Description of a New Mutation in Rhodopsin, Pro23Ala, and Comparison With Electroretinographic and Clinical Characteristics of the Pro23His Mutation

Kean T. Oh, MD; Richard G. Weleber, MD; Andrew Lotery, MD, FRCOphth; Dawn M. Oh, MS; Andrea M. Billingslea, BS; Edwin M. Stone, MD, PhD

Objectives: To report the clinical characteristics of a family with autosomal dominant retinitis pigmentosa caused by a proline-to-alanine mutation at codon 23 (Pro23Ala) of the rhodopsin gene and to compare this phenotype with that associated with the more common proline-to-histidine mutation at codon 23 (Pro23His).

Methods: We examined 6 patients within a single pedigree. The electroretinograms (ERGs) of 35 patients with known Pro23His mutations and of 22 healthy individuals were reviewed. Scotopic dim flash–response amplitudes, maximum combined-response amplitudes, and photopic-response amplitudes from the ERGs of these patients were plotted against age. The ERG indices of 5 individuals in the Pro23Ala family were compared with those of the patients with Pro23His mutations and of healthy individuals. Multiple linear regression was performed to evaluate the effect of age and mutation type on amplitudes. Mutation detection was performed using single-strand conformation polymorphism analysis, followed by automated DNA sequencing.

Results: Patients with the Pro23Ala mutation have a clinical phenotype characterized by onset of symptoms in the second to fourth decades of life, loss of superior visual field with relatively well-preserved inferior fields, and mild nyctalopia. Comparison with patients with the Pro23His mutation demonstrates statistically significant differences (P<.001) in responses to dim flash, maximum combined, and photopic responses between patients with these mutations after controlling for the effects of age. Patients with Pro23Ala mutations were less affected by ERG criteria than patients with Pro23His mutations. Patients with Pro23Ala mutations also differed significantly from healthy patients in all ERG indices examined (P<.001), after controlling for age.

Conclusion: We describe a rare mutation in codon 23 of rhodopsin causing autosomal dominant retinitis pigmentosa. The retinal dystrophy associated with the Pro23Ala mutation is characteristically mild in presentation and course, with greater preservation of ERG amplitudes than the more prevalent Pro23His mutation.

Arch Ophthalmol. 2000;118:1269-1276

Rhodopsin is the photopigment of rod photoreceptors and forms an integral part of the visual cascade. Mutations in the rhodopsin gene account for 25% to 30% of all cases of autosomal dominant retinitis pigmentosa (ADRP).1-3 To date, 96 sequence variations in the rhodopsin gene have been identified in patients with ADRP, and 4 have been associated with autosomal recessive retinitis pigmentosa (RP). Autosomal dominant RP due to rhodopsin mutations has a wide range of clinical presentation and severity. A mutation of codon 23 of the rhodopsin gene in which proline is changed to histidine (Pro23His) accounts for the largest fraction of rhodopsin mutations observed in the United States. The phenotype of the RP associated with the Pro23His mutation is characteristically relatively mild but variable.1-8 A second mutation of proline to leucine at codon 23 (Pro23Leu) has also been described in a single family.3 We herein describe 6 individuals in a single family with RP of a sectorial or altitudinal distribution of disease associated with a documented mutation of proline to alanine at codon 23 (Pro23Ala) in the rhodopsin gene.

REPORT OF CASES

PATIENT 1:2

The proband was a 78-year-old white woman with a lifelong history of nyctalopia. She reported being able to see stars in the sky during her childhood and driving at night until 70 years of age. She began to notice problems with her peripheral vision at 50 years of age. On initial examination, her visual acuity was 20/60 OU. She
**PATIENTS AND METHODS**

**PATIENTS**

We examined 6 individuals from a 4-generation family of Dutch ancestry. Although no one else in the family was known to be affected, the proband’s mother died at 30 years of age. Six clinically affected individuals were examined, and results of molecular analysis and historical data ruled out ADRP in a seventh individual. The pedigree demonstrates autosomal dominant pattern of transmission (Figure 1). The patients with the Pro23His mutation belonged to a 10-generation family that has been studied for 25 years by one of us (R.G.W.).

