• Basic Research •

A novel mutation of *RPGR* in a Chinese family with X-linked retinitis pigmentosa

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Abstract

● **AIM:** To identify potential mutations and elucidate the clinical findings of male patients and female carriers of X-linked retinitis pigmentosa (XLRP) in a Chinese family.

● **METHODS:** A four generation pedigree was collected that consisted of 20 individuals. Genomic DNA was extracted from peripheral blood, and then the target fragments were amplified by PCR and sequenced directly. In addition, all affected patients and female carriers underwent comprehensively ophthalmic evaluation.

● **RESULTS:** A novel mutation c.2865G>A p.W955X in *RPGR* gene was identified of this family, including four affected individuals and eight carriers. All male patients, aging from 7 to 31y, tended to have more various, even potentially deleterious clinical features of RP. At the same time, individuals with heterozygous mutations (carriers) manifested a wide spectrum of clinical features. Herein, only two male patients and three female carriers manifested pathological myopia (PM). Among the female carriers, half of subjects who harbor poor visual acuity suffered esotropia or exotropia. Additionally, 16.7% and 66.7% of carriers had abnormal electroretinogram (ERG) and fundus, respectively.

● **CONCLUSION:** In this study, a novel mutation of the *RPGR* gene is identified, which broadens the spectrum of *RPGR* mutations, and elaborates the relationship between genotype and phenotype.

● **KEYWORDS:** X-linked retinitis pigmentosa; *RPGR*; nonsense mutation; phenotype

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INTRODUCTION

Retinitis pigmentosa (RP), is the most prevalent form of inherited retinal degeneration, which represent a spectrum of eye disorders that primarily resulted by retinal photoreceptor cells malfunctioning, and ultimately leading to blindness[1-2]. In common, at the initial stages of the disease, male patients exhibit nyctalopia. With progressive photoreceptor degeneration, visual field reduces gradually and eventually reaches blindness in midlife. According to previous reports, in general, there were between 1/3000 and 1/4000 cases of RP. Commonly, the mode of inheritance of this disease can be autosomal dominant (30%-40%), autosomal recessive (50%-60%) and X-linked manner (5%-15%)[3-4].

So far, more than 80 genes have been identified which are associated with RP, including 3 genes (*RPGR*, *RP2*, and *OFD1*) for X-linked retinitis pigmentosa (XLRP)[5-6]. Among all the related genes of XLRP, mutations account for over 70% in *RPGR* and approximately 8% to 15% in *RP2*, respectively[7-8]. Up to now, 261 mutaions including deletion translocation, point muations (frameshit, nonsence, splice site) and deep intronic mutations has been detected of *RPGR* (http://www.hgmd). What's more, the majority of variants occurr in the region of *RPGRORF15*[9-10]. Due to *de novo* mutations, variable expression and penetrance, even the manifesting of female carriers, it is a challenge to identify the underlying genetic defect and provide appropriate genetic counseling for the individuals with RP[11].

Herein, we reported a case of Chinese family, consisted of 20 numbers, in which 4 individuals with a novel hemizygous mutation c.2865G>A p.W955X in *RPGR* gene and 8 carriers

