

Compound pathogenic mutation in the USH2A gene in Chinese RP families detected by whole-exome sequencing

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Abstract. Retinitis pigmentosa (RP) is a common form of inherited retinal degeneration that causes progressive loss of vision or adult blindness, characterized by the impairment of rod and cone photoreceptors. At present, mutations in >60 pathogenic genes have been confirmed to cause RP. The predominant modes of inheritance are autosomal dominant, autosomal recessive and X-linked. In addition, other modes of inheritance, including digenic or mitochondrial inheritance, have been reported. In previous decades, with the development of sequencing techniques, significant advances in identifying novel RP pathogenic genes and screening mutations have been made. In the present study, whole-exome sequencing was performed on samples from two Chinese pedigrees diagnosed with RP. A compound heterozygous mutation in the gene usherin 2A (USH2A; c.6,485+5G>A/c.11,156G>A) and a heterozygous X-linked mutation in the gene retinitis pigmentosa 2 (RP2) ARL3 GTPase-activating protein (RP2; c.358C>T) were identified by Sanger sequencing and co-segregation analysis, of which the pathogenic mutation (c.6,485+5G>A) in USH2A has not been previously reported among Chinese patients. The findings of the present study may expand on current knowledge of RP among the Chinese population, providing essential assistance in the molecular diagnosis and screening of RP, and promoting further investigation of the pathogenesis of RP.

Introduction

Retinitis pigmentosa (RP) is an inherited retinal dystrophy that causes adult blindness, and is characterized by the degeneration of rod and cone photoreceptors. Typical symptoms include night blindness at an early age and progressive constriction of the mid-peripheral visual field. Patients with RP eventually become completely blind. There are three major modes of inheritance: Autosomal dominant (ADRP), autosomal recessive (ARRP) and X-linked (XLRP) (1). In addition, other modes of inheritance, including digenic or mitochondrial inheritance, have been reported (2,3).

RP can also occur as a syndrome accompanying other diseases. Usher syndrome is the most frequent form of syndromic RP, characterized by RP accompanied by sensorineural hearing loss. Usher syndrome can be divided into three types based on the assessment of clinical features.

Given the complexity and perniciousness of RP, molecular diagnosis is a potentially effective method of analysis that can be used to diagnose RP and assess disease prognosis. Although the traditional method, Sanger sequencing, can be a valid strategy used to detect genetic abnormalities, analyzing a large number of candidate genes with this method is expensive (4). With the advance of whole-exome sequencing technology, determining full-coding regions has become faster (5). Whole-exome sequencing is particularly useful in the detection of novel disease-causing genes (6).

In the present study, one Chinese pedigree with ARRP and one Chinese pedigree with XLRP were investigated. Whole-exome sequencing was performed on the affected patients, identifying a compound heterozygous mutation in usherin (USH2A) and a heterozygous X-linked mutation in ARL3 GTPase-activating protein (RP2). The mutations co-segregated with the diseases in the pedigrees and, to the best of our knowledge, the pathogenic mutation (c.6,485+5G>A) in USH2A has not been reported previously among Chinese patients.

Materials and methods

Subjects and clinical assessment. The present study involved seven individuals from two Chinese families with typical clinical features of RP. The two pedigrees were labeled Family

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D and Family H and the pedigrees of the two families are shown in Fig. 1A and B. The present study was performed in accordance with the tenets of the Declaration of Helsinki and the ethical guidelines of the Shanghai Renji Hospital, Shanghai Jiaotong University School of Medicine (Shanghai, China). Written informed consent was obtained from all participants. The clinical examinations performed to diagnose RP predominantly included assessing the best-corrected visual acuity (BCVA) using a Snellen chart, capturing images of each fundus, assessing computerized test of central and peripheral visual fields, and optical coherence tomography (OCT).

DNA extraction. Peripheral blood samples were obtained from all subjects, and used for DNA extraction using a kit (Qiagen, Shanghai) according to the manufacturer's protocols. DNA yield was quantified with NanoDrop One (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) and 1.5 μ g of DNA was used for the following assay. The DNA samples were stored at -80°C until use.

