The Phenotype of Severe Early Childhood Onset Retinal Dystrophy (SECORD) from Mutation of RPE65 and Differentiation from Leber Congenital Amaurosis

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PURPOSE. To describe in detail the characteristic clinical phenotype and electrophysiologic features of Severe Early Childhood Onset Retinal Dystrophy (SECORD) caused by mutation of RPE65.

METHODS. Ophthalmological examination, color fundus photography, visual field testing, detailed electrophysiologic assessment, and screening of RPE65 were undertaken in five subjects. Selected patients also had spectral domain optical coherence tomography.

RESULTS. All five patients had life-long, extremely poor night vision. Variable degrees of nyctalopia were present; three cases lacked nyctalopia at the time of assessment. Bilateral disc drusen were evident in three subjects. While case 1 had an undetectable electroretinogram and features supporting a diagnosis of Leber congenital amaurosis (LCA) as an infant, her level of acuity and function into the second decade of life was more consistent with SECORD. In two cases, both vision and electrophysiologic responses were seen to improve into the second decade of life. The objective demonstration of improved retinal function over time, with electrophysiologic testing, has not been previously reported. Cases 4 and 5 had evidence of fine white retinal dots. The authors propose that these represent abnormal accumulations of retinyl esters, as has been demonstrated in animal models, and has also been observed as lipid droplets within the retinal pigment epithelium (RPE). These white dots were seen to fade with time in the patients and were replaced by RPE changes.

CONCLUSIONS. The identification of patients with mutations in RPE65 has attained greater significance now that gene replacement trials have begun. The features presented in this article assist in the recognition of this form of LCA/SECORD. (Invest Ophtalmol Vis Sci. 2011;52:292–302) DOI:10.1167/iovs.10-6106

Leber congenital amaurosis (LCA) is a severe congenital or early infant-onset form of non-syndromic retinal blindness first described by Theodore Leber in 1869.1,2 He characterized the disorder by the presence of a searching nyctalmagia, absence of normal pupil responses, minimal if any vision beyond infancy, and a normal fundus appearance initially, followed by the development of pigmentary changes over time. In a later publication in 1916, Leber described a milder form of the same disease that came on in the 6th and 7th years of life and led to blindness by age 30 years, which he considered to be on the same spectrum as LCA.3 This later-onset retinal disorder has been referred to by several names, including juvenile and early-onset retinitis pigmentosa,4 childhood-onset severe retinitis,9 Early Onset Severe Retinal Dystrophy (EOSRD),6,7 and Severe Early Childhood Onset Retinal Dystrophy (SECORD).8

LCA and SECORD are extremely genetically heterogeneous, being caused by more than 16 genes (AIP1, CEP290, CRX, CRB1, GUCY2D, IQCB1, LCA5, LRAT, MERTK, RD3, RDH12, RPE65, SPATA7, and TULP1).8–10 All exhibit autosomal recessive inheritance except CRX and IMPDH1, where some de novo mutations result in an autosomal dominant trait.8–10

The gene RPE65 encodes the isomerase that converts all-trans retinyl esters to 11-cis retinol. Without sufficient activity of this gene product, no active chromophore can be produced. Notwithstanding a degree of worldwide variation, mutations of RPE65 account for approximately 8% of LCA,5,6,11 and a smaller percentage of patients with SECORD.6,11 The phenotype of LCA/SECORD associated with mutations in RPE65 includes night blindness from birth. Variable levels of vision may be present in childhood and may persist into adulthood.11 The assumption is that differing degrees of residual isomerase activity associated with certain RPE65 alleles and/or variations in alternative 11-cis-retinal regeneration pathways12 allow for greater and/or longer retention of vision.

