RP2 Phenotype and Pathogenetic Correlations in X-Linked Retinitis Pigmentosa

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**Objectives:** To assess the phenotype of patients with X-linked retinitis pigmentosa (XLRP) with RP2 mutations and to correlate the findings with their genotype.

**Methods:** Six hundred eleven patients with RP were screened for RP2 mutations. From this screen, 18 patients with RP2 mutations were evaluated clinically with standardized electroretinography, Goldmann visual fields, and ocular examinations. In addition, 7 well-documented cases from the literature were used to augment genotype-phenotype correlations.

**Results:** Of 11 boys younger than 12 years, 10 (91%) had macular involvement and 9 (82%) had best-corrected visual acuity worse than 20/50. Two boys from different families (aged 8 and 12 years) displayed a choroideremia-like fundus, and 9 boys (82%) were myopic (mean error, −7.97 diopters [D]). Of 10 patients with electroretinography data, 9 demonstrated severe rod-cone dysfunction. All 3 female carriers had macular atrophy in 1 or both eyes and were myopic (mean, −6.23 D). All 9 nonsense and frameshift and 5 of 7 missense mutations (71%) resulted in severe clinical presentations.

**Conclusions:** Screening of the RP2 gene should be prioritized in patients younger than 16 years characterized by X-linked inheritance, decreased best-corrected visual acuity (eg, >20/40), high myopia, and early-onset macular atrophy. Patients exhibiting a choroideremia-like fundus without choroideremia gene mutations should also be screened for RP2 mutations.

**Clinical Relevance:** An identifiable phenotype for RP2-XLRP aids in clinical diagnosis and targeted genetic screening.

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RETINITIS PIGMENTOSA (RP) IS a clinically and genetically heterogeneous group of retinal disorders that causes progressive loss of visual function due to rod and cone photoreceptor degeneration. The X-linked forms of RP (XLRP) account for 10% to 20% of all RP cases.1-3 Two genes have been cloned for XLRP: retinitis pigmentosa GTPase regulator, RPGR (OMIM 312610),4 and RP2 (OMIM 312600),5,6 which together account for more than 80% of XLRP.7-10 Mutations in RP2 are reported to cause 7% to 10% of XLRP.6,11-15 The RP2 gene is composed of 5 exons and encodes a widely expressed protein of 350 amino acids.6,16 The RP2 protein consists of an amino-terminal domain with homology to cofactor C and a carboxyl-terminal domain with homology to nucleoside diphosphate kinase.17 The amino-terminal domain of RP2 binds to a small guanosine triphosphate–binding protein, Arl3, which shows homology with adenosine diphosphate–ribosylation factor and is involved in cellular transport regulation mechanisms.18 Although the 30 amino-terminal residues of RP2 are critical for binding to Arl3, human disease-causing mutations Arg118His and Gln138Gly also reduce the affinity of RP2 to Arl3, indicating a clinically relevant association elsewhere in the protein. Post-translational acyl modifications at the N-terminus of RP2 act to target the protein to the plasma membrane, and disruption of this acylation site ultimately leads to the RP phenotype.19,20 Most pathogenic sequence alterations found in RP2 represent truncating mutations.11 However, missense mutations have been located in the cofactor C–like domain of RP2.17 Given the considerable phenotypic and genetic heterogeneity associated with XLRP14 and the scarcity of patients with RP2 diagnoses, there has been insufficient information to date to predict the clinical phenotype of a patient based on the RP2 mutation. Although there have been studies9,12,14,21 containing correlations of RP2 phe-
Mutational analysis was performed as described by Mears et al. RP2 were screened for variations and mutations in the gene. To facilitate the investigation of a probable or possible diagnosis of XLRP, we undertook the present study to carefully analyze the phenotype in a cohort of patients with RP2 and carriers found at our institution and in previously published articles (Table 1). We correlated the severity of disease with the predicted effect of the mutation on the putative function of RP2.

### METHODS

**PATIENTS**

Mutational analysis was performed on 611 DNA samples as part of a larger screening study from the XLRP Repository of the University of Michigan. Patients with only a clinical diagnosis of XLRP with or without documented RP2 mutations were included. The sequestration of clinical features (age at symptom onset, visual function, electroretinography data, and retinal appearance) of individual patients with RP2 mutations. Data on the 7 identified cases were collected from the literature for inclusion and comparison to supplement the cohort from our institution to delineate the phenotype of RP2 and make genotype to phenotype correlations (Table 1).

