• Basic Research •

Whole-exome sequencing identifies novel mutations in genes responsible for retinitis pigmentosa in 2 nonconsanguineous Chinese families

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Received: 2018-08-18 Accepted: 2018-09-25

Abstract

• AIM: To detect the pathogenetic mutations responsible for nonsyndromic autosomal recessive retinitis pigmentosa (RP) in 2 nonconsanguineous Chinese families.

• METHODS: The clinical data, including detailed medical history, best corrected visual acuity (BCVA), slit-lamp biomicroscope examination, fundus photography, optical coherence tomography, static perimetry, and full field electroretinogram, were collected from the members of 2 nonconsanguineous Chinese families preliminarily diagnosed with RP. Genomic DNA was extracted from the probands and other available family members; whole-exome sequencing was conducted with the DNA samples provided by the probands, and all mutations detected by whole-exome sequencing were verified using Sanger sequencing in the probands and the other available family members. The verified novel mutations were further sequenced in 192 ethnicity matched healthy controls.

• RESULTS: The patients from the 2 families exhibited the typical symptoms of RP, including night blindness and progressive constriction of the visual field, and the fundus examinations showed attenuated retinal arterioles, peripheral bone spicule pigment deposits, and waxy optic discs. Whole-exome sequencing revealed a novel nonsense mutation in FAM161A (c.943A>T, p.Lys315*) and compound heterozygous mutations in RP1L1 (c.56C>A, p.Pro19His; c.5470C>T, p.Gln1824*). The nonsense c.5470C>T, p.Gln1824* mutation was novel. All mutations were verified by Sanger sequencing. The mutation p.Lys315* in FAM161A co-segregated with the phenotype, and all the nonsense mutations were absent from the ethnicity matched healthy controls and all available databases.

• CONCLUSION: We identify 2 novel mutations in genes responsible for autosomal recessive RP, and the mutation in FAM161A is reported for the first time in a Chinese population. Our result not only enriches the knowledge of the mutation frequency and spectrum in the genes responsible for nonsyndromic RP but also provides a new target for future gene therapy.

• KEYWORDS: retinitis pigmentosa; nonsyndromic; whole-exome sequencing; mutation; novel

DOI:10.18240/ijo.2019.06.06

Citation: Hu YS, Song H, Li Y, Xiao ZY, Li T. Whole-exome sequencing identifies novel mutations in genes responsible for retinitis pigmentosa in 2 nonconsanguineous Chinese families. *Int J Ophthalmol* 2019;12(6):915-923

INTRODUCTION

Retinitis pigmentosa (RP) is a group of heterogenous hereditary retinal degeneration diseases, which are characterized by the progressive loss of function in photosensory cells and pigment epithelium[1-2]. RP can manifest in a syndromic or a nonsyndromic form and is typically characterized by night blindness, progressive constriction of the visual field, changes in the fundus and abnormal electroretinogram results. The incidence of nonsyndromic RP worldwide is approximately 1/5000-1/3000. At present, it is estimated that there were approximately 2.5 million RP patients in the world, and RP is an important cause of irreversible blindness due to fundus disease[3-4]. In China, the incidence is approximately 1/3800[5], which means that there are approximately 370 000 patients in China suffering from nonsyndromic RP.

Genetic predisposition plays an important role in the pathogenesis of RP, and RP can be inherited as an autosomal dominant (AD), autosomal recessive (AR) or X-linked trait. Currently, there are 92 genes listed in RetNet (available in the public domain at https://sph.uth.edu/retnet/) that have been identified as being involved in the development of RP. These genes are involved in the transduction cascade of the optical signal and the regulation of the transcription and translation of other retinal genes. It has been reported that these genes

account for only 60% of the nonsyndromic RP patients[6-8]; therefore, more efforts should be made to uncover the genetic basis of RP.

Whole-exome sequencing (WES) has been proven to be an efficient method to detect the genetic defects underlying hereditary diseases, and it primarily captures information about the coding regions of genes, providing a faster and more efficient way to explore the genetic causes of hereditary diseases[9-10]. In this study, WES was adopted to uncover the disease-causing mutations in 2 nonconsanguineous Chinese families with nonsyndromic RP.

SUBJECTS AND METHODS

Ethical Approval The current study was approved by the Ethics Committee of The Central Hospital of Enshi Autonomous Prefecture and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the probands and their family members (or their guardians if they were minors).