Table 1 Summary of primers of *RPGR* gene

Gene	Exon	Position	Length (bp)	Target primers (5'-3')
RPGR	1	chrX:38327185-38327503	319	F: GTGTGGAAGTCTCAGGATCGT
				R: AGGAGCTGTGGGAGGAAGAT
RPGR	2-3	chrX:38322783-38323655	873	F: TTAAACATTGCCAGAGTGGGG
				R: CATTAAAGAACTACACAGTCAACAT
RPGR	4	chrX:38320957-38321152	196	F: TGTCCTGGACTACTGTTCATTTTC
				R: AGCCACGTTACTGGAATGAGAC
RPGR	5	chrX:38318741-38319063	323	F: ATCGCTGCTATACACTGACCTG
				R: AGCAATGCTCCCTTCGGTTTA
RPGR	6	chrX:38317267-38317521	255	F: TTCAGAGCCTGGCTACCTTTTA
				R: AACAACATAGAAGTGGGAGATAACA
RPGR	7	chrX:38310564-38310839	276	F: GACGGTAAGACCAGCTTTTTGTTC
				R: TCATTAGCCACCACAGAACGC
RPGR	8	chrX:38304509-38304904	396	F: CCAGAGGCACTTAACCTTCACT
				R: GGAATTCCATTTTTCTCAGCCATTA
RPGR	9	chrX:38301096-38301491	396	F: CAAGGCAGAATTTGAGGGGGATA
				R: TGACATTTGGCTTTTAGGAAACAA
RPGR	10	chrX:38298808-38299272	465	F: AGAGAGATTCACCAAGCCAGT
				R: AAAGTTTGTTAGCACTCAACTCT
RPGR	11	chrX:38297163-38297578	416	F: AATGTTGTGGAGTGTTGGCAT
				R: TAGGCTCTAACCAGGGAGAGAA
RPGR	12-13	chrX:38290909-38291549	641	F: CTGTCCAGTTGCCTTTCACTTTT
				R: ACTTAAACTGCTCTCACCAACAAT
RPGR	14	chrX:38287711-38288111	401	F: AAAGTAGATAAGTTGTCCTTGTCA
				R: CCTTCTGACTGTGTCCTCCA
RPGR	15a	chrX:38286883-38287230	348	F: AGGAAGGAGCAGAGGATTCA
				R: CCCTCTTCTTCCATTCTTCC
RPGR	15b	chrX:38286527-38286970	444	F: GGGGAGAAAGACAAGGGTAG
				R: TCCTTTCCCCTCCTCTACTT
RPGR	15c	chrX:38285654-38286635	982	F: GGAAGAAGGAGACCAAGGAG
				R: CCCATTTCCCTGTGTGTTAG
RPGR	15d	chrX:38285348-38285762	415	F: GCAGGATGGAGAGGAGTACA
				R: GAGAGAGGCCAAAATTTACCA

F: Forward; R: Reverse.

were investigated. Based on genetic analysis, pedigree analysis and comprehensively clinical features, we characterized the phenotypic manifestation associated with the mutation.

SUBJECTS AND METHODS

Ethical Approval This study observed the tenets of the Declaration of Helsinki. And it was approved by the Ethics Committee of Children's Hospital of Hebei Province. Twenty participating individuals agreed study and signed informed consent.

Clinical Data The family, which is a 4 generation pedigree, comprised 4 affected individuals (Figure 1). The proband IV-1 and family members (II-5, III-3, III-5, III-8, III-9, IV-2, IV-3, IV-4, IV-5, IV-6), totally 11, underwent a complete ophthalmic examination. It includes best-corrected visual acuity (BCVA), slit lamp biomicroscopy, intraocular pressure (IOP), cycloplegic refraction by compound tropicamide, detailed fundus photography, full-field electroretinogram (ERG) according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards and optical coherence tomography (OCT), that were performed by ophthalmologists.

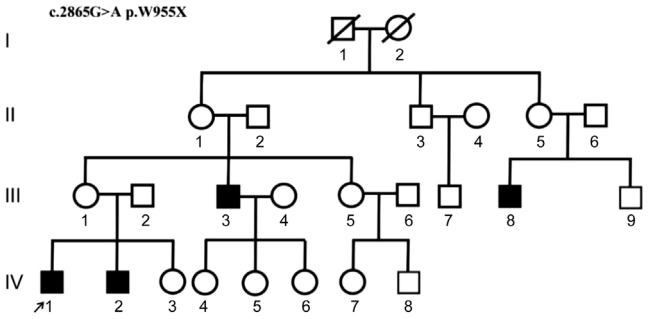

Figure 1 Pedigree of the Chinese family with X-linked retinitis pigmentosa Square: Male; Circle: Female; Arrow: Proband.

DNA Extraction and Sequence Peripheral blood sample of 20 subjects was collected into sample tube which contains ethylenediamine tetraacetic acid (EDTA). Genomic DNA was extracted from peripheral blood leukocytes. Primers were designed from GenBank (OMIM 312610) and adopted the published exon 15 sequence (Table 1)[5]. The reaction mixture was set up with high fidelity Taq polymerase (Invitrogen, USA). Cycling conditions of the PCR were conducted as

follows: (95□ 30s, 57.5□ 45s, 72□ 45s) ×32 cycles, 72□ 5min (Bio-Rad, USA). The products were sequenced by company (TSINGKE, China) and visualized by the Finch TV software (Geospiza, USA). The presence of the mutations in *RPGR* gene was assessed by comparing the patient's sequence with the reference sequence (Figure 2).

Statistical Analysis An analysis of the data using SPSS18.0 software was carried out. Two-tailed Student's *t*-test was used to determine whether there were significant differences between two groups. The criterion for statistical significance was $P<0.05$.