**DNA EXTRACTION**

DNA was extracted from the whole blood of patients. Primers for amplifying RP2 exons 2-5 were used as previously reported. The sequences for the RP2 exon 1 forward and reverse primers were 5’ CTCTGATTGGCTCACAAGGC and 5’ GTCTAAGAGATGCGGCA, respectively. These primers amplified 447-base pair polymerase chain reaction (PCR) fragments.

**PCR CONDITIONS AND SEQUENCING**

DNA was used at approximately 100 ng per PCR. All the exons except exon 2 were amplified with Ex Taq Polymerase (TaKara Bio Inc, Shiga, Japan). Exon 2 was amplified with AccuPrime high-fidelity polymerase (Invitrogen, Carlsbad, California). The annealing temperature for exons 1 and 2 was 59°C; for exons 3, 4, and 5, it was 64°C. All PCR volumes were made to 25 µL, and PCR products were run on 2% agarose gels to verify the sizes and quality of amplification. Before submitting the samples for sequencing, the DNA concentration was measured using a spectrophotometer (NanoDrop 1000; Thermo Scientific, Wilmington, Delaware). The PCR amplicons were then diluted (1-3 ng/µL in distilled water) as required by the sequencing core at the University of Michigan Medical School.

### Table 1. Mutations for Families Included in This Study

<table>
<thead>
<tr>
<th>Family No.</th>
<th>Location</th>
<th>Type of Mutation</th>
<th>Nucleotide Change</th>
<th>Protein Change</th>
<th>Severity of Associated Phenotype</th>
<th>Predicted Effect of Mutation on Protein</th>
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</thead>
<tbody>
<tr>
<td>1090</td>
<td>Exon 1</td>
<td>Missense</td>
<td>c.8G&gt;C</td>
<td>p.Cys33Ser</td>
<td>Less severe</td>
<td>Mislocalization of protein</td>
</tr>
<tr>
<td>148</td>
<td>Exon 1</td>
<td>Insertion/frameshift</td>
<td>c.77insCA</td>
<td>p.Gln28fs71</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
<tr>
<td>1015</td>
<td>Intron 1</td>
<td>Splice</td>
<td>IVS1 + 1G&gt;A</td>
<td>Splice5</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
<tr>
<td>528</td>
<td>Intron 1</td>
<td>Splice</td>
<td>IVS1 + 3A&gt;G</td>
<td>Splice14</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
<tr>
<td>951</td>
<td>Exon 2</td>
<td>Missense</td>
<td>c.260C&gt;T</td>
<td>p.Thr87Ile</td>
<td>Less severe</td>
<td>Protein alteration</td>
</tr>
<tr>
<td>933</td>
<td>Exon 2</td>
<td>Missense</td>
<td>c.352C&gt;T</td>
<td>p.Arg118Gly</td>
<td>Severe</td>
<td>Protein alteration</td>
</tr>
<tr>
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<td>Missense</td>
<td>c.353G&gt;A</td>
<td>p.Arg118His</td>
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<tr>
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<td>p.Arg118His</td>
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<td>Protein alteration</td>
</tr>
<tr>
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<td>Exon 2</td>
<td>Deletion</td>
<td>c.409-411delATT</td>
<td>p.Ile137del</td>
<td>Severe</td>
<td>Protein instability</td>
</tr>
<tr>
<td>1029</td>
<td>Exon 2</td>
<td>Nonsense</td>
<td>c.450G&gt;A</td>
<td>p.Trp150Stop27</td>
<td>Severe (carrier)</td>
<td>Loss of function</td>
</tr>
<tr>
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<td>Insertion/frameshift</td>
<td>c.515_516insG</td>
<td>p.Ser172fsTer17314</td>
<td>Severe</td>
<td>Loss of function</td>
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<td>548</td>
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<td>p.R225fsTer23414</td>
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<td>Loss of function</td>
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<tr>
<td>1167</td>
<td>Exon 2</td>
<td>Missense</td>
<td>c.758T&gt;C</td>
<td>p.Leu253Pro</td>
<td>Severe</td>
<td>Protein instability</td>
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</tbody>
</table>

