RP2 Phenotype and Pathogenetic Correlations in X-Linked Retinitis Pigmentosa

Thiran Jayasundera, MD; Kari E. H. Branham, MS; Mohammad Othman, PhD; William R. Rhoades, BS; Athanasios J. Karoukis, BS; Hemant Khanna, PhD; Anand Swaroop, PhD; John R. Heckenlively, MD

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 electrophotography, 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.

Arch Ophthalmol. 2010;128(7):915-923

OPHTHALMIC MOLECULAR GENETICS

SECTION EDITOR: JANEY L. WIGGS, MD, PhD
notypes 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.

**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’s Center for Retinal and Macular Degeneration (outside samples not reported). Samples from patients affected with a probable or possible diagnosis of XLRP or X-linked cone-rod dystrophy (as described by Breuer et al) were screened for variations and mutations in the RP2 gene. Mutational analysis was performed as described by Mears et al (n = 51), by Breuer et al (n = 234), or herein (n = 326).

Included in this genotype-phenotype correlation study are the 18 patients with previously identified RP2 mutations who were clinically evaluated in the Retinal Dystrophy Clinic at the University of Michigan’s Kellogg Eye Center or at the University of California, Los Angeles’ Jules Stein Eye Institute. All the patients gave informed consent, and the research was approved by the institutional review board at the University of Michigan. Patients with only a clinical diagnosis of XLRP without documented RP2 mutations were excluded. Patients are identified throughout with a family identification number and an individual identification number (i.e., family number-individual number).

A comprehensive literature search was performed to identify publications containing unambiguous and adequate descriptions 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’TCTTGATGGCCTAACGACCC and 5’ GTTCAAGAGAGTGCGGCAG, 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.
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.

**RESULTS**

**MUTATIONAL ANALYSIS**

Mutational screening identified 13 families with mutations in RP2 (Table 1). Of these, we previously reported the genotype of 4 individuals31,14; 5 mutations we identified have been reported by others, and 4 are novel changes. The locations of these mutations in relation to all previously reported RP2 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.

**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 RGR 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 14e 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° OU). When data were analyzed for patients younger than 16 years, all 8 had severe constriction of the visual field when tested with the 14e 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 and were found to be negative. The clinical features of patient 971-2490 are illustrated in Figure 4B. There is 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 2. Phenotypic Differences in 25 Patients With XLRP and Proven Mutations