Animal models of retinal blindness from deficiency of RPE65 have either been engineered via targeted disruption of the gene in the mouse,13 or available due to a naturally occurring mutation in the Briard dog.14,15 Pharmacologic bypass of the metabolic block has resulted in rapid restoration of visual pigment and function in the mouse.16,17 Gene replacement, using a normal copy of the gene in an adeno-associated virus (AAV) vector administered subretinally, has successfully restored electroretinograms (ERGs) and retinal function in both
the murine\textsuperscript{13,18–20} and the canine models.\textsuperscript{21–24} These experiments have resulted in substantial, durable recovery of vision as determined by ERGs, pupillary responses, and behavioral measures. This success has led to human clinical trials, both in the USA and UK, involving a subretinal injection of AAV-vector delivering a wild-type copy of RPE65. These have all reported good safety, with early results of varying degrees of efficacy.\textsuperscript{25–31}

Recognition of the clinical features of LCA/SECORD due to mutations of RPE65 is important to direct molecular testing and properly counsel patients about treatment options. To facilitate the diagnosis of this form of early onset retinal dystrophy, we report five cases emphasizing the characteristic clinical and electrophysiological findings.

**Patients and Methods**

All five patients were ascertained by the first author (RWG) in the Ophthalmic Genetics Clinic at Casey Eye Institute (CEI). All were consented for molecular testing through the Carver Nonprofit Genetic Testing Laboratory in Iowa City. The protocol of the study adhered to the provisions of the Declaration of Helsinki and was approved by the local Ethics Committee.

A full medical and family history was taken and ophthalmological examination performed. All subjects underwent color fundus photography, visual field testing, and electrophysiological assessment.

Selected subjects were examined with kinetic visual field testing using a projection perimeter (Octopus 101, Haag-Streit, Inc., Kóniz, Switzerland). In addition, white-on-white static perimetry was performed using the projection perimeter (Octopus 101), utilizing either the Dynamic or the GATE strategy.\textsuperscript{52} Goldmann stimulus size III or V, and a 148-, 164-, or 174-test-point full-field grid of radial-oriented and centrally-condensed design. Selected patients also had spectral domain optical coherence tomography (OCT; Spectralis OCT Heidelberg Retina Angiograph [HRA], Heidelberg Engineering, Heidelberg, Germany).

Electrophysiological assessment included full-field electroretinograms (ffERG) according to a previously described protocol\textsuperscript{53,54} that complied with the standards published by the International Society for Clinical Electrophysiology of Vision (ISCEV).\textsuperscript{55}

**Results**

Table 1 summarizes the clinical findings for ease of comparison, including the age at presentation, the age at last review, the visual acuities at first and most recent visit, and the findings on ERG and visual field testing.

**Case 1**

This patient was diagnosed with “retinitis pigmentosa” at 11 months of age on the basis of poor vision, nystagmus, sluggish pupils, and an “abnormal” ERG that, on review, showed no discernible ERG above noise. She did not develop a social smile during infancy and was unable to see at night. Her nystagmus improved over time and her vision was sufficient that she attended regular school and was able to slowly read normal print. She saw well enough to play competitive soccer at the club level until, after an 8-day hospitalization for presumed viral meningitis at age 13 years, her vision deteriorated and she developed more notable nystagmus, balance difficulties, and a significant decrease in her peripheral vision. Although her balance improved after two months, because of the nystagmus and change in vision, she was never able to return to playing soccer.

After age 13, she was able to read large, high-contrast print. At age 16, the visual acuity (VA) with both eyes open was 20/160 at distance and 20/70 at near. Her vision continued to deteriorate over the next two years, after which time she depended on an optical reading machine. Since age 16, she had been light sensitive and preferred sunglasses outdoors. She described very poor color vision throughout life and has always been emmetropic. There was no family history of eye disease or consanguinity.

When evaluated at CEI at age 18, the VA at distance was counting fingers (CF) at 6 feet in the right eye and 20/400 in the left eye, and 20/400 at near (12 inches) in each eye. Medical history included a small, pedunculated extra thumb surgically removed at age 4 months and an enlarged thyroid with normal thyroid function. She was found to have sluggish pupils, latent nystagmus, and a right exotropia. Fundus examination showed bilateral waxy disc pallor with disc drusen, severely attenuated retinal vessels, yellowish pattern-like retinal pigment epithelium (RPE) lesions and wrinkling of the internal limiting membrane in the macular regions, and peripheral fine RPE mottling with clumping and rare bone spicules (Fig. 1). There were isolated atrophic lesions nasally and supra-nasally in the left eye. Kinetic perimetry using the size 4\textsuperscript{e} test target disclosed responses only with motion, with no static presentations recognized. The nystagmus precluded valid estimates of monocular field, but moving test targets were recognized within the central 30° in the right eye and in scattered areas in the left. Full-field ERG disclosed no discernible responses of rods or cones to single flash, even with computer summation and sub-microvolt 30 Hz flicker responses using computer summation and digital filtering.

