Novel Mutations in the NRL Gene and Associated Clinical Findings in Patients With Dominant Retinitis Pigmentosa

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Objectives: To search for mutations in the neural retina leucine zipper (NRL) gene in patients with dominant retinitis pigmentosa and to compare the severity of disease in these patients with that observed previously in patients with dominant rhodopsin mutations.

Methods: Single-strand conformation analysis was used to survey 189 unrelated patients for mutations. The available relatives of index patients with mutations were also evaluated. In our clinical examination of patients, we measured visual acuity, final dark-adaptation threshold equivalent visual field diameter, and electroretinogram amplitudes among other parameters of visual function. We compared the clinical findings with those obtained earlier from similar evaluations of a group of 39 patients with the dominant rhodopsin mutation Pro23His and a group of 25 patients with the dominant rhodopsin mutation Pro347Leu.

Results: We identified 3 novel missense mutations in a total of 4 unrelated patients with dominant retinitis pigmentosa: Ser50Pro, Ser50Leu (2 patients), and Pro51Thr. Each mutation cosegregated with dominant retinitis pigmentosa. None of these mutations were found among 91 unrelated control individuals. The visual acuities among the 4 index patients and 3 relatives with NRL mutations who were clinically evaluated ranged from 20/20 (in a 9-year-old patient) to 20/200 (in a 73-year-old patient). All patients had bone-spicule pigment deposits in their fundi. Average rod-plus-cone and cone-isolated electroretinogram amplitudes were both decreased by 99% or more compared with normal amplitudes. The dark-adaptation thresholds, equivalent visual field diameters, and electroretinogram amplitudes (all corrected for age and refractive error) indicated that the disease caused by the NRL mutations was more severe than that caused by the dominant rhodopsin mutation Pro23His and was similar in severity to that produced by the rhodopsin mutation Pro347Leu.

Conclusion: The 3 novel NRL mutations we discovered bring the total number of reported mutations in this gene to 6. Five of the 6 mutations affect residues 50 or 51, suggesting that these residues are important in a structural or functional domain of the encoded protein.

Clinical Relevance: Rod and cone function is affected to a similar degree in patients with these mutations. The disease caused by NRL mutations found in this study appears to be more severe than that caused by the rhodopsin mutation Pro23His and is similar in severity to that caused by the rhodopsin mutation Pro347Leu, even after correcting for age.

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Approximately 40% of patients with retinitis pigmentosa (RP) are from families exhibiting a dominant mode of inheritance. Evidence suggests that at least 11 different genes can cause dominant RP, only 4 of which have been identified: rhodopsin (RHO), retinal degeneration slow (RDS), neural retinal leucine zipper (NRL), and RP1. At each of these loci except NRL, numerous pathogenic mutations have been discovered. NRL is distinctive because only 3 mutations in this gene have been reported. One mutation, Ser50Thr, was found to be associated with RP in 4 families, all of which originated in southeast England and were likely descended from the same ancestor. The mutation Pro51Leu was found in 1 Spanish family with dominant RP, and the mutation Gly122Glu was a new germline mutation in a simplex case of RP, also from Spain.

Our original purpose in conducting this study was to search for additional examples of dominant RP caused by NRL mutations. On discovering such cases, we subsequently investigated the clinical findings of these patients because the specifics of the phenotype produced by NRL mutations have not been previously documented. Furthermore, we compared the clinical findings in these patients with those in patients with the dominant...
SUBJECTS AND METHODS

This study involved human subjects and conformed to the tenets of the Declaration of Helsinki. Patients and controls were recruited by one of the investigators (E.L.B.). All 189 index patients came from families with a consecutive transmission of RP for 2 successive generations, and many showed transmission across 3 or more generations. Patients known to have mutations in the rhodopsin, RDS, or RPL genes were not included in the search for mutations in the NRL gene; however, some patients had not been screened for mutations in these other genes. To be specific, 156 of the 189 patients had been evaluated for mutations in the rhodopsin gene, 86 for mutations in the RDS gene, and 173 for mutations in the RPL gene. Normal controls had no symptoms of RP and no family history of the disease; most normal controls did not have ocular examinations. All participants gave their informed consent before donating 10 to 50 mL of venous blood. Leukocyte DNA was purified using standard phenol-chloroform extraction methods.

