Clinical Course and Visual Function in a Family With Mutations in the RPE65 Gene

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Objective: To evaluate the phenotype of affected and carrier members of a family with mutations in RPE65 (a retinal pigment epithelium gene).

Methods: RPE65 mutation screening was performed on DNA from 2 affected brothers, 1 unaffected brother, both parents, and 3 surviving grandparents using cycle sequencing. Ophthalmic examinations included ophthalmoscopic fundus examination; visual function testing; 2-color, static, dark-adapted threshold perimetry; and rod electroretinographic a-wave phototransduction analysis.

Results: The 2 affected brothers carried RPE65 mutations in compound heterozygous form: a maternal Y368H (1156T → C) missense mutation and a paternal IVS1+5g → a splice-site mutation. Severe visual deficits and an absence of rod and cone electroretinographic responses were diagnosed in both affected boys before the age of 5 years. Visual acuities of about 20/100 during grade school declined to hand movements by the teenage years, and only a rudimentary peripheral temporal visual field remained by the ages of 25 and 29 years. Both parents had normal central visual function, as measured by visual acuity, contrast sensitivity, color vision, and Humphrey 10-2 fields. However, the 50-year-old father showed hundreds of tiny whitish hard drusen in both eyes and had abnormal peripheral function on dark-adapted perimetry, with extended field defects of 15 to 20 dB outside 30° eccentricity. His rod photoreceptor sensitivity and amplitude, calculated by fitting the rod a waves by a model of activation of phototransduction, were normal, but the flicker electroretinographic response was delayed.

Conclusions: The RPE65 mutations Y368H and IVS1+5g → a present in compound heterozygous form cause severe visual compromise in childhood and progress to nearly total vision loss by the second to third decades of life. The retinal and functional changes in the father carrying a presumed functional null allele suggest that some RPE65 heterozygous carriers may manifest visual symptoms.

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GENETIC mutations that disrupt vitamin A processing in the retinal pigment epithelium (RPE) result in a spectrum of retinal dystrophies and dysfunctions. Among the known disease genes in this pathway, mutations in RPE65 seem to be the most common and are associated with a severe phenotype. The RPE65 gene encodes a unique protein necessary for the conversion of vitamin A to 11-cis retinal, the chromophore of the visual pigments. Mutations in RPE65 result in autosomal recessive retinal degeneration that is often diagnosed as Leber congenital amaurosis type II. However, a range of disease severity has been reported, from congenital blindness to adult-onset retinitis pigmentosa. Genetic defects in vitamin A metabolism are, in theory, attractive targets for therapeutic intervention in several ways, including gene therapy, RPE transplantation, and survival factor therapy. In the case of RPE65, advances in such treatments are hoped to be rapid, because mouse and dog models of the disease are available. Characterization of patients with RPE65 mutations is, therefore, an important goal, because these patients may be expected to be candidates for future therapeutic trials. We report findings in a family with 2 different RPE65 disease alleles that together result in early-onset, severe, and rapidly progressing retinal degeneration. One of the affected family members was followed up from the age of 5 years until the present (age 29 years) and shows a prominent macular component to the disease. In addition, there is evidence of macular changes in one of the heterozygous carrier parents.
SUBJECTS AND METHODS

SUBJECTS

Four individuals were studied in a family in which an RPE65 splice-site mutation (IVS1 + 5g→a) and a missense mutation (Y368H [1156T→C]) segregate in compound heterozygous form with a retinal degeneration phenotype (Figure 1). Two brothers, aged 25 and 29 years, carry both mutations and are visually impaired. Their mother, aged 49, carries the Y368H mutation, and their father, aged 50, carries the IVS1 + 5g→a mutation. The RPE65 mutations in this family have been reported serologically, we describe the associated phenotype. Informed consent was obtained from all participating subjects in accordance with the University of Michigan Medical School Institutional Review Board.

MUTATION SCREENING

DNA from the 2 affected brothers, 1 unaffected brother, both parents, and 3 surviving grandparents was screened for sequence changes in all RPE65 exons and adjacent intronic regions by cycle sequencing using primer pairs and conditions published previously.

VISUAL EXAMINATION

The best-corrected distance visual acuity was determined using the Early Treatment Diabetic Retinopathy Study Vision standard, beginning after 1 hour of dark adaptation. Inferior vision (estimated as is equivalent to a distance of 1 m and at a luminance of 85 candela (cd)/m². The central visual field was examined using standard Humphrey 10-2 automated perimetry. Color vision, Goldmann visual fields, and standard clinical electroretinograms (ERGs) were measured according to methods previously described. Color vision was evaluated using the Ishihara plates and the Farnsworth dichotomous D-15 test.

RESULTS

Findings in the 2 affected brothers and their parents are summarized in the Table.