Molecular testing disclosed compound heterozygosity for the previously described RPE65 disease-causing alleles, IVS1+5 G>C and Leu541Ser.\textsuperscript{4,56} She was also heterozygous for a novel Ser203Arg mutation of unknown significance in RDH12.\textsuperscript{2}

In summary, this patient fulfills some of the criteria for LCA, having poor vision since infancy, nystagmus, sluggish pupil responses, and an undetectable ERG at age 11 months. However, her disorder is remarkable for the development and retention of relatively good central vision well into the second decade of life. Thus, her condition better fits the definition of SECORD.

**Case 2**

He was first seen at CEI at age 6.5 years for evaluation of “retinitis pigmentosa.” He reached normal early milestones, sitting at six months and walking at 12 months. At age 18 months, he was noted to have very limited vision in dim illumination but, in retrospect, night vision had probably always been poor, given his fear of darkness since early infancy. Retinitis pigmentosa was diagnosed at age three years, when he was noted to have poor side vision and a subsequent ERG was found to be nearly undetectable. Since age 4 years, his color vision had been poor. At 4 years 10 months, his acuity was 20/30 in the right eye and 20/60 in the left. At age 6 years, his acuity was 20/60 and 20/50, with minimal refractive error.

At home, he requested that the lights be always on and used a golf club as a mobility aid in dim illumination.

When examined at CEI at age 6.5 years, the VA was 20/200 in each eye at distance, and 20/50, J5 size letters, in the right eye, and 20/100, J10 size letters, in the left, at near. Examination disclosed normal pupillary responses, no nystagmus, and 0.25 diopters of myopia. Fundus examination showed bilateral waxy, full optic discs with buried drusen, mildly attenuated retinal vessels, normal foveal reflexes, and mild desaturation of the fundus coloration but minimal, if any, discernible pigmentary disturbance (Fig. 2). There was an isolated area of choroidal thinning nasally in the right eye (Fig. 2). An ERG, performed using intravenous (IV) propofol sedation, disclosed no detectable responses of rods and severely subnormal but clearly detectable, delayed responses of cones to single flash

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<table>
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<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at Presentation</th>
<th>Current Refractive Error</th>
<th>Presenting ERGs (Age)</th>
<th>Latest ERGs (non-sedated) (Age)</th>
<th>Latest Kinetic VFs (Age)</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>11 months</td>
<td>Emmetropia</td>
<td>No discernible ERG above noise (11 months)</td>
<td>No discernible responses of rods or cones to single flash; 30 Hz flicker responses using computer summation and digital filtering were 0.8 µV OD and 0.9 µV OS (18 years)</td>
<td>The size V4e test target disclosed responses only with motion, with no static presentations recognized. Moving test targets were recognized within the central 30 degrees in the OD and in scattered areas in the OS (18 years)</td>
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<tr>
<td>2*</td>
<td>M</td>
<td>3 years</td>
<td>-1.75 + 0.50 × 90° OU</td>
<td>Sedated ERG disclosed no detectable responses of rods and severely subnormal but clearly detectable, delayed responses of cones to single flash and 30 Hz flicker (6.5 years) (Figure 3)</td>
<td>Sedated ERG was abnormal and very similar to that of his brother, case 2 (18 months) (Figure 3)</td>
<td>(continues)</td>
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<tr>
<td>3*</td>
<td>M</td>
<td>18 months</td>
<td>Emmetropia</td>
<td>Sedated ERG was abnormal and very similar to that of his brother, case 2 (18 months) (Figure 3)</td>
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Table 1 (continued). Summary of Clinical Findings

