Homozygosity for a Recessive Loss-of-Function Mutation of the NRL Gene Is Associated With a Variant of Enhanced S-Cone Syndrome

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PURPOSE. To investigate the genetic basis for severe visual complaints by Bukharan Jewish patients with oculopharyngeal muscular dystrophy (OPMD).

METHODS. Polymerase chain reaction amplification and direct sequencing were used to test for NRL, PABPN1, and NR2E3 mutations. Complete ophthalmic examination included best-corrected visual acuity, biomicroscopic examination, optical coherence tomography, and fundus autofluorescence. Detailed electroretinography (ERG) testing was conducted including expanded International Society for Clinical Electrophysiology of Vision protocol for light-adapted and dark-adapted conditions, measurements of S-cone function, and ON-OFF light-adapted ERG.

RESULTS. The index patients were homozygotes for both a dominant mutation of the PABPN1 gene, (GCN)13, and a recessive mutation of the NRL gene, p.R31X, on chromosome 14q11.1, leading to early-onset OPMD accompanied by night blindness and reduced visual acuity. No mutations were found in the NR2E3 gene. Both patients were of Bukharan Jewish origin, but from unrelated families. Electroretinography responses of both patients were dominated by short-wavelength-sensitive mechanisms, with no detectable rod function, similar to the ERG responses of individuals with enhanced S-cone syndrome (ESCS) due to NR2E3 mutations. Heterozygotes for the PABPN1 and NRL mutations demonstrated normal fundi and ERG responses.

CONCLUSIONS. Homozygosity for the recessive NRL mutation described here appears to be associated with a distinct retinal phenotype, demonstrating ERG characteristics similar to those of ESCS patients. This report expands the spectrum of NRL recessive mutations, as well as the genetic spectrum of ESCS, and indicates a new syndrome of OPMD with an ESCS-like phenotype.

Keywords: NRL, enhanced S-cone syndrome, retinal degeneration

The NRL gene encodes neural retina leucine zipper factor, a transcription factor, driving photoreceptor precursors to a rod fate and suppressing a cone fate. An immediate action of NRL is the induction of retinal orphan photoreceptor-specific nuclear receptor NR2E3,1 which together with NRL, induces rod genes and suppresses cone genes.2–5 Consequently, Nrl and Nr2e3-knockout mice have no rod photoreceptors, but have a large excess of S-cone-like photoreceptors, which are generated from postmitotic photoreceptor precursor cells instead of the early-born rod precursor population.6,7

Mutations in the NR2E3 gene cause a rare, slowly progressive autosomal recessive inherited retinal dystrophy (IRD) that is characterized by night blindness and increased sensitivity to blue light.8,9 Electroretinography (ERG) shows no rod function, depressed function of M- and L-cones, and enhanced S-cone function, leading to the diagnosis of enhanced S-cone syndrome (ESCS).10–12 A histologic report of a patient with ESCS supports the ERG-based diagnosis, showing a degenerate retina with no rods and twice the usual number of cones, most of which express the short-wavelength opsin.13

A variety of fundus appearances have been described in ESCS, the most typical being nummular pigmentary deposition at the level of the retinal pigment epithelium (RPE), usually outside the vascular arcades.14 Children with ESCS may initially manifest a normal fundus appearance, but later develop mottled RPE changes along the arcades, followed by the appearance of white dots in the same distribution.15 Additional features may include foveal scitic changes, whitish retinal deposits, hyperpigmented lesions, torpedo-like atrophic lesions, posterior pole circumferential scars, and yellow dots in areas of relatively normal-appearing retina.14,16,17 Hyperautofluorescence may occur within the arcades, associated with
small areas of hyperpigmentation. Optical coherence tomography (OCT) findings are variable and may include a thickened outer nuclear layer (ONL), cystic macular changes, disorganized retinal structure with splitting of the outer retinal layers, ONL rosette formation, or thin retinas with normal structure. 9-14,18-20