Subjects and DNA Specimens Two nonconsanguineous families with RP and 192 ethnicity matched healthy controls were recruited in the Ophthalmologic Centre of the Central Hospital of Enshi Autonomous Prefecture in Southwest China. The diagnosis of nonsyndromic RP was made based on the patients' medical histories, symptoms and physical examinations. All the patients underwent autorefraction (Topcon KR-8000, Paramus, NJ, USA), subjective optometry, slit-lamp biomicroscopic examination, IOL master (Carl Zeiss Meditec AG, Jena, Germany), fundus photography (Canon CF-60UD, Tokyo, Japan), optical coherence tomography (Heidelberg Engineering HRA+OCT, Heidelberg, Germany), fundus autofluorescence (Cirrus HD-OCT 4000, Carl Zeiss Meditec Inc., Jena, Germany), static perimetry (Humphrey Field Analyser, Carl Zeiss Meditec Inc., Dublin, CA, USA), and full field electroretinography (Roland Electrophysiological Test Unit RETI-Scan 21, Roland Consult, Berlin, Germany). The electroretinogram was conducted in accordance with the standards of the International Society for Clinical Electrophysiology of Vision[11]. DNA specimens were collected from peripheral venous blood using a blood DNA extraction kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions and stored in TE buffer.

Whole-exome Sequencing WES was conducted with the DNA samples from the probands by a commercial service (Macrogen Inc., Seoul, Korea). The genomic DNA of each proband was enriched with an Agilent SureSelect Human All Exon Enrichment Kit V5 array (Agilent Technologies, Santa Clara, CA, USA), and the enriched DNA fragments were sequenced with an Illumina HiSeq4000 system (Illumina, Santiago, CA, USA). The sequencing depth of every sample was greater than 125-fold. Burrows Wheeler Aligner software was used to map short reads to the hg19 reference genome (available in the public domain at http://bio-bwa.sourceforge.net/bwa.shtml). Variant calling and filtering was conducted with GATK (Genome Analysis Toolkit) software (available in the public domain at https://www.broadinstitute.org/gatk/). Variant annotation was performed with SnpEff (available in the public domain at http://snpeff.sourceforge.net/SnpEff.html).

Data Analysis WES data were selected for the known causative genes of nonsyndromic RP. Variants in these genes were filtered by the following criteria: 1) those with a minor allele frequency (MAF) greater than 0.005 in the 1000 Genomes database (available in the public domain at http://www.internationalgenome.org/), the Exome Aggregation Consortium database (ExAC, available in the public domain at http://exac.broadinstitute.org/), the Genome Aggregation database (gnomAD, available in the public domain at http://gnomad.broadinstitute.org/), the NHLBI GO Exome Sequencing Project database (ESP, available in the public domain at http://evs.gs.washington.edu/) and the database of single nucleotide polymorphisms (dbSNP, available in the public domain at https://www.ncbi.nlm.nih.gov/snp) were filtered out; 2) variants located in the intron region that did not affect the splicing site were filtered out; 3) synonymous variants that did not affect the splicing site were filtered out; and 4) variants predicted to be benign or tolerated by Polymorphism Phenotyping v2 (PolyPhen2, available in the public domain at http://genetics.bwh.harvard.edu/pph2/), Sorting Intolerant From Tolerant (SIFT, available in the public domain at http://sift.jcvi.org/) or PROVEN (available in the public domain at https://provean.jcvi.org/index.php) were filtered out. After the data were filtered, only nonsynonymous variants remained for further verification.

Sanger Sequencing for Variant Verification and Segregation Analysis The following verification and segregation analyses were conducted on the mutations that remained after the data were filtered. Polymerase chain reaction (PCR) was carried out to amplify the fragments containing the variants. Primers were designed with the Primer3 online website (available in the public domain at http://primer3.ut.ee/), and all the primers are listed in Table 1. The amplicons were sequenced with an ABI BigDye Terminator v3.1 Cycle Sequencing kit using an ABI 3100 and a 3500xL Dx Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The genomic DNA reference sequences were downloaded from the NCBI GenBank (available in the public domain at https://www.ncbi.nlm.nih.gov/), and the sequencing data were analysed using the SeqMan II programme of the Lasergene software package (DNAStar Inc., Madison, WI, USA). The DNA samples of all the probands and their available family members were Sanger sequenced, and the segregation analysis was conducted in accordance with the respective inheritance mode. Further verification was

Table 1 PCR primers used for FAM161A and RP1L1

Gene	Mutation	Sequence	Primer length (bp)	Amplicon size (bp)
FAM161A	c.943A>T	F: 5'-TGGACAGACTTTTGTGTTGAGG-3'	22	689
		R: 5'-TCAAAATCAGGAGTTGGGCAC-3'	21	
RP1L1	c.56C>A	F: 5'-GCACCTCTAGAAAGACGGGA-3'	20	291
		R: 5'-GGCGCTGAAGGTCTTAAAGG-3'	20	
	c.5470C>T	F: 5'-GAGACAAAGATCCCAAACTCGG-3'	22	698
		R: 5'-GGTCTCCACTTCAACCTCCA-3'	20	