**Mutations From Published Cases in the Literature**

<table>
<thead>
<tr>
<th>Case</th>
<th>Location</th>
<th>Mutation</th>
<th>Nucleotide Change</th>
<th>Protein Change</th>
<th>Severity of Associated Phenotype</th>
<th>Predicted Effect of Mutation on Protein</th>
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</thead>
<tbody>
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<td>1</td>
<td>Exon 1</td>
<td>Deletion</td>
<td>c.12_18 del</td>
<td>p.Ser66del</td>
<td>Less severe</td>
<td>Mislocalization of protein</td>
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<td>2</td>
<td>Exon 1</td>
<td>Nonsense</td>
<td>c.76C&gt;T</td>
<td>p.Gln265Stop</td>
<td>Severe</td>
<td>Loss of function</td>
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<tr>
<td>3</td>
<td>Exon 2</td>
<td>Nonsense</td>
<td>c.358C&gt;T</td>
<td>p.Arg120Stop23</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
<tr>
<td>4</td>
<td>Exon 2</td>
<td>Nonsense</td>
<td>c.358C&gt;T</td>
<td>p.Arg120Stop23</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
<tr>
<td>5</td>
<td>Exon 2</td>
<td>Nonsense</td>
<td>c.358C&gt;T</td>
<td>p.Arg120Stop23</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
<tr>
<td>6</td>
<td>Exon 2</td>
<td>Missense</td>
<td>c.758T&gt;G</td>
<td>p.Leu253Arg</td>
<td>Severe</td>
<td>Protein instability</td>
</tr>
<tr>
<td>7</td>
<td>Exon 2</td>
<td>Deletion/frameshift</td>
<td>c.798delGACA</td>
<td>p.Gln266fs21</td>
<td>Severe</td>
<td>Loss of function</td>
</tr>
</tbody>
</table>

**Change**aReferences cited in this column denote the first mention of the change in the literature.

**Novel change.**

Nototypes with visual function data, no large study exists, to our knowledge, in which clear clinical distinctions have been identified to help make it a recognizable entity to ophthalmologists. A recognizable phenotype would help narrow the differential diagnosis for candidate gene mutational screening for RP2. We undertook the present study to carefully analyze the phenotype in a cohort of patients with RP2 and carriers found at our institution and in previously published articles (Table 1). We correlated the severity of disease with the predicted effect of the mutation on the putative function of RP2.
Sequencing was performed with either forward or reverse primers for exons 1, 3, 4, and 5 and with 4 primers for exon 2.

MUTATIONAL DATA ANALYSIS

Sequences were downloaded from the sequencing core server and were analyzed using a 4.8 demo version of Sequencher (Gene Codes Corp, Ann Arbor). The sequences were read by 2 people (M.O. and A.J.K.) independently, and mutations were tabulated. The mutations were reconfirmed by running an independent PCR on the samples.

CLINICAL DATA

All medical records were reviewed for the following clinical features: age at onset of visual disturbance; best-corrected visual acuity (BCVA); refractive error (spherical equivalence); macular, pericentral, peripheral retinal, and optic disc appearance (color fundus photographs were also analyzed to supplement the written description in the medical record); Goldmann visual field (GVF) data; and standardized electroretinography amplitudes and implicit times. The same information was gathered from previously published cases identified by the literature search. Clinical data were recorded for each patient visit when available; however, not all outcome measures were available at every patient visit.

CLINICAL SEVERITY GRADING

We devised a novel grading approach to subdivide patients according to 2 severity categories: less severe and severe (no patients were mild). A patient was considered less severe if he or she had relatively late onset of severe macular dysfunction. The BCVA was used as a surrogate for macular function and was considered severe if worse than 20/50 at 20 years or younger, worse than 20/100 from age 21 to 30 years, worse than 20/200 from age 31 to 40 years, and worse than 20/400 after age 41 years.

MUTATIONAL ANALYSIS

Mutational screening identified 13 families with mutations in RP2 (Table 1). Of these, we previously reported the genotype of 4 individuals; 5 mutations we identified have been reported by others, and 4 are novel changes. The locations of these mutations in relation to all previously published mutations are shown in Figure 1. Four novel mutations were identified in these families, including 1 missense change (Cys3Ser) identified in exon 1, 2 missense changes identified in exon 2 (Thr87Ile and Leu253Pro), and 1 splice site change (IVS1 + 1 G>A). None of these changes have previously been identified in patients or controls. The chromatograms of these mutations are found in Figure 2. In 3 of the families, the mutation was also detected in at least 1 other affected male family member or a carrier female.

Eighteen patients from 13 families were included from our institution (Table 1). Seven additional patients with well-identified phenotypes were added from previously published articles, giving a total of 25 patients (22 affected males and 3 female carriers) for genotype-phenotype correlations.