<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>Optical Nerve</th>
<th>Fundus Appearance</th>
<th>Visual Field Constriction</th>
<th>Phenotypic %</th>
<th>Implicit Time, s</th>
<th>Scotopic %</th>
</tr>
</thead>
<tbody>
<tr>
<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/choroid atrophy</td>
<td>normal</td>
<td>granular</td>
<td>14e&lt;25°, IV4e&lt;25°</td>
<td>34</td>
<td>60 (D)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plano</td>
<td>20/20</td>
<td>Unaffected</td>
<td>none</td>
<td>RPE/choroid atrophy</td>
<td>normal</td>
<td>granular</td>
<td>14e&lt;25°, IV4e&lt;25°</td>
<td>36</td>
<td>60 (D)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA</td>
<td>15</td>
<td>20/30</td>
<td>Unaffected</td>
<td>none</td>
<td>RPE/choroid atrophy</td>
<td>normal</td>
<td>granular</td>
<td>14e&lt;15° OU with equatorial scotomata OU</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA</td>
<td>3</td>
<td>20/70</td>
<td>Atrophy</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>148-2240</td>
<td>3</td>
<td>NA</td>
<td>20/70</td>
<td>Atrophy</td>
<td>NA</td>
<td>Atrophy</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>-14</td>
<td>14</td>
<td>20/100</td>
<td>Atrophy</td>
<td>PCS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Constricted</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>-12</td>
<td>20/100</td>
<td>Atrophy</td>
<td>PCS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>951-2420</td>
<td>12</td>
<td>-7.875</td>
<td>20/200</td>
<td>Atrophy</td>
<td>NA</td>
<td>Atrophy</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>944-2437</td>
<td>12</td>
<td>-7.125</td>
<td>20/60</td>
<td>PMA</td>
<td>None</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy, pigment ++</td>
<td>14e&lt;20° with equatorial islands</td>
<td>2</td>
<td>49 (D)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/60</td>
<td>PMA</td>
<td>None</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy, pigment ++</td>
<td>14e&lt;20° with equatorial islands</td>
<td>2</td>
<td>50 (D)</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/400</td>
<td>Atrophy</td>
<td>FRS</td>
<td>Atrophy</td>
<td>TA</td>
<td>Atrophy, pigment ++</td>
<td>14e&lt;20° with equatorial islands</td>
<td>0</td>
<td></td>
<td></td>
<td></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, pigment ++</td>
<td>14e&lt;20° with equatorial islands</td>
<td>0</td>
<td></td>
<td></td>
<td></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>Age at Exam, y</th>
<th>Visual Acuity</th>
<th>Fundus Appearance</th>
<th>Scotoma</th>
<th>Macular Features</th>
<th>Pericentral Features</th>
<th>Optic Nerve</th>
<th>Peripheral Features</th>
<th>ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>948-2443, Carrier</td>
<td>2 NA</td>
<td>6 20/50</td>
<td>Unaffected</td>
<td>None</td>
<td>Mild Atrophy</td>
<td>Mild Staphyloma</td>
<td>Normal</td>
<td>Normal</td>
<td>Granulopathy</td>
<td>Granulopathy</td>
</tr>
<tr>
<td>652-1694, Carrier</td>
<td>2 −7.625</td>
<td>10 20/60</td>
<td>BE</td>
<td>CS</td>
<td>RPE DP</td>
<td>RPE DP</td>
<td>Normal</td>
<td>Normal</td>
<td>RPE/choroidal DP</td>
<td>CHM-like</td>
</tr>
<tr>
<td>1029-2585, Carrier</td>
<td>20s −6.00</td>
<td>45 4/200</td>
<td>Atrophy</td>
<td>CS</td>
<td>PPA, TA</td>
<td>PPA, TA</td>
<td>Mild Atrophy</td>
<td>Mild Atrophy</td>
<td>RPE/choroidal DP</td>
<td>CHM-like</td>
</tr>
<tr>
<td>971-2490</td>
<td>5 −8.750</td>
<td>8 20/40</td>
<td>G</td>
<td>None</td>
<td>RPE DP</td>
<td>RPE DP</td>
<td>PPA, TA</td>
<td>PPA, TA</td>
<td>RPE/choroidal DP</td>
<td>CHM-like</td>
</tr>
<tr>
<td>548-1358</td>
<td>3 −7.5</td>
<td>9 20/70</td>
<td>Mild PMA</td>
<td>CS</td>
<td>MILD DP</td>
<td>MILD DP</td>
<td>TA</td>
<td>TA</td>
<td>MILD DP</td>
<td>Mild Atrophy</td>
</tr>
<tr>
<td>1167-2760</td>
<td>25 −8.375</td>
<td>28 20/80</td>
<td>G</td>
<td>None</td>
<td>RPE DP</td>
<td>RPE DP</td>
<td>TA</td>
<td>TA</td>
<td>Unaffected</td>
<td>Unaffected</td>
</tr>
<tr>
<td>Case 1 (IV-2, 00322)</td>
<td>18 −5.25</td>
<td>34 0.8</td>
<td>NA</td>
<td>RS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Preserved RPE/choriocapillaris with mild pigment</td>
<td>4e contracted, IV4e insignificant constriction</td>
</tr>
<tr>
<td>Case 2 (IV-2, 200307)</td>
<td>6 −4.25</td>
<td>18 6/66</td>
<td>Atypical central reflexes</td>
<td>CS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Patchy DP at posterior pole</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Case 3 (Ellie)</td>
<td>7 −2.75</td>
<td>25 30/50</td>
<td>Unaffected</td>
<td>NA</td>
<td>Atrophy</td>
<td>NA</td>
<td>NA</td>
<td>Pigment +</td>
<td>V4, 20°-30°</td>
<td>0 0</td>
</tr>
<tr>
<td>Case 4</td>
<td>7 0.4</td>
<td>0.3</td>
<td>CRA</td>
<td>NA</td>
<td>CRA</td>
<td>NA</td>
<td>CRA, no pigment</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Case 5 (II-1)</td>
<td>5 −2.75</td>
<td>24 0.08</td>
<td>CAS, CRA</td>
<td>NA</td>
<td>CRA, no pigment</td>
<td>NA</td>
<td>NA</td>
<td>Pigment, bone spicule clumping</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Case 6 (II-4)</td>
<td>6 −6.5</td>
<td>29 20/100</td>
<td>Atrophy</td>
<td>OA</td>
<td>OA</td>
<td>NA</td>
<td>NA</td>
<td>Superior loss</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Case 7 (III-2)</td>
<td>12 −13</td>
<td>45 5/300</td>
<td>Atrophy</td>
<td>OA</td>
<td>OA</td>
<td>Ocular, no pigment</td>
<td>NA</td>
<td>NA</td>
<td>Superior loss</td>
<td>Reduced</td>
</tr>
</tbody>
</table>

Abbreviations: BE, bull’s eye; CAS, central areolar sclerosis; CCRD, choriocapillaris/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; NLP, no light perception; NR, nonrecordable; OA, optic nerve atrophy; PCS, pericentral scotoma; +, mild pigment deposits; +++, moderate pigment deposits; CHM-like severe disease; 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.

(REPRINTED) ARCH OPHTHALMOL/VOL. 128 (NO. 7), JULY 2010   WWW.ARCHOPHTHALMOL.COM

©2010 American Medical Association. All rights reserved.

Downloaded From: https://archophth.jamanetwork.com/ by a Non-Human Traffic (NHT) User  on 08/12/2019
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 alone). Previous studies have been either case reports with phenotype descriptions12-26,34 or comparative analyses of many XLRP gene subtypes.12,21 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 al25 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 studies12,21 have shown macular atrophy atypical of classic RP34 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 al12 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.35 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,17 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 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.
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 1) 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.

Submitted for Publication: August 5, 2009; final revision received October 6, 2009; accepted November 2, 2009.
Correspondence: John R. Heckenlively, MD, Kellogg Eye Center, University of Michigan, 1000 Wall St, Ann Arbor, MI 48105 (jheck@umich.edu).

Author Contributions: Dr Jayasundera and Ms Branham contributed equally to this investigation.

Financial Disclosure: None reported.

Funding/Support: This research was supported by grants from the Foundation Fighting Blindness and the National Eye Institute Intramural Research Program (Dr Swaroop) and by grant R01 EY007961 from the National Eye Institute.

Additional Contributions: Richard Hackel, MA, assisted with fundus photography and illustrations; Paul Sieving, MD, PhD, evaluated several patients; Naheed Khan, PhD, assisted with electroretinographic illustrations; and Jill Oversier, BS, assisted with patient coordination.

REFERENCES