Table 2 Clinical information regarding probands in the EQT33 and EQT38 families

Family No.	Gender	Age at exam (y)	Refraction error, OD/OS (D)	BCVA, OD/OS	Fundus, OD/OS	OCT, OD/OS	ERG, OD/OS	Visual field, OD/OS
EQT33	F	20	+0.5/+0.75	HM/HM	Affected/affected	MA/MA	RRCS/RRCS	NA/NA
EQT33II-2	M	19	-11.5/-11.0	0.15/0.12	Affected/affected	MA/MA	RRCS/RRCS	TVF/TVF
EQT33I-1	F	50	0/0	1.0/1.0	NA/NA	NA/NA	NA/NA	NA/NA
EQT33I-2	M	50	-0.5/-0.5	0.8/0.8	NA/NA	Normal/normal	NA/NA	NA/NA
EQT38	F	26	-3.75/+0.5	0.8/HM	Normal/affected	Normal/MT	NA/NA	Normal/NA

BCVA: Best corrected visual acuity; D: Diopter; ERG: Electroretinogram; OD: Right eye; OS: Left eye; HM: Hand movement; MA: Macular atrophy; MT: Macular thinning; TVF: Tubular visual field; RRCS: Reduced rod and cone response; NA: Not available.

carried out in 192 ethnicity matched healthy controls. The amino acid sequences of different species were acquired from the NCBI website (available in the public domain at https://www.ncbi.nlm.nih.gov/), and the conservation analysis was conducted using the MegAlign programme of the Lasergene software package (DNAStar Inc., Madison, WI, USA).

RESULTS

Clinical Manifestations and Pedigree Information In the current study, we investigated 2 nonsyndromic RP families, EQT33 and EQT38. The proband of EQT33 was a 19-year-old male whose chief complaint was night blindness with decreased vision. The main complaint was decreased dark adaptation 13 years ago, and over the past 13y, his visual acuity slowly decreased. When he first visited our ophthalmologic centre, his anterior ocular segment was normal and BCVA revealed that he could perceive hand movements with both eyes. The mydriasis fundus examination demonstrated peripheral pigment bone spicule deposits, slightly waxy optic discs, attenuated retinal arterioles and macular degeneration in both eyes. The OCT showed blurring of inner and outer segment layers (IS/OS). The electroretinogram (ERG) was severely reduced in all six tests (Figure 1). His visual field was not measured due to his poor visual acuity. The proband's younger sister (EQT33 II-2) also suffered from RP, but with milder symptoms. She had sensed a decrease in dark adaptation at the age of 10, and her BCVA was 0.15 for the right eye and 0.12 for the left at her first visit. The mydriasis fundus examination showed slightly attenuated retinal arterioles, a small number of retinal pigment deposits in the peripheral retina and tigroid fundus. Other family members did not exhibit any symptoms of RP, and their physical examinations revealed normal results for both eyes; therefore, we concluded that the most likely mode of inheritance in EQT33 was AR. The clinical information for EQT33 is summarized in Table 2.

The proband EQT38 was a 26-year-old female. Her left eye had suffered from poor visual acuity since birth, and for as long as she could remember, she had been unable to see anything at night with her left eye. In the last 26y, her left eye gradually developed exotropia. Unlike her left eye, her right eye had a normal visual acuity. When she first came to our ophthalmologic centre, her BCVA was 0.8 for her right eye and hand movements for her left eye. Slit-lamp microscopic examination revealed normal anterior segments for both eyes. The fundus examination demonstrated severe signs of RP in the left eye, including panretinal dense pigment deposits (in some places, the pigments connected to form flakes), waxy optic discs, attenuated retinal arterioles and macular degeneration. Interestingly, the retina of her right eye was normal without any sign of pigment deposits, waxy optic disc or retinal arterioles. The macular OCT of her left eye exhibited marked thinning and blurring of all retina layers, while the OCT of her right eye was normal. The visual field was not measured in her left eye due to the poor BCVA, and the visual field in her right eye was normal. She stated that her parents did not suffer from ocular diseases, and her child did not exhibit any signs of night blindness or poor visual acuity; therefore, we speculated that the inheritance mode in this pedigree was also AR. Unfortunately, due to economic difficulties, she did not return to our centre for follow-up, so we could not perform an ERG to generate more evidence supporting the diagnosis.