RESULTS

CLINICAL DATA

Male Cohort Results—Predominant Male RP2 Phenotype

Fifteen male patients were identified during mutational screening. We assessed these patients’ macular function.
based on macular appearance, BCVA, and presence of central scotoma on GVF testing (Table 2). Reported denominators varied slightly with data availability. Of these patients, 12 had adequate fundus photographs. Eleven of 12 patients (92%) had manifestations of macular involvement in the form of granularity, atrophy, or a bull’s eye appearance on fundus examination (Figure 3), with 10 of 11 patients (91%) showing macular involvement before age 12 years. Nine of 11 patients had a BCVA of 20/50 or worse by age 12 years. Four patients (148-2239, 528-115, 948-2743, and 1167-2760) for whom BCVA was not available from an examination performed before age 12 years all developed severe vision loss (worse than 20/200) by the third to seventh decade of life. Tapetal or golden macular sheens were not seen in the patients with RP2, a finding more typical of RPGR X-linked patients. The GVF testing revealed central scotomata in 50% (5 of 10) of all male patients for whom testing was performed, including 38% (3 of 8) of the patients younger than 12 years. Figure 4 shows examples of RP2-XLRP phenotypes.

Measurable peripheral GVF data for boys younger than 12 years were found in 8 cases. Six of these 8 patients (75%) had constriction of the visual field when tested with the I4e target (median visual field size, 25° OD and 25° OS) and only mild constriction when tested with the IV4e target (median visual field size, 55° OD and 17.5° OS). When data were analyzed for patients younger than 16 years, all 8 had severe constriction of the visual field when tested with the I4e target (median visual field size, 12.5° OD and 17.5° OS) and still only mild constriction when tested with the IV4e target (median visual field size, 50° OU).

Data on refractive errors were available for 11 of 15 patients (range, plano to −14 diopters [D]; mean, −6.55 D). Nine of these 11 patients (82%) were found to be myopic (mean, −7.97 D), with most (78%, 7 of 9) of those affected classified as high myopes with greater than −6.00 D (mean, −8.91 D).

Electroretinography was performed on 10 of 15 patients; 90% (9 of 10) of the patients demonstrated severe rod-cone dysfunction. One patient (1167-2760) showed cone-rod dysfunction. The degree of cone dysfunction was further represented by the delayed photopic b-wave implicit times in all 9 male patients, with mean implicit times of 47.2 milliseconds OD and 46.8 milliseconds OS (mean [SD] reference range, 32.3 [1.2] milliseconds).

Choroideremia-like Phenotype

Two patients (933-2420 and 971-2490) with different mutations (Arg118Cys and Ser172fs, respectively) had peripheral choroideremia-like atrophy. Both patients were tested for mutations in CHM. Data were found to be negative. The clinical features of patient 971-2490 are illustrated in Figure 4B. There was significant choriocapillaris atrophy in the midperiphery and the posterior pole with no notable pigment deposition. Two male patients demonstrated characteristic superior visual field loss similar to the visual field changes attributed to retinal phototoxicity in patients with RHO mutations. As an example, patient 1167-2760 is illustrated in Figure 4C.