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant late-onset myopathy, characterized by early selective involvement of the eyelids and pharyngeal muscles, producing ptosis and dysphagia, followed by proximal limb weakness. 21 Oculopharyngeal muscular dystrophy is caused by an expansion of a trinucleotide repeat, (GCN)10 to (GCN)12-17, within the PABPN1 gene. 22 One of the world’s largest patient clusters is found among Bukhara Jews, who segregate the (GCN)13 allele (formerly called (GCG)9). 23 Most OPMD patients are heterozygotes for the expansion. However, owing to a high rate of consanguinity, several homozygous Bukhara Jewish OPMD patients have been described. These patients have an earlier onset of disease, faster progression, cognitive impairment, and a reduced life span. 24

Here we reported the case of two unrelated Bukharan Jewish patients with early-onset OPMD, who also complained of severe, slowly progressing visual loss including night blindness and reduced visual acuity. They were identified, genetically and electroretinographically, as suffering from a variant of ESCS due to a novel recessive mutation of the NRL gene, in close proximity to PABPN1.

**Materials and Methods**

**Subjects**

The study was approved by the National Helsinki Committee for Genetic Research in Humans and by the local Ethics Committees at Hillel Yaffe Medical Center and Tel Aviv Sourasky Medical Center. A written informed consent was obtained from all participants. The described research adhered to the tenets of the Declaration of Helsinki.

**Genetic Analysis**

Genomic DNA was extracted from venous blood samples according to a standard protocol. 25 PABPN1 mutation testing was performed as previously described. 22 Primer sequences used for amplification and sequencing of NRL and NR2E3 coding exons are listed in Supplementary Table S1.

**Ophthalmic Evaluation**

Ophthalmic examination included measurement of best corrected visual acuity (BCVA) using Snellen visual acuity charts, biomicroscopy, and fundus examination after pupillary dilation. Fundus photography was obtained with a fundus camera (FF450 plus fundus camera; ZEISS, Jena, Germany), and cross-sectional images were obtained by using spectral-domain OCT (SD-OCT; Heidelberg Engineering, Heidelberg, Germany). Blue laser fundus autofluorescence (FAF) was obtained with HRA/Spectralis (Heidelberg Engineering).

**Electroretinogram**

Full-field ERG was conducted according to an expanded protocol of the ERG guidelines of the International Society for Clinical Electrophysiology of Vision (ISCEV), 26 using the Espion E3 Electrophysiology System (Diagnosys LLC, Lowell, MA, USA). Electroretinography responses were recorded from both eyes with bipolar Burian-Allen corneal electrodes (Hansen Ophthalmic Development Lab, Coralville, IA, USA), applied with methylcellulose after pupil dilation and topical corneal analgesia.

After preparing the patient for ERG recording under normal room light, the light-adapted ERG was first recorded under white background illumination of 30 cd/m², using white light stimuli of different energies covering approximately 3.3 log units (0.1–200 cd·s/m²) and 30-Hz flicker (3 cd·s/m²). Each response was an average of three consecutive stimuli separated by 0.5 seconds for dim stimuli and by 1 second for bright ones. ON-OFF ERG responses were recorded under the same background conditions (30 cd/m²), using white light stimuli of 200-ms duration and different luminance, namely, 100, 150, 250, 500, and 600 cd/m². Each response was an average of six consecutive stimuli separated by 1 second for the dimmest stimulus and by 5 seconds for the brightest. Following 20 minutes of dark adaptation, scotopically matched dim blue and bright red light stimuli were used to record isolated rod response and cone response. Then, a series of white light stimuli of increasing energy covering 4.6 log units (0.005–200 cd·s/m²) were used to allow construction of the response–log stimulus energy relationship. Each response was an average of three consecutive responses separated by 1 to 30 seconds depending upon stimulus energy. The brighter the stimulus the longer was the time delay between consecutive stimuli. S-cone responses were recorded under scotopic conditions by using a paired blue flash protocol composed of a bright (34 cd/m²) blue (445 nm) stimulus of 200 ms in duration to saturate the rod system, which was followed after 750 ms by another blue (445 nm) light stimulus of 4 ms and energy of 0.6, 1.2, or 1.8 cd·s/m² to elicit the isolated S-cone ERG response. For each test light stimulus, the protocol was repeated five times, separated by 5-second intervals, in order to obtain the average S-cone response.

