A novel mutation of \textit{RPGR} in a Chinese family with X-linked retinitis pigmentosa

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Abstract

\textbf{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.

\textbf{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 opthalmic evaluation.

\textbf{RESULTS:} A novel mutation c.2865G>A p.W955X in \textit{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.

\textbf{CONCLUSION:} In this study, a novel mutation of the \textit{RPGR} gene is identified, which broadens the spectrum of \textit{RPGR} mutations, and elaborates the relationship between genotype and phenotype.

\textbf{KEYWORDS:} X-linked retinitis pigmentosa; \textit{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\cite{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%)\cite{3-4}. So far, more than 80 genes have been identified which are associated with RP, including 3 genes (\textit{RPGR}, \textit{RP2}, and \textit{OFD1}) for X-linked retinitis pigmentosa (XLRP)\cite{5-6}. Among all the related genes of XLRP, mutations account for over 70% in \textit{RPGR} and approximately 8% to 15% in \textit{RP2}, respectively\cite{7-8}. Up to now, 261 mutations including deletion translocation, point mutations (frameshit, nonsence, splice site) and deep intronic mutations has been detected of \textit{RPGR} (http://www.hgmd). What’s more, the majority of variants occur in the region of \textit{RPGRORF15}\cite{9-10}. Due to \textit{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\cite{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 \textit{RPGR} gene and 8 carriers
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.

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)

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

Individual III-3, available records of the oldest of patients were found at the age of 31. Initial symptoms include poor visual acuity (BCV A 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 fundusscopic, 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
An **RPGR** mutation in a Chinese family with XLRP

**Table 2 Clinical characteristics of individuals in the Chinese family**

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

**IOP:** Intraocular pressure; **OD:** Right eye; **OS:** Left eye; **OU:** Binocular; **DS:** Spherical diopter; **DC:** Cylinder diopter; **BCVA:** Best-corrected visual acuity; **NA:** Not available; **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 carriers (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. 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
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\textsuperscript{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\textsuperscript{[14-15]}. Previous analysis revealed that the RPGR\textsuperscript{ORF15} variants are associated with significantly dysfunction of retinal defects, ranging from RP to cone-rod or macular degeneration\textsuperscript{[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\textsuperscript{[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\textsuperscript{[9,18]} Breuer et al\textsuperscript{[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\textsuperscript{ORF15} variant transcript interacts relatively well with other proteins\textsuperscript{[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\textsuperscript{[20-21]}. In summary,
we found a mutation in the RPGR<sup>ORF15</sup> region that may have potentially more deleterious clinical features.

According to previous researches, the mutational hotspot of the RPGR<sup>ORF15</sup> has been reported to cause pathological myopia (PM) in Asians almost exclusively<sup>[22-23]</sup>. Zhang et al<sup>[4]</sup> 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. 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. 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. 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. EM and fundus showed abnormalities in 16.7% and 66.7% of female carriers, respectively, which is lower than previous reports. 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. As discussed above, RP exhibits a higher degree of phenotypic heterogeneity and variability. Nanda et al 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. Hence, it’s significant to discover novel RP mutations, generalize phenotype and assess the process, so that patients can undergo genetic therapy treatment timely.

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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