• Investigation •

Myopia with X-linked retinitis pigmentosa results from a novel gross deletion of RPGR gene

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Abstract

● **AIM:** To identify mutations with whole exome sequencing (WES) in a Chinese X-linked retinitis pigmentosa (XLRP) family.

● **METHODS:** Patients received the comprehensive ophthalmic evaluation. Genomic DNA was extracted from peripheral blood and subjected to SureSelect Human All Exon 6+ UTR exon capture kit. The exons were sequenced as 100 base paired reads on Illumina HiSeq2500 system. Only mutations that resulted in a change in amino acid sequence were selected. A pattern of inheritance of the RP family was aligned to identified causal mutation.

● **RESULTS:** We analysed the data of WES information from XLRP family. The analysis revealed a hemizygous large genomic deletion of RPGR c.29_113del was responsible for this XLRP. The gross deletion lead to a frame-shift mutation and generate stop codon at 7 animo acid behind Asp (D10Afs*7), which would serious truncate RPGR protein. The novel frame-shift mutation was found to segregate with retinitis pigmentosa (RP) phenotype in this family. Bilateral myopia was present on the male patients, but carrier female showed unilateral myopia without RP.

● **CONCLUSION:** Our study identifies a novel frame-shift mutation of RPGR in a Chinese family, which would expand the spectrum of RPGR mutations. The geno-phenotypic analysis reveals a correlation between RP and myopia. Although exact mechanism of RP related myopia is still unknown, but the novel frame-shift mutation will give our hit on studying the molecular pathogenesis of RP and myopia.

● **KEYWORDS:** retinitis pigmentosa; myopia; retinitis pigmentosa GTPase regulator; whole exome sequencing

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INTRODUCTION

Retinitis pigmentosa (RP) is a genetically heterogeneous retinal dystrophies and characterized by progressively night blindness, decreased visual acuity, and constricted visual fields, that results from the loss of rod photoreceptor followed by the cone photoreceptor dysfunction. RP is inherited as autosomal recessive, autosomal dominant, X-linked trait. Among these, X-linked retinitis pigmentosa (XLRP) is the most serious type of RP, account for 15% of RP and more than 80% of XLRP have mutations in the gene RPGR[1-3], which is also responsible for cone-rod dystrophy, and atrophic macular degeneration[4]. Moderate and high myopia is often secondary to RP phenotype both on patients and carrier[3,5-6], especially for XLRP[7]. In this study, we applied whole exome sequencing (WES) to identify a novel frameshift mutation of RPGR gene evoked by a gross deletion of exon2 and part of intron between exon1 and exon2 in a Chinese family with XLRP, which is X-linked recessive RP family with high myopia in male patients, and female carriers was only presented unilateral high myopia with no RP appearance. This study will expand the genotype-phenotype spectrum of RPGR mutations.

SUBJECTS AND METHODS

Ethical Approval The research was adhered to the principle

of the Declaration of Helsinki and was approved by the Ethics Committee of the People's Hospital of Ningxia Hui Autonomous Region. All participants involved in this study was signing the informed consent before examination.

Clinical Observations and Diagnosis Thoroughly ophthalmic examination was performed on patients, including best-corrected visual acuity (BCVA) according to Snellen E chart, refractometry (Topcon KR8100, Topcon Inc.), intraocular pressure (IOP) which was measured by Goldman applanation tonometer, slit-lamp examination, fundoscopy (TRC- 50DX, Topcon Inc, Japan), color sensitivity was tested by Ishihara plates, the macular structure was observed by spectral-domain optical coherence tomography (SD-OCT, Cirrus HD-OCT, Zeiss Meditec, German). Perimetry of central 30 degrees was used for visual field function analysis (750i Humphrey VF automated perimetry, Zeiss, German); Visual electrophysiological test including pattern visual evoked potentials and full-field flash electroretinography were performed by Retiscan/Retiport system (Roland Consult, German), which were performed and complied with the standard published by the International Society for Clinical Electrophysiology of Vision. The diagnosis of RP was followed by symptoms of night blindness and progressive loss of peripheral vision, and typical sign of ocular fundus, visual field, and visual electrophysiological examination was also considered. The diagnosis of high myopia was abide by the spherical equivalent refractive error was more than -6.00 diopters or axial eye length >26 millimeters.