<table>
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<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at Presentation</th>
<th>Age at Last Clinic Visit</th>
<th>BCVA First Visit OD/OS (Age)</th>
<th>BCVA Last Visit OD/OS (Age)</th>
<th>Current Refractive Error</th>
<th>Presenting ERGs (Age)</th>
<th>Latest ERGs (non-sedated) (Age)</th>
<th>First VFs (Age)</th>
<th>Latest Kinetic VFs (Age)</th>
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<tr>
<td>4</td>
<td>F</td>
<td>3 years, 2 months</td>
<td>16 years, 4 months</td>
<td>20/80 OU (distance)</td>
<td>20/60 OU (near) (3 years, 2 months)</td>
<td>+0.25 +3.25 × 102° OD +1.00 +3.50 ×85° OS</td>
<td>Sedated ERG disclosed minimal, if any, discernible rod responses, and cone responses that were markedly subnormal, but normal in timing for cone b-wave implicit times (3 years 2 months) (Figure 3)</td>
<td>No significant change in the photopic cone responses, but a 112% increase in the scotopic response to a 0.6 log cd/s/m² maximal intensity white flash (14 years 10 months) (Figure 5)</td>
<td>Kinetic fields were reasonably intact, whereas the static fields documented mild to moderate reductions of retinal sensitivity throughout the central 30° test field (12 years 10 months)</td>
<td>The area encompassed by the I4e and I2e test targets had decreased by 25% and 75%, respectively, indicating significantly reduced sensitivity. Also, a paracentral scotoma was evident for the left eye with the I2e test target (14 years, 10 months) Kinetic perimetry was relatively intact; full-field static perimetry disclosed moderate to marked reductions of sensitivity throughout the central field (16 years, 4 months) (Figure 8)</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>4 years, 10 months</td>
<td>10 years, 9 months</td>
<td>20/30 OU (distance) (4 years, 10 months)</td>
<td>20/30 OU (distance)</td>
<td>Emmetropia</td>
<td>A sedated ERG disclosed no discernible rod responses and severely subnormal and mildly prolonged cone responses (4 years 10 months) (Figure 3)</td>
<td>Compared to the previous sedated ERG, the rod b-wave amplitudes were clearly present (54 μV), amplitudes for the scotopic bright flash were 154 μV (previously 55 μV) and photopic single flash amplitudes were 108 μV (previously 53 μV). These increases are in keeping with the improved responses in Case 4 (10 years 9 months)</td>
<td>Kinetic fields were normal. Full-field static perimetry documented mild sensitivity losses in each eye that were greater for the central 30° field and greater for the left eye. (12 years 10 months)</td>
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* Patients 2 and 3 are brothers. BCVA, best-corrected visual acuity; ERG, electroretinogram; VF, visual fields; CF, counting fingers.
and 30 Hz flicker (Fig. 3). A diagnosis of SECORD was made. At age 7 years 8 months, with a myopic correction of −1.75 + 0.50 × 90° in both eyes, his VA was 20/60 in the right eye and 20/50 — in the left at distance, and 20/200 and 20/70, respectively, at near. At 8 years 3 months, the VA was 20/60 in the right eye and 20/50 in the left at distance, and 20/80 and 20/40, respectively, at near. At age 8 years 9 months, the VA (without correction) was 20/100 in the right eye, 20/125 in the left at distance, and J7 in each eye at near. With the Titmus test, he achieved 140 seconds of arc of stereopsis (fly, 3/3 animals, 4/9 circles).

Molecular testing disclosed compound heterozygosity for novel disease-associated alleles Arg118Ser and Val443Ala.9

In summary, as may be seen in other children with SECORD, the VA in this patient has fluctuated greatly between examinations. Although his recorded VA is better with a moderate myopic correction, he prefers to not use his glasses, claiming that they are not helpful. This suggests that lighting and contrast may be more important factors than refractive error.

Case 3

This is the younger brother of case 2. At age 18 months, he had minimal nystagmus, sluggish pupils, and a reduction in night vision. He was holding books very close, and turning his head to the left, using right gaze to see at near. On examination, the fundus appearance was of very fine mottling of the RPE in the periphery. An ERG using IV propofol sedation was abnormal and very similar to that of his brother’s (Fig. 3). At age 2 years, he developed more visible nystagmus, head bobbing, unsteadiness, and limited side vision. Evaluation disclosed delayed motor skills and occupational therapy was started. An MRI was normal. The head bobbing worsened slowly until age 3, when it began to improve. At age 3 years 8 months, the VA was 20/125 in the right eye and 20/100 in the left, 20/60 with both eyes open. He was emmetropic. Fundus appearance was unchanged.