Female Carrier Cohort Results

Two female carriers manifested a phenotype similar to that of the affected males, exhibiting atrophic macular changes, poor visual acuity, and central scotomata. The third female carrier (1015-2553) demonstrating asymmetrical disease had anisometropia of approximately 8.00 D, with the severely affected eye being myopic (Figure 4D), further supporting the association of myopia with RP2 retinal disease. In fact, all 3 female carriers had macular atrophy in 1 or both eyes, and all 3 were myopic (mean, −6.23 D).
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age at Onset, y</th>
<th>Refraction</th>
<th>Age at Exam, y</th>
<th>Visual Acuity</th>
<th>Fundus Appearance</th>
<th>Scotoma</th>
<th>Pericentral Features</th>
<th>Optic Nerve</th>
<th>Fundus Appearance</th>
<th>Visual Field Constriction</th>
<th>Photopic, Implicit, Scotopic,</th>
<th>ERG</th>
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<td>1090-2262</td>
<td>4</td>
<td>plano</td>
<td>8</td>
<td>20/25</td>
<td>Unaffected</td>
<td>None</td>
<td>RPE/chorioretinal atrophy</td>
<td>Normal</td>
<td>Granular pigment ++</td>
<td>14e 25°, IV 4e 55°</td>
<td>34 60 (D) 0</td>
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<td>1090-2262</td>
<td></td>
<td>plano</td>
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<td>20/20</td>
<td>Unaffected</td>
<td>None</td>
<td>RPE/chorioretinal atrophy</td>
<td>Normal</td>
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<td>20/30</td>
<td>Unaffected</td>
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<td>RPE/chorioretinal atrophy</td>
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<td>Granular pigment ++</td>
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<td>NA</td>
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<td>NA</td>
<td>Atrophy</td>
<td>NA</td>
<td>Posterior pole degeneration</td>
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<td>Posterior pole degeneration</td>
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<td>Atrophy</td>
<td>14e 30°</td>
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<tr>
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<td>None</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy</td>
<td>14e 30°</td>
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<td>IV 4e &lt;10°</td>
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<td>TA</td>
<td>RPE/chorioretinal atrophy</td>
<td>14e 25°, IV 4e 60°</td>
<td>8 45 (D) 0</td>
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<td>20/80</td>
<td>CS</td>
<td>Mild staphyloma</td>
<td>TA</td>
<td>RPE/chorioretinal atrophy</td>
<td>14e 25°, IV 4e 60°</td>
<td>14 45 (D) 0</td>
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<td>−7.125</td>
<td>12</td>
<td>20/60</td>
<td>PMA</td>
<td>None</td>
<td>Atrophy</td>
<td>TA</td>
<td>Contracted 4e, IV 4e</td>
<td>2 49 (D) NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>−7.125</td>
<td>20/80</td>
<td>PMA</td>
<td>None</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy</td>
<td>Near full 4e, IV 4e</td>
<td>2 50 (D) NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>16</td>
<td>20/400</td>
<td>Atrophy</td>
<td>FRS</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy</td>
<td>14e not seen, IV 4e 20° with equatorial islands</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/200</td>
<td>Atrophy</td>
<td>FRS</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy</td>
<td>14e not seen, IV 4e 20° with equatorial islands</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
### Table 2. Phenotypic Differences in 25 Patients With XLRP and Proven Mutations (continued)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age at Onset, y</th>
<th>Refraction</th>
<th>Age at Exam, y</th>
<th>Visual Acuity</th>
<th>Fundus Appearance</th>
<th>Scotoma</th>
<th>Pericentral Features</th>
<th>Optic Nerve</th>
<th>Fundus Appearance</th>
<th>Visual Field Constriction</th>
<th>ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>948-2443, Carrier</td>
<td>2</td>
<td>NA</td>
<td>6</td>
<td>20/50</td>
<td>Unaffected</td>
<td>None</td>
<td>Mild staphyloma/Mild staphyloma</td>
<td>Normal Normal</td>
<td>None</td>
<td>Granularty Granularty</td>
<td>Near full 14e, IV4e</td>
</tr>
<tr>
<td>948-2743</td>
<td>7</td>
<td>NA</td>
<td>31</td>
<td>HM</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>OA</td>
<td>TA</td>
<td>NA</td>
</tr>
<tr>
<td>652-1694</td>
<td>2</td>
<td>7–8.25</td>
<td>10</td>
<td>20/60</td>
<td>BE</td>
<td>CS</td>
<td>RPE DP, RPE DP</td>
<td>Normal Normal</td>
<td>OA</td>
<td>TA</td>
<td>Atrophy Atrophy</td>
</tr>
<tr>
<td>1029-2585, Carrier</td>
<td>20s</td>
<td>NA</td>
<td>45</td>
<td>4/200</td>
<td>Aperture Atrophy</td>
<td>CS</td>
<td>RPE DP, RPE DP</td>
<td>Normal Normal</td>
<td>OA</td>
<td>TA</td>
<td>Mild atrophy</td>
</tr>
<tr>
<td>971-2490</td>
<td>5</td>
<td>8</td>
<td>8</td>
<td>20/40</td>
<td>G</td>
<td>None</td>
<td>RPE DP, RPE DP</td>
<td>Normal Normal</td>
<td>OA</td>
<td>TA</td>
<td>Mild atrophy</td>
</tr>
<tr>
<td>548-1358</td>
<td>3</td>
<td>7–7.625</td>
<td>9</td>
<td>20/70</td>
<td>MILD PMA</td>
<td>Atrophy</td>
<td>CS</td>
<td>CS</td>
<td>Atrophy</td>
<td>Mild DP</td>
<td>Unaffected</td>
</tr>
<tr>
<td>1167-2760</td>
<td>25</td>
<td>8–8.375</td>
<td>28</td>
<td>20/80</td>
<td>G</td>
<td>None</td>
<td>RPE DP, RPE DP</td>
<td>Normal Normal</td>
<td>OA</td>
<td>TA</td>
<td>Mild RPE DP</td>
</tr>
</tbody>
</table>

