CASE REPORT

A novel USH2A gene mutation in a family with retinitis pigmentosa: A case report

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Abstract

We report a novel variant of the USH2A gene in a family affected by retinitis pigmentosa (RP). Genomic DNA was obtained from a 55-year-old woman (the proband) with night blindness who was diagnosed with non-syndromic RP. We identified the compound heterozygous variants c.8559-2A>G and c.151A>T (p.Ile51Phe) in the USH2A gene as the underlying cause in the proband; the former variant, instead of the latter variant, has been reported in the literature. The proband’s mother carried the c.151A>T variant, while her father and daughter carried the c.8559-2A>G variant. In this family, the proband and her mother developed RP; however, her father and daughter did not develop the disease. Although in silico tools predicted that c.151A>T is benign, segregation analysis suggested that this variant could be potentially harmful. The identification of c.151A>T (p.Ile51Phe) variant is a novel finding, and this variant might be a potentially harmful variant of USH2A gene. This finding also further expands the mutation spectrum of this gene in the Chinese population.

Keywords: Gene mutation; Retinitis pigmentosa; USH2A

1. Background

Retinitis pigmentosa (RP), a progressive hereditary dystrophic, and degenerative eye disease, occur in approximately 1 in every 3000 – 7000 individuals[1]. The earliest symptom of this disease is a night blindness, followed by progressive defect of the visual field, eventually decline in the central vision. This disease can be further subdivided into syndromic RP and non-syndromic RP, depending on whether there is defect in the hearing or not.

Usher syndrome, also known as hereditary deafness-RP syndrome, is the most common type of syndromic RP, which is characterized by reduced visual field and visual impairment caused by congenital sensorineural hearing loss and progression of RP[2]. Usher syndrome can be further subdivided into three types according to the symptom severity: (i) type I that manifests as severe congenital hearing loss, vestibulo-ocular reflex loss, and RP onset during adolescence; (ii) type II that manifests as mild to moderate congenital hearing loss and RP, without vestibular dysfunction; and (iii) type III (rare type) that manifests as progressive hearing loss and RP[3]. Among them, the type II Usher syndrome is the most common type and is typically caused by mutations in the Usherin
(USH2A) gene\textsuperscript{41}. Therefore, this study was conducted to screen and analyze the USH2A gene mutations in a Chinese family with RP.

2. Case presentation
2.1. Clinical findings
The family in this study has family members who had developed RP. Including the proband, all family members are of Han Chinese ethnic group, and the age of the proband and her father, mother, and daughter were 55, 89, 84, and 29 years, respectively. The proband, who had an approximately 37-year history of night blindness, was diagnosed with non-syndromic RP, whereas her mother was diagnosed with type II Usher syndrome. In contrast, her father and daughter had no clinical manifestations of RP and presented with a normal phenotype (Figure 1). The proband had a history of night blindness of 18 years with no symptoms of hearing loss. She had a visual acuity level of 0.1 and 0.2 in the right and left eyes, respectively. Fundus photography revealed a yellowish waxy optic disk, thin retinal blood vessels, and proliferation of a large amount of osteocyte-like pigments in the central and peripheral retina. In addition, atrophic changes and proliferative membranes were observed in the macula, as shown in Figure 2A. Optical coherence tomography (OCT) showed the absence of a normal fovea in the macula of both eyes, with the presence of cystoid edema between the layers and hyper-reflective continuum of the epiretinal membrane. Further, both the outer nuclear layer and the nerve fibers around the optic disk were thinned (Figure 2D and F). In contrast to the proband, her mother showed sensorineural high- and low-frequency hearing loss in both ears, as shown in Figure 2H according to the pure-tone audiometry examination. However, no obvious abnormality was noted in the vestibular function examination. Thus, the proband’s mother was clinically diagnosed as type II Usher syndrome. In contrast, no abnormality was noted in the ophthalmic, vestibular function, and pure-tone audiometry examination in the proband’s father and daughter.

2.2. Identification of the USH2A gene mutations
Whole-exome sequencing of the genomic DNA of the proband led to the identification of two mutations in the USH2A gene, namely, the heterozygous variants c.8559-2A>G and c.151A>T (p.Ile51Phe) (NM_206933), which were compared with the UCSC hg19 human genome reference sequence to identify the genetic variations. On average, the mean coverage of the target regions was 182.13X, and for each sample, more than 99.77% of target regions were covered. Sanger sequencing for the identified regions was performed, and the presence of these two variations in the proband family members was, further, determined and verified. The verification results were consistent with the Illumina sequencing results, as shown in Figure 3. The c.8559-2A>G genetic mutation has been reported previously in multiple studies. Interestingly, c.151A>T (p.Ile51Phe) is a novel genetic mutation which was identified for the first time. The c.151A>T (p.Ile51Phe) variant located at chr1:216595528 with a nucleotide switch, that is, A to T at position 151 of the second exon of the USH2A gene, results in an amino acid change from isoleucine to phenylalanin at position 51 of the corresponding peptide.

