Early-Onset Severe Rod–Cone Dystrophy in Young Children with RPE65 Mutations

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Purpose. To describe the ocular phenotype of patients with RPE65 mutations in infancy and young childhood.

Methods. Four children from three families with severe early-onset visual impairment related to electrophysiologically detectable retinal dystrophy were screened for mutations in the RPE65 gene. Visual function from infancy to the age of 10 years was assessed with age-adapted methods. Clinical examinations and electroretinograms (ERGs) were also performed on the six parents.

Results. In all three families, patients were compound heterozygous for mutations of the RPE65 gene (ins144T/IVS1+5G→A, R91W/Y368H, 1114delA+T457N/IVS1+5G→A). Visual acuity was measurable in all patients at the age of 6 to 10 years, despite severe visual impairment noted during infancy and congenital nystagmus in three of the four patients. Photophobia was not a feature. Funduscopic changes were discrete, the most prominent finding being increased granularity in the macula and the periphery. Peripheral vision was well preserved, measured by Goldmann perimetry. Rod ERGs were not recordable, whereas cone ERGs were detectable in early childhood. All features taken together suggest a specific form of Leber congenital amaurosis (LCA) distinguishable on clinical grounds. ERGs were normal in five of the six parents. One father had an ERG compatible with congenital stationary night blindness unrelated to his heterozygous state for the RPE65 mutation.

Conclusions. RPE65 mutations on both alleles may be associated with early-onset severe rod–cone dystrophy. Visual functions of the four patients were better than is usually seen in LCA, in particular in cases associated with retGC1 mutations. RPE65 mutations should be suspected in infants who appear to be blind in dim surroundings but react to objects in bright illumination and have nonrecordable rod ERGs and residual cone ERGs. (Invest Ophthalmol Vis Sci. 2000;41:2735–2742)

The human cDNA encoding RPE65, an abundant 65-kDa microsomal protein expressed exclusively in retinal pigment epithelium (RPE), was identified in 1993 by Hamel et al.1 The gene was assigned to chromosome 1p31 1 year later by the same group.2 Nicoletti et al.3 characterized the exon–intron structure of the gene, which spans 20 kb and is divided into 14 exons. The transcript is approximately 2.9 kb and consists of approximately 0.1% of the total mRNA isolated from the RPE. The deduced 533-amino acid human protein is evolutionarily highly conserved and does not show significant similarity to any other entry in the databases. Recent data obtained by biochemical analysis of RPE65-deficient (knock-out) mice suggest that the protein is necessary for the isomerization of 11-cis-retinol.4 Expression of RPE65 begins just before photoreceptors are developing in the retina, whereas there is no detectable RPE65 expression in the iris pigment epithelium (Thompson DA, written communication, March 1999). To date, a number of different RPE65 mutations have been reported in patients with retinal dystrophies classified as Leber congenital amaurosis (LCA), autosomal recessive childhood-onset severe retinal dystrophy, and autosomal recessive retinitis pigmentosa (arRP).5–10

LCA is a clinically and genetically heterogeneous condition with autosomal recessive (ar) inheritance, although a few families with autosomal dominant (ad) inheritance have also been reported.11 In general, LCA is diagnosed when there is marked visual impairment from birth on, whereas the disease is considered juvenile RP if vision is lost during the first 2 years of life.11 Mutations in the gene of the retina-specific guanylate cyclase (retGC1) on chromosome 17p13.1 have been identified in a group of patients with arLCA (LCA1, Mendelian Inheritance in Man [MIM] 204000).12 In addition, mutations of three other genes have been associated with an ocular phenotype designated LCA, those of RPE65 (LCA2, MIM 204100),7,9,10 AIPL1, a recently identified gene with homology to aryl-hydrocarbon receptor-interacting protein (AIP; LCA4, MIM 604393),13 and the cone–rod homeobox gene (CRX) on chromosome 19q13.3 (MIM 602225). In this latter case, heterozygous CRX mutations have been found in sporadic cases as well as in families with adLCA.14,15 Recently, a child with LCA and
a homozygous CRX mutation has also been reported. Both parents were heterozygous and had only minor cone abnormalities on electroretinogram (ERG) testing. An additional arLCA locus (LCA3, MIM 604232) has recently been assigned to chromosome 14q24.