Molecular testing disclosed the same two novel alleles present in his brother. Testing of the parents revealed that each parent had one of the two alleles. This case exemplifies the variability in phenotype that can often be seen within families who harbor the same disease-associated alleles; presumably other genetic and environmental modifying factors account for these differences.

Case 4

This child was first evaluated at CEI at age 3 years 2 months with the referring diagnosis of possible congenital stationary night blindness. Her parents felt that she had essentially no night vision since early infancy and that she was virtually “blind” in dim illumination. However, during the day, they were not aware of any visual limitations. Her developmental milestones were mildly delayed. The parents noted that her pupils did not dilate in the dark, as did their other children’s. With a +4.00 sphere correction in both eyes, her VA was 20/80 at distance and 20/60 at near in each eye. With both eyes open, her VA was 20/40 at distance. She had no nystagmus. Fundus examination disclosed bilateral waxy, pink optic nerves, possible mildly attenuated retinal vessels, increased reflex off the internal limiting membrane in the temporal raphe, and very fine granularity to the peripheral retina. An ERG using IV sedation disclosed minimal, if any, discernible rod responses, and cone responses that were markedly subnormal, but normal in timing for cone b-wave implicit times (Fig. 3).
At age 10 years 9 months, she still showed mild developmental delay. Her vision was good during the day, at which time there were no noticeable limitations of color vision or peripheral vision, but she had “no vision” at night. Her best-corrected VA (+0.50 +3.00 × 90° both eyes) was 20/30− in the right eye, and 20/25− in the left; at near she read J1+ print with each eye. With the Titmus test, she achieved 140 seconds of arc of stereopsis (fly, 3/3, and 4/9 circles). Confrontation fields were essentially normal in good lighting but were constricted with dimmer illumination. Fundus examination (Fig. 4A) disclosed mild waxy disc pallor, mildly attenuated retinal vessels, unremarkable macular regions, whitish punctate lesions in the temporal raphe, and mild but definite choriocapillaris atrophy throughout the fundus, with fine granularity to the mid and far retinal periphery. Unsedated ERG testing showed no discernible rod-specific responses, severely subnormal dark-adapted cone and scotopic bright flash responses, mildly to moderately subnormal 30 Hz flicker, and normal to near normal amplitudes of light-adapted cones (Fig. 5). Cone b-wave implicit times were normal. A 45-minute dark-adapted psychophysical threshold was elevated into the high cone range at −1.3 log cd/m² in the right eye and −1.4 log cd/m² in the left (normal rod mean −5.35, normal rod upper limit −5.05 log cd/m²; normal cone mean −2.90, normal cone upper limit −2.60 log cd/m²).

At age 12 years 10 months, with a refractive error of +0.25 +3.25 × 102° in the right eye, and +1.00 +3.50 × 83° in the left, her VA was 20/25− at distance and J1+ at near in each eye. The bilateral, discrete white lesions present previously were nearly identical in the temporal raphe but were now present also around the disc and elsewhere (Fig. 6A and Fig. 6B). Octopus 101 kinetic and static visual fields were performed. The kinetic fields were reasonably intact, whereas the static fields, using the G2 grid and the Dynamic strategy (not shown) documented mild to moderate reductions of retinal sensitivity throughout the central 30° test field. The 45-minute dark-adapted retinal psychophysical threshold was elevated into the low cone range (−2.90 log cd/m² in the right and −2.77 log cd/m² in the left). The ERGs showed, at most, only mild progression (Fig. 5).
At age 14 years 10 months, the VA was 20/25 in the right and 20/20 in the left. The area encompassed by the isopters on kinetic perimetry had decreased minimally for larger and brighter test targets, by 5% and 4% for the V4e and III4e test targets, respectively. However, the area encompassed by the I4e and I2e test targets had decreased by 25% and 75%, respectively, indicating significantly reduced sensitivity. Also, a para-central scotoma was evident for the left eye with the I2e test target. The white retinal dots were more discrete than at age 10 years 9 months (Fig. 4B and Fig. 7). SDOCT imaging revealed deposits at the level of the RPE and photoreceptor outer segments that corresponded to the white retinal lesions (Fig. 7). The ERG showed no significant change in the photopic cone responses, but a 112% increase in the scotopic response to a 0.6 log cd-s/m² maximal intensity scotopic white flash was still severely subnormal but had increased by >100%. At 10 years 9 months the cone responses to 30 Hz flicker were mildly to moderately subnormal, with normal to near normal amplitudes to photopic single bright white flash with normal b-wave implicit times. The photopic cone responses have remained reasonably stable during serial testing.

