A novel de novo frameshift mutation of RPGR ORF15 is associated with X-linked retinitis pigmentosa in a Chinese family

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Purpose: To identify the genetic basis of disease in a Chinese family with retinitis pigmentosa (RP).

Methods: Linkage analysis was performed for 15 family members in the RP family using microsatellite markers flanking candidate genetic loci for known autosomal dominant RP (adRP) and markers covering the entire X chromosome by every 10 cM. To screen for a mutation causing RP, PCR and DNA sequence analyses of the complete coding region (including ORF15) and exon-intron boundaries of the retinitis pigmentosa GTPase regulator (RPGR) gene associated with X-linked RP (xIRP) were carried out for the proband in the RP family. After the mutation was identified, direct DNA sequence analysis was performed for all 15 family members and 101 controls to determine whether the mutation co-segregated with RP in the family and whether it was present or absent in the controls.

Results: Linkage analysis excluded all known adRP loci. However, positive linkage was identified with two markers on the X chromosome, DXS993 and DXS1068, where the RPGR gene is located. Direct DNA sequence analysis revealed a hemizygous mutation, g.ORF15+1166delA (c.2919delA), in affected males. The deletion results in a frameshift leading to early termination of RPGR. The g.ORF15+1166delA mutation arose de novo and co-segregated with all male patients, but was not present in normal family members and 101 controls. The clinical features of the mutation carriers showed intrafamilial variability.

Conclusions: The novel g.ORF15+1166delA mutation of RPGR causes X-linked RP in a four generation Chinese family. The deletion arose de novo. An interesting feature of mutation g.ORF15+1166delA is that it was associated with RP in all hemizygous males and four of five heterozygous female carriers in the Chinese family. These results revealed the broader xIRP genotypic and phenotypic spectrum of RPGR mutations.

X-linked retinitis pigmentosa (xIRP, OMIM 268000) is a severe form of inherited progressive retinal degeneration, which is characterized by night blindness, constriction of the visual field, reduced visual acuity, and eventually complete blindness. To date, six xIRP loci, including RP2 (OMIM 312600), RP3 (OMIM 312610), RP6 (OMIM 312612), RP23 (OMIM 300424), RP24 (OMIM 300155), and a new locus RP34 (OMIM 300605) on chromosome Xq28, have been found [1-6]. The genes for RP2 and RP3 [7,8] have been cloned. RP3 accounts for more than 70% of xIRP cases [3], but mutations of the original RP3 gene, retinitis pigmentosa GTPase regulator (RPGR) were found only in 10%-27% of families with xIRP [7,9,10]. Vervoort et al. identified a new RPGR exon called open reading frame 15 (ORF15, Genbank accession number AF286472) that encodes a 567 amino acid polypeptide with a repetitive domain rich in glutamic acid residues [11]. They found that 80% of RPGR mutations occurred in ORF15, and suggested that exon ORF15 of RPGR was a mutation hotspot [11]. Several follow-ups studies identified additional new disease-causing mutations within ORF15 [12-15].

It is interesting that some mutations in ORF15 cause typical RP, but others cause cone dystrophy, cone-rod dystrophy or atrophic macular degeneration [16-19]. ORF15 mutations were found not only in the families with X-linked recessive RP [11,12,14,20], but also in incomplete X-linked dominant RP families [21-25]. The previously-reported RP15 family described as having X-linked cone-rod degeneration [26] was re-mapped to the RP3 region, and a causative de novo insertion in ORF15 was identified [23]. Affected females in this family showed variable and milder expressivity of the disease. A female who had been previously described as unaffected and carrying the heterozygous mutation was later diagnosed as affected due to fundus changes and abnormal results on electroretinography [23]. Rozet et al. reported nine ORF15 mutations in 14 dominant xIRP families. Within these families, the onset of disease in the females from nine previously reported families [27] was delayed. The female patients some-
times showed milder symptoms than hemizygous males, but some female carriers from additional five families were as severely affected as male patients [24].

In the present study, we identified a seemingly dominant RP family. Both females and males were affected and there was variation in age of onset and severity in clinical symptoms. By using linkage analysis and mutation detection, we found a novel 1 bp deletion in ORF15 of RPGR on the X chromosome. Our data broaden the genotypic and phenotypic spectrum of RPGR mutations.

METHODS

Study subjects and clinical evaluations: A four generation Chinese RP family was identified and characterized in Tiantai, Zhejiang Province of China (Figure 1 and Table 1). Eight affected family members were identified and clinically evaluated, and the medical records of two deceased unaffected family members (I:1 and I:2) were also reviewed. Both males and females were affected in this family, which suggests a dominant inheritance pattern. However, there was no evidence of male-to-male transmission in the family to exclude the X-linked dominant inheritance pattern. A full medical history was obtained for each participant in the family. We recruited 101 general healthy individuals from the Chinese Han ethnic population to serve as controls, including 42 females and 59 males at the age of 19 to 35 years.