Autosomal recessive childhood-onset severe retinal dystrophies are also a heterogeneous group of diseases affecting rods and cones. The most severe cases are termed LCA, whereas later-onset forms are termed juvenile RP. Gu et al. estimated that RPE65 mutations account for approximately 3% of all ar retinal dystrophy cases. Morimura et al. found mutations in the RPE65 gene in approximately 16% of cases of LCA and in 2% of cases of arRP.

In the current study, we identified the ocular phenotype of four affected children from three unrelated families with autosomal recessive childhood-onset severe retinal dystrophy and mutations in the RPE65 gene.

METHODS

Four children from three families with severe visual handicap from infancy on and phenotypic features of retinal dystrophy were studied by clinical, electrophysiological, and molecular genetic means. All six parents and a paternal uncle of subject H.J. were examined clinically and with electrophysiological methods to detect carrier signs eventually present in heterozygotes. Informed consent was obtained according to the Declaration of Helsinki, and the study was approved by the ethics board of the University of Regensburg.

Clinical and Electrophysiological Examinations

Clinical examinations included evaluation of visual function with age-adapted functional tests such as preferential-looking testing with Teller Acuity Cards (TACs), Cambridge Crowding Cards (CCCS), children pictures, illiterate E test, and numbers. In infants aged more than 5 years, kinetic photopic visual fields were measured with Goldmann perimetry. Pupillary reaction and presence of nystagmus were evaluated. The anterior segment and optical media were examined with a portable slit lamp (SL 14; Kowa, Tokyo, Japan) up to the age of 3 years, and with a stationary slit lamp (20 SL; Zeiss, Oberkochen, Germany) thereafter. Funduscopy was performed by monocular and binocular indirect ophthalmoscopy. Fundus photographs were taken with a fundus camera (TRC 50×; Topcon Optical, Tokyo, Japan), and also with a handheld fundus camera (Kowa). Data from the first ERG recordings performed under general anesthesia in patients H.J., L.H., and L.J. (Fig. 1) up to the age of 1 year were taken from external medical records. Detailed information on the anesthetics applied are not available. Follow-up ERGs in those patients and the ERG in patient B.R. (Fig. 1) were recorded by us with an ERG examination unit (Spirit; Nicolet, Madison, WI) according to the International Standard (International Society for Clinical Electrophysiology of Vision [ISCEV]) in patients under general anesthesia (propofol bolus injection 1 mg/kg followed by propofol perfusion 2 mg/kg · h, spontaneous breathing), with sedation (chloral hydrate, 60 mg/kg) or without sedation, depending on the child’s compliance. In one patient (L.H.), multifocal ERG (MERG) was performed at the age of 10 years (setup: 103 stretched hexagon pattern, filter settings 10–500 Hz, recording time 3 hours 38 minutes, Burian-Allen electrode). Kinetic visual fields were tested on a Goldmann perimeter (Haag-Streit, Bern, Switzerland). Color vision was tested with Matsu-bara, Ishihara, and Ichikawa color plates and with the Lanthony’s panel-D15 test.

Molecular Genetic Analysis

Genomic DNA samples of the four patients and their relatives were screened by single-stranded conformational polymorphism (SSCP) analysis for sequence changes and analyzed by restriction enzyme digestion and/or by direct sequencing of the 14 RPE65 gene exons. Each amplicon was analyzed under at least two different SSCP conditions. For experimental details see Gu et al. 6

RESULTS

Figure 1 shows the pedigrees and RPE65 genotypes of the three families, Figure 2 the fundi of the four patients, and
Family H