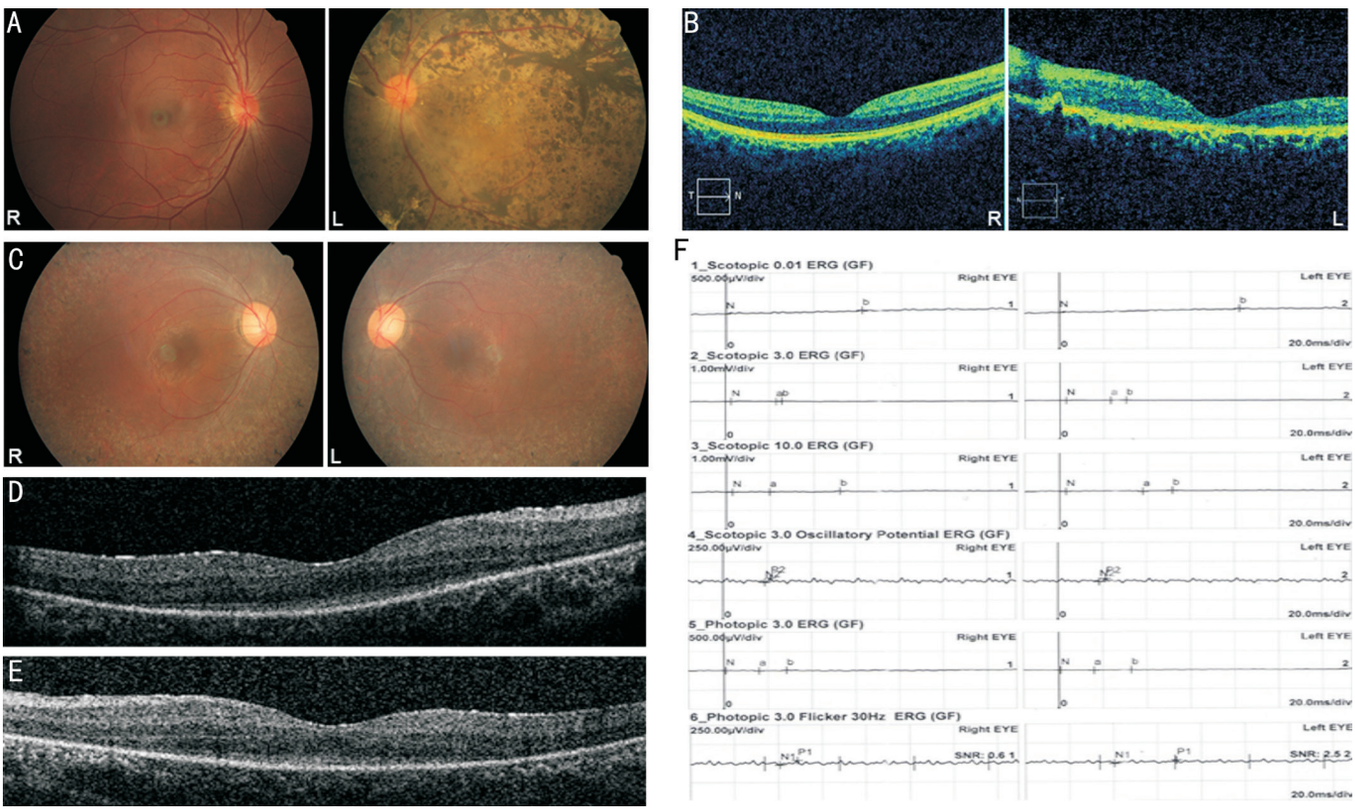

Figure 1 Ophthalmic examinations of the probands from two RP families A: Fundus photograph of the proband in EQT38. Fundus photograph showed panretinal dense pigment deposits, waxy optic disc, attenuated retinal arterioles and macular degeneration in the left eye, while the retina of her right eye was normal. B: OCT image of the proband in EQT38. OCT of the macula in the left eye exhibited marked thinning and blurring of all retinal layers, while the OCT in the right eye was normal. C: Fundus photograph of the proband in EQT33. Fundus photograph showed peripheral pigment bone spicule deposits, slightly waxy optic discs, attenuated retinal arterioles and macular degeneration in both eyes. D, E: OCT image of the proband in EQT33. OCT image of the maculae in both eyes showed blurring of the inner and outer segment layers (IS/OS). D: Right eye; E: Left eye. F: ERG of the proband in EQT33. The ERG was severely deduced in all six tests.

Table 3 Summary of WES results

Sample	Total read bases (bp)	Total reads	On-target reads	Q20 (%)	Q30 (%)	Coverage >30× (%)	Mean depth of target regions (×)	Total SNP	Synonymous variant
EQT33	8246785744	81651344	56539425	95.9	90.5	90.3	95.6	81401	11390
EQT38	9163448412	90727212	54079044	98.1	95.3	90.3	89.6	84166	11481

Whole-exome Sequencing Results On average, 8.7 billion read bases were obtained from the WES for EQT33 and EQT38, and the number of total reads was 86.2 million per sample; 97.0% of the read bases reached Q20, while 92.9% of the read bases reached Q30. The throughput depth of target regions reached 172.7×. After being mapped to the human reference genome sequence (hg19), the mean depth of target regions reached 92.6×. Then the base quality score recalibration, indel realignment, duplicate removal, SNP and INDEL discovery and genotyping were conducted, and we obtained 82783 variants. After the filtration described in the Methods section, a total of 478 variants remained for further analysis. The WES results are summarized in Table 3, and the histogram of the depth distribution in target regions is shown in Figure 2. Then, we mapped these 478 variants to the genes responsible for nonsyndromic RP listed in RetNet. Ultimately, we obtained a homozygous nonsense mutation in FAM161A (c.943A>T, p.Lys315*) in EQT33, and a compound heterozygous mutation in RP1L1 (c.56C>A, p.Pro19His; c.5470C>T, p.Gln1824*) in EQT38. The nonsense mutation p.Lys315* in FAM161A and p.Gln1824* in RP1L1 were not included in the 1000 Genomes database, ExAC, gnomAD, ESP, dbSNP or Human Gene Mutation Database (HGMD, available in the public domain at http://www.hgmd.cf.ac.uk/ac/index.php), and no published papers have reported these 2 mutations. The missense mutation p.Pro19His in RP1L1 has an allele frequency of 0.0005243 in East Asian populations in gnomAD. The effect of the amino acid substitution in the missense (c.56C>A, p.Pro19His) mutation in RP1L1 was predicted using SIFT, PolyPhen2 and Proven, and all the tools