RESULTS

Verification of a Novel Mutation of *RPGR* As a result of the detailed family history and the clinical presentation of the patients, an X-linked genetic defect was inferred. Hence, direct Sanger sequence was conducted to verify the possible disease-causing mutations of *RPGR* and *RP2* (data not shown). In the event, no variants were identified, whole exome sequencing (WES) would be conducted. Fortunately, a novel mutation (c.2865G>A p.W955X) of *RPGR* was identified in the proband, which was not reported in either public databases. What's more, mutated individuals and carriers can be classified separately according to the same criteria.

In *RPGR*, there is a novel nonsense mutation W955X (c.2865G>A) that leads to premature termination codons, resulting in truncated proteins.

Clinical Presentation of Affected Male Subjects Among 20 participating individuals, 4 affected patients with hemizygous mutation and 8 carries with heterozygous mutation were confirmed eventually (Table 2). Unfortunately, it's difficult to obtain the clinical data of II-1 and III-1, because of living so far away. There was, however, a detailed medical history that had shown signs of visual dysfunction of RP.

The patient (IV-1), a 10-year-old boy, he exhibited typical manifestations of RP. Such as a significant decline in visual acuity (OD: +0.25 DS, -0.75 DC×5°=0.4; OS: -0.50 DS, -1.50 DC×170°=0.4), with initial symptoms being nyctalopia, which presented in the decade of his life. Bilateral fundus changes revealed tilted optic disc, optic disc drusen, myopic maculopathy, macular atrophy, even hyperpigmented deposits in the periphery. There was a drastic reduction in the amplitude of rods and cones on ERGs. During OCT imaging, it was a common to see the degeneration and loss of the outer retinal bands in the peripheral retina, especially in regions near ellipsoid zone (EZ) as well as the inner/outer segments (IS/OS), while relative structure and function was conserved sparing of the central macula (Figure 3C).

Meanwhile, the identical mutation was confirmed in his consanguineous brother (IV-2). He showed poor visual acuity, with mild myopia and myopic astigmatism (OD: -2.50 DS, -2.5 DC×5°=0.3; OS: -2.5 DS, -2.50 DC×175°=0.3). Myopic maculopathy, Bull's eye, attenuated arteries and bone-spicule hyperpigmentation in retina of fundus were also discovered beginning in the first decade, accompanied by obviously flat ERG and atrophy of outer retinal bands at the (para)fovea of OCT (Figure 3D).

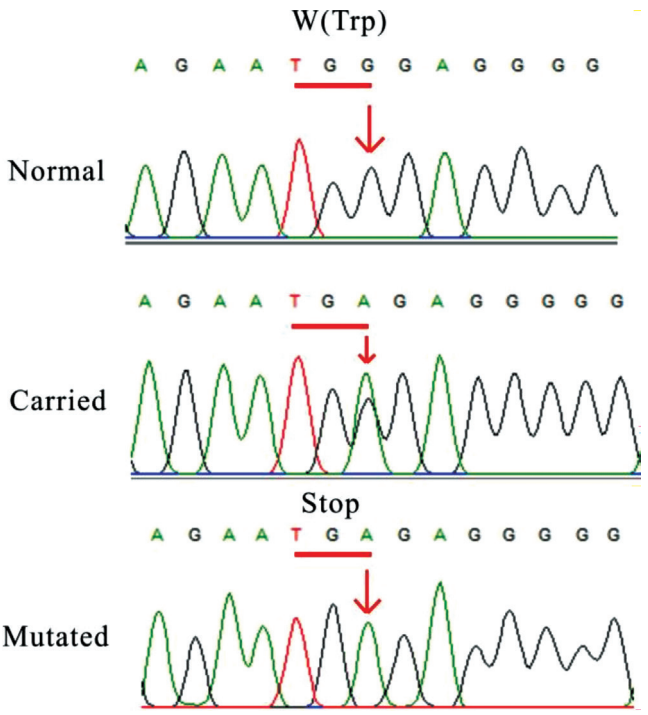

Figure 2 Sanger sequence of *RPGR* of participants in a Chinese family Vertical arrows indicate the mutation site in sequencing chromatograms. The mutaion (c.2865G>A p.W955X) was confirmed by sanger sequence in all patients (III-3, III-8, IV-1, and IV-2) and heterozygou variant in carriers (II-1, II-5, III-1, III-5, IV-3, IV-4, IV-5, IV-6).