Family H has been presented briefly in a previous communication. H.J. was the only child of nonconsanguineous parents. Pregnancy and birth were unremarkable. At 3 months of age, the parents noted a visual impairment. At 4 months, the patient was referred to our department with suspected blindness. In bright surroundings, some reaction to light could be elicited. No photophobia was noted. Pupillary reaction was sluggish. A fine rotatory nystagmus was present. On retinoscopy, moderate hypermetropia was noted (+4.5 dpt OU). On funduscopy, a slightly increased granularity in the macula, together with a decreased macular reflex, moderate thinning of the retinal arteries, and increased granularity of the RPE in the periphery, were noted. At 6 months, the patient could follow objects and faces at high illumination. At 10 months, binocular visual acuity (VA) tested with TACs was 1.3 cyc/deg in 55 cm. The ERG performed at the age of 1 year with the patient under general anesthesia was nonrecordable under scotopic conditions but was residual under photopic conditions. At 17 months, binocular VA tested with TACs was 4.8 cyc/deg in 55 cm. At 20 months, cutoff filters were prescribed (Perfalit L 400; Rodenstock, Munich, Germany) to protect the cones from incident blue light. When the patient was 3 years of age, VA was 0.1 OU. The nystagmus was found to be manifest-latent pendular-jerk and remained so up to the last follow-up at the age of 8 years. When the patient was aged 5 years, VA was essentially the same. ERG (in sedation) was nonrecordable under both scotopic and photopic conditions. Funduscopy showed some optic disc pallor, bull’s eye maculopathy, thinned retinal arteries, and increased granularity of the RPE in the periphery of both eyes. Compared with changes noted at the age of 4 months, the changes were more prominent (Fig. 2A). Blood levels of vitamin A and retinol-binding protein were within normal range at the age of 5.5 years. When the patient was aged 6 years, VA was 0.1 OD. and 0.2 OS. Goldmann kinetic photopic visual fields showed relatively preserved outer limits for target III/4, with relative scotomata for targets II/4 and smaller (Fig. 3A). There was still no photophobia. The cutoff filters were less accepted by the patient, compared with acceptance in earlier years. When the patient was aged 8 years, visual fields and monocular VA were unchanged. On binocular testing, VA was 0.3.
With the panel-D15 test, there was a tritan axis in the desaturated version, and multiple errors without clear axis in the saturated version. The patient attends regular primary school.

H.K., the father of H.J., was examined at age 39 years. His distant VA was 1.0 OD and 1.2 OS without glasses. Anterior segments were normal. On funduscopy, the optic nerve head, the fovea, and retinal vessels were normal in both eyes. However, fine white-yellowish deep intraretinal dots were present all over the posterior pole, compatible with either hard drusen or fundus albipunctatus. ERGs showed residual rod responses, negative shape in the maximal response with normal a-wave and low amplitude b-wave, nonrecordable oscillatory potentials (OPs), normal cone flicker response, and a delayed a-wave in the single-cone response with normal amplitudes, compatible with congenital stationary night blindness. The patient was unaware of any major dark adaptation problems but admitted some minor problems. However, he reported that one of his brothers H.W. had at least as many difficulties during dark adaptation as he did. Therefore, H.W. was also examined clin-
### Table 1. Clinical Findings

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient Age</th>
<th>Vision</th>
<th>Nystagmus</th>
<th>Pupillary Reaction</th>
<th>Photophobia</th>
<th>Fundus</th>
<th>ERG</th>
<th>Visual Fields</th>
<th>Color Vision</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.J.</td>
<td>Infancy</td>
<td>Severe VI, suspected blindness, positive reaction to light, improvement follows objects and faces in bright surroundings</td>
<td>Fine rotatory</td>
<td>Sluggish</td>
<td>No</td>
<td>Macula: minor changes; periphery: increased granularity; retinal arteries: minor thinning</td>
<td>Toddler up to 8 years</td>
<td>Up to 0.3 BIN</td>
<td>Pendular → manifest-latent, pendular-jerk</td>
</tr>
<tr>
<td>B.R.</td>
<td>Infancy</td>
<td>Severe VI</td>
<td>By history</td>
<td>Fine oscillatory → fine horizontal pendular-jerk</td>
<td>NA</td>
<td>Normal</td>
<td>No</td>
<td>Figure 2B</td>
<td>At 4 y: scotopic and photopic nonrecordable</td>
</tr>
<tr>
<td>L.H.</td>
<td>7 m</td>
<td>Fixation to light only</td>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>At 7 m: residual (GA); at 7.5 y: scotopic and photopic nonrecordable; at 10 y: MERG, no distinct signals</td>
</tr>
<tr>
<td>L.J.</td>
<td>7 m to 1 y</td>
<td>VI particularly in darkened environment</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>At 1 y: residual (GA)</td>
</tr>
</tbody>
</table>