#### Literature Cohort

- **Case 1 (IV-2, 0032)**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

- **Case 2 (IV-2, 200307)**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

- **Case 3 (El8)**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

- **Case 4**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

- **Case 5 (Il1)**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

- **Case 6 (Il-4)**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

- **Case 7 (Il-2)**
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A
  - N/A

**Abbreviations:** BE, bull’s eye; CAS, central areolar scarring; CCRD, choriodocapillaris/retinal pigment epithelium (RPE) degeneration; CF, count fingers; Ch, childhood; CHM, choroideremia; CRA, chorioretinal atrophy; CS, central scotoma; D, delayed photopic implicit time (≥33 ms); DP, degeneration; ERG, electroretinography; exam, examination; FRS, full ring scotoma; G, granular macula; HM, hand motions; ID, identification number; LP, light perception only; NA, not available; NLF, no light perception; NR, nonrecordable; OA, optic nerve atrophy; PCS, pericentral scotoma; +, mild pigment deposits; ++, moderate pigment deposits; ++++, heavy pigment deposits; PMA, perimacular atrophy; PPA, peripapillary atrophy; RS, ring scotoma; TA, temporal atrophy; XLRP, X-linked retinitis pigmentosa.

*Patients with novel mutations being characterized for the first time.
*Treated with systemic immunosuppression for secondary autoimmune retinopathy.
*Original case report references are included in parentheses to facilitate comparisons.
Correlation of Severity to Genotype

When the severity grading criteria described previously herein was applied to all 25 patients examined, only 3 (12%) (including cases from the literature) exhibited a less severe phenotype characterized by a relatively older age at onset of macular dysfunction (patient 1090-2262, patient 951-2448, and published case 122), whereas most (88%) were considered to have a severe phenotype. Table 1 lists the disease severity and the predicted effect of the mutation on RP2 function. All the patients with premature truncations (9 of 9 patients with frameshift or nonsense mutations) fell into the severely affected group. Interestingly, 5 of 7 patients (71%) with missense mutations predicted to be hypomorphic (reduced protein function) also exhibited a relatively severe phenotype.

This study represents the largest comprehensive clinical analysis of patients with causative RP2 mutations (the cohort from our institution alone). Previous studies have been either case reports with phenotype descriptions or comparative analyses of many XLRP gene subtypes. We gathered supplemental information from previously published cases yielding meta-analysis-type data on the RP2 clinical phenotype. The present article describes a recognizable phenotype consisting of early onset of macular atrophy and poor visual acuity combined with high myopia. This phenotype runs contrary to the typical forms of RP, in which the macula is often spared until late in the disease course. We propose that screening of RP2 should be prioritized in male patients with an X-linked pedigree, high myopia, poor visual acuities, and early-onset macular atrophy.

In addition, screening for RP2 mutations is appropriate in the rare male patients who fail CHM mutation screening. Consistent with the fundus findings in patient 933-2420 with an Arg118Cys mutation, Vorster et al noted a similar phenotype in a male patient with an Arg120Stop mutation. Patient 971-2490 also has a similar choroideremia-like phenotype, and the mutation (Ser172Fs) shares the same exon (2) and functional Arl3-binding domain. These data suggest that mutations in this domain (Arg118Cys, Arg120Stop, and Ser172Fs) can lead to a choroideremia-like phenotype.

Female patients from XLRP pedigrees who have high myopia, asymmetrical retinal involvement, macular atrophy, or reduced central visual acuity may also have RP2 mutations. Mutational screening of RP2 is warranted in these cases, which are exemplified by female carrier patients 1015-2553, 1029-2585, and 948-2443.

Although previously published studies have shown macular atrophy atypical of classic RP with poor visual acuity in patients with RP2 mutations, a clear clinical phenotype for RP2 mutations has not been described. Most patients (10 of 11, 91%) in the cohort of male patients from our institution demonstrated macular atrophy starting at an early age (before age 12 years). This atrophy progressed into central scotomata in 50% of the patients and runs counter to the typical RP presentation, in which the macula is spared until late in the natural history of disease progression. The present results indicate that early macular involvement is a distinguishing clinical feature of disease due to RP2 mutations.