Oligonucleotide primers based on the flanking intron sequences for each of the 3 exons of the NRL gene were designed with the Primer3 program (Whitehead Institute for Biomedical Research/MIT Center for Genome Research, Cambridge, Mass). The primer pairs were as follows (sense primer, antisense primer, both written in the 5'-3' direction):

- exon 1, CACAGATTAGCTTACAGAGCCCTGCTTTA, CACAGATTAAAGGGGGTTCTAGTGAGC;
- exon 2, ACCATCCCTCTGCTTTCAAAAATCTCTTCTG, GATCTGATTTCTACAAGGACCTTTCTCC; and
- exon 3, GACCTGCGCCGTACCCCGTTTCTGATCCTT, GCCCAACCCACGCCCCACTACACCA.

The polymerase chain reaction was used to amplify exon fragments from 20 ng of leukocyte DNA in a solution of 20 mM of Tris-HCl (pH, 8.4); 1.5 mM (for exon 1), 1.0 mM (for exon 2), or 2.0 mM (for exon 3) of MgCl₂; 50 mM of KCl; 0.1 mg/mL of bovine serum albumin; 0.02 mM each of dATP, dCTP, and dGTP; and 0.02 mmol of dCTP including 0.6 µCi (2.2 x 10⁸ Bq) of [α-³²P]dCTP, 1% dimethyl sulfoxide; and 0.25 units of Taq DNA polymerase (Perkin Elmer, Norwalk, Conn). The temperatures used during the polymerase chain reaction were as follows: for exons 1 and 2, 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds and 67°C for 1 minute, with a final extension at 70°C for 5 minutes; for exon 3, 93°C for 1 minute followed by 30 cycles of 94°C for 30 seconds and 70°C for 3 minutes, with a final extension of 70°C for 5 minutes. The DNA fragments derived from the amplification of exons 2 and 3 were digested with the restriction endonucleases StuI and HphI, respectively. The amplified DNA fragments were then heat-denatured and loaded on to acrylamide gels to separate the sense and antisense strands. Anomalously migrating fragments were subsequently analyzed using direct sequencing with an ABI PRISM 377 DNA sequencer using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer’s protocol (Applied Biosystems, Foster City, Calif).

As an additional assay for the previously published NRL mutations Ser50Thr and Pro31Leu, we amplified the DNA fragment containing exon 2 separately without radiolabeled dCTP. After digestion with HphI, the resulting DNA fragments were separated using electrophoresis through a 2% agarose gel with 0.4 µg/mL of ethidium bromide. DNA samples that were not cleaved by HphI were easily detectable by inspection.

Ocular examinations were performed according to techniques previously described. Specifically, dark-adapted thresholds after 95 minutes of dark adaptation were measured with the Goldmann-Weekers darkadaptometer using an 11° white test light projected either centrally or, if the patient’s visual field was sufficiently large, 7° below fixation. Kinetic perimetry was performed with the V4e white test light of the Goldmann perimeter, bringing the test light from the nonseeing to the seeing areas. Visual field areas were determined after plotting the fields with a desktop planimeter or by scanning images of the visual fields into a computer. Equivalent visual field diameters were calculated as twice the square root of the visual field area divided by π, that is, 2(area/π)².²²

After 45 minutes of dark adaptation, full-field electroretinograms (ERGs) were elicited in darkness to single, 10-μsecond flashes (0.5 Hz) of white light (3.5 log foot-lambert) and then to 30-Hz white, 10-μsecond flashes of the same luminance in a Ganzfeld dome. Responses were recorded without computer averaging for amplitudes greater than 10 µV or with computer averaging for amplitudes less than 10 µV. Amplitudes were measured from the trough of the a-wave (or from the baseline if the a-wave was absent) to the peak of the b-wave for responses to 0.5-Hz light flashes, and from trough to peak for the responses to 30-Hz flashes. Nondetectable responses, defined as amplitudes less than 1 µV for responses to 0.5-Hz light flashes or less than 0.05 µV for responses to 30-Hz light flashes, were coded as 1 µV or 0.05 µV, respectively, because these are the limits of detectability. The reproducibility of sub-microvolt signals in response to 30-Hz light flashes has been documented previously.¹⁰,¹¹