Individual III-3, available records of the oldest of patients were found at the age of 31. Initial symptoms include poor visual acuity (BCVA OD: 0.4; OS: 0.3), which typically appears in the third decade of life, compared to others. He diagnosed with RP and displayed typical disease characteristics like bone-spicule pigment deposition. Temporal yellow dots in fundus and flat or barely extinguishing ERG were also found. A significant alteration in the organization of the retina, characterized by multiple low reflective cystic spaces and distortion of layers, was also seen in both eye by OCT (Figure 3A). Meanwhile, the results of III-8 ophthalmic examination showed poor visual acuity (OU 0.4) and ametropia (OD: -5.00 DS, -0.75 DC×180°; OS: -4.75 DS, -0.75 DC×180°). The funduscopic, structural and functional changes were also detected, consist with affected individuals (Figure 3B). Exams of both eyes of patients were normal in terms of intraocular pressure and anterior segment (Table 2).

Clinical Presentation of Female Carriers Combined with their family history, genetic testing and detailed ophthalmic

Table 2 Clinical characteristics of individuals in the Chinese family

Patient ID	Type	Age	Gender	IOP (mm Hg)	Refraction	BCVA	Macular/retinal appearance	ERG/OCT findings	Other complications
II-5	Carried	35	Female	16/18	OD: +0.50 DS, -0.50 DC×90°; OS: +0.50 DS	OD 0.5, OS 1.0	Widespread loss of RPE and tessellated fundus	NA; thinning stratification of outer layers	Esotropia
III-3	Mutated	31	Male	8/10	OD: +1.25 DS, -1.25 DC×90°; OS: +1.00 DS	OD 0.4, OS 0.3	Normal vessels, bone-spicule hyperpigmentation	Lowness of amplitude; atrophy of retinal layers, bilateral cystoid macular edema and multiple low reflective cystic spaces in both eyes	NA
III-5	Carried	28	Female	20/20	OD: +0.25 DS; OS: +0.5 DS	OU 1.0	Widespread loss of RPE	NA	NA
III-8	Mutated	12	Male	12/13	OD: -5.00 DS, -0.75 DC×180°; OS: -4.75 DS, -0.75 DC×180°	OU 0.4	Optic disc pallor, drusen, attenuated vessels, macular atrophy	Lowness of amplitude; bilateral cystoid macular edema and degeneration of the outer retinal layers	Myopia
III-9	Normal	12	Male	17/18	OD: -3.5 DS, -0.50 DC×180°; OS: -3.5 DS, -1.25 DC×180°	OD 1.0, OS 0.8	NA	NA	Astigmatism
IV-1	Mutated	10	Male	19/19	OD: +0.25 DS, -0.75 DC×5°; OS: -0.50 DS, -1.50 DC×170°	OD 0.4, OS 0.4	Bull's eye, tessellated fundus,bone-spicule pigmentation	Lowness of amplitude; bilateral cystoid macular edema, atrophy of outer retinal layers	Myopia, astigmatism
IV-2	Mutated	7	Male	17/17	OD: -2.50 DS, -2.5 DC×5° OS: -2.5 DS, -2.50 DC×175°	OD 0.3, OS 0.3	Tilted optic disc, macular atrophy, hyperpigmented deposits	Lowness of amplitude; degeneration of the outer retinal layers	Myopia astigmatism amblyopia
IV-3	Carried	12	Female	16/17	OD: +1.0 DS, -0.50 DC×180°; OS: +1.25 DS, -0.75 DC×180°	OU 0.8	NA	NA	NA
IV-4	Carried	10	Female	19/18	OD: -5.0 DS, -5.0 DC×5°; OS: -5.5 DS, -6.5 DC×5°	OU 0.5	Retinal atrophy, attenuated retinal vessels, pathological fundus of myopia	Lowness of amplitude, disordered the outer retinal bands	Myopia, exotropia
IV-5	Carried	8	Female	14/15	OD: +1.25 DS; OS: -0.75 DS, -0.5 DC×175°	OD 0.8, OS 0.4	Large discs with a C/D ratio of 0.6	NA	Myopia anisometropia amblyopia
IV-6	Carried	6	Female	18/17	OD: -0.5 DS, -2.75 DC×180°; OS: -1.5 DS, -2.00 DC×180°	OD 0.6, OS 0.4	Widespread loss of RPE	NA	Myopia astigmatism amblyopia