Whole Exome Analysis The genomic DNA was collected from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen). Then DNA samples were subjected to WES for family members I-1, 2; II-1, 3, 4, 5, 7; III-3. The libraries of DNA samples were established using an exon capture kit (SureSelect ver. 6+ UTR, Agilent Technologies). The exons were sequenced as 100 base paired reads on Illumina HiSeq2500 system (Illumina, SanDiego, CA, USA). Raw sequencing reads were mapped against the human reference genome database (1000 Genomes, phase 2 reference, hs37d5) using the Burrows-Wheeler alignment tool[8]. The Genome Analysis Toolkit (GATK) applied for calling mutations. ANNOVAR was used for variant functional annotation[9]. Mutations were filtered by hereditary pattern to obtain mutations with removing known and frequent single nucleotide polymorphisms (SNPs) or benign polymorphisms. Variants which were to be disease-associated in the Human Gene Mutation Database (HGMD) and the Retinal Information Network Database or with frequency less than 0.1% in the population were selected. Variants and indels which located at coding regions, splice acceptor and donor site or lead to nonsynonymous mutation or frameshift indels were considered for the further analysis. The pathogenic variants were subjected to online reference databases, such as, 1000 Genome Project, ESP-6500, and Exome Aggregation Consortium (ExAC) databases. Publicly available servers, such as SIFT (https://omictools.com/sift-tool), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2) were used for mathematical predictions. Totally 3 mutations in 3 gene on X chromosomal were predicted to be responsible mutation. Among them a large deletion in exon2 of RPGR was identified, which was a well-known causative gene for RP. The public servers, such as MutationTaster2, Mutalyzer, Variant Effect Predictor, PSIPRED, CADD, Human Splicing Finder, were further investigated for predict mechanisms of disease onset. The Phyre2[10] was used to predict 3D structure change of the gross deletion of RPGR. Modeling was subsequently confirmed using Chimera version 1.11.2 software[11]. The breakpoints of RPGR deletion and co-segregation status were detected by Sanger sequencing using the primer pairs (Fw:5'-CCAGTTTCTATTAGGTGGAAAAGA-3', Rv:5'-GACATCTACTTGGTGACAGCAGA-3') and (Fw: 5'-TGCTCTTGATGACTTATTTTGTGA-3', Rv:5'-AGATGACTGCCTTCCTTGTGA-3') which covering the suspected borders areas, and further confirmed by gel electrophoresis.

RESULTS

Clinical Findings Eight subjects were enrolled in the study and three persons in the family were affected by RP (Figure 1). In this family only males affected, the proband (case III-3) and his uncles (case II-1 and II-7) began to show night blindness at 6 years old, and high myopic refractive error with best-corrected visual acuity (BCVA) decline in their teenage (Table 1), while the carrier took on myopic anisometropic amblyopia in left eye with no sign of RP appearance. The fundus of patients appeared typical RP sign including waxy and pale optic disc with attenuated retinal arteries, bone spicule-shaped deposits and tapetal reflex also pathological myopia degenerative changes characterizing with tilted optic disc, retinal pigment epithelium (RPE) and choriocapillary degeneration was shown on patients, but the left eye of carrier with myopic anisometropic amblyopia didn't appear such severe signs even with long axial length of 25.91 mm (Figure 2).

Genetic Findings WES was performed on the male proband, his parents, his grandparents, his two affected uncles, and non-affected aunt. The analysis revealed a hemizygous large genomic deletion of RPGR: c.29_113del was responsible for this XLRP. The sanger sequencing was used for polymerase chain reaction (PCR) products to localize both the proximal and distal deletion breakpoints located at g.38182807 and g.38182692 respectively, including intron-exon boundary and within the splice site (Figure 1B, 1D). A genomic