Many patients were clinically evaluated a decade or more before the genetic analysis. When patients had more than 1 clinical evaluation, data from the initial visit were used for analysis. Test results from both eyes were averaged. Mean values for ocular function (visual acuity, dark-adapted threshold elevation, equivalent visual field diameter, log ERG amplitude with 0.5-Hz flashes, log ERG amplitude with 30-Hz flashes, and ERG implicit time with 30-Hz flashes) were compared with data obtained previously with the same conditions from patients who had the dominant rhodopsin mutations Pro23His and Pro347Leu.¹³,¹² Multiple regression analyses were performed with each measure of ocular function as the dependent variable and with age, refractive error (spherical equivalent), and genetic class (NRL, Pro23His, or Pro347Leu) as the independent variables. We controlled for age and refractive error because these factors contribute to the variation in ocular function measured in patients with RP. Differences by genetic class (NRL vs Pro23His, and NRL vs Pro347Leu) were assessed using linear contrast. In addition, multiple regression analyses were also performed based on ranks to better approximate normal distributions for the measures of ocular function. The ocular function variables were converted to ranks and then to the normal form based on the cumulative probability distribution function for the normal distribution.¹³ Statistical analyses were performed using the software JMP, version 3.2 (SAS Institute Inc, Cary, NC).
rhodopsin mutations Pro23His and Pro347Leu. These 2 rhodopsin mutations were selected for this comparison because they are the 2 most frequent dominant rhodopsin mutations in North America and because they cause RP that is roughly at the least severe (Pro23His) and most severe (Pro347Leu) ends of the disease spectrum caused by dominant rhodopsin mutations.5

RESULTS

We surveyed 189 unrelated patients with dominant RP for mutations in the NRL gene. Three novel mutations were discovered, all of which were missense (Figure 1): Ser50Pro (1942 T to C), Ser50Leu (1943 C to T), and Pro51Thr (1945 C to A). The mutations Ser50Pro and Pro51Thr were found in 1 patient each; the mutation Ser50Leu was found in 2 unrelated index patients. None of these mutations were found among 91 unrelated control individuals without RP. Each of the mutations cosegregated with RP in the families of the index patients (Figure 1). No other sequence anomalies were found in the coding sequence or the intron splice acceptor or donor sites.

To be certain that the previously reported NRL mutations Ser50Thr and Pro51Leu were not missed because of an insensitivity of the single-strand conformation analysis used for the mutation screen, we specifically searched for these mutations by analyzing the amplified fragments containing codons 50 and 51 (exon 2) after digestion with the restriction endonuclease HphI. A recognition sequence at which this enzyme cleaves DNA is normally present within codons 50 and 51 and is eliminated by the mutations Ser50Thr and Pro51Leu and by other mutations of these codons. Although this analysis failed to uncover any instances of Ser50Thr or Pro51Leu, it did detect, as expected, the 3 novel mutations described here.

Figure 1. A, Sequences of novel NRL mutations. For each of the 3 mutations, the sequence from an affected index patient is shown above the sequence of the same region from a control patient with the wild-type sequence. The numbers 001-083, 001-338, and 001-172 are the identification numbers of the index patients. Not shown is the sequence of index patient 001-122, who had the same Ser50Leu mutation as patient 001-338. The electropherogram depicting the Ser50Pro mutation in patient 001-083 was from a sequencing run in the antisense direction; the image was flipped for this figure to show the sense sequence, and the nucleotide colors were correspondingly changed. Pro indicates proline; Ser, serine; Thr, threonine; and Phe, phenylalanine. B, Schematic pedigrees of 4 families, illustrating the cosegregation of the NRL mutations with dominant retinitis pigmentosa. Filled symbols indicate affected individuals; open symbols, unaffected individuals. Arrows point to the index patient in each family (patient 001-083 in family 5715, 001-338 in family E481, 001-122 in family 5763, and 001-172 in family 5677). The NRL genotype of each individual whose DNA was evaluated is shown below the corresponding symbol. The plus sign indicates the wild-type sequence of codons 50 and 51, and A, B, and C are the mutations indicated at the bottom of the figure.
We clinically evaluated the 4 index patients and 3 of their affected relatives with the Ser50Pro, Ser50Leu, and Pro51Thr mutations. The patients ranged in age from 9 to 73 years. Most of these patients reported difficulty with night vision starting in the first decade of life and limitation of peripheral vision by the third decade (Table 1). Posterior subcapsular cataracts were evident in 3 of the 7 patients examined, including the 2 oldest patients (aged 34 and 73 years).