After informed consent was obtained, blood samples (3 ml/participant) were drawn from family members and controls for DNA extraction. Genomic DNA was prepared with the DNA isolation kit for mammalian blood (Roche Diagnostics). Genotyping was performed with markers at the locus confirmed this positive linkage result and age to RP in this family. Further haplotype analysis with other markers on the X chromosome mapped the disease locus distal to DXS1226 and proximal to DXS993 as the flanking markers for the disease interval. The RP3 gene, RPGR, is located between markers DXS1226 and DXS993, and became a strong candidate gene for RP in this family (Figure 1).

RESULTS

The proband and other affected males in the family displayed night blindness and/or high myopia as their initial symptoms of RP. The proband in the family showed waxy-pale discs and bone spicule-like pigment clumping from peripheral retina to the middle and macular region. He had obvious attenuation of retinal vessels, and his visual acuity was progressively decreased over two years to hand movement at a distance of 30 cm (Figure 2). Male family members displayed similar clinical features of RP with onset of night blindness in the second decade or later, followed by progressive loss of visual acuity. III:1 and III:4 declined to finger counting in about their fifth decade. The initial RP symptom in affected females was myopia that appeared at the different age of 13 to 35 years (Table 1). Symptom severity for all affected individuals showed high intrafamilial variability (Table 1).

Genetic linkage analysis excluded the 14 known adRP genes as the disease-causing gene in this family. Linkage analysis was carried out with markers spanning the entire X chromosome. Marker DXS1214 in Xp11.4 showed positive linkage to RP in this family. Further haplotype analysis with other markers at the locus confirmed this positive linkage result and defined DXS1226 and DXS993 as the flanking markers for the disease interval. The RP3 gene, RPGR, is located between markers DXS1226 and DXS993, and became a strong candidate gene for RP in this family (Figure 1).

All coding exons of RPGR were then screened for a mutation in affected family members. A 1-bp deletion at the 1166th nucleotide of exon ORF15 (Genbank accession number AF286472) of RPGR (g.ORF15+1166delA) was identified. This mutation results in a frameshift that truncates the RPGR protein by 65 amino acid residues at the C-terminus and replaces them with an aberrant 113 amino acid polypeptide enriched in lysine (Figure 3). The g.ORF15+1166delA mutation was identified in all male RP patients, but was not found in 101 normal controls. The mutation was also present in four female family members with clear diagnosis of RP (II:2, III:2, III:6, IV:5 in Figure 1). IV:2, a female family member who also carried the deletion, did not present the RP phenotype, although she did have myopia.

DISCUSSION

In this study, we investigated a Chinese family, who presented with a range of clinical severity of RP. By linkage analysis, we excluded all known adRP loci on autosomes. However, further genotype and haplotype analysis with microsatellite markers on the X chromosome mapped the disease locus distal to marker DXS1226 and proximal to DXS993, where a major
xIRP disease gene, RPGR, is located. Direct DNA sequence analysis of 19 RPGR exons in the proband did not reveal any causative mutation, but a g.ORF15+1166delA mutation was detected in ORF15 of RPGR. The deletion was present in all affected members. When compared with wild type RPGR, the g.ORF15+1166delA mutation was expected to cause a severe alteration in the predicted protein, including truncation of 65 C-terminal amino acid residues and the addition of an aberrant RPGR C-terminus enriched in lysine residues. These results strongly suggested that the g.ORF15+1166delA mutation causes RP in the family.

RP3 accounts for 15% to 20% of all RP cases, which is higher than any other single RP locus identified to date [12,14,15,28]. It is considered to be the most severe type of RP with its early age at onset [28,29]. Mutations in the RPGR gene have been identified in 80% to 90% of families with RP3 [12,29].