Exome capture and sequencing. Whole-exome sequencing was performed by Beijing Youle Fusheng Technology Co., Ltd. (Beijing, China). Sample libraries of gDNA were prepared using the TruSeq DNA Sample Preparation kit (Illumina, Inc., San Diego, CA, USA) and the exomic regions were enriched using the Roche Nimblegen SeqCap EZ v5.1 kit (Roche Applied Science, Madison, WI, USA) according to manufacturers' protocols. Paired-end short read sequencing was performed on an Illumina platform with a PE150 read length (Illumina, Inc.).

Identification of pathogenic mutations from exome data. Bioinformatics analyses were performed using an in-house pipeline. Briefly, sequencing reads that passed quality filtering were aligned with the human reference genome, hg19, using the Burrows-Wheeler Aligner program (BWA version 0.7.15) (7). Variants were identified using a combination of the FreeBayes (version 0.9.21) (8), Genome Analysis Toolkit (GATK version 3.8) (9-11), CNVnator (version 0.2.7) (12) and in-house software programs.

The in-house software program, Langya, was used for variant annotation and filtering. The variants were prioritized based on variant frequency, pathogenicity, inheritance pattern and how closely a gene associated with the given phenotype. The variant frequency was obtained from the 1,000 Genomes Project, Single Nucleotide Polymorphism database (<http://www.1000genomes.org>) and Exome Aggregation Consortium databases (<http://exac.broadinstitute.org>). Predicted pathogenicity data were obtained using the SNPEff (<http://snpeff.sourceforge.net>), Ensembl Variant Effect Predictor (<http://asia.ensembl.org/info/docs/tools/vep/index.html>), Sorting Intolerant From Tolerant, Polymorphism Phenotyping version 2 (<http://genetics.bwh.harvard.edu/pph2/>) and dbSNP databases (<https://sites.google.com/site/jpopgen/dbNSFP>). Gene-phenotype association was characterized from structured resources including the online Mendelian Inheritance in Man database (<https://www.omim.org>), and unstructured resources, including literature, utilizing natural language processing techniques. The average sequencing depth in Family D was $>100\times$, whereas it was

$\sim 85\times$ in Family H. The average coverage of the two families was $\sim 99.8\%$.

Sanger sequencing. The candidate variants in USH2A and RP2 identified in the exome data were validated by Sanger sequencing.

Results

Clinical assessment of families with RP. The clinical features of the affected patients in the two pedigrees are listed in Table I. The male patient D-II-2 suffered from decreased visual acuity and night blindness at the age of 14 years. The best-corrected visual acuity (BCVA) of D-II-2 decreased to light perception as the patient aged. Although the central vision of the affected female individual, D-II-3, was 20/20, the visual field of the eyes of D-II-3 exhibited severe mid-peripheral scotomata due to a coalescence of scotomata. All of the clinical features fit with the diagnostic criteria of RP. The results of fundus examinations revealed that the patients exhibited a typical RP appearance: Pale optic discs, attenuated retinal arterioles and 'bone spicule' pigmentation. The OCT indicated that marked thinning and disruption of the retinal nerve fiber layer, and the retinitis pigment epithelium was evident (Fig. 2A and B). The hearing examinations performed on the patients were normal.

In Family H, a 48-year-old female patient suffered from visual difficulties at night at the age of 20 years; visual field loss and decreased visual acuity progressed markedly from the age of 30 years. In the present study, the BCVA of the patient was finger count (FC)/5 cm. The visual field of the patient's eyes demonstrated mid-peripheral scotomata due to a coalescence of scotomata. The deceased father of the patient had been diagnosed with RP. The patient's son (H-III-1) was diagnosed with RP at the age of 7 years due to night blindness. The visual acuity of H-III-1 decreased significantly following diagnosis. The detailed ophthalmic examination results are shown in the Fig. 3A and B. The fundus examinations indicated that H-III-1 exhibited a typical RP appearance, including 'bone spicule' pigmentary deposits, retinal vessel attenuation and waxy optic discs. The OCT revealed that the retinal thickness was decreased and that the foveal and retinal layers were atrophied. The unaffected individuals exhibited normal phenotypes in all of the ophthalmological examinations.