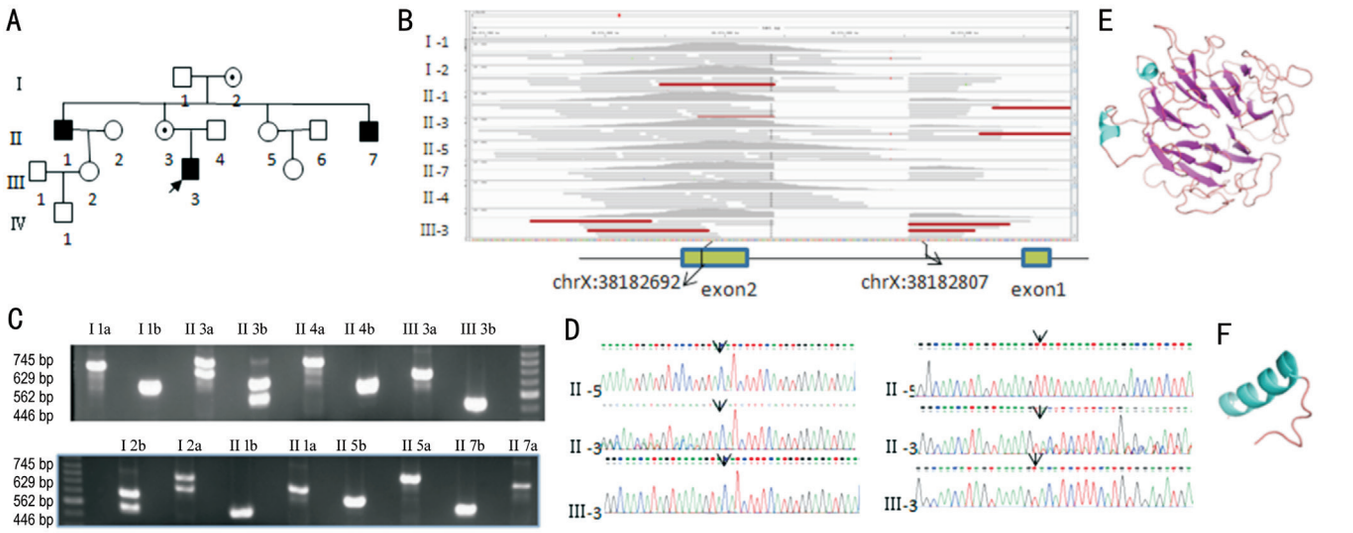

Figure 1 A Pedigree of RP family with a novel frame-shift mutation of the RPGR gene Black squares refer to affected male patients, unfilled circles or squares represent unaffected ones, and dotted circles represent female carriers. Arrow refers to the proband (B, D). Male patients (II-1, II-7, III-3) lack of read between chrX:38182692 and chrX:38182807. Gel electrophoresis of male patients show a single lane 116 bp deletion compare to normal persons. Female carrier show double bands. Wild type of RPGR 3D structure (E) was predict to severe truncated (F) by the gross deletion.