### Table 1. Clinical features of affected individuals in a family with X-linked retinitis pigmentosa

<table>
<thead>
<tr>
<th>Family member</th>
<th>Gender/age</th>
<th>Age of onset</th>
<th>Visual acuity OD/OS</th>
<th>Visual acuity OS</th>
<th>ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:2</td>
<td>M/78</td>
<td>NA</td>
<td>HM</td>
<td>HM</td>
<td>NA</td>
</tr>
<tr>
<td>III:1</td>
<td>F/58</td>
<td>22</td>
<td>CF</td>
<td>CF</td>
<td>extinguished</td>
</tr>
<tr>
<td>III:2</td>
<td>F/55</td>
<td>35</td>
<td>0.3</td>
<td>0.3</td>
<td>NA</td>
</tr>
<tr>
<td>III:3</td>
<td>M/52</td>
<td>18</td>
<td>CF</td>
<td>CF</td>
<td>extinguished</td>
</tr>
<tr>
<td>III:4</td>
<td>F/45</td>
<td>26</td>
<td>0.2</td>
<td>0.2</td>
<td>NA</td>
</tr>
<tr>
<td>IV:1</td>
<td>M/35</td>
<td>30</td>
<td>0.5</td>
<td>0.4</td>
<td>NA</td>
</tr>
<tr>
<td>IV:2</td>
<td>F/19</td>
<td>18</td>
<td>0.3</td>
<td>0.2</td>
<td>NA</td>
</tr>
<tr>
<td>IV:5</td>
<td>F/15</td>
<td>13</td>
<td>0.1</td>
<td>0.08</td>
<td>NA</td>
</tr>
</tbody>
</table>

The following abbreviations were used: male (M), female (F), right eye (OD), left eye (OS), count finger (CF), hand movement (HM), electroretinography (ERG), and not available (NA).

Figure 1. Pedigree of a Chinese family with X-linked retinitis pigmentosa. Blackened squares and circles represent affected males and females, respectively, and empty geometric shapes denote normal individuals. Slashed shapes mark deceased. Female carrier IV:2, is shown with a grey symbol, had myopia, but no full manifestation of retinitis pigmentosa. Arrow points to the proband (III:4). Genotyping data and results from haplotype analysis for four markers (DXS1226, DXS1214, DXS1068, and DXS993) are shown below each symbol.
gene are responsible for X-linked recessive RP [11,14,20,22] and incomplete X-linked dominant RP [21-25]. In some families with RPGR mutations, female carriers showed a wide spectrum of phenotypes, including delayed disease onset, sometimes milder symptoms than hemizygous males, but some times severe symptoms as in male patients [23-25]. Therefore, RP3 was considered to be a partial X-linked dominant RP [24]. It has been proposed that random X chromosome inactivation is responsible for the broad range of phenotypes in female carriers [22,24,25], although no preferential X chromosome inactivation was found by analyzing the peripheral blood samples in female carriers [22,24]. It is possible that the conclusion drawn from indirect assessment could not represent the X-inactivation patterns in retina [25].

Interestingly, all female mutation carriers in the family except for IV:2 showed the RP phenotype. As IV:2 was 18 years old and did not display other features of RP (Figure 2), we consider the inheritance in the family under study to be an incomplete X-linked dominant pattern. It should be noted that IV:2 was affected with severe myopia, which was the first clinical observation found in female RP patients in the family. It is also interesting to note that female family members may exhibit even more severe phenotypes than male patients at comparable ages. One female carrier, II:2, had a complete loss of vision. The other carrier, IV:5, was a 15 year old female patient who was found to have high myopia two years ago. Fundus examinations have already confirmed a diagnosis of RP. In the family, III:8 and IV:1 are males who carry the ORF15.
mutation, however, IV:1 showed milder RP symptom in comparison to III:2 III:6 and IV:5, and III:8 was diagnosed five years ago with RP.

The g.ORF15+1166delA mutation of RPGR could be a de novo mutation. Based on the medical records of I:1 and I:2, they had normal vision until their death in their seventh decade. Although affected II:2 and her younger sister II:5 with normal phenotype share the same disease haplotype (black vertical bar, Figure 1), the 1 bp deletion in ORF15 was identified in II:2, but was not present in II:5. Thus, the 1-bp deletion of ORF15 arose de novo in II:2. II:2 then transmitted the disease haplotype and the 1 bp deletion to all affected individuals in the next generation. ORF15 of RPGR was predicted to have a high mutation rate because of its special composition and the nature of 3’ DNA sequence [20], thus it was not surprising to detect de novo mutations in this exon.

In summary, in this study, we identified a novel de novo g.ORF15+1166delA mutation of RPGR in a Chinese family with interfamilial variability of RP. The affected females showed no signs of delayed onset, they appear to be as severely affected as the males in this family. The findings extend the range of phenotypes associated with the RPGR mutations, and expand our knowledge about the genetic heterogeneity underlying this disease.

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Figure 3. Identification of the g.ORF15+1166delA mutation in the RPGR gene. Shown are sequence chromatograms for a normal individual (top), a male patient (middle), and a female mutation carrier (bottom). Note that the sequences were derived from the reverse primer. Arrow marks the deletion point. The amino acid sequences for the normal and mutant RPGR proteins are shown on the right.
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