The severe degree of cone photoreceptor dysfunction in RP2 mutations is further supported by the electroretinography data demonstrating large delays in the photopic b-wave implicit times in all 12 patients in the combined male and female cohorts for whom data were available. These data corroborate the implicit times found by Sharon et al in patients with RP2 mutations. However, only 1 patient had a clear cone-rod dysfunction pattern on electroretinographic testing, suggesting that rod photoreceptor degeneration is still a prominent feature in this disease.

Predilection for superior visual field loss (inferior retinal disease) attributed to retinal phototoxicity has been described in autosomal dominant RP associated with RHO mutations. We encountered a similar superior field loss in 4 patients, 2 evaluated at our institution and 2 in the cohort of published cases, but the role of sunlight in the disease mechanism for RP2 mutations is currently unknown.

The association of high myopia with RP2 mutations has been demonstrated in another study, and we confirmed this finding in our group of patients. The female carrier (1015-2553) manifesting asymmetrical disease had anisometropia of approximately 8.00 D, with the severely affected eye having myopia (Figure 4D), further supporting the concomitance of myopia and RP2.

Correlating the wide spectrum of clinical phenotypes in patients with RP2 to their genotypes has been an intriguing puzzle. In general, missense or in-frame deletion mutations are considered hypomorphic because they may result in a mutant protein with reduced function, whereas truncation mutations in RP2 (frameshift or splice site defects) cause severe phenotypes likely due to loss of protein function. However, examination revealed that missense RP2 mutations are also associated with a severe phenotype. Because most of the truncation mutations in published cases, but the role of sunlight in the disease mechanism for RP2 mutations is currently unknown.
tion mutations are found in the amino-terminal domain of RP2, the carboxyl-terminal region may be involved in providing stability to the protein or is important for maintaining a functional conformation of RP2.

The Arg118His and Arg118Cys mutations are associated with a severe phenotype, although previous in vitro biochemical studies predict that mutations at Arg118 result in residual, but not abolished, activity of RP2 and its affinity to Arl3. On the other hand, RP2 Cys3Ser or Ser6del mutations have previously been shown to affect the localization of RP2 to plasma membrane in cultured cells. In fact, RP2 Ser6del mutant protein is present at relatively low levels likely due to decreased stability. These results demonstrate that the localization of RP2 to plasma membrane may not be critical for its function. Clinically, we successfully correlated the genotypes from a patient with a Cys3Ser mutation (1090-2262) and a patient from the published literature with a Ser6del mutation (case 12) with a less severe phenotype. It is also possible that alternative localization of RP2 in the cells may be affected by some of the mutations. Because Arl3 localizes to photoreceptor sensory cilium and the mouse mutant of Arl3 develops a ciliary phenotype, RP2 may be involved in the targeting of Arl3 or in modulating its activity at the cilium. Further studies are necessary to resolve these issues.

Splice mutations represent another level of complexity associated with the prediction of the phenotype. Such mutations can result in a severe phenotype if they occur early in the gene, resulting in premature truncation.

Taken together, these data provide a platform for clinical identification of patients with XLRP and RP2 mutations that can assist in better disease management and genetic counseling. We propose that RP2 be the first gene screened in male patients with an X-linked pedigree, high myopia, poor visual acuities, and macular atrophy in childhood. Future therapeutic modalities for RP2-XLRP should carefully consider the quality and character of the mutant protein expressed in the diseased photoreceptors. Resolving the crystal structure of RP2 has increased our understanding of the role of different amino acid residues in the protein’s function and the probable effect of disease-associated mutations on its 3-dimensional structural and putative function. This genotype-phenotype analysis shows that a mutant RP2 protein with reduced activity can result in the same severe phenotype caused by mutations that result in protein degradation. Because the biochemical activity of RP2 has not been demonstrated in vivo, further investigations are necessary to carefully analyze the correlation between RP2 mutations and their associated phenotypes, which will aid in the design of appropriate clinical treatments.

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Figure 4. Clinical phenotype variations in X-linked retinitis pigmentosa secondary to patients with RP2. CHM indicates choroideremia.
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Author Contributions: Dr Jayasundera and Ms Brantham contributed equally to this investigation.

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REFERENCES


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