BIN, binocular testing; OU, in each eye; RE, right eye; LE, left eye; GA, general anesthesia; MERG, multifocal ERG; VI, visual impairment; NA, not available.
ically, electrophysiologically, and genetically. His VA and fundi were normal in both eyes. However, ERGs were identical with the recordings in his brother. The IVS1+5G→A mutation was not present. The findings in the clinical examination of the mother of H.J. were unremarkable in both eyes, as were the scotopic and photopic ERGs.

**Family B**

B.R. was the only child of nonconsanguineous parents. The father had a healthy daughter from a second marriage. Pregnancy and birth were unremarkable. By history, significant visual impairment and nystagmus had been noted since early infancy. In surroundings with reduced illumination, the boy was considered to be blind. The patient was first seen by an ophthalmologist at the age of 3.5 years. At that time, a fine oscillatory nystagmus was described. There was a moderate hypermetropic astigmatism in both eyes (+3.0/−3.0 × 0°). VA was noted to be 0.2 OU, and 0.3 on binocular testing (with children’s pictures). Some form of ocular albinism was suspected. Findings in magnetic resonance imaging and an ear, nose, and throat examination were unremarkable. The patient was first seen by us when he was aged 4 years. At that time, VA on binocular testing was 6/18 (CCC, single). In darkened surroundings, the child behaved as though he was blind, and no eye movements to light or objects could be elicited, whereas in bright surroundings the eyes followed promptly. No photophobia was observed. In agreement with these findings, the patient’s mother also reported that his visual performance was much better with bright light. There was a fine horizontal pendular jerk nystagmus. Some abnormal head posture was noted with chin-up, tilt, and turn to the left of approximately 15°. Pupillary reaction was noted to be normal. Anterior segment and optical media were normal in both eyes. On funduscopy, a slightly increased granularity in the macula, together with a decreased macular reflex, minimal thinning of the retinal arteries, and increased granularity of the RPE in the periphery were noted, together with a very blond fundus in the circumpapillary area (Fig. 2B). A tentative diagnosis of rod-cone dystrophy was made. Cutoff filters (Clarlet F 560; Zeiss) were fitted to protect the cones from short-wavelength light damage. ERG without sedation was unsuccessful when the patient was aged 4 years. When he was 6 years of age, the ERG registered without sedation according to the ISCEV standard was nonrecordable under both scotopic and photopic conditions. VA was 0.1 OD, 0.08 OS, and 0.1 OU on binocular testing (numbers). Head posture continued to be abnormal (mostly chin up 20°). The fundus appearance was unchanged. Visual fields could not be tested reliably at that time. When the child was aged 7 years, VA was unchanged. Goldmann kinetic photopic visual fields showed only slight eccentric constriction for target III/4. Target II/4 could be detected only with the right eye with relative scotomata for all smaller targets. The blind spot could not be registered. Testing accuracy was hampered by nystagmus, and the isopters are therefore only approximate values (Fig. 3B). With Ishihara and Ichikawa color plates, no numbers were detected, but with the panel-D15 desaturated test, multiple errors in all three axes were present. Ophthalmologic examination findings in both parents, aged 27 and 29 years, were unremarkable, as were the scotopic and photopic ERGs.