IOP: Intraocular pressure; OD: Right eye; OS: Left eye; OU: Binocular; DS: Spherical diopter; DC: Cylinder diopter; BCVA: Best-corrected visual acuity; NA: Not avaliable; RPE: Retinal pigment epithelium; ERG: Electroretinogram; OCT: Optical coherence tomography; C/D: Cup to disc ratio.

examination were performed to confirm *RPGR* mutation carrier status. Then we looked into the clinical features of carriers. In summary, individuals with heterozygous mutation had various symptoms, ranging from completely normal condition to slight or mild retinal changes to obvious complaints. Compared to 4 male patients (III-3, III-8, IV-1, IV-2), the visual function of 6 female carries (II-5, III-5, IV-3, IV-4, IV-5, IV-6), was much better. All female carriers (age range from 6 to 35y), had a minimum of 20/50 vision in one eye (BCVA ranged from 0.4 to 1.0). Three female carriers (IV-4, IV-5, IV-6) suffered from myopia, which the power of spherical equivalent (SE) range was -1.0 to -8.75 diopters (D). What's more, one of the female carriers (IV-4) had high myopia (SE OD -7.5 D, OS -8.75 D). Anisometropia, denoted by >1.5 D of spherical degree or >1.0 D of cylindrical degree, was observed in 2 female carriers (IV-4, IV-6). In addition, half of female carriers (II-5, IV-4) who harbor poor visual acuity suffered esotropia or exotropia, this might also be a factor to influence BCVA.

Remarkably, it was noting that a total of 66.7% (8 of 12 eyes) of eyes of female carriers exhibited patchy pigment clumping in the peripheral retina, however, in 4 of the 12 eyes with carriers, the fundus appeared fairly normal (Figure 4). Additionally, there was a dramatic reduction in rod and cone amplitude for affected males, while 83.3% of female carriers were relatively stable, within a relatively normal range (Figure 5). What's more, 2 female carriers subjects (33.3%) had abnormal OCT findings, such as slight disruption of IS/OS or thinning of the outer retinal layer. Negative traits of OCT were detected from 4 carriers (III-5, IV-3, IV-5, IV-6). The detailed clinical features are summarized in Table 2.

DISCUSSION

The development of DNA sequencing technology has made genetic testing more accessible to patients. It is conventional that scholars are passionately interested in next-generation sequencing (NGS) to explore the novel causative gene even unknown mutations[12-13]. Surely, it offers great benefits to diagnosis. At the same time, it also has limitations, such as long time consuming, high costing and false error. Herein, we used the direct Sanger sequence and it was reliable and cost-effective to focus on the hot point of mutation. History and clinical characteristics had further revealed the true identity of the disease. As a consequence, the result forcefully suggested that the direct sequence is still an effective detection method of special diseases.

Based on mutagenesis analysis, an exon 15 mutation in *RPGR* gene has been identified. The gene, which is located on the X