**CLINICAL METHODS**

**Examination**

All patients completed a retinal degeneration questionnaire developed at the Casey Eye Institute, Portland, Ore. Best-corrected visual acuities were obtained using projected Snellen charts. Goldmann perimetry was performed using multiple isopters according to standard technique. Visual fields were digitized, and the area of each isopter (seeing field minus any scotomas) was expressed in steradians. In all patients, final dark adaptation threshold was measured with an 11° test target directed 10° below fixation, or wherever sensitivity was best, after 45-minute dark adaptation using a Goldmann-Weekers adaptometer (Haag-Streit, AG, Bern, Switzerland). A standard, comprehensive ophthalmic examination was performed, including tonometry, slitlamp biomicroscopy, and direct and indirect ophthalmoscopy. Fundus photography was used to document abnormal fundus findings. Table 1 presents the clinical patient information.

**Ganzfeld electroretinography (ERG)** was performed using previously described techniques that adhere to the standards set by the Standardization Committee for the International Society for Clinical Electrophysiology of Vision. Patient responses (Figure 2) were compared with published reference ERG ranges. The records of 35 patients with Pro23His mutations and 22 healthy patients from the practice of one of us (R.G.W.) were reviewed for ERG data. Amplitudes for b waves under scotopic conditions for dim white flashes and scotopic and photopic bright white flashes were recorded. The amplitudes of the b wave from each of the patients’ eyes were averaged for data analysis. We used commercially available statistical software (JMP IN; SAS Inc, Cary, NC, and Systat Inc, Chicago, Ill) to compare the amplitudes of the 6 ERGs from patients with the Pro23Ala mutation with the amplitudes from healthy subjects and with those from patients with the Pro23His mutation, using multiple linear regression to control for age. The regression was repeated for each of the following responses: scotopic bright flash, scotopic dim flash, and photopic bright flash. Consistent with other studies, our data and larger groups of normal data (K.T.O., R.G.W., D.M.O., unpublished data, July 1999) confirm a non-Gaussian distribution for ERG b-wave amplitude. We used a square root transformation on amplitude to normalize the data for analysis and to obtain normal residuals for the regression model. Amplitude data were also plotted as a scattergram with respect to age, and a best-fit line was drawn showing the difference in amplitudes with aging for all 3 populations.

**Molecular Methods**

Informed consent was obtained from all study patients or their legal guardians. We extracted DNA from peripheral blood using a previously described protocol. The proband, and in many cases all those at risk, in each family underwent screening for mutations in the coding sequence of the rhodopsin gene by means of single-strand conformation polymorphism analysis. Primer sequences and polymerase chain reaction (PCR) conditions are available on request from the authors. The PCR amplification products were denatured for 3 minutes at 94°C and then electrophoresed on 6% polyacrylamide–3% glycerol gels at 25 W for about 3 hours. The gels were then stained with silver nitrate. The PCR products from samples with aberrant migration patterns were then sequenced bidirectionally with fluorescent deoxyxynucleotides on an automated sequencer (ABI model 377; PE Biosystems, Foster City, Calif).

had mild nuclear sclerotic and posterior subcapsular cataracts in both eyes. Results of dilated fundus examination revealed bone spiculelike pigmentation and retinal pigment epithelial (RPE) atrophy in the inferior and nasal distributions of the midperiphery. The far periphery was minimally affected. There was RPE atrophy in both maculas and mild attenuation of retinal vessels predominantly in the inferior distribution (Figure 3, A). Goldmann perimetry revealed superior field loss and mild constriction of isopters in the inferior hemifields (Figure 3, B).

Her final dark-adapted threshold was severely elevated at −2.25 log candelas (cd)/m² OD and −2.05 log cd/m² OS (normal, −5.00 log cd/m²; normal upper limit, −4.55 log cd/m²). This indicates that the subject’s final retinal psychophysical threshold was elevated slightly less than 3 log units above normal rod level, which is into the cone range. Results of ERG testing demonstrated moderately subnormal scotopic b-wave amplitudes with markedly prolonged rod and cone b-wave implicit times (Table 2 and Figure 2).