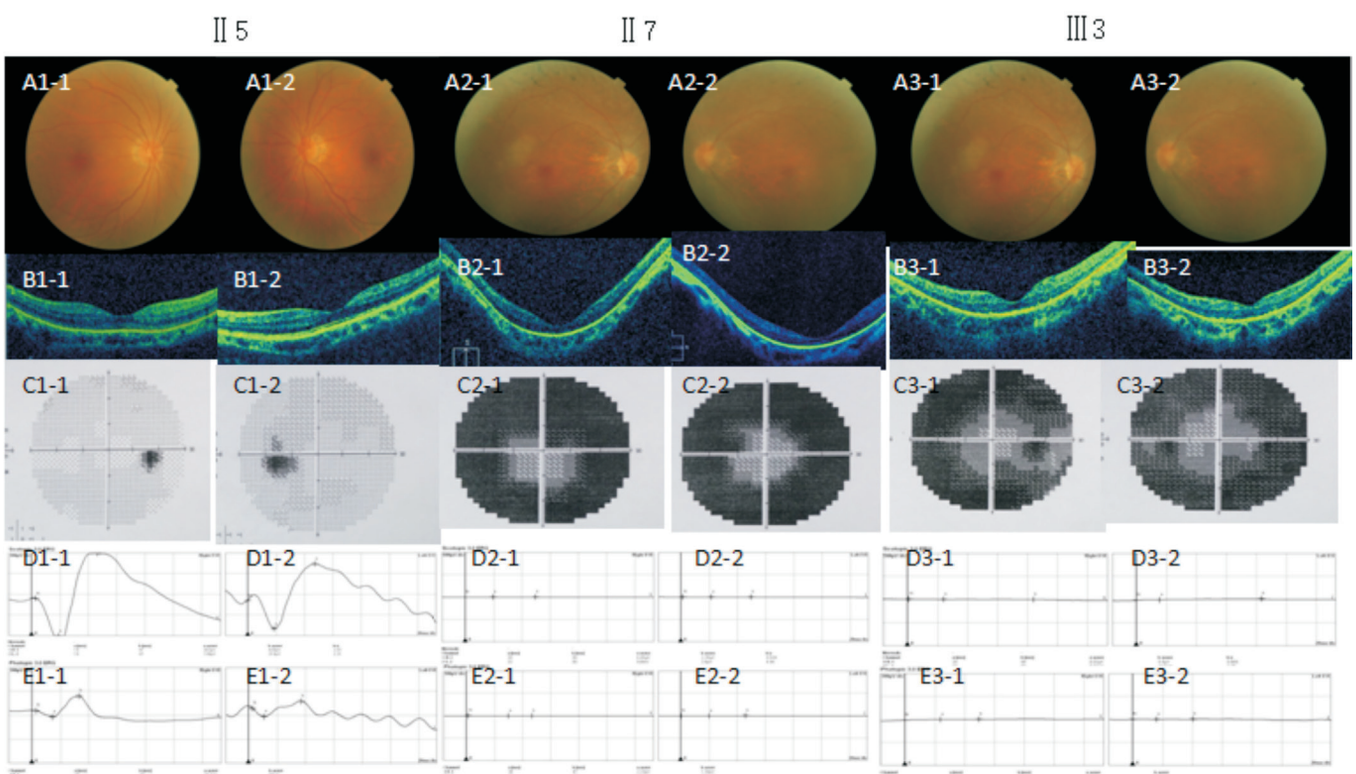

Figure 2 Fundus photographs of the one carrier case II-3 (A1-1, A1-2) and two patients case II-7 (A2-1, A2-2) and case III-3 (A3-1, A3-2) Case II-7 and case III-3 bilaterally show the typical sign of RP accompanied with myopia change; Case II-5 show no RP sign except for smaller and tilted optic disc with peripapillary atrophy on her left eye. SD-OCT examination of case II-5 (B1-1, B1-2), case II-7 (B2-1, B2-2) and case III-3 (B3-1, B3-2), reveals retinal thinning along with both ellipsoid band and outer segment atrophy. Myopic changes of posterior staphyloma were observed and more serious in case II-7. Computerized visual field test reveals a central reduction of retinal sensitivity and narrowing of the vision field in patients (C2-1, C2-2, C3-1, C3-2). No obvious abnormal is found on carrier (C1-1, C1-2). Full field f-ERG shows rod (D2-1, D2-2, D3-1, D3-2) and cone (E2-1, E2-2, E3-1, E3-2) response were extinguish on two patients , and carrier (D1-1, D1-2 E1-1, E1-2) was normal in ERG.

deletion in the RPGR gene detected by WES alignment data show male patients (II-1, II-7, III-3) lack of read between chrX:38182692 and chrX:38182807. Female carrier (I-2 and II-3) show heterozygous mutation, which lead to frame-shift

Table 1 General information of X-link RP family

ID	Age (y)	Sex	RP (phenotype)	Onset age	BCVA (Snellen), OD/OS	Refraction (SE), OD/OS	Axial length (mm), OD/OS
II1	59	M	Yes	6	20/400/20/400	-10.00/-9.75	26.90/26.77
II3	55	F	No	Normal	20/20/20/40	-1.25/-8.25	22.54/25.92
II5	53	F	No	Normal	20/20/20/20	-0.75/-0.50	23.24/23.18
II7	50	M	Yes	6	20/200/20/200	-9.25/-8.75	26.63/26.52
III3	32	M	Yes	6	20/60/20/60	-5.75/-6.50	25.46/25.58

RP: Retinitis pigmentosa; SE: Spherical equivalent; BCVA: Best corrected visual acuity. Due to poor health condition of I-1 and I-2, we only get the blood sample from them, no clinical data obtained.

mutation[12]. Gel electrophoresis of PCR using the primer pairs (Fw: 5'-CCAGTTTCTATTAGGTGGAAAAGA-3', Rv:5'-GACATCTACTTGGTGACAGCAGA-3' 745 bp and Fw: 5'-TGCTCTTGATGACTTATTTTGTGA-3', Rv:5'-AGATGACTGCCTTCCTTGTGA-3' 562 bp) to verify the deletion (Figure 1C). Male patients (II1a, II7a, III3a and II1b, II7b, III3b) show a single lane on 629 bp and 446 bp position, 116bp deletion compare to normal persons (I1a, II4a, II5a and I1b, II4b, II5b) at 745 bp and 562 bp respectively. Female carrier (I-2 and II-3) show double bands. The RawScore of CADD was 3.11, and the PHRED score of CADD was 23.9 (http://cadd.gs.washington.edu/info). The MutationTaster (http://www.mutationtaster.org/) predicts that this gross deletion was disease causing mutation. The frameshift mutation generates stop codon at 7 animo acid behind Asp (D10Afs*7), which would serious truncate RPGR protein[12]. When termination codons appear in the front part of an mRNA, the premature stop of translation would lead to the incomplete polypeptide chain, which elicit nonsense-mediated mRNA decay (NMD)[12]. Due to the mutation occurs in the early exonic positions, exonic splicing silencers ESS), exonic splicing enhancers (ESE) and the acceptor splice sites could be also affected by the mutation[13]. Besides, the deletion was neighbor to open chromatin region[14], which were the regions of spaced out histones, making them accessible to protein interactions and the histone modifications was relative to X inactivation[15]. The regulator of chromosome condensation 1 (RCC1) motif was also affected by this frame-shift mutation. The RCC1 motif was the conserved guanine nucleotide exchange factors and was responsible for nuclear assembly and regulation of spindle formation during mitosis, besides during interphase, the RCC1 motif participate in transport of macromolecules across the nuclear membrane[16-17]. This deletion appears as a hemizygous variant in the male probands and his two maternal uncles with RP, they were also affected by myopia. The proband's mother and grandmother were the carrier of this deletion, which was further confirmed by gel electrophoresis (Figure 1). There were double bands of carrier, the upper band was normal and lower band including the gross deletion. This deletion co-segregated with the RP phenotype in the pedigree. There was interesting phenomenon, bilateral myopia was present on the male patients with RP, but carrier female showed unilateral myopia without RP.