Supplementary Figure S1 demonstrates the repeatability of the ERG responses that were recorded during one recording session in patients A-1, B-1, and ESCS. Electroretinography repeatability between recording sessions is demonstrated for patient A-1, who was tested on two occasions, 5 months apart.

**Results**

**Genetic Analysis and Clinical Description**

A Bukharan Jewish patient (patient A-1) with early-onset OPMD, who was confirmed to be a homozygote for the PABPN1 (GCN)13 mutation, also complained of impaired vision. He was subsequently diagnosed with IRD, a feature which is not part of the OPMD phenotype (Fig. 1A, family A). We hypothesized that the (GCN)13 dominant mutant allele of PABPN1 was linked to a recessive mutation of an IRD-causative gene. Examination of the locations of all known IRD-causing genes (http://www.sph.uth.tmc.edu/Retnet/; provided in the public domain by the University of Texas Health Science Center, Houston, TX, USA) indicated that the NRL gene is located only 800 kb away from PABPN1 on chromosome 14q11.2. Sequence analysis of the two coding exons of NRL (exons 3 and 4) in patient A-1 identified a homozygous C to T transition at position 91 of NRL CDNA (GeneBank accession number NM_006177.3), located in exon 3, leading to the substitution of a codon for arginine by a stop codon at position 51 of the NRL protein (c.91C>T; p.R31X) (Fig. 1B). This mutation has not been previously reported in patients with IRD. It is not present in Single Nucleotide Polymorphism Database (dbSNP) (http://www.ncbi.nlm.nih.gov/projects/snp/; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD, USA) or the 1000 genomes database (http://www.1000genomes.org/; pro-
and in the choriocapillaris layer (Figs. 2D–F). Hyperreflective foci were evident in the retina, above the RPE, and ellipsoid zones), but no definite parafoveal thinning. OCT revealed flattening of the foveal contour with some

ecscence in the posterior pole (Figs. 2B, 2C). Spectral-domain

evident as well. There were atrophic RPE changes in the

eyes. Fewer small, round, hyperpigmented lesions were

around the optic nerve, and along the vascular arcades in both

numerous large yellow pigment clumps in the posterior pole,

open iridectomy.

The anterior segment was normal in the RE with a posterior

right eye (RE) and finger counting (10 cm) in the left eye (LE).

At the age of 51 years, when he was tested in our ophthalmic

assessed. Communication with the patient was quite difficult,

vertical and horizontal gaze. Blood vitamin levels were not

dysphonia, bilateral ptosis, and severe ophthalmoplegia in both

51 years, he had severe dysphagia for solids and liquids, nasal

glary myotomy, attempting to improve swallowing. At the age of

years. Consequently, he had undergone ptosis repair, followed

by cataract surgeries. Recently, he underwent a ciscopharyngeal

myotomy, attempting to improve swallowing. At the age of

51 years, he had severe dysphagia for solids and liquids, nasal
dysphoria, bilateral ptosis, and severe ophthalmoplegia in both

vertical and horizontal gaze. Blood vitamin levels were not

assessed. Communication with the patient was quite difficult,

and it was impossible to obtain reliable ophthalmologic history.

At the age of 51 years, when he was tested in our ophthalmic

clinic, he complained of reduced central vision, and in

response to specific questioning ascertained that he suffered

from visual difficulties at night. His BCVA was 20/240 in the

right eye (RE) and finger counting (10 cm) in the left eye (LE).

The anterior segment was normal in the RE with a posterior

chamber intraocular lens (IOL), while the LE examination

revealed an anterior chamber IOL, an irregular pupil, and an

open iridectomy.

Funduscopy demonstrated mild pallor of the optic discs and

numerous large yellow pigment clumps in the posterior pole,

around the optic nerve, and along the vascular arcades in both
eyes. Fewer small, round, hyperpigmented lesions were

evident as well. There were atrophic RPE changes in the

macula in both eyes (Fig. 2A). Fundus autofluorescence

revealed hyperautofluorescent spots mainly along the vascular

arcades, and hyperreflective PR–RPE thickening compatible

with fibrosis (Figs. 2I–K).