**PATIENT II:2**

A 57-year-old man had received a diagnosis of RP 5 years earlier. He denied nyctalopia but reported peripheral field loss beginning at about 50 years of age. On examination, his visual acuity was 20/25 OD and 20/40+2 OS. Results of fundus examination demonstrated RPE depigmentation in the inferior midperiphery and nasal quadrants. Occasional bone spiculelike pigmentation was seen in this region, but the choroidal vasculature appeared normal. Final dark adaptation threshold was elevated at −3.0 log cd/m² OD and −3.1 log cd/m² OS (normal rod mean, −5.00; normal rod upper limit, −4.65 log cd/m²). Electroretinographic testing demonstrated moderate impairment of scotopic responses with normal photopic am-
PATIENT III:2

A 35-year-old woman had noticed “blanks” in her visual field as early as high school and nyctalopia in her early 20s. Her uncorrected visual acuity was 20/20 OU. Results of fundus examination demonstrated symmetric bone spiculelike pigmentation, RPE atrophy, and vascular attenuation inferiorly and nasally (Figure 5, A). Final dark adaptation thresholds were elevated at −3.7 log cd/m² OU. Goldmann perimetry demonstrated superior field loss with a rim of preserved field to the IV4e and III4e isopters (Figure 4, B). Results of the fundus examination were largely unchanged (Figure 4, A) and a second ERG showed no evidence of progression during the 2 years.

PATIENT III:3

A 35-year-old man, the fraternal twin of patient III:2, noticed difficulty with his vision on very dark nights while serving in the army at 23 years of age. Retinitis pigmentosa was diagnosed at 33 years of age, when he failed a commercial driver’s license examination. On examination, his visual acuity was 20/20 OU. Results of fundus examination demonstrated RPE and choroidal atrophy in the inferior midperiphery with normal retina present anteriorly. There was greater involvement of the nasal periphery. Final dark adaptation thresholds were elevated at −4.00 log cd/m² OD and −4.25 log cd/m² OS (normal, −5.15 log cd/m²; normal upper limit, −4.81 log cd/m²). Goldmann perimetry showed superior hemifield loss with slight extension below the horizontal meridian temporally. There was a rim of residual field remaining superiorly to the III4e and V4e isopters in both eyes. Electroretinography demonstrated moderately subnormal scotopic responses with lesser involvement of cones under photopic conditions (Table 2). Cone implicit times were prolonged to 30-Hz flicker and single bright flash.

PATIENTS IV:1 AND IV:2

Both children of the patients in generation III were asymptomatic. Patient IV:1 was a boy aged 14 years (son of patient III:2) with minimal fundus changes and relatively normal Goldmann visual fields. Electroretinographic testing demonstrated mildly reduced scotopic and maximum combined responses (Table 2, Table 3, and Figure 2). Patient IV:2 was a girl aged 9 years (daughter of patient III:3) with inferiorly distributed RPE mottling and shallow superior field defects on Goldmann visual field testing (Table 3 and Figure 6).

STATISTICAL ANALYSIS

The ERGs of 22 healthy individuals, 35 patients with Pro23His mutations, and the 5 patients with Pro23Ala mutations were analyzed with regard to age and amplitude. Ages in the healthy

Table 1. Patient Basic Data

<table>
<thead>
<tr>
<th>Patient No./Age, y</th>
<th>Current BCVA</th>
<th>Manifest Refraction</th>
<th>Goldmann Perimetry Results</th>
<th>Reduction Scotopic Dim Flash ERG, %</th>
<th>Fundus Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/2/79.4 50</td>
<td>20/70 OD</td>
<td>−1.50 + 2.00 × 180 OD</td>
<td>Superior loss, inferior constriction</td>
<td>31.8</td>
<td>Bone Spic and atrophy in Inf/nasal</td>
</tr>
<tr>
<td>II/2/58.6 50</td>
<td>20/80 OS</td>
<td>−1.50 + 1.00 × 175 OS</td>
<td>Superior loss with rim</td>
<td>31.3</td>
<td>Bone Spic and atrophy in Inf/nasal</td>
</tr>
<tr>
<td>III/2/35.6 16</td>
<td>20/25 OS</td>
<td>−0.75 × 0.25 × 028 OS</td>
<td>Superior loss with rim</td>
<td>30.2</td>
<td>Bone Spic and atrophy in Inf/nasal</td>
</tr>
<tr>
<td>III/3/35.6 23</td>
<td>20/20 OU</td>
<td>−0.75 × 0.75 × 155 OD</td>
<td>Superior loss with island</td>
<td>35.4</td>
<td>Bone Spic and atrophy in Inf/nasal</td>
</tr>
<tr>
<td>IV/1/14.3</td>
<td>20/20 OD</td>
<td>−1.75 sphere OD</td>
<td>Mild superior constriction</td>
<td>51.0</td>
<td>RPE stippling inferior; right eye &gt; left eye</td>
</tr>
<tr>
<td>IV/2/9.4</td>
<td>20/20 OU</td>
<td>−1.75 sphere OD</td>
<td>Superior scotoma</td>
<td>NA</td>
<td>RPE clumping and atrophy in Inf/nasal</td>
</tr>
</tbody>
</table>