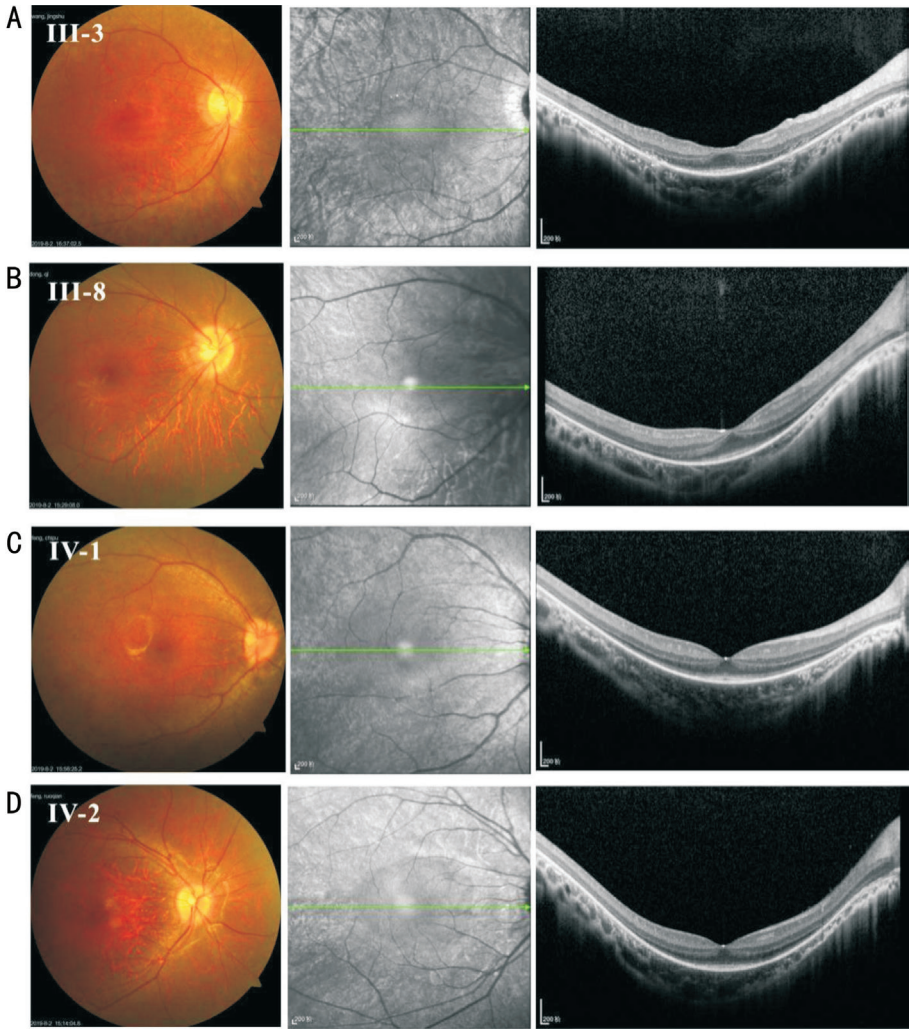

Figure 3 Photographs of the fundus and optical coherence tomography images of *RPGR*-mutated patients A: Bone-spicule hyperpigmentation, retinal arteriole attenuation in fundus and multiple low reflective cystic spaces and distortion of outer retina in OCT; B: Pathologic myopia changes in fundus and degeneration of outer segment in OCT; C: Tilted optic disc, optic disc drusen, attenuated retinal vessels and hyperpigmented deposits in fundus and atrophy of retinal layers in OCT; D: A Bull's eye appearance, bone-spicule-like deposits in fundus and degeneration across the peripheral retina in OCT.

chromosome and approximately accounts for 5%-15% of all cases of RP[3]. Commonly, multiple isoforms of RPGR were detected in functional performance. The RPGR^{ORF15} is one of isoforms, which consists of 15 exons coding 1152-aa protein, enriches in retina and is thought to facilitate the connecting and trafficking of rod and cone photoreceptors[14-15]. Previous analysis revealed that the RPGR^{ORF15} variants are associated with significantly dysfunction of retinal defects, ranging from RP to cone-rod or macular degeneration[16]. In the present study, nearly all the patients suffered from visual disturbance and tended to be worse with increasing age. That was consistent with previous reports[17].

Meanwhile, it is noting that ORF15, which is the terminal exon of the *RPGR* containing repetitive glutamic acid and glycine-rich sequence, the product that is considered to assist in regulating molecules between the inner and outer segments[9,18]. Breuer *et al*[19] described that the special sequence region creates a mutational hotspot, the incidence rate has reached 60% roughly, and majority of these occurs in the region between codons 801 and 1070. The novel mutation in this study was further validating the region of high mutation.

Some scholars believed that patients with XLRP who have frameshift mutations or nonsense mutations within exons 1 to 14 may develop severe clinical features, it is possibly because of this reason that truncated protein from the RPGR^{ORF15} variant transcript interacts relatively well with other proteins[20]. However, in the current study, not only mutated males but also some carried females with the W955X (c.2865G>A) mutation had a more significantly severe visual impairment, more varied fundus (including tilted optic disc, bone-spicule pigment deposition, retinal arteriole attenuation and optic disc pallor, even retinal atrophy), faster loss of ERG amplitudes and more thinning of OCT than patients with the mutations in exon 1 to 14 that described in some past reports[20-21]. In summary,