We tested the NRL mutation in a third unrelated Bukharan

Jewish patient (patient C-1) with late-onset OPMD who was

heterozygote for the PABPN1 mutation. He was found to be

heterozygote for the NRL mutation as well (Fig. 1A, family C).

Patient C-1 was diagnosed with OPMD at the age of 59 years.

Individuals A-2 and A-3 are the daughters of patient A-1 (Fig.

1A). They were not tested for the PABPN1 mutation, but since

their father is heterozygote for this mutation, they are obligate

heterozygotes. As expected, both were found to be heterozy-

gotes for the NRL mutation. At the ages of 27 and 18 years,

respectively, they were not yet affected by OPMD and did not

have any ocular symptoms. Best correct visual acuity of the

older sibling was 20/30 in the RE and 20/25 in the LE, while

the younger sibling’s BCVA was 20/20 in both eyes. Ophthal-
mic examination revealed no retinal abnormality in either

sibling.

Patient B-1 is an unrelated Bukharan Jewish individual, who

was referred to our clinic owing to chorioretinal scarring,

known since childhood. She reported nyctalopia and reduced

vision since early childhood. At the age of 35 years, she had

signs suggestive of OPMD, including ptosis and dysphonia, and

a family history of OPMD from both paternal and maternal

sides (Fig. 1A, family B). Genetic testing proved that she was

homozygote for the same PABPN1 and NRL mutations as those

of patient A-1. Her BCVA was 20/480 in the RE and 20/100 in

the LE. On examination, nystagmus was noted, the anterior

segments were normal, and there were a few cells in the

anterior vitreous in both eyes. Funduscopy demonstrated mild

tallor of the optic nerves, subretinal scars in the temporal

macula, and extensive patches of retinal atrophy along the

arcades and around the optic discs with pigmentary clumps.

There were numerous white dots in the peripheral retina and

fewer yellow dots in the posterior pole surrounding the

macula (Fig. 2G). In FAF, the atrophic lesions and the scars

appeared as hypoautofluorescent patches, while the yellow

dots appeared hyperautofluorescent (Fig. 2H). Optical coer-

ence tomography demonstrated perifoveal thinning and

irregularity of the outer retinal layers, some hyperreflective

foci above the RPE, patches of PR–RPE atrophy along the

arcades, and hyperreflective PR–RPE thickening compatible

with fibrosis (Figs. 2I–K).

We tested the NRL mutation in a third unrelated Bukharan

Jewish patient (patient C-1) with late-onset OPMD who was

heterozygote for the PABPN1 mutation. He was found to be

heterozygote for the NRL mutation as well (Fig. 1A, family C).

Patient C-1 was diagnosed with OPMD at the age of 59 years. At

vided in the public domain by the European Bioinformatics

Institute, Cambridge, UK), and is found heterozygously on 1/

120,240 alleles in the ExAC browser (http://exac.
broadinstitute.org/, provided in the public domain by the

Broad Institute, Cambridge, MA, USA).

Patient A-1, who is homozygous for both the PABPN1 and

the NRL mutations, was diagnosed with OPMD at the age of 30

years. Consequently, he had undergone ptosis repair, followed

by cataract surgeries. Recently, he underwent a ciscopharyngeal

myotomy, attempting to improve swallowing. At the age of

51 years, he had severe dysphagia for solids and liquids, nasal
dysphoria, bilateral ptosis, and severe ophthalmoplegia in both

vertical and horizontal gaze. Blood vitamin levels were not

assessed. Communication with the patient was quite difficult,

and it was impossible to obtain reliable ophthalmologic history.

At the age of 51 years, when he was tested in our ophthalmic

clinic, he complained of reduced central vision, and in

response to specific questioning ascertained that he suffered

from visual difficulties at night. His BCVA was 20/240 in the

right eye (RE) and finger counting (10 cm) in the left eye (LE).

