery (b). Visual field tests demonstrated centripetal narrowing of both eyes with significant nasal side defects (c). GDX demonstrated bilateral micro-ocular inferior visual field defect with central dark spot.
Figure 2

A: Pedigree of the patient's family. The proband is a male with retinitis pigmentosa and his parents are carriers of pathogenic mutations.

B-D: DNA Sanger sequencing of USH2A and DHX38 genes. B. Sequences of the heterozygous splicing variant c.8559-2A>G and the correct sequence. C. Sequences of the heterozygous missense variant c.2802T>G and the correct sequence. D. Sequences of the heterozygous frameshift variant c.3056delA (p.(Lys1019fs)) and the correct sequence.
Figure 3

Prediction of protein molecular models. Structures of DHX38 (A) wild type protein and (B) mutant protein (p.Lys1019fs) was predicted by Pymol software, the DHX38 mutation protein loss of the partial P-loop domain and has a different structure compared to the wild-type.