THE 29-YEAR-OLD PROPOSITUS (III:1)

This affected brother related a lifelong history of severe vision loss but completed normal school and obtained a university degree despite progressive vision loss and blindness by his early 20s. Clinical records from the age of 5 years indicate 20/100 visual acuities with 3 diopters of hyperopic correction. Intermittent nystagmus was seen. Both fundi had micropigment clumping in the posterior pole and a grayish retinal sheen in the middle and far periphery, with attenuated arterioles. Optic nerve heads were unremarkable. He was diagnosed as having Leber congenital amaurosis at the age of 22 months, and his vision was markedly diminished; acuities were not specifically tested.

When examined at the present age of 29, his visual acuity in both eyes was reduced to hand movements. Nystagmus was noted. The anterior segments were normal, and the lenses and media were clear. The retina had a whitish gliotic reticular RPE appearance across the entire fundus (Figure 2A). The vessels were markedly attenuated. The central macula had a 1.5-disc diameter area of RPE atrophy. On visual field testing, he perceived only the V-4-e target in the inferotemporal region between 50° and 70° for each eye. This measurement is approximate, as nystagmus was present, although care was taken to monitor the eye position during field testing. Electroretinographic testing showed no rod or cone responses, except for 0.3-µV responses to a 32-Hz flicker on computer-averaged recordings. This is at or barely above noise levels. Further details of ophthalmic examination results at the age of 29 years are given in the Table, and Figure 3 summarizes the visual fields between the ages of 12 and 29 years.

THE 25-YEAR-OLD AFFECTED BROTHER (III:2)

This affected brother was given the clinical diagnosis of Leber congenital amaurosis at the age of 22 months, and ophthalmic examination records from that time indicate “cellophane maculopathy.” His visual function was markedly diminished; acuities were not specifically tested. He recalls that he could play basketball in sunlight during grade school. Despite limited vision, he completed normal school and is pursuing a postgraduate degree. He reports that he can see only the edge of the sidewalk highlighted against the grass, and this is seen more easily on overcast days than in direct sunlight.

On ophthalmic examination, the RPE appeared thinned across the entire fundus (Figure 2B), and both maculae had a 1-disc diameter central atrophic area. The
vessel caliber was greatly constricted, and the optic cup showed gliotic filling. Sparse fine bone spicule pigment was present in the periphery, but the predominant feature was a gliotic white appearance across most of the fundus. Results of the ophthalmic examination at the age of 25 years are found in the Table.

**THE 50-YEAR-OLD FATHER (II:2)**

The father carries the IVS1+5g→a splice-site mutation on 1 RPE65 allele. He reports no vision complaints. No relatives are known to have vision complaints. He is in good health but had a myocardial infarction at the age of 42 years. His medications include amiodarone hydrochloride, amlodipine besylate, alprazolam, and aspirin, none of which are known to affect the full-field ERG. Both fundi had normal discs, but the retinal vessels had slight hypertensive narrowing. Both maculae were abnormal, having many tiny, hard, drusenoid RPE lesions that extended beyond the macular arcade vessels into the near peripheral retina (Figure 2C). Such lesions are not typically associated with residual from a myocardial infarction. Results of the ophthalmic examination are given in the Table. Functional abnormalities were found in dark-adapted thresholds for 44% of these locations, based on the difference values of the 500- and 650-nm data. The pattern of findings was similar for both eyes. Despite this, the rod ERG amplitudes were normal, as were cone single-flash responses. However, cone function on 32-Hz flicker testing showed a 5-millisecond timing delay of the fundamental component to 32 milliseconds despite normal amplitudes (Table). The normal t wave to the brightest flash was fitted first, allowing \( R_{max} \) and \( S \) to be free parameters. The remaining individual curves for dimmer intensities were fitted, allowing \( S \) to vary but with \( R_{max} \) and \( t_d \) held constant.

Rod phototransduction of the father was evaluated in 2 ways. First, the peak a-wave amplitude to the brightest flash intensity (4.7 log scotopic td) was normalized as shown in Figure 4, and the leading edge of the rod a wave was within the normal range, indicating that rod phototransduction was not affected. However, the onset of the rising phase of the b wave was delayed beyond the envelope of the 11 healthy subjects. Second, the rod a wave was analyzed with the phototransduction model (given in the “Visual Examination” subsection of the “Subjects and Methods” section), which gave a maximum amplitude \( R_{max} \) of 427 µV, which was not different from the normal mean of 365 µV (SD, 80 µV). The sensitivity, as determined from the phototransduction model, was within the normal range and was not reduced (log sensitivities, based on the difference values of the 500- and 650-nm data. The pattern of findings was similar for both eyes. Despite this, the rod ERG amplitudes were normal, as were cone single-flash responses. However, cone function on 32-Hz flicker testing showed a 5-millisecond timing delay of the fundamental component to 32 milliseconds despite normal amplitudes (Table). The normal t wave to the brightest flash was fitted first, allowing \( R_{max} \) and \( S \) to be free parameters. The remaining individual curves for dimmer intensities were fitted, allowing \( S \) to vary but with \( R_{max} \) and \( t_d \) held constant.