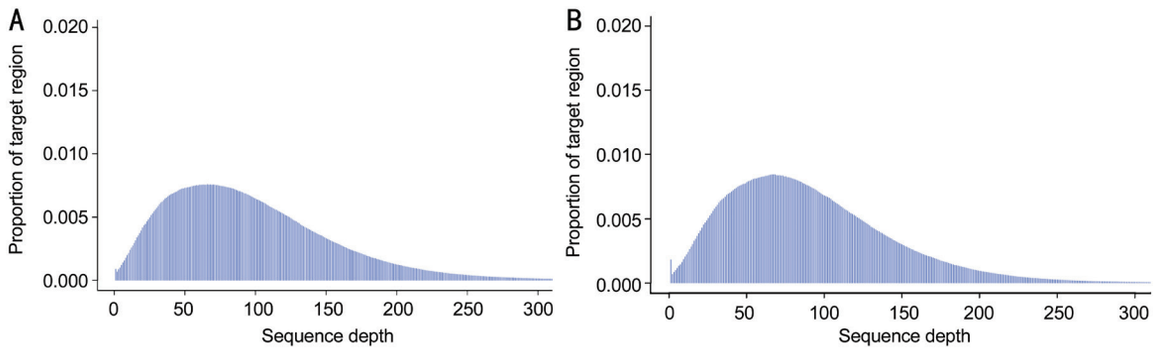

Figure 2 Histogram of the depth distribution in target regions On average, 8.7 billion read bases were obtained from WES for EQT33 (A) and EQT38 (B), and the number of total reads was 86.2 million per sample; 97.0% of the read bases reached Q20, while 92.9% of the read bases reached Q30. The throughput depth of target regions reached 172.7×.