*BCVA indicates best corrected visual acuity; ERG, electroretinogram; Bone Spic, bone spiculelike pigmentation; Inf/nasal, inferior and nasal distributions of midperiphery; RPE, retinal pigment epithelium; and NA, not available.
group ranged from 13 to 67 years (mean, 37.1 years); in the Pro23His group, 6 to 73 years (mean, 32.1 years); and in the Pro23Ala group, 14 to 80 years (mean, 47.1 years). The difference in mean ages between groups required the inclusion of age into our multiple regression model for testing.

The scattergrams of ERG amplitudes with respect to age showed some overlap between the Pro23Ala and Pro23His groups, but the best-fit regression lines drawn to show the difference in amplitudes with aging were clearly different for all 3 populations (Figure 7).

For statistical analysis, all b-wave amplitudes were first transformed, using a logarithm transformation that has been used by others. A constant of 1 was added to accommodate 0-level responses. The amplitudes ranged from 0 to 837 µV for maximum combined response, 0 to 315 µV for photopic responses, and 0 to 631 µV for scotopic responses. When the multiple regression model was run with logarithm transformed amplitude as the outcome and age and group (healthy, Pro23His, and Pro23Ala) as the variables, the residuals were signifi-

Figure 2. Electroretinographic waveforms. Waveforms are prolonged and reduced but recordable in all patients. The x waves (short arrows) in the red flash waveforms are prominent in patient IV:1, while in other patients, they form a sharp shoulder. OP indicates oscillatory potential; cd, candela.

Figure 3. Patient I:2. A, Montage of posterior pole, right eye, at age 78 years. Mild vascular attenuation is seen in the inferior temporal arcades, as well as optic disc pallor. Bone spiculelike pigmentation is noted inferiorly, and the macula demonstrates retinal pigment epithelial atrophy and clumping. B, Goldmann perimetry. The right eye can only see to the I4e isopter. The superior hemifield is much more severely affected in both eyes.
cantly skewed. We then attempted a square root transformation with the amplitude, which had equal success at normalizing the distribution, but also provided normal residuals. The results of that model, repeated for each of the 3 ERG measurement types, are given in Table 4.

The model considered the effects of age and the Pro23Ala mutation, comparing this relationship with amplitudes for the healthy and Pro23His groups. All of the models (scotopic bright and dim white flashes and photopic bright flash) showed the effect of age on amplitude to be significant. Further, all 3 models showed the effect of group type to be significant, and showed the Pro23Ala amplitudes to vary significantly from the healthy and Pro23His amplitudes. Although compared with the healthy population, the Pro23Ala group had significantly reduced waveform in all cases, and their amplitudes were significantly higher than those of the Pro23His population. The P values and means for these comparisons are listed in Table 4.