**FIGURE 5.** Serial unsedated electroretinograms for patient 4, performed at 10 years 9 months, 12 years 10 months, and 14 years 10 months. No discernible responses of rods were elicited at 10 years 9 months. A small amplitude rod response was recorded at 12 years 10 months, which increased to approximately 50% of normal at 14 years 10 months. Marked blink artifact limits the accuracy of measuring implicit times. Over this same period of serial testing the mixed rod-cone response to a 0.6 log cd-s/m² maximal intensity scotopic white flash was still severely subnormal but had increased by >100%. At 10 years 9 months the cone responses to 30 Hz flicker were mildly to moderately subnormal, with normal to near normal amplitudes to photopic single bright white flash with normal b-wave implicit times. The photopic cone responses have remained reasonably stable during serial testing.

**FIGURE 6.** (A) Color fundus montages of case 4 at age 14 years 10 months showing bilateral mild waxy disc pallor, mildly attenuated retinal vessels, unremarkable macular regions, whitish punctate lesions in the temporal raphe and around the optic disc, and mild but definite choriocapillaris atrophy throughout the fundus, with fine granularity to the mid and far retinal periphery. (B) Stereo pair of inferior-temporal raphe region of left eye of case 4 at age 14 years 10 months, showing the diversity of shapes of the deposit. Some have broad sloping bases, whereas others are wide based with a spiral anterior tip.
At age 16 years 4 months, the visual acuity was stable. Blink artifacts precluded assessment of the longer latency rod ERG responses but the scotopic bright flash ERG amplitude was decreased to 42% and the photopic single flash amplitude to 76% of the previous values (data not shown). Kinetic perimetry was relatively intact; however, full-field static perimetry, using the GATE strategy and stimulus size III disclosed moderate to marked reductions of sensitivity throughout the central field (Fig. 8).

Molecular testing disclosed compound heterozygosity for novel disease-causing alleles Gly484Asp and Trp460Cys. In summary, this patient is remarkable for the preservation of vision with adequate lighting. The profound disturbance of vision in dim illumination is consistent with a major defect in generation of 11-cis-retinal for rod function. The preservation of cone function is consistent with the proposed alternative biochemical pathways for regeneration of 11-cis-retinal for cone photoreceptors. The discrete white punctate lesions in the temporal raphe and elsewhere may reflect an abnormal accumulation of retinal products within the RPE/outer retina. The diffuse RPE granularity and apparent greater visibility of the choroidal vessels, suggesting atrophy of the choriocapillaris and/or RPE, could reflect secondary damage from this accumulation. The objective demonstration of improved retinal function over time, with electrophysiological testing, has also not been previously reported.

Case 5

This child was first referred at age 4 years 10 months for evaluation of night blindness and mild pigmentary retinopathy. A social smile was not evident until 2 months of age, with other developmental milestones being normal. Since age 1.5 years, her parents described notable difficulty in adjusting to dim illumination. In good lighting, there was no discernible loss of peripheral vision. Her VA varied from 20/30 to 20/40+/ in each eye with minimal refractive error. She had no nystagmus. Dilated ophthalmoscopy revealed mild waxy pallor of the optic discs, minimal attenuation of retinal vessels, and very fine nummular RPE mottling inferiorly. In the temporal macula and raphe, there were myriads of tiny white dots distributed in a lace-like pattern (Fig. 9). An ERG was performed without sedation. Compared to the previous sedated ERG, the rod b-wave amplitudes were clearly present at 54 μV (previously indiscernible from noise), the amplitudes for the scotopic bright flash were 134 μV (previously 55 μV), and photopic single flash amplitudes were 108 μV (previously, 53 μV). The degree of amplitude increase was greater than that expected in comparison to ERGs performed using IV propofol sedation. This increase was considered in keeping with the improved responses elicited with unsedated ERG testing in case 4.