**Family L**

In family L, the two elder children, L.H. and L.J., were affected (Fig. 1). Parents were nonconsanguineous. Pregnancy and birth were unremarkable for both children. In L.H., only fixation to light could be elicited at the age of 7 months. Pupillary reaction was documented to be normal at that time. The boy was first seen by us at the age of 4 years. Best corrected VA at that age was 0.3 OD (+3.5/−1.75 × 5°) and 0.1 OS (3.5/−1.25 × 7°) and was 0.2 OU (E at 5 m) when the patient was 7.5 years of age. Manifest nystagmus was present at both examinations. Night blindness was noted by the parents. No photophobia was present. Funduscoppy showed slightly pale optic discs, slightly increased granularity in the macula, normal retinal vessels, and increased granularity of the RPE in the periphery of both eyes (Fig. 2C). ERG was documented as residual at 7 months (with the patient under general anesthesia) and nonrecordable both under scotopic and photopic conditions at 7.5 years (ISCEV standard). Goldmann kinetic photopic visual fields when the patient was aged 7.5 years showed only slight eccentric constriction for targets V/4 and III/4, marked constriction for target II/4, and relative scotomata for targets II/3 and smaller (Fig. 3C). Blood vitamin A level was normal when the patient was 10 years of age. MERG at that age did not show any distinct signals. Color vision with the panel-D15 saturated test was greatly disturbed. The child attends a school for the visually handicapped. He has minor neuromotor problems, but his mental development is normal.

The mother, being aware of the visual handicap of her elder son, reported visual impairment of her 7-month-old daughter L.J., mostly in darkened environment. With the patient under general anesthesia at 1 year of age, ERG was documented as residual. No nystagmus or photophobia was seen. When the girl was first seen by us at the age of 3 years,
VA tested with TACs in 55 cm was 9.8 cyc/deg OD and 3.2 cyc/deg OS. Funduscopy revealed a retinal aspect similar to that in her brother (Fig. 2D). Scotopic ERG was nonrecordable, whereas photopic ERG showed residual responses to a single flash (general anesthesia, 12 μV OD, 20 μV OS, normal >70 μV). Blood vitamin A and retinol-binding protein levels were normal when the patient was aged 6 years. The patient could not yet undergo Goldmann perimetry. Visual acuity was 0.2 normal when the patient was aged 6 years. The patient could flash (general anesthesia, 12 μV OD, 12 μV OS). Photopic ERG showed residual responses to a single flash (general anesthesia, 12 μV OD, 12 μV OS). Color vision was clearly disturbed, according to results of the Mutsu-bara test. Because of moderate psychomotor retardation, the child attends a special school. Whether this feature is associated with RPE65 mutations is unclear to date. Findings in clinical and electrophysiologival examinations of the father and mother were unremarkable at age 37 and 33 years, respectively.

**DISCUSSION**

We identified the ocular phenotype of four patients from three families with RPE65 mutations from infancy to the ages of 6 to 10 years. Patients H.J. and B.R. are compound heterozygous for a splice site mutation repeatedly found in patients with retinal dystrophy and a small rearrangement of 1 bp. In each case, a complete absence of the gene product is predicted (functional null alleles). The disease-causing mechanism of the missense mutations R91W and Y368H detected in family L is not yet known. R91W may be pathogenic by the changing of the tertiary structure of the protein after the replacement of an arginine, a positively charged, linear, strong proton donor, by a tryptophan, an uncharged, aromatic proton donor. The fact that R91W has already been described in other unrelated patients with retinal dystrophy suggests that this alteration is indeed pathogenic. Similarly, a significant change in the tertiary structure of RPE65 is the likely consequence of the Y368H mutation, due to the replacement of a negatively charged tyrosine by a positively charged histidine, that usually serves as counterion in complexing cations in enzymes. In family L, the two patients carry two mutations (1114delA and Thr457Asn) on their paternal alleles. Although Thr457 is evolutionarily conserved among all species studied so far, its replacement by asparagine may represent a rare neutral variant. In the case of L.H. and L.J., it is very likely that 1114delA, predicting a functional null allele, is the primary defect on the paternal allele.

In all four patients, severe visual impairment was noted in infancy, with visual responses elicited only in bright surroundings. Of note, visual performance improved during the first year of life with some minor decline over the next 6 to 10 years of follow-up. The early improvement may reflect postnatal physiological cone maturation. Although visual function was greatly reduced from early infancy, visual performance was clearly better than usually seen in LCA. Best measurable VA ranged from 0.1 to 0.3 in all four patients up to the age of 6 to 10 years (Table 1). Similarly, peripheral photopic kinetic visual fields were measurable and still well preserved at the age of 6 to 7.5 years in all three patients who were compliant with the test (Fig. 3). Nystagmus was noted during infancy in H.J. (at 4 months, together with sluggish pupils), B.R., and L.H. Of note, L.J. has never manifested nystagmus.