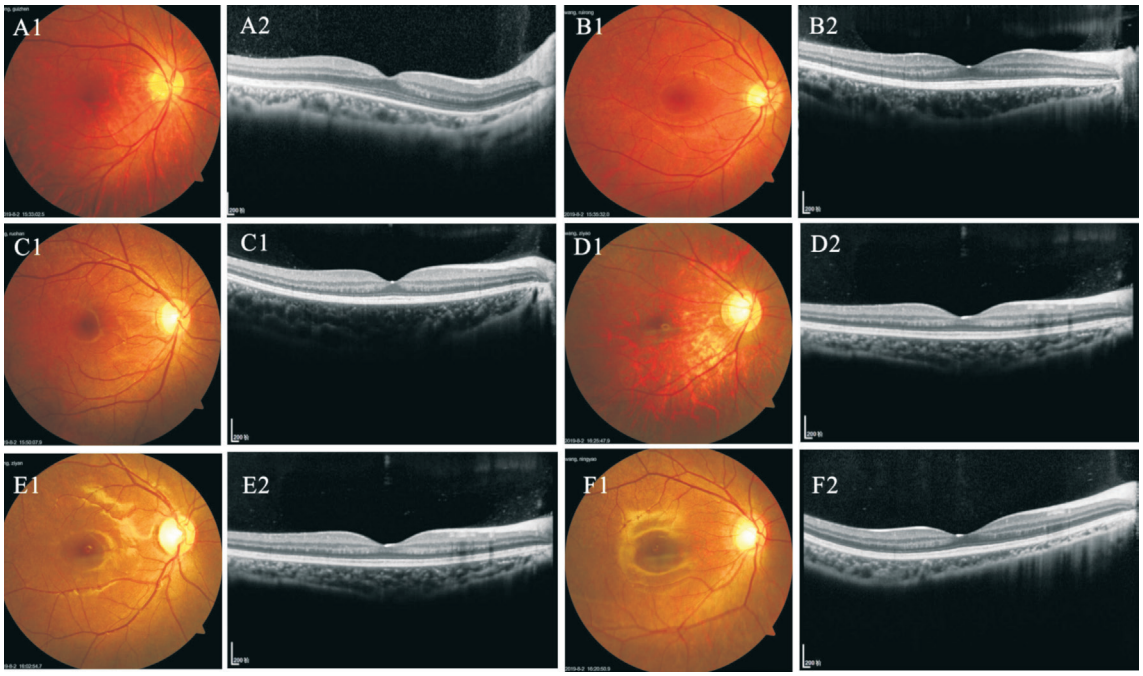

Figure 4 Color fundus photographs and optical coherence tomography images of female carriers A: The fundus of II-5 showed bone-spicule hyperpigmentation in the retina and thinning stratification of outer layers was detected by OCT; B: III-5's fundus showed widespread loss of RPE and normal layers in OCT; C: IV-3's fundus and OCT performed normal; D: IV-4 showed retinal atrophy, attenuated retinal vessels and tessellated fundus. OCT showed slightly atrophy of retinal layers. E: IV-5's fundus showed large discs with a C/D ratio of 0.6 and normal in OCT; F: Bone-spicule-like deposits were observed in fundus of IV-6's right eye.