Pathogenic mutation detection. Whole-exome sequencing was performed on samples from two Chinese families with RP. In Family H, a heterozygous mutation (c.358C>T) in RP2 was identified as a pathogenic mutation, co-segregating with the disease. The c.358C>T mutation in RP2 was demonstrated to be involved in pathogenic mutation in XLRP. The two patients in Family H possessed the heterozygous variants, whereas the unaffected individual did not. The two patients in Family D possessed the same compound heterozygous mutation (c.6,485+5G>A/c.11,156G>A) in the USH2A gene, which co-segregated with the disease (Table II). The c.6,485+5G>A mutation is a pathogenic mutation in ARRP, which has not been reported previously in Chinese patients. The results of Sanger sequencing of the validated pathogenic mutations is shown in Fig. 4A and B. Based on the clinical assessments and mutation analyses, the present study concluded that the three

Table I. Clinical phenotype of the patients in Families D and H.

Family	ID	Age (years)	Gender	Age at onset (years)	BCVA	Slit lamp	Refraction
D	D-II-2	49	Male	14	R: LP/LP L: LP/LP	Mild cataract	R: -2.50DS/-1.75DC*165 L: -2.00DS/-2.00DC*10
	D-II-3	47	Female	20	R: 20/20 L: 20/20	Mild cataract	R: -7.00DS/-1.00DC*160 L: -7.25DS/-0.75DC*5
H	H-II-2	53	Female	20	R: FC/5 cm L: 12/200	Mild cataract	R: -4.50DS/-0.75DC*175 L: +0.50DC*95
	H-III-1	28	Male	7	R: 12/200 L: 12/200	Normal	R: -1.25DS L: +1.25DS

BCVA, best corrected visual acuity; R, right eye; L, left eye; LP, light perception; FC, finger count; DS, diopter of scope; DC, diopter of cylinder.

Table II. Pathogenic mutations of patients in Families D and H diagnosed with retinitis pigmentosa.

Family	Gene	Chromosome position	cDNA mutation	Type of mutation	Hom/Het	Amino acid change	Inheritance	Origin
D	USH2A	1:215933077	c.6485+5G>A		Het		Autosomal recessive	Father
	USH2A	1:215933077	c.11156G>A	Missense variant	Het	Arg3719Leu	Autosomal recessive	Mother
H	RP2	X:46713166	c.358C>T	Stop-gain	Het	Arg120Ter	X-linked	Mother

USH2A, usherin; P2, ARL3 GTPase-activating protein; Hom, homozygous; Het, heterozygote.

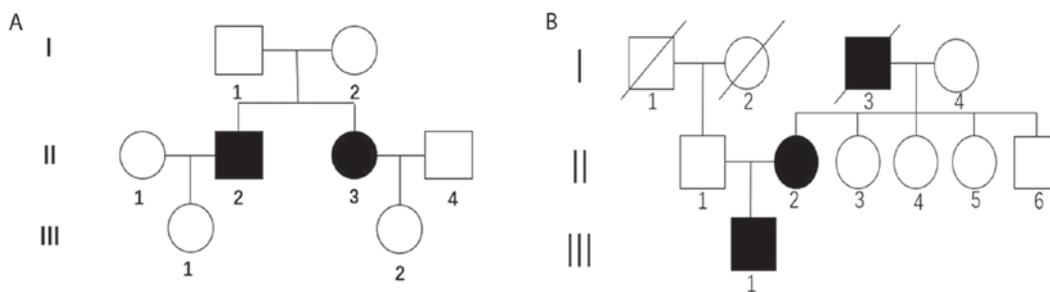


Figure 1. Pedigree of two families with retinitis pigmentosa. Pedigree of (A) Family D and (B) Family H. Closed symbols represent affected patients and open symbols represent unaffected individuals. The squares mean male, the circles mean female and the slashes mark deceased.

heterozygous mutations were the pathogenic variants involved in RP in the two families.