Fundus examinations revealed intraretinal bone-spicule pigment deposits in all patients; however, these deposits were present in only one eye of the youngest patient, aged 9 years. As an example of the fundus appearance of these patients, Figure 2 shows the fundi of patient 001-338 with the Ser50Leu mutation at age 21 years. The macula shows attenuation of the perifoveal retinal pigment epithelium (RPE). The arterioles are narrowed, and there are prominent intraretinal pigment deposits in the periphery. The symmetric involvement of the two eyes is also evident. The progression of the disease is suggested in Figure 3, which shows the fundi of 3 patients, aged 9, 42, and 73 years, all with the Pro51Thr mutation. The vascular attenuation is minimal in the 9-year-old patient. There are patches of RPE atrophy in the 42-year-old patient and large areas of RPE atrophy in the 73-year-old patient. In addition, the 73-year-old patient has atrophy in the central macula.

Table 2 lists the visual acuities, refractive errors, and additional measures of ocular function of the patients who underwent examination. The mean visual acuity was 20/35 or better in every patient except the oldest one, aged 73 years, who had a mean visual acuity of 20/200. (All acu-
ities are expressed as the mean between the two eyes.) There was no apparent tendency toward myopia or hyperopia (mean spherical equivalent, +0.13 diopters). The dark-adaptation thresholds were elevated 1.5 to 3.5 log units above normal (mean, 2.6 log units), consistent with the symptom of night blindness in most patients. Visual fields were constricted in all patients (mean equivalent field diameter, 57°; normal diameter, 120°).

The geometric mean 0.5-Hz (rod-plus-cone) and 30-Hz (cone) ERG amplitudes were both reduced to 1% or less of the lower limit of normal; specifically, the geometric mean 0.5-Hz ERG amplitude was 2.7 µV (only 0.8% of the lower limit of normal; normal amplitude, ≥350 µV), and the mean 30-Hz ERG amplitude was 0.53 µV (approximately 1% of the lower limit of normal; normal amplitude, ≥50 µV). The similarly reduced amplitudes of both the 0.5-Hz and 30-Hz ERGs indicate that rod and cone function were decreased by an approximately equal percentage. The cone ERG implicit time was prolonged in every patient, even in the youngest patient (aged 9 years), who had the largest (but still subnormal) amplitude. The average cone ERG implicit time, 46 milliseconds, was 14 milliseconds longer than the outer normal limit of 32 milliseconds.

We compared the mean retinal function in our set of 7 patients who had NRL mutations with that in 39 patients with the Pro23His rhodopsin mutation and 25 patients with the Pro347Leu rhodopsin mutation who were previously included in a study of patients with rhodopsin mutations (Table 3). The mean age of the patients with the NRL mutations (30 years) was different from the mean ages of the sets of patients with the Pro23His and Pro347Leu mutations (40 and 31 years, respectively); mean refractive errors were also slightly different in the 3 groups (the mean spherical equivalent was +0.13, −0.60, and −0.33 diopters in the patients with the NRL, Pro23His, and Pro347Leu mutations, respectively). To adjust for these dif-

Figure 3. Fundus photographs of 3 patients with the NRL mutation Pro51Thr, aged 9 years (A and B, views of the posterior pole and nasal periphery, respectively, of the right eye), 42 years (C and D, views of the nasal periphery and posterior pole, respectively, of the left eye), and 73 years (E and F, different views of the posterior pole of the right eye). The extent of retinal pigment epithelial atrophy and number of retinal pigment deposits are greater in the older individuals. The macula of the 73-year-old patient has a mitten-shaped region of atrophy not present in the younger patients.
The original designation of NRL as a cause of dominant RP was based on a single missense mutation, Ser50Thr, that cosegregated with dominant RP. This mutation could have been interpreted as a rare nonpathogenic variant that happened to be in phase with a pathogenic mutation in another closely linked gene. This alternative explanation is unlikely because the NRL protein with the Ser50Thr mutation functioned normally when studied in vitro. Our discovery of 3 novel mutations together with the recent report of 2 NRL mutations (Pro51Leu and Gly122Glu) in patients with RP from Spain provides additional evidence confirming NRL as a cause of dominant RP. Our newly discovered mutations all cosegregate with RP and were not found among normal controls, as would be expected for dominant pathogenic mutations.