The anterior segment was normal in the RE with a posterior

chamber intraocular lens (IOL), while the LE examination

revealed an anterior chamber IOL, an irregular pupil, and an

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Funduscopy demonstrated mild pallor of the optic discs and

numerous large yellow pigment clumps in the posterior pole,

around the optic nerve, and along the vascular arcades in both
eyes. Fewer small, round, hyperpigmented lesions were

evident as well. There were atrophic RPE changes in the

macula in both eyes (Fig. 2A). Fundus autofluorescence

revealed hyperautofluorescent spots mainly along the vascular

arcades and periphery, with milder diffuse hyperautofluores-
cence in the posterior pole (Figs. 2B, 2C). Spectral-domain

OCT revealed flattening of the foveal contour with some

segments of an epiretinal membrane and irregularity of the

retinal surface. There was waviness of the photoreceptor–RPE

segments of an epiretinal membrane and irregularity of the

outer retinal layers, some hyperreflective

foci evident in the retina, above the RPE, and in the choriocapillaris layer (Figs. 2D–F).
the age of 66 years, he had severe dysphagia, dysphonia, and mild tongue and proximal weakness in four limbs. He recently underwent a cricopharyngeal myotomy with subsequent swallowing improvement but no change in the other clinical parameters. His BCVA was good (20/25 RE, 20/20 LE) and his funduscopy revealed only minimal extrafoveal RPE changes in the LE.

Electroretinography

Complete ERG testing was conducted on patients A-1 and B-1, both homozygotes for PABPN1 and NRL mutations; individuals A-2, A-3, and C-1, who are heterozygotes for both mutations; and an unrelated ESCS patient, harboring a homozygous mutation of NR2E3 (c.932G>A; p.R311Q). Representative ERG responses of these five individuals and a normal individual are compared in Figure 3.

In the light-adapted state (background of 30 cd/m²), the single flash cone ERG (energy of 3.0 cd-s/m²) responses (Fig. 3, first column) of patients A-1 and B-1 (first and second rows, respectively) were characterized by a prolonged a-wave implicit time with normal amplitude, and a prolonged b-wave implicit time and subnormal amplitude. The ERG responses to the bright (30 cd/s/m²) white light stimulus (Fig. 3, second column) were of supernormal amplitudes and prolonged implicit times of the a-wave and the b-waves relative to the normal response (Fig. 3, seventh row). These were qualitatively similar to the corresponding responses of the ESCS
patient (Fig. 3, third row), except the latter had larger amplitudes of the photopic b-waves. Flicker responses (Fig. 3, third column) of A-1, B-1, and the ESCS patients were delayed and markedly subnormal, smaller than the a-wave amplitude of the light-adapted ERG response to 3.0 cd-s/m^2, which is a typical finding in ESCS patients.9–12 The isolated rod response (Fig. 3, fourth column), elicited by a dim blue stimulus in the dark-adapted state, was nonrecordable in A-1, B-1, and ESCS patients. The ISCEV standard mixed rod–cone responses26 in A-1, B-1, and ESCS patients (Fig. 3, fifth column) were of small amplitude and prolonged implicit times, very similar to their ERG responses for the same stimulus in the light-adapted state (Fig. 3, first column). The dark-adapted ERG responses to bright (30 cd-s/m^2) white light stimuli (Fig. 3, sixth column) of these patients were of large amplitudes and delayed implicit times of both a-waves and b-waves, but were characterized by different waveform. In patients A-1 and B-1, the a-wave dominated the waveform, and the b-wave was difficult to identify reliably. In fact, we selected the peak b-wave according to a small notch in the rising phase of the large a-wave. In the ESCS patient, the ERG to bright flash had a normal a-wave to b-wave waveform. Another ERG criterion that has been suggested as typical for ESCS patients is reduction in the function of M- and L-cones. This criterion was met in patients A-1 and B-1, as evident by the single flash and flicker responses in the light-adapted state (Fig. 3, first and