2.3. Pathogenicity analysis of the gene mutations
The c.8559-2A>G variant, known as splice-site mutation, is located at chr1:216051224. It has an allele frequency of
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Figure 2. Clinical data of the proband (left) and her mother (right). Fundus photography (A and B), optical coherence tomography (OCT) of the macula (C and D), thickness of the nerve fibers around the optic disk (E and F), and pure-tone audiometry (G and H). The pictures (C₁, D₁, E₁, F₁) represent the right eyes, and pictures (C₂, D₂, E₂, F₂) the left eyes. Fundus photography showed that the optic disk was yellowish and waxy, the retinal blood vessels were thin, and a large number of osteocyte-like pigments proliferated in the central and peripheral retina. OCT of the macula revealed the absence of a normal fovea in the macula of both eyes, with cystoid edema between the layers. Hyper-reflective continuum of the epiretinal membrane was visible. Both the outer nuclear layer and the nerve fibers around the optic disk were thinned. Pure-tone audiometry indicated that the hearing of both ears of the proband was within the normal range. In contrast, her mother had hearing loss at both the high- and low-frequencies in both the ears.
0.0005 in gnomAD_exome (EAS), 0.0003 in ExAC (EAS), and 0.001 in 1000 genomes. The MutationTaster and Combined Annotation Dependent Depletion (CADD) tools predicted that the mutation was harmful. In addition, the GERP score (+++) indicates that the affected amino acids were highly conservative.

In addition, the c.151A>T (p.Ile51Phe) variant was not found in the gnomAD_exome (EAS), ExAC (EAS), and 1000 genomes databases. Sorting Intolerant From Tolerant (SIFT), PolyPhen2, CADD, and Rare Exome Variant Ensemble Learner (REVEL) tools predicted that the novel missense mutation located at chr1-216595528 is benign, while the MutationTaster tool predicted that the variant is a polymorphism, and the GERP score (+++) showed that the affected amino acids were non-conservative.

2.4. Segregation analysis

The proband carried both the heterozygous variants, c.8559-2A>G, and c.151A>T (p.Ile51Phe). Although her father and daughter carried c.8559-2A>G variant, neither of them developed RP. In contrast, her mother who carried the novel c.151A>T (p.Ile51Phe) variant suffered from RP. The result of segregation analysis suggested that USH2A c.151A>T was consistent with the characteristics of cosegregation (Figure 1).

3. Discussion

In the family of this study, the proband was a 55-year-old woman, who has a history of night blindness with the first symptom started to appear at the age of 18 years. She had no obvious hearing impairment; therefore, she was clinically diagnosed with non-syndromic RP. Meanwhile, her mother has both RP and hearing loss; therefore, she was clinically diagnosed with type II Usher syndrome. The whole-exome sequencing of the genomic DNA collected from the proband led to the identification of two variants in the USH2A gene, with c.151A>T (p.Ile51Phe) being a novel variant of unknown clinical significance, while the c.8559-2A>G variant had been reported in the previous studies[5,6].

RP can be inherited in autosomal dominant, autosomal recessive, X-linked, or mitochondrial manner[7]. To date, more than 150 pathogenic gene mutations leading to RP have been discovered. Among these mutations, c.8559-2A>G is the most common variant in the Chinese population[5,6]. It is a splice-site mutation located in the 42nd intron of the USH2A gene[8]. Researchers found that through its inactivation of splice donor and splice acceptor sites, the c.8559-2A>G variant led to the skipping of exon 43, and may result in the deletion of 41 amino acids, thereby producing pathogenic effect[9]. In the family of this study, the father and daughter of the proband who carried the heterozygous variant c.8559-2A>G did not show clinical manifestations of RP. The result is consistent with the previous studies, where c.8559-2A>G variant followed an autosomal recessive inheritance pattern, and a heterozygous c.8559-2A>G genotype is not necessarily pathogenic[8].

In addition, the c.151A>T (p.Ile51Phe) variant located at chr1-216595528 was a novel missense mutation of the USH2A gene family. For this variant, the A nucleotide is replaced by T nucleotide at position 151 of the second exon...
of the USH2A gene, leading to the replacement of isoleucine to phenylalanine at position 51 of the corresponding peptide. SIFT, PolyPhen2, CADD, and REVEL tools predicted that this mutation is benign. In addition, MutationTaster predicted that c.151A>T (p.Ile51Phe) is a polymorphism, whereas GERP score (+++) showed that the amino acids were non-conservative. However, it was noted through the segregation analysis that the proband and her mother, who displayed the clinical manifestations of typical RP, carried this novel variant. In contrast, her father and daughter did not carry this mutation, and neither of them developed RP. Therefore, it was concluded that this novel variant is likely to follow an autosomal dominant inheritance pattern. However, there is a limitation in our study, where only Sanger sequencing was used to identify the variants in the proband’s mother; therefore, we could not determine whether the mother also carries some other mutations in the USH2A gene family.

4. Conclusions
In this report, we describe two heterozygous variants that may potentially affect the development of RP in a Chinese family. The c.151A>T (p.Ile51Phe) variant was identified as a novel mutation of the USH2A gene, which could be potentially harmful. The specific mechanism of this variant in the development of RP needs to be further elucidated by future research. The finding of this study further expands the mutation spectrum of the USH2A gene in Chinese population.

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Conflicts of interest
All authors have no conflicts of interest to declare.

Authors’ contributions

Conceptualization: Yalong Dang
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Ethics approval and consent to participate
The study was approved by the Ethics Committee of Sanmenxia Central Hospital. All subjects signed the informed consent forms.

Consent for publication
The subjects had given written consent for the case report to be published.

Availability of data
Data will be available on contacting the corresponding author.

References
   https://doi.org/10.1097/MAO.0000000000002054
   https://doi.org/10.1016/0021-9681(83)90147-9
   https://doi.org/10.1016/j.bbadis.2014.11.020
   https://doi.org/10.1159/000016167
   https://doi.org/10.1167/iovs.17-23312
   https://doi.org/10.1038/jhg.2011.45
   https://doi.org/10.1101/cshperspect.a017111
   https://doi.org/10.1038/jhg.2014.65
   https://doi.org/10.1038/jhg.2010.83