Molecular testing disclosed a novel Val136Phe mutation on one chromosome. Complete sequencing through the entire coding sequence of RPE65 failed to identify the second variant. However a novel point mutation in intron 1, of uncertain significance, was demonstrated. In summary, although only a single disease-causing variant was found, the clinical history, white dots in the fundus, and ERGs are virtually identical with those for case 4.

DISCUSSION

The cases presented herein delineate several important characteristic clinical, psychophysical, and electrophysiological fea-

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**Figure 7.** Color fundus photographs, fundus reflectance images, and spectral domain optical coherence tomography (SDOCT) of the left temporal raphe of case 4. The left-hand column pair of color photos illustrates the change that has occurred over 16 months (age 14 years 10 months, upper left; age 16 years 4 months, upper center and upper right), with the deposits becoming less apparent and more granular in appearance. The SDOCT horizontal and vertical scans show deposits at the level of the RPE and photoreceptor outer segments corresponding to the retinal lesions seen on the reflectance images.
tures of RPE65-associated retinal dystrophy. Specifically, all five had life-long extremely poor night vision. Case 1 had an undetectable ERG and features supporting a diagnosis of LCA as an infant, but her level of acuity and function into the second decade of life was more consistent with SECORD. Cases 2 and 3 presented with early childhood symptoms more typical of SECORD and had similar sedated ERGs with no detectable rod responses and severely reduced and delayed cone responses. These three cases had variable degrees of nystagmus and differing substantial vision loss that, for a period, even appeared to improve, in keeping with previous reports. Case 4, arguably the most remarkable subject, was initially thought to have congenital stationary night blindness because of the normal appearing fundus and good day vision. Her vision continued to improve and her light-adapted cone responses were, at age 10 years, normal for the left eye and mildly subnormal for the right eye, with normal b-wave implicit times bilaterally. The clinical and electrophysiological findings in case 5 were very similar to those observed in case 4. Cases 4 and 5, despite harboring different sequence variants, also have some similarities to the recent case reported by Lorenz et al.7 with a homozygous P25L mutation. However, our patients showed an even greater preservation of cone responses.

Why is the phenotype for these 5 patients with SECORD milder than typical LCA? The allele with the greater remaining gene product function for autosomal recessive disease disproportionately determines the phenotype. Indeed, since the known alleles for our patients have already been reported in compound heterozygosity with the more severe phenotype of LCA, the novel mutations in these subjects most likely transcribe a gene product that has enough residual function to alter the phenotype. Future studies on the function of the gene product of these novel mutations of RPE65, similar to those that have been already done on 11 missense mutations,37 may provide more definitive answers to these questions. In addition, non-allelic variations (i.e., the genetic background) may underlie some of this phenotypic variation. A more or less active “alternative” 11-cis-retinal regeneration pathway would be the most obvious example of such a background effect.

Expression or function of mutations for genetic diseases can also be modified by epigenetic regulatory mechanisms38 that are induced by hormonal changes during puberty. If it occurred, this could account for the improvement in the rod function in two of our patients.

The identification of patients with mutations in RPE65 has attained greater significance now that clinical gene replacement trials have begun. The features presented in this article should assist in the recognition of this form of SECORD. The hallmark clinical feature is minimal, if any, night vision from birth. Because day vision is normal or near normal initially, diagnosis may be delayed beyond infancy, as observed in our case series. The presentation may be similar to typical LCA with severe delay in visual development during the first 6 to 8 years of life.