The ocular phenotype described does not fit the classification scheme proposed by Heckenlively for LCA and juvenile RP. According to that classification, patients with uncomplicated LCA (group 1) have an onset of symptoms before 6 months, VA below 20/400, a searching nystagmus, sluggish pupillary reactions, extinguished ERGs, and no measurable visual fields. LCA group 2 comprises syndromic forms. Patients with juvenile RP (group 3) have onset of symptoms before 2 years, VA higher than 0.2, occasionally a latent nystagmus, normal pupillary responses, an extinguished ERG, and constricted or tubular visual fields. Our patients showed features intermediate between groups 1 and 3. Most reports published so far on patients with RPE65 mutations and the clinical diagnosis of LCA do not provide detailed ophthalmologic information. In particular, data are unavailable on visual performance and nystagmus in early childhood. In a recent article, a comparative description of the phenotype of patients with RPE65 mutations and retGC1 mutations is given. The authors concluded that although onset of disease and fundus appearance in childhood were similar in the two groups, visual function in early childhood was better in patients with RPE65 mutations. VAs ranged between 0.1 and 0.2, visual fields were measurable, although constricted. Neither rod nor cone ERGs were recordable. However, because night blindness was a constant feature and cone function was measurable, Perrault et al. suggest that RPE65 mutations result in rod–cone dystrophy.

We also suggest that the RPE65 phenotype is a rod–cone dystrophy that can be distinguished by clinical means from other types of LCA, in particular LCA1, segregating with retGC1 mutations, that appears to be a cone–rod dystrophy. Classification of LCA1 as a cone-rod dystrophy has become even more appropriate in the light of recent publications reporting heterozygous retGC1 mutations in autosomal dominant cone-rod dystrophy with early onset and loss of central vision before the age of 7 years. This finding is in line with the fact that retGC1 is more abundantly expressed in cones than in rods. The presence of photophobia in these patients may also be explained by that fact. In contrast, infants with RPE65 mutations perform best in bright conditions as indicated by our data and by those of Perrault et al.

A recent study showed that the phenotype of different RPE65 mutations may be variable (i.e., either compatible with LCA or with RP). This may be explained by the different functional consequences of the various mutations. Nevertheless, congenital night blindness and macular dystrophy in the second decade of life were common findings in all patients. Detailed data on the phenotype in infancy and early childhood were not presented.

The analysis of RPE65−/− knock-out mice clearly showed that rods were affected predominantly. In these animals, the rod ERG was absent from early on, whereas the cone ERG was well preserved even after weeks. Heterozygous mice did not show any ERG changes up to the same age. This was paralleled by normal histology up to this age in contrast to clear atrophic changes in the RPE65−/− mice. As a consequence of the absence of RPE65 in the knockout mice, the rhodopsin level was reduced to approximately half in heterozygous mice, whereas in RPE65−/− animals, no rhodopsin absorption could be measured. Affected animals in a Swedish strain of Briard dogs are homozygous for a naturally occurring null mutation of the RPE65 gene. The preservation of their cone function, which seems to be much better than that in human, at least in infancy and young adulthood, is unexplained today.
The normal ERG in heterozygous knockout mice is in agreement with the electrophysiological findings in five of the six heterozygous parents of the families reported in the present study. All had normal scotopic and photopic ERGs. The abnormal ERG together with an abnormal fundus appearance in one of the parents is unrelated to his heterozygous state; his brother had the same abnormal ERG (with normal fundus) but did not carry the mutation of the RPE65 gene. In both brothers, the ERG is compatible with congenital stationary night blindness.

In conclusion, patients with RPE65 mutations appear to have a specific type of autosomal recessive severe childhood-onset rod–cone dystrophy. The classification LCA2 (MIM 204100) may be misleading, in that many of the patients with RPE65 mutations retain some useful visual functions at age 10, and others may have an even later onset of symptoms.

Acknowledgments

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References