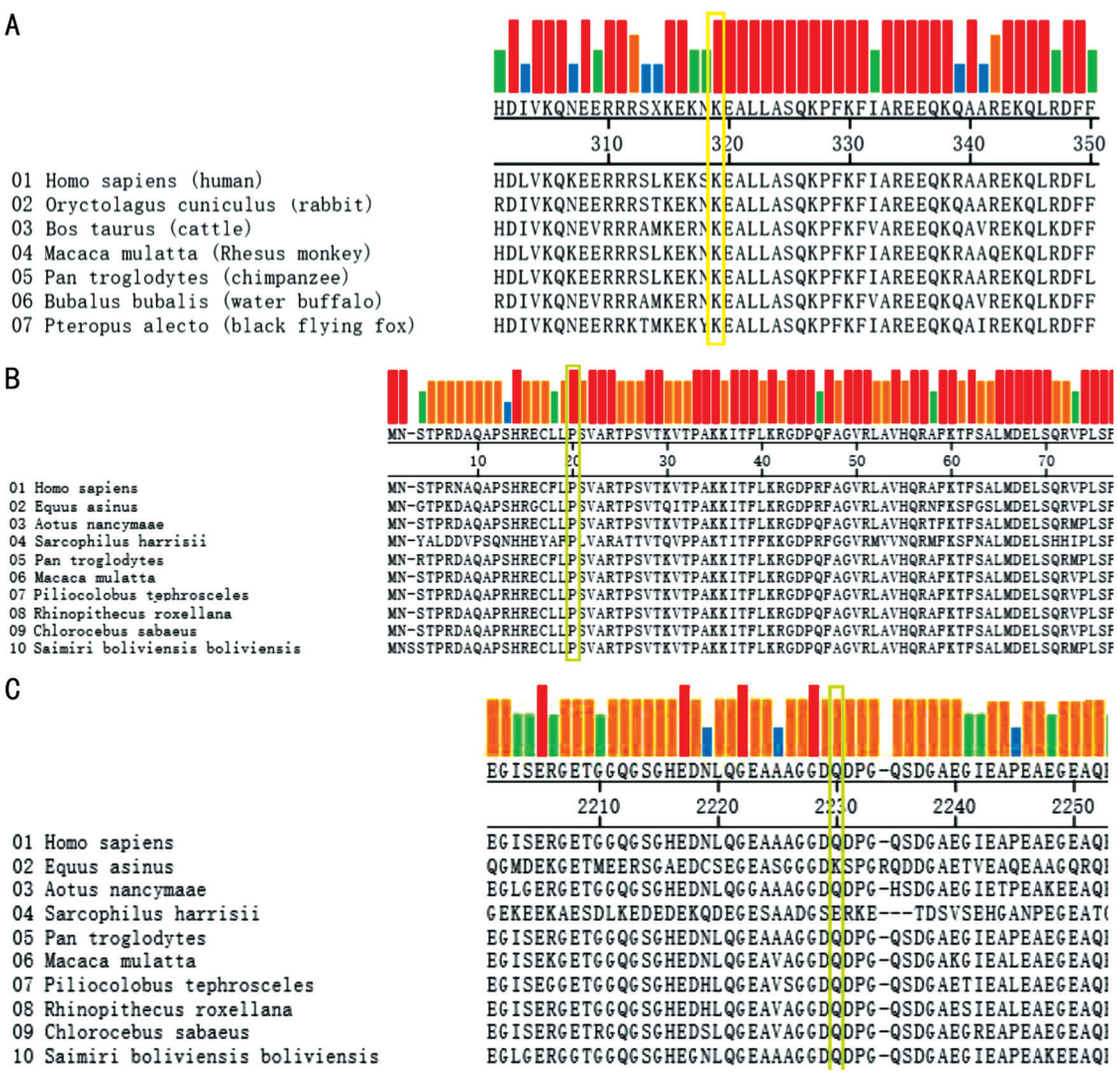

Figure 3 Amino acid sequences alignment results in different species A, B: The p.Lys315* mutation in FAM161A and p.Pro19His in RP1L1 is highly conserved in different species; C: The p.Gln1824* mutation in RP1L1 is highly conserved in primates.

predicted a damaging impact of the mutation on the protein function. The detailed information is summarized in Table 4. Conservation analysis demonstrated that all 3 mutations are highly conserved. The Conservation analysis result is shown in Figure 3.

Sanger Sequencing for Mutation Verification and Segregation Analysis To verify the mutations detected by WES, Sanger sequencing was performed with the samples from the probands and their available family members. The results demonstrated the co-segregation of the mutation with the disease phenotype in the EQT33 pedigree. The proband of the EQT38 pedigree did not return for follow-up due to economic difficulties, and therefore we could not perform the segregation analysis. The segregation analysis results and the family tree are shown in