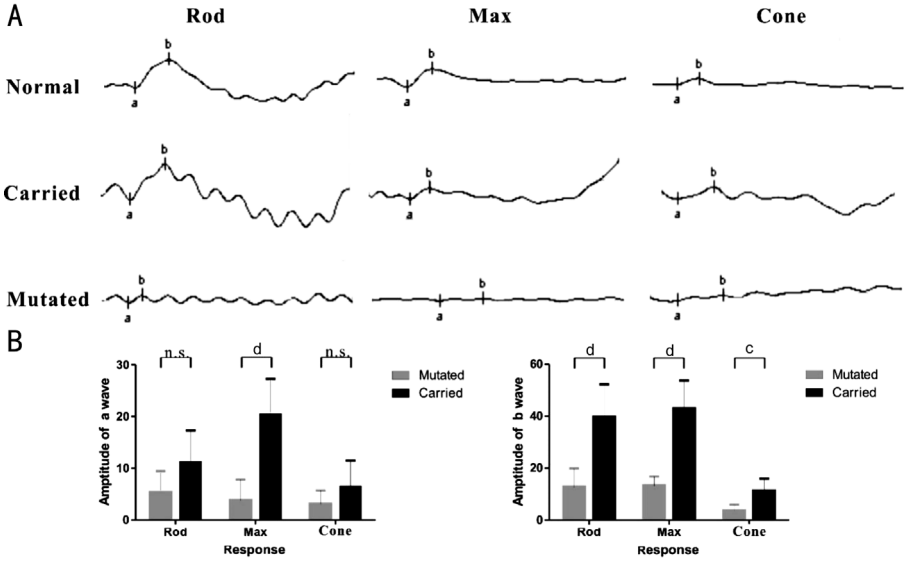

Figure 5 Electroretinography (ERG) of the affected, carried and normal individuals A: As for III-9, a 12-year-old boy, the amplitudes of a-wave and b-wave, that response to scotopic rod-specific, maximal and photopic cone-specific ERG, were 4.01 and 71.79 μV, 42.48 and 92.37 μV, 0.24 and 15.15 μV, respectively. III-5 carrying heterozygou mutation, the amplitudes of a-wave and b-wave were 22.22 and 25.61 μV, 1.67 and 48.98 μV, 15.19 and 19.25 μV. IV-1, it showed 3.92 and 8.72 μV, 6.61 and 14.35 μV, 2.54 and 6.57 μV, respectively. B: The amplitudes of a-wave and b-wave for four affected individuals (III-3, III-8, IV-1, IV-2, n=4) and five carried individuals (II-5, III-5, IV-3, IV-4, IV-5, IV-6, n=6). In rod and cone system, the amplitude of b wave was frequently and severely reduced, while the amplitude of a wave was not readily apparent. $^{c}P<0.05$, $^{d}P<0.01$.

we found a mutation in the RPGR^{ORF15} region that may have potentially more deleterious clinical features.

According to previous researches, the mutational hotspot of the RPGR^{ORF15} has been reported to cause pathological myopia (PM) in Asians almost exclusively[22-23]. Zhang *et al*[5] even suggested that PM appears may be a distinct phenotype that associated with ORF15 nonsense mutations (c.2833G>T p.E945X). There is a possibility that the rehabilitation of cell

degeneration may be involved in PM in XLRP patients[24]. In comparison, patients with RP manifested a refractive error ranging from +1.0 DS to -5.0 DS (media -2.22 D), in which the percent of subjects with moderate myopia is 50%, without high myopia. Meanwhile, myopia was also performed in normal individuals (III-9) and female carriers (IV-4, IV-5, IV-6), in which just only one carrier (IV-4) has moderate myopia and severe astigmatism. In a word, the incidence of PM in affected male and female carriers was lower than ever reports[25]. Except for myopia, esotropia and exotropia are also obvious association with BCVA. In this study, BCVA deterioration of affected subjects did not correlate with increasing of age, that conclusion was disagree with previous report[17]. Small sample is to a great extent a limitation of this study.

A cone-rod or cone dystrophies due to mutations in the *RPGR* gene, in which a truncation mutant tends to override endogenous RPGR proteins to affect photoreceptor function. Consistent with previous notion, multiple cystics and distortion of outer layers (external limiting membrane, EZ and IS/OS) were detected in the peripheral retina of affected male subjects, with relative structure of IS/OS line sparing of the macula. In terms of ERG, it showed minimal cone response and extinguished rod response. The results of all those revealed extensive loss of photoreceptors, especially in the peripheral retina rather than the macula, which could account for the remaining visual acuity and flat ERG response.

Compare with 4 patients, carriers showed a wide range of clinical features. ERG and fundus showed abnormalities in 16.7% and 66.7% of female carriers, respectively, which is lower than previous reports[16-17]. Additionally, normal layers of OCT were also more apparent in female carriers. Variability of phenotypic severity in female carriers is attributed to a variety of factors, such as age, environment, skewed X-activation, and genetic modifiers[26]. As discussed above, RP exhibits a higher degree of phenotypic heterogeneity and variability. Nanda *et al*[27] advocated female carriers who suffer from severe visual impairment and display a retinal phenotype should be considerate to process therapeutic intervention. Specifically, phenotypic follow-up of female carriers must be conducted for a longer period of time[28]. Hence, it's significant to discover novel RP mutations, generalize phenotype and assess the process, so that patients can undergo genetic therapy treatment timely[29-30].

In summary, we reported the clinical features of individuals with RP and carriers caused by a novel mutation (c.2865G>A p.W955X) in *RPGR*, and broadened the spectrum of mutations, which is valuable for provide future genetic counselling and specific gene therapy.

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Authors' contributions: Sun HH and Chen L conceived the idea; Sun HH, Zhao JC, Yang SL, Shi JD, and Wei YS collected clinical message and performed the experiments; Sun HH, Wang JC performed data analyses; Sun HH, Gu F and Chen L wrote the manuscript. All authors approved the manuscript.

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