MOLECULAR RESULTS

All affected patients from the Pro23Ala family harbored the same sequence variation, a cytosine-to-guanine change in the first nucleotide of codon 23; patient II:1 had clinically normal results of examination, with negative findings for the mutation. This sequence change results in the substitution of alanine for proline at the protein level. This sequence variation has not been observed previously in 3277

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Table 2. Electroretinography Values*

<table>
<thead>
<tr>
<th>Patient No./ Age at ERG, y</th>
<th>DA Dim Flash Amplitude, µV (Range)</th>
<th>DA Dim Flash Implicit Time, ms</th>
<th>DA Bright Flash Amplitude, µV (Range)</th>
<th>DA Bright Flash Implicit Time, ms</th>
<th>Photopic Bright Flash Amplitude, µV (Range)</th>
<th>Photopic Bright Flash Implicit Time, ms</th>
<th>Scotopic OP Index OP2-5 (Amplitude), µV</th>
<th>30-Hz Flicker Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>I:2/79.4</td>
<td>74/101 (175-378)</td>
<td>109/120 (73-92)</td>
<td>151/153 (372-647)</td>
<td>68/70 (42-57)</td>
<td>54/42 (48-246)</td>
<td>43.4/40.2 (25.9-33.2)</td>
<td>22/22 (245)</td>
<td>Reduced both eyes, prolonged both eyes</td>
</tr>
<tr>
<td>II:2/57.9</td>
<td>67/75 (187-411)</td>
<td>106/103 (73-92)</td>
<td>148/184 (385-660)</td>
<td>66/64 (42-57)</td>
<td>53/76 (58-256)</td>
<td>35.4/34.6 (25.9-33.2)</td>
<td>8/10 (245)</td>
<td>Reduced both eyes, prolonged both eyes</td>
</tr>
<tr>
<td>II:2/59.6</td>
<td>75/112 (187-411)</td>
<td>105/110 (73-92)</td>
<td>113/191 (385-660)</td>
<td>66/64 (42-57)</td>
<td>46/62 (58-256)</td>
<td>34.0/33.0 (25.9-33.2)</td>
<td>15/16 (245)</td>
<td>Reduced both eyes, prolonged both eyes</td>
</tr>
<tr>
<td>III:2/35.6</td>
<td>127/95 (260-473)</td>
<td>96/88 (73-92)</td>
<td>211/175 (450-725)</td>
<td>60/60 (42-57)</td>
<td>106/93 (111-309)</td>
<td>31.8/31.8 (25.9-33.2)</td>
<td>39/52 (245)</td>
<td>Reduced both eyes, normal timing both eyes</td>
</tr>
<tr>
<td>III:3/35.6</td>
<td>131/130 (260-473)</td>
<td>106/115 (73-92)</td>
<td>212/245 (450-725)</td>
<td>64/64 (42-57)</td>
<td>68/77 (111-309)</td>
<td>35.6/35.0 (25.9-33.2)</td>
<td>47/53 (245)</td>
<td>Reduced both eyes, prolonged both eyes</td>
</tr>
<tr>
<td>IV:2/14.3</td>
<td>173/247 (300-524)</td>
<td>100/82 (73-92)</td>
<td>268/428 (501-777)</td>
<td>53/56 (42-57)</td>
<td>157/256 (153-351)</td>
<td>32.6/32.8 (25.9-33.2)</td>
<td>96/146 (245)</td>
<td>Reduced right eye, normal timing both eyes</td>
</tr>
</tbody>
</table>

* ERG indicates electroretinogram; DA, dark adapted; and OP, oscillatory potential. Data are expressed as right eye/left eye.

Figure 4. Patient II:2. A, Montage of posterior pole, right eye, at age 59 years. There are no macular changes or drusen in either eye. There is mild vascular attenuation inferiorty and nasally. The far inferior peripheral retina appears normal. B, Goldmann perimetry. There is greater involvement of the superior hemifields with an arc of preserved field to the IV4e and III4e isopters bilaterally.
unrelated patients with retinal degeneration who underwent screening for rhodopsin mutations in our laboratory. The Pro23His mutation found in the 10-generation family segregated correctly with disease status.8 This mutation has been documented as being pathogenic in other studies.4-6,17-20

COMMENT

Retinitis pigmentosa affects between 50,000 and 100,000 people in the United States.3 Autosomal dominant RP accounts for approximately 15% of these cases. In 1990, the Pro23His mutation of the rhodopsin gene was reported as the first mutation associated with RP.1,2,4-6 This mutation has been described only in the United States, where it continues to be the most commonly described gene defect in RP. Most mutations described in rhodopsin, most notably the Pro23His mutation, appear to result from point mutations in a single founder individual.1-7,13,21 However, a mutational “hot spot” at codon 347 has been identified with multiple unrelated families exhibiting the Pro347Leu mutation, and 3 other documented mutations have been identified at this particular codon.3 We suspect that our fam-