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**Figure 3.** Representative ERG responses of patients A-1 and B-1 (homozygotes for both the PABPN1 and the NRL mutations), a patient with ESCS (homozygote for an NR2E3 mutation), and individuals A-2, A-3, and C-1 (heterozygotes for the PABPN1 and the NRL mutations). For comparison, ERG responses of a healthy volunteer with no visual complaints are shown (lower row of responses). For each individual, ERG responses that were recorded in the light-adapted state by using a single white light stimulus of 3.0 cd-s/m^2 energy (LA 3) or of 30 cd-s/m^2 energy (LA 30), and the response to a 30-Hz white light flicker of 3.0 cd-s/m^2 energy (LA flicker) are shown (columns 1, 2, and 3, respectively). Dark-adapted responses include the isolated rod response to dim blue stimulus (DA blue), and the mixed rod–cone response to white light stimuli of 3.0 cd-s/m^2 energy (DA 3), and to 30 cd-s/m^2 energy (DA 30) (columns 4, 5, and 6, respectively).
second columns). Furthermore, we typically record the dark-adapted ERG response to a red stimulus that elicits a characteristic X-wave, reflecting cone function, almost exclusively that of L-cones. The amplitudes of the X-wave in patients A-1, B-1, and ESCS were subnormal—26 μV, 15.4 μV, and 43.5 μV, respectively—while our lowest limit for the normal range was 50 μV. The ERG responses of the three heterozygotes (individuals A-2, A-3, and C-1) (Fig. 3, rows 4–6) were very similar to the corresponding ERG responses of the normal subject (Fig. 3, seventh row) for all recording conditions.

The ERG responses of patients A-1 and B-1 (Fig. 3, first and second rows) were qualitatively similar, but not identical, to those of the ESCS patient (Fig. 3, third row). To gain a more quantitative comparison, we plotted the amplitudes and implicit times of the ERG a-wave and b-wave in Figures 4 and 5 for the light-adapted state and dark-adapted state, respectively. The ERG data of our patients were compared to the normal range (Figs. 4, 5; dashed lines), which represents the ERG data of two individuals with normal retinal function, representing the minimal and maximal values of our normal range that was estimated from data of 50 individuals with normal retinal function.

The light-adapted a-wave amplitude of A-1, B-1, and ESCS patients were in the lower normal range for dim stimuli, and of supernormal amplitudes for bright stimuli (Fig. 4A, left; filled symbols). The implicit times of the light-adapted a-wave were longer than the normal range for all light stimuli used here (Fig. 4A, right; filled symbols). The light-adapted b-wave amplitudes of A-1, B-1, and ESCS patients showed a monotonic increase in amplitude as stimulus energy was increased (Fig. 4B, left; filled symbols), in contrast to the typical “photopic hill” curve of individuals with normal retinal function. The normal relationship between photopic b-wave amplitude and log stimulus energy is termed the “photopic hill” curve because following a peak amplitude that is reached with mid-energy stimulation (typically 3–10 cd·s/m²), further increases in stimulus energy lead to a monotonic decline in the b-wave amplitude. The b-wave implicit times were longer in patients A-1, B-1, and ESCS compared to the normal range of our laboratory for all stimulus energies used here (Fig. 4B, right). The light-adapted a-wave and b-wave of the three heterozygotes (Fig. 4, open symbols) were within the normal range with regard to both amplitude and implicit time, and exhibited the typical “photopic hill” pattern of the relationship between photopic b-wave amplitude and log stimulus energy (Fig. 4).

The dark-adapted a-wave amplitudes of patients A-1, B-1, and ESCS were at the lower limit of the normal amplitude range for dim stimuli, and of normal amplitudes for bright stimuli (Fig. 5A, left; filled symbols). The implicit times of the dark-adapted a-wave were stable in the range 30 to 40 ms, and showed very little dependency upon stimulus energy, while the normal range exhibited a steep decline from approximately 25 to 40 ms for dim stimuli to 8 to 12 ms for bright ones (Fig. 5A, right). The dark-adapted b-wave amplitude of patients A-1, B-1, and ESCS were considerably smaller than the normal range for dim and moderate light stimuli and were prolonged for bright light stimuli (Fig. 5B, left; filled symbols). The b-wave implicit times of patients A-1, B-1, and ESCS were within the normal range for dim to moderate stimuli and were prolonged for bright light stimuli (Fig. 5B, right; filled symbols). The dark-adapted a-wave and b-wave of the three heterozygous individuals (Fig. 5, open symbols) were within the normal range (dashed lines) with regard to both amplitude and implicit time.