Discussion

In the present study, three mutations associated with RP were reported. In humans, XLRP is one of the most severe forms of RP, with six associated genetic loci. It is estimated that ~20% of patients with XLRP have pathogenic mutations in RP2. RP2 has been reported as an activator of small GTPase ADP-ribosylation factor like-3, and has been implicated in cell signaling and vesicular transportation by linking the cell membrane with the cilia and cytoskeleton in photoreceptor cells (13,14). Patil *et al* (15) revealed that RP2 was crucial in photoreceptor development, and distinct mutations in RP2

caused dysfunctions in cone and rod photoreceptors, resulting in a wide-spectrum of clinical phenotypes. In the present study, the c.358C>T mutation in exon 2 resulted in a truncated RP2 protein through a change of the arginine codon to a nonsense codon. c.358C>T was first reported by Mears *et al* (16) in 1999. With the development of sequencing techniques, whole-exome sequencing has allowed for significant advances to be made in the identification of novel RP pathogenic genes and screening of mutations. In Family H, the affected male (H-III-1) exhibited more severe clinical features compared with the affected female (H-II-2); H-III-1 had an earlier age of onset, rapid progression of visual field constriction and loss of visual acuity. RP is generally determined by random X-chromosome inactivation, the process responsible for silencing one of the X chromosomes in females. The X-inactivation center produces