Table 3. Visual Field Data*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age, y</th>
<th>Isopters, Steradians</th>
</tr>
</thead>
<tbody>
<tr>
<td>I:2</td>
<td>78.3</td>
<td>V4e: 0.891/0.229, IV4e: 0.400/0.311, III4e: 0.080/0.140, I4e: 0.021†, NS</td>
</tr>
<tr>
<td>II:2</td>
<td>59.6</td>
<td>NA</td>
</tr>
<tr>
<td>III:2</td>
<td>35.6</td>
<td>2.724/2.592, NA</td>
</tr>
<tr>
<td>III:3</td>
<td>35.6</td>
<td>1.607/1.777, NA</td>
</tr>
<tr>
<td>IV:1</td>
<td>14.4</td>
<td>3.417/3.565, NA</td>
</tr>
<tr>
<td>IV:2</td>
<td>9.4</td>
<td>3.419/3.379, NA</td>
</tr>
<tr>
<td>Healthy subjects, mean ± SD</td>
<td>3.686 ± 0.213, 3.592 ± 0.212</td>
<td>3.358 ± 0.254, 2.614 ± 0.237, 1.781 ± 0.288, 0.728 ± 0.226</td>
</tr>
</tbody>
</table>

*NA indicates not available; NS, not seen; and ellipses, not applicable. Unless otherwise indicated, data are given as right eye/left eye.
†Left eye only.

Figure 5. Patient III:2. A, Montage of posterior pole, right eye, at age 35 years. Bone spiculelike pigmentation is present inferiorly and nasally. No macular changes are apparent. B, Goldmann perimetry. There is a rim of visual field remaining in the far periphery with disproportionately greater loss in the superior hemifield.

Figure 6. Patient IV:2. A, Montage of right fundus, at age 9 years. Retinal pigment epithelial depigmentation is seen inferiorly and nasally. Early pigment clumping is also apparent in this distribution. B, Goldmann perimetry. There is a superior scotoma in the right eye to the I4e isopter and the constriction of the superior field of the left eye.

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ily descended from a single Dutch ancestor who harbored a point mutation at codon 23. Although patient I:2 had the first documented case of RP in this family, her mother died at 30 years of age, and no information is available regarding earlier ancestors. This family demonstrates the third identified mutation at codon 23, underscoring its functional significance to the rhodopsin molecule.

Multiple studies have demonstrated that the degree of severity of a given mutation in the rhodopsin gene is based in large part on its position in the rhodopsin molecule—intradiscal, transmembrane, or cytoplasmic. Intradiscal mutations tend to be less severe, whereas mutations affecting cytoplasmic domains and retinol binding sites, such as the region flanking codon 135, tend to be very severe. The severity of cytoplasmic mutations such as those at the C-terminus encoded by codon 347 may result from inappropriate intracellular transport of the molecule. Based on its intradiscal location, the Pro23Ala mutation should be relatively less severe than other forms of ADRP.

Our patients' symptoms first began in their second to fourth decades of life. Perceived impairments ranged from visual field defects alone to nyctalopia alone. The proband, at 78 years of age, retained 20/70 to 20/80 central visual acuity and some inferior visual field. Furthermore, her ERG still exhibited recordable rod-mediated responses that were comparable to those of her son. Responses in both were only slightly worse than in the 2 patients in generation III, suggesting a very slow progressive course. This concept is further supported by the lack of significant change in the ERG of patient II:2 during a 2-year period. Because of the associated subnormal and prolonged rod b-wave response, a prominent dark-adapted cone x-wave response with red flash testing is a finding that is typical and characteristic of early or mild forms of RP and is suggestive of a rod-specific gene defect but is not specific for a particular mutation (R.G.W., unpublished observation, July 1994). Results of fundus examination demonstrated a striking nasal predilection for the retinal degeneration associated with the Pro23Ala mutation. The inferior midperiphery also was symmetrically involved with sparing of the far inferior periphery.