The ERG data, discussed above, indicate that patients A-1 and B-1 share many common features with ESCS patient, raising the possibility that they represent a variant of ESCS. To test this possibility, we recorded the S-cone ERG responses and the ON-OFF light-adapted ERG responses, and compared the results to those of the ESCS patient, a heterozygote patient (C-1), and a normal volunteer (Fig. 6). The S-cone response of the ESCS patient had a b-wave to a-wave waveform shape similar to that of the normal individual but the response was considerably larger in amplitude (both a- and b-waves) and was of prolonged implicit time as compared to the normal individual. The S-cone responses of patients A-1 and B-1 were delayed in their implicit times, and have an electronegative pattern with a supernormal amplitude a-wave, while the b-wave amplitudes were within our normal range. The S-cone response of the heterozygote patient was similar to the normal one (Fig. 6, first column, fourth row). The ON component of the light-adapted ON-OFF ERG responses (Fig. 6, second column) varied between patients A-1, B-1, and ESCS, having an electronegative waveform in patients A-1 and B-1, but not in the ESCS patient. The OFF-response had a similar waveform in all three patients (A-1, B-1, ESCS). It was composed of a slow rate of depolarization toward a plateau. The typical peak of the d-wave was missing. The ON-OFF ERG response of the heterozygote patient (C-1) was similar to the normal one.

Since for most ESCS cases that have been studied until now the causative gene is NRZE3, we sequenced the eight exons of NRZE3 in patients A-1 and B-1. No mutations were found.

**Discussion**

Oculopharyngeal muscular dystrophy is an autosomal dominant myopathy, leading to ptosis and dysphagia, followed by proximal limb weakness. Since retinal dysfunction is not a characteristic finding in OPMD, we aimed to investigate the genetic cause of severe visual complaints, including night blindness and reduced visual acuity, in two patients with early-onset OPMD (patients A-1 and B-1). We found that the OPMD-causative mutation of the PABPN1 gene, (GCN)13, was linked to a nonsense mutation of the NRL gene, p.R31X. Since visual complaints were expressed only by homozygotes for both the PABPN1 and the NRL mutations (patients A-1 and B-1) and not by the heterozygotes (patients A-2, A-3, C-1), we concluded that the genetic cause of their visual complaints was a loss of function recessive mutation in the NRL gene.

Patients A-1 and B-1, whom we found to be homozygotes for an NRL mutation, presented common ERG characteristics (Figs. 3–6) that included the following: (1) rod ERG was undetectable in the dark-adapted state; (2) the photopic and scotopic responses to the same white light stimulus had similar delayed waveform; (3) the amplitude of the photopic ISCEV standard 30-Hz flicker was smaller than that of the a-wave in the single flash photopic ERG (ISCEV standard); (4) the photopic b-wave of the transient responses to bright white stimuli was prolonged, and increased in amplitude with increasing stimulus energy, in contrast to the “photopic hill” behavior in volunteers with normal photopic ERG; (5) short-wavelength cone (S-cone) ERG responses had delayed implicit times and larger a-wave amplitudes than those of normal subjects; and (6) function of L- and M-cones was significantly reduced. These ERG characteristics are very similar to those reported for ESCS patients, suggesting that patients A-1 and B-1 represent a variant of ESCS.

The S-cone responses of the NRL-mutant patients differed in waveform from that of our ESCS patient (Fig. 6). While the S-cone ERGs of the NRL-mutant patients had an electronegative pattern with abnormally large a-waves, the S-cone ERG of the NRZE3-mutant patient was characterized by normal a-wave amplitude with a b-wave of supernormal amplitude. However,
S-cone ERGs with electronegative waveform have been reported before in other ESCS patients.9 The photopic ON-OFF ERG responses of patients A-1 and B-1 were qualitatively similar to that of the ESCS patient (Fig. 6). While the ON responses had electronegative waveform in the NRL-mutant patients with reduced (A-1) or nonexisting (B-1) b-wave, the NR2E3-mutant patient presented an ON-response of normal b-wave to a-wave relationship. However, other ESCS patients, reported in the literature, show large variability in the ON-response, including an electronegative waveform with reduced b-wave, similar to our NRL-mutant patients. The OFF-responses had similar waveform in the three patients, composed of a slow depolarization and absence of a transient peak, in agreement with previous reports on ESCS patients.9,14