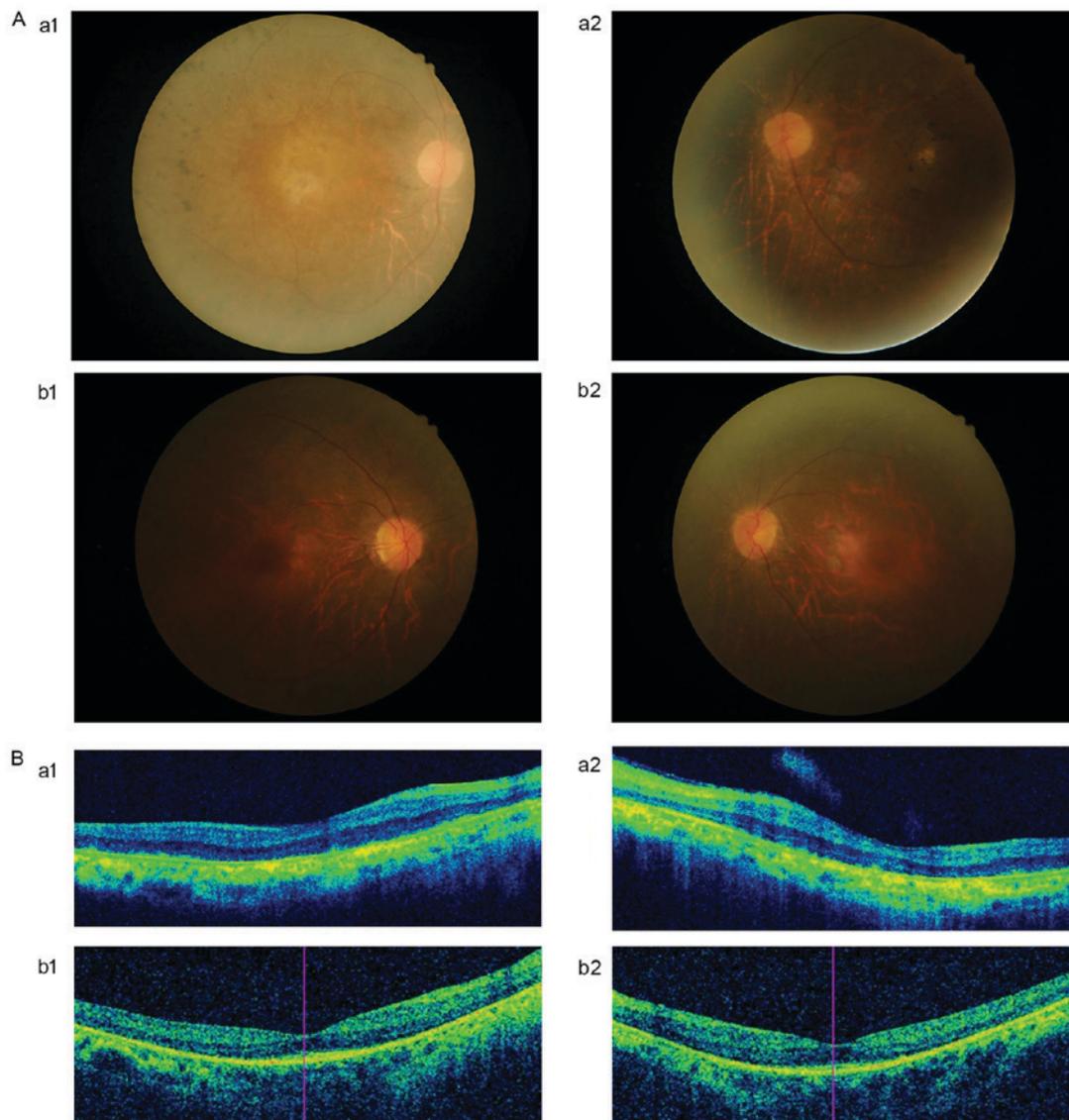


Figure 2. Clinical examination of Family D. (A) Fundus examinations of the (a1) right and (a2) left eyes of D-II-2, and the (b1) right and (b2) left eyes of D-II-3. (B) Optical coherence tomography of the (a1) right and (a2) left eyes of D-II-2, and the (b1) right and (b2) left eyes of D-II-3.

a long functional non-coding RNA, X inactive specific transcript (Xist), which is directly involved in the mediation of chromosome silencing (17). However, the mechanism of Xist silencing requires further investigation.

RP can also occur as a syndrome, which accompanies other diseases. Usher syndrome is the most frequent form of syndromic RP associated with hearing loss (18). It can be divided into three types based on the assessment of clinical features. At present, ~500 coding variants in USH2A have been reported to be pathogenic. However, Jacobson *et al* (19) analyzed photoreceptors and the retinal pigment epithelia of individuals with different mutations in USH2A using OCT, demonstrating consistent results between the individuals with the same USH2A genotype. USH2A is located on chromosome 1q41 and is the most commonly mutated splice variant of USH2, which consists of two main spliced transcripts. The shorter splice variant consists of 21 exons and encodes a 170 kDa protein. The longer splice variant consists of 72 exons and encodes a 600 kDa protein, which contains a membrane-spanning segment followed by an intracellular

PDZ-binding domain at the C-terminus (20). The expression of the longer splice variant is restricted to photoreceptors and developing cochlear hair cells (21). Liu *et al* (22) disrupted the expression of USH2A in mice, resulting in the progressive degeneration of photoreceptors and non-progressive hearing impairment, suggesting that USH2A may be essential for the maintenance and development of photoreceptors and cochlear hair cells.

In Family D, D-II-3 and D-II-2 possessed the same compound heterozygous mutation (c.6,485+5G>A/c.11,156G>A) in USH2A. Although the two shared the same mutations, D-II-3 had a central visual field, whereas D-II-2 exhibited almost complete visual field loss. It has been suggested that the same mutations can cause a variety of intra-familial phenotypes. Previously, it was reported that mutations in USH2A can cause patients to suffer from RP without hearing loss. The missense mutation in USH2A, c.11,156G>A, affects the long splice variant of USH2A, resulting in a substitution of arginine for leucine at protein position 3,719 (23). This change may affect the insertion of the protein into the membrane,