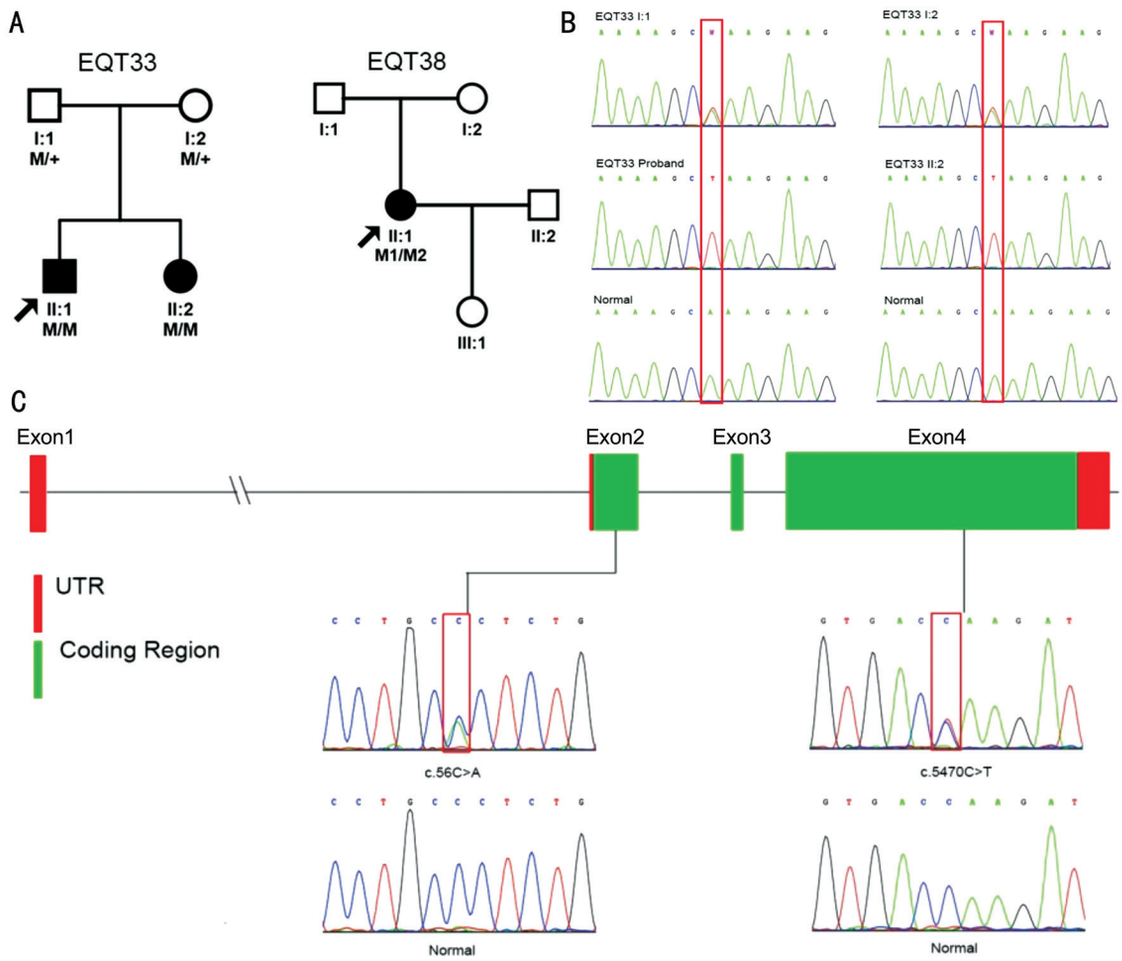

Figure 4 The segregation analysis results and the family tree A: RP pedigrees presented in this study. The probands are indicated by the black arrows. +: Wild-type; M: Mutation. B: Mutation verification of the FAM161A gene in the EQT33 family. Sanger sequencing demonstrated that the mutation c.943A>T in FAM161A co-segregated with the phenotype. The proband's normal parents (EQT33 I:1 and EQT33 I:2) carried heterozygous c.943A>T mutations in FAM161A, while the affected proband and his affected sister (EQT33 II:2) harbored homozygous c.943A>T mutations in FAM161A. C: Genomic structure of exons encoding the open reading frame of RP1L1. Three out of four exons are translated (green), while exon 1, portions of exon 2 and exon 4 are untranslated (red). The Sanger sequencing results of the compound heterozygous mutations in RP1L1 (c.56C>A, p.Pro19His; c.5470C>T, p.Gln1824*) are shown.

Table 4 Mutations detected in the EQT33 and EQT38 pedigrees

Family	Gene	Position	Exon	DNA change	Protein change	Status	Mutation type	Note	Allele frequency in control Poly-Phen2 SIFT proven
EQT33	FAM161A	62067196	3	c.943A>T	p.Lys315*	HOM	Nonsense	Novel	0/192, NA, NA, NA
EQT38	RP1L1	10480656	2	c.56C>A	p.Pro19His	HET	Missense	Reported[a]	NA, probably, damaging, damaging, deleterious
	RP1L1	10466138	4	c.5470C>T	p.Gln1824*	HET	Nonsense	Novel	0/192, NA, NA, NA

HOM: Homozygous; HET: Heterozygous; NA: Not available. [a]The missense mutation p.Pro19His in RP1L1 has an allele frequency of 0.0005243 in East Asian populations in gnomAD.

Figure 4. We next screened 192 ethnicity matched healthy controls for the 2 novel mutations (c.943A>T, p.Lys315* and c.5470C>T, p.Gln1824*) by Sanger sequencing, and neither mutation was detected in the control group.

DISCUSSION

RP is a group of heterogenous hereditary retinal degeneration diseases, characterized by the progressive loss of function of the photosensory cells and pigment epithelium. In the current study, we investigated 2 families with nonsyndromic AR RP, pedigrees EQT33 and EQT38. All the patients presented with typical RP symptoms and signs, namely night blindness with decreased vision, peripheral pigment bone spicule deposits revealed by a mydriasis fundus examination, waxy optic discs, attenuated retinal arterioles and macular degeneration. It is worth noting that the proband in EQT38 had suffered from RP in only the left eye. WES and the subsequent Sanger

sequencing identified a novel nonsense mutation in FAM161A (c.943A>T, p.Lys315*) and compound heterogeneous mutations (c.56C>A, p.Pro19His; c.5470C>T, p.Gln1824*), of which c.5470C>T, p.Gln1824* was determined to be novel.

FAM161A was first reported to cause nonsyndromic RP by Langmann *et al*[12] in an Indian population in 2010; since then, several articles have reported a few more mutations in different ethnicities[13-15]. The mutation frequency of FAM161A in North America is approximately 1%[13]. Van Schil *et al*[14] concluded that mutations in FAM161A were responsible for 2% of AR RP cases in the Dutch and Belgian populations, while Bandah-Rozenfeld *et al*[15] determined that mutations in FAM161A were responsible for approximately 12% of AR RP families in a cohort from Israel and Palestine. To date, mutations in FAM161A have not been reported to cause RP in the Chinese population; thus, our study is the first concerning the role of mutations in FAM161A in the development of RP in a Chinese population.