The proportion of patients with both dominant RP and NRL mutations is low. Summing our data, based on a survey of 189 unrelated patients mostly from North America, with the published surveys of 200 and 130 patients from England and Spain, respectively, a total of 8 of 519 unrelated patients with dominant RP have the disease because of NRL mutations. After correcting for the fact that patients with mutations in the rhodopsin and RDS genes were generally excluded from these surveys (together these genes account for about 18%-28% of dominant RP cases), we estimate the proportion of dominant RP caused by mutations in the NRL gene to be approximately 1%.

Table 2. Visual Function in Patients With NRL Mutations

<table>
<thead>
<tr>
<th>Identification No.</th>
<th>Family No.</th>
<th>NRL Mutation</th>
<th>Age, y</th>
<th>Sex</th>
<th>Snellen Visual Acuity</th>
<th>Spherical Equivalent, † D</th>
<th>Dark Adaptation‡</th>
<th>Visual Field Diameter,§ degrees</th>
<th>Full-Field Electroretinograms¶</th>
<th>0.5-Hz Amplitude, μV</th>
<th>30-Hz Amplitude, μV</th>
<th>30-Hz Implicit Time, ms</th>
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<tr>
<td>226-1705</td>
<td>5677</td>
<td>Pro51Thr</td>
<td>9</td>
<td>M</td>
<td>20/20</td>
<td>0.75</td>
<td>2.0</td>
<td>−60.0§</td>
<td>20.0</td>
<td>3.49</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>001-122</td>
<td>5783</td>
<td>Ser50Leu</td>
<td>21</td>
<td>M</td>
<td>20/35</td>
<td>2.25</td>
<td>2.8</td>
<td>53.5</td>
<td>1.65</td>
<td>0.17</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>001-338</td>
<td>E481</td>
<td>Ser50Leu</td>
<td>21</td>
<td>M</td>
<td>20/27</td>
<td>−0.75</td>
<td>2.5</td>
<td>58.5</td>
<td>1.03</td>
<td>0.36</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>226-1692</td>
<td>5715</td>
<td>Ser50Pro</td>
<td>25</td>
<td>F</td>
<td>20/30</td>
<td>−0.19</td>
<td>1.5</td>
<td>123.0</td>
<td>8.98</td>
<td>0.81</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>001-172</td>
<td>5677</td>
<td>Pro51Thr</td>
<td>26</td>
<td>F</td>
<td>20/27</td>
<td>−3.12</td>
<td>2.8</td>
<td>64.0</td>
<td>3.74</td>
<td>0.61</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>001-083</td>
<td>5715</td>
<td>Ser50Pro</td>
<td>34</td>
<td>F</td>
<td>20/30</td>
<td>0</td>
<td>3.0</td>
<td>22.5</td>
<td>1.64</td>
<td>0.49</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>218-427</td>
<td>5677</td>
<td>Pro51Thr</td>
<td>73</td>
<td>F</td>
<td>20/200</td>
<td>2.00</td>
<td>3.5</td>
<td>15.5</td>
<td>1.0</td>
<td>0.24</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Averages#</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/21, 0.13</td>
<td>2.6</td>
<td>56.7</td>
<td></td>
<td></td>
<td>2.7</td>
<td>53.3</td>
<td>46</td>
</tr>
</tbody>
</table>

*Values for visual acuity, spherical equivalent, visual field diameter, and electroretinogram implicit time are expressed as the mean between the two eyes. NRL indicates neural retina leucine zipper gene; ellipses, not applicable.
†Spherical equivalent = sphere + 1/2 cylinder.
‡Log threshold above normal after 45 minutes of dark adaptation.
§Equivalent diameter derived from visual field area to a V4e white test light; normal diameter is ≥120°.
¶Normal 0.5-Hz amplitude is ≥50 μV; normal 30-Hz amplitude, ≥50 μV; and normal 30-Hz cone implicit time, ≥32 milliseconds.
#Averages are arithmetic means except for the 0.5-Hz and 30-Hz electroretinogram amplitudes, which are geometric means; the values in this table are not corrected for age or refractive error.