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The mother carries the Y368H missense mutation on 1 RPE65 allele. She has no vision complaints except for a vague statement of worse vision at night, but this could not be documented objectively. She is healthy and takes no medications. There is no history of eye disease in her family. The macula, disc, vessels, and retinal periphery appeared normal, with the sole exception of a collection of 8 to 10 tiny hard drusen inferonasal to the disc in the right eye only, with none seen in the left eye. The RPE appearance was otherwise normal in both eyes across the macula and the peripheral fundus. Her ophthalmic examination yielded normal results for visual acuity, light- and dark-adapted visual fields, and ERGs (Table).

**THE PATERNAL GRANDFATHER (I:4)**

Visual testing was not possible in the paternal grandfather because he is deceased. Optometric records at the age of 72 years indicate a visual acuity of 20/20 OU, but a retinal and macular examination was not done.

More than 50 disease-causing mutations in the RPE65 gene have been reported, with estimates indicating that RPE65 mutations are responsible for 7% to 15% of the cases of early-onset autosomal recessive severe retinal dystrophy. Although it is generally agreed that most patients are seen with a relatively homogeneous severe phenotype in early childhood, few clinical details are given.

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**Clinical Results**

<table>
<thead>
<tr>
<th>Patient Description/Age, y</th>
<th>Acuity and CS</th>
<th>ERG Findings</th>
<th>Dark-Adapted Thresholds</th>
<th>Visual Fields</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affected male (III:1)/29</strong></td>
<td>ETDRS acuity: temporal projection only for each eye</td>
<td>Photopic 32-Hz flicker: fundamental amplitude, 0.3 µV; and markedly delayed timing</td>
<td>Dark adaptation: unable to see the brightest stimulus (&gt;5-log unit loss)</td>
<td>Goldmann: V-4-e inferotemporal peripheral crescent in both eyes and cannot see III-4-e</td>
<td>Moderate nystagmus and lens clear in both eyes</td>
</tr>
<tr>
<td><strong>Affected male (III:2)/25</strong></td>
<td>ETDRS acuity: LP for both eyes</td>
<td>Photopic 32-Hz flicker: fundamental amplitude, 0.15 µV; and markedly delayed timing</td>
<td>Dark adaptation: &gt;4-log unit loss perceives the brightest stimulus after 75 min in the dark</td>
<td>Goldmann: cannot identify V-4-e target at any field position</td>
<td>Moderate nystagmus and lens has a 0.5-mm PSC in the right eye and posterior sheen in the left eye</td>
</tr>
<tr>
<td><strong>Carrier mother (II:1)/49†</strong></td>
<td>ETDRS acuity: OD, −0.06 (20/17); and OS, −0.10 (20/16)</td>
<td>Scotopic b wave, 312 µV (nl); photopic b wave, 120 µV (nl); and photopic OPs normal</td>
<td>Dark adaptation: normal cone-rod break and normal threshold at fixation</td>
<td>Goldmann: full and normal in both eyes to V-4-e, II-4-e, and I-4-e targets</td>
<td>Color vision: normal by Ishihara plates and the Farnsworth dichotomous D-15 test</td>
</tr>
<tr>
<td><strong>Carrier father (II:2)/50‡</strong></td>
<td>ETDRS acuity: OD, −0.12 (20/15); and OS, −0.21 (20/12)</td>
<td>Scotopic b wave, 252 µV (nl); photopic b wave, 168 µV (nl); and photopic OPs normal</td>
<td>Dark adaptation: normal cone-rod break and normal threshold at fixation</td>
<td>Goldmann: full and normal in both eyes to V-4-e, II-4-e, and I-4-e targets</td>
<td>Color vision: normal by Ishihara plates and the Farnsworth dichotomous D-15 test</td>
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</table>

*CS indicates contrast sensitivity; ERG, electrotetinographic; ETDRS, Early Treatment Diabetic Retinopathy Study; LP, light perception; PSC, posterior subcapsular cataract; nl, normal; and OP, oscillatory potentials.
†The mother carries the Y368H (1156T→C) missense mutation on one RPE 65 allele.
‡The father carries the IVS1+5g→a splice-site mutation on one RPE 65 allele.
in the literature. Our study of a family carrying 2 different RPE65 disease alleles further documents the association of RPE65 mutations in compound heterozygous form with severely compromised vision due to rod-cone involvement at young ages. In addition, we report the finding of macular drusen and peripheral rod visual dysfunction in a parent carrying a presumed RPE65 functional null allele in heterozygous form.