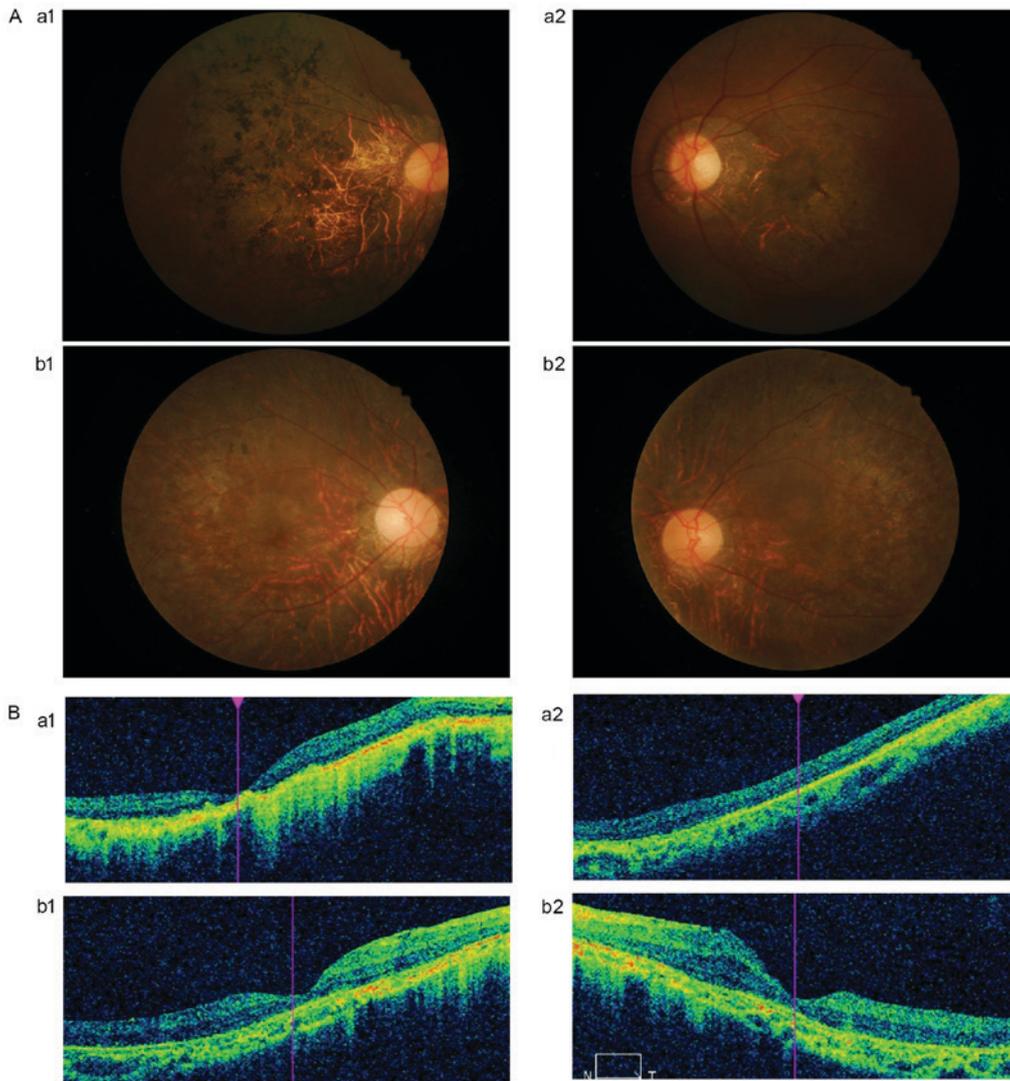


Figure 3. Clinical examination of Family H. (A) Fundus examinations of the (a1) right and (a2) left eyes of H-II-2, and the (b1) right and (b2) left eyes of D-II-3. (B) Optical coherence tomography of the (a1) right and (a2) left eyes of H-II-2, and the (b1) right and (b2) left eyes of H-III-1.