DISCUSSION

In the present study, we identified novel gross deletions of 116 bp (RPGR: c.29_113del) was co-segregated with the RP phenotype in the Chinese family. Khateb *et al*[18] had reported the genomic deletion of exon 2-5 which was expected to cause an in-frame deletion and lead to early onset RP with mild myopia in a Ashkenazi Jewish family, but with better BCVA compare to our study. The deletion of exon 2-3 for RPGR gene display a severe rod-cone pattern in first decade, but whether the patients accompanied by refractive error was not mentioned[19]. The previous reported deletion areas were overlap with the RPGR: c.29_113del but the breakpoint was different. Mutations located in exons 1-14 usually lead to rod-cone phenotype, and variants at the 3' end of ORF15 more opt to cause cone-rod dystrophy[20-21]. The patients in the family exhibited a rod-cone pattern of degeneration agrees with previously. The frame-shift mutations located in various exons of RPGR gene could show RP phenotype with occurrence of high myopia among the patients and heterozygous female carriers. The members of XLRP family was apt to affect by mild-to-high myopia[22], which indicating that there may be a specific correlation between RP and myopia[4]. XLRP male patients with RPGR gene mutation in ORF15 region in a higher frequency to suffer from myopia compare to those with mutation in exon 1-14[21,23-24]. But as for female carrier with mutations located at exon 1-14 was more likely to have high myopia and worse vision[4,25], was not due to retinal pigmentary changes[6]. The deletion of this Chinese family located in exon2 lead to frame-shift mutation from 10th Asp and generate stop codon at 7 animo acid behind Asp (D10Afs*7), which would serious truncate RPGR protein. The termination codons appear in the early part of an mRNA would generate the incomplete polypeptide chain, which elicit nonsense-mediated mRNA decay (NMD). NMD pathway participates in the regulation of X chromosome inactivation (XCI)[26-27], which

was considered as a biomarker of severity of clinical symptom in female carriers of RPGR-associated XLRP[28]. Besides, XCI was critical for the wide diversity of the expression of myopic traits both inter-individuals and inter-occulars[6,29]. In this RP recessive pedigree, a unique phenomenon was that, all male patients manifested refractive error was ranging from -6 D to -10 D, but the carrier females showed high myopic anisometropia with no photoreceptor dysfunction on electroretinogram. One possible explanation is skewed XCI may contribute to the dissimilar growth between eyes in female carrier. The N-terminal of RPGR protein contains the RCC1-like domain (RLD), which play a key role on RPGR protein interaction network[30]. The deletion was neighbor to the RCC1 motif and open chromatin region, which were the regions of spaced out histones, making them accessible to protein interactions. Besides, the stop codon generated by this frame-shift mutation was located at RLD region and expect to disrupt the RPGR protein interaction network, which was regards as a common feature of missense mutations in RLD region of RPGR[30]. But for the female carrier, the less interaction with endogenous factors means less mutant protein complex produced, which would be benefit for RPGR normal function, because one normal allele would fulfill the role of RPGR gene. The carrier in the study exhibit no RP symptom may be benefit from this. Although the mechanism for the association of RPGR mutations and high myopia is unknown, but whether the mutation elicit the XCI and the interaction network change should be pay more attention for further study.

In conclusion, our study identified a novel frame-shift mutation result from a large genomic deletion of RPGR: c.29_113del in a Chinese family, which is the pathogenic mutation for XLRP in recessive pattern. Meanwhile the deletion was related to myopia of male RP patient and no symptomatic carrier. These findings expand the genetic map of RP and provide a new site for genetic counseling. XCI result from NMD which was elicit by the deletion may contribute to symmetric myopia in male patients and asymmetric myopia in female carrier. Further study needed to verify the relationship between RPGR: c.29_113del and myopia from XCI view.

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