Table 3. Comparisons of Visual Function in Patients With NRL Mutations vs Patients With the Rhodopsin Mutations Pro23His and Pro347Leu

<table>
<thead>
<tr>
<th>Ocular Function</th>
<th>NRL (n = 7)</th>
<th>Pro23His (n = 39)</th>
<th>Pro347Leu (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual acuity</td>
<td>0.59 ± 0.08 (20/34)</td>
<td>0.80 ± 0.03 (20/25)</td>
<td>0.53 ± 0.04 (20/38)</td>
<td>.02</td>
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<tr>
<td>Log elevation of the dark-adapted threshold</td>
<td>2.8 ± 0.3</td>
<td>1.3 ± 0.1</td>
<td>3.3 ± 0.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Equivalent visual field diameter, degrees</td>
<td>44.8 ± 12.9</td>
<td>90.1 ± 5.5</td>
<td>54.0 ± 7.0</td>
<td>.002</td>
</tr>
<tr>
<td>Log 0.5-Hz ERG amplitude (geometric mean), μV</td>
<td>0.25 ± 0.21 (1.8)</td>
<td>1.42 ± 0.09 (26.3)</td>
<td>0.32 ± 0.12 (2.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Log 30-Hz ERG amplitude (geometric mean), μV</td>
<td>−0.51 ± 0.25 (0.31)</td>
<td>1.02 ± 0.11 (10.5)</td>
<td>−0.18 ± 0.14 (0.66)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>30-Hz ERG implicit time, ms</td>
<td>46.7 ± 2.2</td>
<td>39.2 ± 1.0</td>
<td>40.1 ± 1.2</td>
<td>.003</td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SEM unless otherwise indicated and are adjusted for age and refractive error. NRL indicates neural retina leucine zipper gene; ERG, electroretinogram.
The protein NRL is a member of the v-maf family of transcription factors. The protein is 237 amino acid residues in size and is encoded by a gene with 3 exons. The protein can form with another transcription factor, CRX. Data reported by Mitton et al suggest that NRL forms a homodimer mediated by its leucine zipper motif and that this homodimer may form the structure recognized by CRX. NRL is expressed in the brain and retina, whereas CRX is expressed in the pineal body and retina. Both of these DNA-binding proteins recognize sequences in the promoters of photoreceptor-specific genes such as those encoding rhodopsin and interphotoreceptor retinoid binding protein. In vitro, the NRL-CRX complex can activate the rhodopsin promoter approximately 10 times as well as CRX alone and 80 times as well as NRL alone. In vivo, the onset of NRL expression during retinal development is later than that of CRX, indicating that the NRL-CRX complex is probably not necessary for certain stages of photoreceptor development and differentiation.

It is notable that the 3 novel mutations and the 3 previously reported mutations are all missense mutations and that 5 of the 6 mutations affect amino acid residues 50 or 51. This observation supports the notion that residues 50 and 51 are part of an important functional or structural domain. One of the 4 mutants, Ser50Thr, when expressed in vitro together with CRX, has an increased ability to activate the rhodopsin promoter compared with wild-type NRL, also expressed together with CRX. It will be interesting to see if the other mutants share this property or if some other functional abnormality shared by the 4 mutants could explain their toxic effects on photoreceptor cells.

Fundus abnormalities, elevated dark-adaptation thresholds, constricted visual fields, and reduced and delayed ERGs were all present in patients with NRL mutations, as is typical of dominant RP. We observed that rod and cone function, as monitored by the full-field ERG, were comparably reduced in patients with NRL mutations. Although we observed an apparent progression of retinal degeneration by comparing patients of different ages with the same NRL mutation (Figure 3), an accurate description of the course of disease associated with NRL mutations would require longitudinal follow-up of large patient cohorts.

Even after correcting for age, patients with NRL mutations exhibited on average more severe disease than patients with the rhodopsin mutation Pro23His, based on every measure of retinal function we examined. As examples, the patients with the NRL mutations had about a 2-fold smaller mean visual field diameter and about a 30-fold lower geometric mean cone ERG amplitude. In contrast, retinal function of the patients with NRL mutations was, for the most part, similar to that of patients with the rhodopsin mutation Pro347Leu, which causes RP at the severe end of the spectrum of disease produced by dominant rhodopsin mutations. The comparisons between the RP caused by NRL vs rhodopsin mutations are tentative, however, because they are based on a small group of patients with the NRL mutations.

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