![Image](Fig8.png)
Figure 9. Fundus montage of the right eye of case 5 at age 10 years 9 months, demonstrating a lace-like accumulation of numerous fine white deposits in the temporal raphe.

months, but infants with mutations of RPE65 will often show improved visual function beginning at approximately 9 to 12 months.

Patients with retinal dystrophy from mutations of RPE65 will have a normal appearing fundus at birth but in later infancy and early childhood may develop hypopigmentation and fine granularity. Paunescu et al. noted that the hypopigmentation is characteristically in a peripapillary distribution surrounding and inferior to the optic nerve (as in cases 2, 4, and 5). Not infrequently, such as in our cases 4 and 5, the retina may display fine white dots. We propose that these may represent abnormal accumulations of retinyl esters, as has been demonstrated in animal models, and has also been observed as lipid droplets within the RPE. These white dots appear to reside deep in the retina, can have soft edges initially, may wax and wane, and eventually fade with time (Figs. 4, 6), being replaced initially by RPE dropout and atrophy, with more substantial mottling and eventual pigment clumping occurring over time. Interestingly, Paunescu et al. described “peripapillary areas of a glinting aspect”, that might represent more subtle accumulation of retinyl esters. They also reported multiple circular patches of chorioretinal atrophy in the periphery in later stages or in more advanced disease. The small area of chorioretinal thinning present nasally in the right eye of case 2 is therefore likely to represent an early stage of this process.

Redmond et al. reported in 1998 that rod function, as measured by the ERG, was abolished in rpe65 knock-out mice. However, a more recent study suggested that the source of remaining vision in these mice may yet be from rods. The ERG features of retinal disease in patients with RPE65 mutations associated with SECORD include absence, to profound reduction of rod-isolated responses (as in cases 4 and 5). The dark- and light-adapted cone responses may be near normal or moderately reduced, with little or no delay in b-wave implicit times. Moreover, in two of our subjects, the ERG cone responses improved with time and in one, rod responses became discernible after being not recordable earlier in life. However, some of the earlier studies were conducted using IV propofol sedation/ anesthesia, which is known to reduce the rod and, to a lesser degree, cone responses (RGW, unpublished observations, 2002).

It is believed that approximately 3700 individuals in the United States have LCA and that approximately 8 to 15% are associated with mutations of RPE65. Assuming a figure of 10%, then approximately 370 individuals with LCA in the United States have mutations of RPE65. Patients with SECORD may have mutations of several of the same genes associated with LCA, including an as yet undetermined percentage of patients with mutations of RPE65. A further small number of patients who carry the diagnosis of “retinitis pigmentosa” with early-onset may also have mutations of RPE65 as the cause of their disease.

Gene therapy trials are being conducted for patients with LCA/SECORD from mutations of RPE65. Because these early trials were Phase I safety studies, they have principally enrolled patients with more severe stages of disease with advanced vision loss. Nonetheless, improvement in VA, subjective night vision, and trends suggestive of an improvement in visual fields, have been reported at the 12- and 18-month timepoints. Phase 2 studies of increasing dose and enrollment of younger subjects with earlier stages of disease are currently underway, thereby making the clinical and electrophysiological findings reported herein of cases with early stage disease even more pertinent.

Paunescu and colleagues reported the longitudinal and cross-sectional VA in 51 patients and visual field (VF) in 29 patients in a natural history study of patients with both LCA and later-onset severe childhood dystrophy from mutations of RPE65. On cross-sectional observations, VA and VF both deteriorated notably during the second and third decades of life, with very little field remaining up to age 30, with either residual central or peripheral islands of field being observed. They found on longitudinal observations, that the vast majority, if not virtually all patients, experienced a rapid and marked loss of VA and VF in the middle of the second decade of life. Our two patients who have retained good cone function are thereby at a high risk of such vision loss over the next five years. Indeed, the ERG has begun to become reduced and the full-field static perimetry disclosed significant decreases of sensitivity throughout the central portion of the field for patient 4 at age 16 years 4 months. The substantial loss of vision during the second decade of life serves as further evidence of the importance of timely identification of patients with milder forms of SECORD from mutations of RPE65 and the consideration of these patients for gene replacement or potential future pharmacological therapies that may forestall this expected decline in visual function.

References