The IVS1 + 5g→a splice-site mutation carried by the father of the 2 affected brothers in our study is the most common of the known RPE65 mutations, occurring on at least 2 genetic backgrounds.14 Although the father has good corrected visual acuity and normal visual fields, our studies show subtle changes in his rod absolute dark-adapted threshold sensitivities and in his cone ERG flicker responses. In addition, both his maculae were covered with hundreds of tiny hard drusen extending into the rod-rich retina beyond the macular arcades. His history of severe heart disease may have contributed to these lesions. Hypertensive vascular narrowing is associated with cardiac disease; however, a myriad of tiny hard drusen typically are not associated with cardiac disease. In addition, he is too young to ascribe these drusen to aging changes. Such RPE level abnormalities in an individual carrying one copy of a presumed RPE65 null allele suggest that the resulting decrease in expression may produce mild pathogenic effects. Interruption of the visual cycle by mutations in the RDH5 (a retinol dehydrogenase gene) that encodes for the visual cycle enzyme 11-cis retinol dehydrogenase29 results in fundus albipunctatus.6,30 in which the principal findings are a myriad of tiny yellowish drusenlike RPE deposits across the fundus and impaired dark adaptation.

The Y368H mutation carried by the mother of the 2 affected brothers was previously reported as pathogenic in another patient in whom the mutation was presumed to be associated with retinitis pigmentosa.

Figure 2. Fundus photographs. A, Right eye of the 29-year-old propositus. B, Right eye of the 25-year-old affected brother. C, Macula of the right eye of the 50-year-old father. D, Dark-adapted visual field abnormalities from the carrier mother (top) and the carrier father (bottom), obtained with a 500-nm target. Data for left eyes are shown on the left; and for right eyes, on the right. A comparison of the 500- and 650-nm data indicated that the detection threshold was mediated by rod photoreceptors in the mother, but cones mediated some of the peripheral sensitivity in the father. The color bar indicates deviations from the normal mean. S indicates superiorly; N, nasally; I, inferiorly; and T, temporally.
ent in compound heterozygous form. The mother had only a few tiny drusenlike lesions in 1 eye, suggesting the possibility that her missense mutation may not be fully inactivating or may affect a different aspect of RPE65 function. Consistent with this notion are reports of 2 patients with relatively mild forms of retinal degeneration associated with compound heterozygous RPE65 missense mutations. This does not seem to be the case for RPE65 missense mutations in general, however, as a large-scale study failed to find differences in the clinical descriptions and best visual acuities of several age-matched patients carrying 2 presumed null mutations, 2 missense mutations, or a combination of both.

Both affected brothers in our study had profound night blindness, impaired cone function, and unrecordable ERG responses at young ages. Their residual visual function, however, was sufficient to succeed in regular school. This clinical picture is similar to 4 other patients diagnosed as having RPE65 mutations in early childhood, in whom rod ERGs were undetectable and cone ERGs were severely diminished even at the earliest ages tested (approximately 1 year); cone ERGs were unrecordable by the age of 5 to 7 years. Both brothers experienced a progressive disease course that culminated in complete loss of central vision and most peripheral vision before the ages of 25 and 29 years. Their fundus appearance showed severe peripheral degeneration and prominent atrophic macular scars indicative of extensive macular degeneration in the middle- to late-stage disease process. These findings, and the macular involvement present in their carrier father, suggest that, over time, loss of RPE65 function resulting in decreased 11-cis retinal synthesis, accumulation of vitamin A metabolic intermediates, or both has a major effect in the central retina, possibly due to unique metabolic requirements and physiological features.

Previous genetic studies of the adenosine triphosphate–binding cassette transporter (ABCR) that is involved in transport of all-trans retinal in the photoreceptors demonstrated a correlation between disease severity and mutation type, with different classes and combinations of ABCR mutations shown to result in autosomal recessive Stargardt disease, fundus flavimaculatus, cone-rod dystrophy, and autosomal recessive retinitis pigmen-tosa. In addition, although it is controversial, certain ABCR missense mutations are proposed to increase susceptibility to age-related macular degeneration. The extent of phenotypic variability associated with mutations in RPE65 is certainly less than that associated with mutations in ABCR. Our findings, however, add to the evidence that the RPE65 mutation type may factor into disease phenotype, and further suggest that RPE65 mutations may be linked to macular abnormalities in affected and carrier individuals. It remains to be determined whether RPE65 heterozygous individuals, in general, are at increased risk for vision loss in later life, especially in association with aging. Sorting out these possibilities, and validating the connections of defects in vitamin A metabolism to late-onset disease, will require mutation studies in many more patient families. Nevertheless, the hope of progress toward therapeutic intervention in RPE diseases affecting vitamin A metabolism provides impetus for additional observational studies.

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