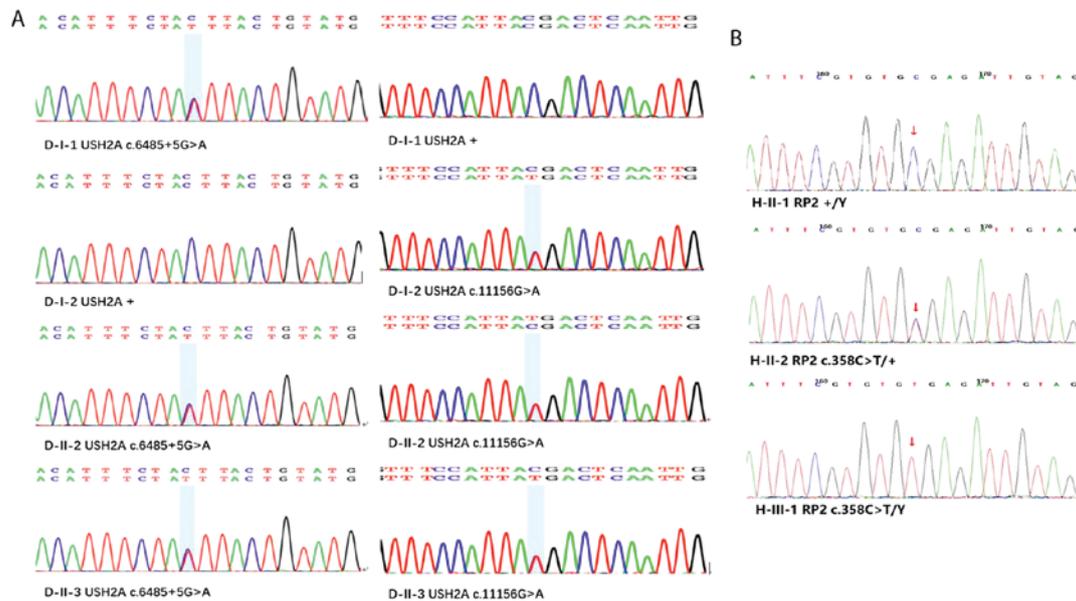


Figure 4. Sanger sequencing. Sanger sequencing results of pathogenic mutations in Families (A) D and (B) H. The blue shaded areas in figure A and red arrows in figure B represent the mutant nucleotide. USH2A, usherin; P2, ARL3 GTPase-activating protein.

leading to the degeneration of photoreceptors. As the mRNA expression levels of the long splice variant in the cochlea are low, the function of USH2A has been demonstrated to persist in cochlea cells (22). The mutation c.6,485+5G>A in USH2A was previously reported in a Japanese patient with Usher syndrome type 2, however, it was only identified in one individual without families involvement (24). In the present study, the two patients shared the same compound mutation in family H, co-segregating with clinical features. In addition, it demonstrated pleiotropy when patients in family H did not suffer from hearing impairment.

In conclusion, the present study described two Chinese families comprising individuals diagnosed from RP. Through the use of whole-exome sequencing, a compound heterozygous mutation co-segregating with the disease was identified as the pathogenic mutation in ARRP in Family D. To the best of our knowledge, this is the first identification of compound mutation c.6,485+5G>A in USH2A as a pathogenic mutation among Chinese patients with RP. However, the pathogenic mechanisms resulting from c.6,485+5G>A require further investigation. In Family H, the XLRP mutation (c.358C>T) in RP2 was confirmed to be a pathogenic mutation caused by random X-chromosome inactivation. Future investigations aim to analyze more pedigrees with RP, and examine the pathogenic mechanism of the gene mutation.

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Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YF contributed to conception and design of the study, blood sample collection, data acquisition, statistical analysis, data interpretation and drafting of the manuscript; NC contributed to data acquisition, data interpretation and critical revision of the manuscript for scientific and factual content; ZQ contributed to critical revision of the design of the study; JS contributed to supervision, conception and design of the study, data interpretation and critical revision of the manuscript; LL contributed to supervision, conception and design of the study and critical revision of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Ren Ji Hospital [reference number (2016) 219 (2)]. Informed consent was obtained from all the patients involved.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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