Preferential degeneration of the inferior retina has been reported in multiple families with other mild phenotypes, including patients with the Pro23His mutation. However, none of our Pro23Ala group demonstrated diffuse disease, which can sometimes be

Table 4. Statistical Analysis Results*

<table>
<thead>
<tr>
<th>Group</th>
<th>P for Difference</th>
<th>Mean Square Root Transformation for Amplitude†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotopic bright white flash, normal vs Pro23Ala</td>
<td>&lt;.001</td>
<td>24.4 vs 14.2 (595 vs 202)</td>
</tr>
<tr>
<td>Scotopic bright white flash, Pro23His vs Pro23Ala</td>
<td>&lt;.001</td>
<td>9.3 vs 14.2 (86 vs 202)</td>
</tr>
<tr>
<td>Scotopic bright white flash, age</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Scotopic dim white flash, normal vs Pro23Ala</td>
<td>&lt;.001</td>
<td>19.4 vs 10.7 (376 vs 114)</td>
</tr>
<tr>
<td>Scotopic dim white flash, Pro23His vs Pro23Ala</td>
<td>&lt;.001</td>
<td>4.9 vs 10.7 (24 vs 114)</td>
</tr>
<tr>
<td>Scotopic dim white flash, age</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Photopic bright white flash, normal vs Pro23Ala</td>
<td>&lt;.001</td>
<td>14.0 vs 9.2 (196 vs 85)</td>
</tr>
<tr>
<td>Photopic bright white flash, Pro23His vs Pro23Ala</td>
<td>&lt;.001</td>
<td>8.9 vs 9.2 (79 vs 85)</td>
</tr>
<tr>
<td>Photopic bright white flash, age</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

* Normal indicates healthy subjects; Pro23Ala, patients with proline to alanine substitution at codon 23; and Pro23His, patients with proline to histidine substitution at codon 23.
† The values in parentheses are the amplitudes in microvolts before transformation to a square root.
seen with the Pro23His mutation. Evaluation of b-wave amplitudes demonstrated that patients with the Pro23Ala mutation showed moderate impairment of rod and cone function when compared with findings in healthy patients. Although the superior retina in Pro23Ala disease appears to allow retention of better visual field than the inferior retina, we believe even the superior retina is abnormal because the dark adaptation thresholds were elevated and cone ERG implicit times were prolonged. However, the ERGs from the Pro23Ala group consistently demonstrated less impairment than those from the 35 patients with Pro23His mutations. The ages in the Pro23Ala family spanned 7 decades (14-79 years), and their pattern of visual loss with respect to age suggests a slower progression of disease than with Pro23His patients. This impression, however, cannot be verified without longitudinal data. Nevertheless, from an ERG standpoint, the patients with Pro23Ala are less severely affected than patients with Pro23His mutations.

Fundamental differences in the configuration of the molecule between substitution of histidine and alanine at codon 23 may account for this milder phenotype. With only 6 affected individuals, however, differences in the genetic background of this particular family cannot be discounted as the underlying cause of the apparently less severe phenotype of ADRP. Furthermore, despite similar charge and size characteristics of the amino acids alanine and proline, retinal degeneration still developed, implying the structural importance to rhodopsin of the bend caused by proline at position 23 in the tertiary structure of the protein.

Before 1991, phenotypic evidence pointed to different subsets of ADRP with varying prognoses. Molecular classification of ADRP and further subclassification based on the region of the mutation in the rhodopsin gene allowed better prediction of a particular disease course. Evaluation of this family with a Pro23Ala mutation suggests that even within these specific subsets, the prognosis is influenced by the specific mutation itself.

Accepted for publication May 12, 2000.

Kean T. Oh, MD, is a Heed Foundation Ophthalmic Fellow and a Ronald G. Michels Foundation Fellow for 1999-2000. This research was supported by the Foundation Fighting Blindness, Hunt Valley, Md (Drs Weleber and Stone and Ms Billingslea); The Carver Charitable Trust, Muscatine, Iowa; The Grousbeck Family Foundation, Boston, Mass; and, in part, by unrestricted funds from Research to Prevent Blindness, New York, NY (Drs Weleber and Stone).

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REFERENCES