Although most of the mutations detected so far have been nonsense mutations located in exon 3, the largest exon in FAM161A[16-17], the RP phenotypes differ in distinct populations, even within the same family[18-21]. In North America, Venturini *et al*[13] observed that patients with FAM161A mutations exhibit early-onset RP with relatively good visual acuity and greatly reduced cone response on ERG tests, while Bandah-Rozenfeld *et al*[15] reported extinguished rod-cone ERG responses in a majority of their patients. Other researchers found that some RP patients have cataracts or myopia in Israeli, Palestinian, Dutch and Belgian populations[14-15,22]. The 2 patients in EQT33 both showed early-onset symptoms of RP and were found to carry the same p.Lys315* mutation in FAM161A but with different disease severity and phenotypes. The proband in this family suffered from night blindness, and the test of visual acuity revealed that both eyes could perceive hand movements, with minor refraction errors. The OCT showed blurring of the inner and outer segment layers (IS/OS). The ERG was severely reduced in all six tests. His visual field was not measured due to his poor visual acuity, while his younger sister had milder symptoms and signs. Her BCVA was 0.15/0.12 for the right and left eyes, respectively, with refraction errors of about -10 diopter. Her fundus showed less pigmentation than that of her brother. Neither patient suffered from cataracts or other ocular or systemic diseases.

Animal models have proven that the FAM161A protein localizes at the base of the connecting cilium of photoreceptor cells and is mainly involved in ciliopathy[23-24]. FAM161A is involved in the stabilization of microtubules, so it is essential for molecular transport from the inner to the outer segment of the cilium, a function that is critical for the formation of the outer segment disk[25-26]. Gene-trapped mice exhibited disorganized discs in their photoreceptor cells and early loss of photoreceptor function[25]. The mutation c.943A>T identified in pedigree EQT33 introduces a termination codon, resulting in the translation of a truncated protein, which severely affects its function. In addition, this mutation is rated as “likely pathogenic” according to the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for the interpretation of sequence variants[27]. Segregation analysis revealed co-segregation of the mutation with the disease phenotype. In summary, we consider the nonsense mutation in FAM161A to be a disease-causing mutation in this family.

The human RP1L1 gene is on chromosome 8p and consists of 4 exons. It encodes the RP1L1 protein, which is 2400 amino acids long[28]. RP1L1 strongly resembles RP1, mostly within the first 350 amino acids, including the doublecortin domains[29]. Animal models have already proven that, like RP1, RP1L1 is specifically expressed in the retina, especially in the cone and rod photoreceptors, and that it has fundamental roles in maintaining the photosensitivity and outer segment morphogenesis of rod photoreceptors[30-31]. Since RP1 has been identified to cause 5.5% and 1% of dominant and recessive RP, respectively, it is reasonable to deduce that RP1L1 is also a main causative gene of RP. However, until now, only a few articles have reported a causative relationship between RP1L1 and RP[32-36]. Most of the mutations in RP1L1 have been identified as causing occult macular dysfunction (OMD)[28,31,36-42]. Like most hereditary ocular diseases, the relationship between RP1L1 genotypes and retinal dystrophy phenotypes is highly heterogenous. Okuno *et al*[40] observed a late and nonsynchronous onset of OMD, while Hayashi *et al*[42] reported an early onset of OMD in a Japanese cohort. The mutation frequency for RP among different ethnicities also differs greatly. Bowne S.J. et al. did not discover any disease-causing mutations in RP1L1 among 60 AR RP patients in the USA[28], Haer-Wigman *et al*[35] detected one mutation causing RP in 266 Dutch visually impaired patients, and Patel *et al*[34] revealed one RP-causing mutation in a Saudi Arabian cohort of 292 families, while Japanese researchers demonstrated that mutations in RP1L1 are responsible for 7.8% of the AR RP cases in a Japanese population[36]. In our study, we revealed an AR RP pedigree harbouring compound heterogeneous mutations (c.56C>A, p.Pro19His; c.5470C>T, p.Gln1824*) in RP1L1, of which the missense mutation (c.56C>A, p.Pro19His) is predicted to be probably damaging, to be deleterious and to have a damaging impact on protein function by Poly-Phen2, PROVEN and SIFT, respectively; the nonsense mutation (p.Gln1824*) leads to the expression of a truncated protein or, more likely, results in nonsense-mediated decay. As in to the report by Okuno *et al*[40], the fundus

phenotype of one proband was asymmetrical, but since she was only 26 years old, we speculate that the second eye may exhibit signs of RP in the future, otherwise there is occurrence of de novel mutation or germline mosaic. This proband came from Southwest China, which is an extremely impoverished region. It is unfortunate that this proband temporarily decided not to participate in follow-up, but we will not cease tracing the advance of the disease or verifying the unique phenotype exhibited by this family.

In summary, we identified 2 novel mutations in genes responsible for AR RP, and the mutation in FAM161A is reported for the first time in a Chinese population to cause AR RP. Due to limited data about the RP1L1 mutation, more studies are required to provide more evidence regarding the role of this mutation. Nevertheless, our study enriches the knowledge of the mutation frequency and spectrum in the genes responsible for RP and provides a new target for future gene therapy.

ACKNOWLEDGEMENTS

We sincerely thank all the subjects participated in this study.

Foundation: Supported by the National Natural Science Foundation of China (No.81360154).

Conflicts of Interest: Hu YS, None; **Song H,** None; **Li Y,** None; **Xiao ZY,** None; **Li T,** None.

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