The simplest explanation for the abnormal OFF-response of the ESCS patients and our NRL-mutant patients is based on a suggested model attributing the transient peak of the OFF-response to OFF-center bipolar cells, and the slow depolarization to the recovery of the cones from the light stimulus.31 There is still debate in the literature about the existence of S-cone OFF bipolar cells, but if they exist they are very sparse.32,33 Accordingly, as suggested before,34 the waveform of the OFF-response in ESCS patients and in our NRL-mutant patients (Fig. 6) reflects mainly the recovery of the S-cones from the light stimulus in the absence of S-cone OFF-center bipolar cells.

The sparsity of S-cone OFF-center bipolar cells can also account for the abnormal response–stimulus energy relation-
ship of the photopic b-wave amplitude in ESCS patients and our NRL-mutant patients (Fig. 4B). The "photopic hill" curve of the photopic b-wave was attributed to the summation of a bell-shape relationship for the OFF-pathway, and a monotonic increase of the ON-pathway. When the bell-shape contribution of the OFF-pathway is reduced or even absent, a monotonic increasing photopic b-wave amplitude with increasing stimulus energy is expected, reaching very large amplitudes with bright stimuli due to the abundance of S-cones.

Our NRL-mutant patients did not present with the typical nummular pigmentation of ESCS, but do share retinal findings with other ESCS patients reported in the literature. Patient A-1 had yellow pigment clumps, which appeared as hyperreflective foci in the retina and above the RPE in SD-OCT and as hyperautofluorescent dots in FAF (Fig. 2). Indeed, yellow pigment dots have been previously described in ESCS due to NR2E3 mutations, and also in one patient with a heterozygous NRL mutation. In addition, whitish subretinal dots, that appear hyperautofluorescent, have been described in ESCS. Furthermore, the intraretinal hyperreflective foci demonstrated in OCT of patient A-1 seem similar to the "rosette" formation described in that report. Patient B-1 had atrophic lesions along the arcades, fibrotic scars in the macula, as well as white and yellow dots. All of these findings have recently been described as part of the expanded clinical spectrum of ESCS. The FAF pattern and the OCT findings of subretinal

**FIGURE 5.** Amplitude and implicit time relationships to stimulus energy for the a-wave (A) and the b-wave (B) as derived from the dark-adapted ERG responses of the NRL-mutant patients (A-1 and B-1; filled squares and circles, respectively), ESCS patient (filled triangles), and three individuals who are heterozygotes for the PARPNT1 and the NRL mutations (A-2, A-3, and C-1; open squares, circles, and triangles, respectively). The normal range (thick dashed lines) for each dark-adapted ERG parameter is composed of data from two individuals, out of a group of 50, having the minimal and maximal values of our normal range.
fibrosis and retained ellipsoid zone subfoveally in that report are also similar to those encountered in our patient.

*NRL* encodes neural retina leucine zipper factor, a transcription factor that induces the expression of the orphan nuclear receptor NR2E3, acting together with NRL to activate rod genes and to suppress cone genes. In humans, dominant mutations in the *NR2E3* gene are associated with retinitis pigmentosa (RP), while recessive *NR2E3* mutations are associated with three different but overlapping phenotypes: ESCS, Goldmann–Favre syndrome, and clumped pigmentary retinal degeneration. Thus, mutations in the *NRL* gene are expected to be associated with retinal disorders.

Most *NRL* pathogenic mutations reported to date are dominant and are associated with an RP phenotype (Table). Electroretinography is consistent with a severe generalized rod–cone dysfunction, typical for RP, and may have an electronegative pattern. Only two cases of recessive retinal dystrophies due to *NRL* mutations have been reported to date (Table). In one case, a homozygous patient for the c.444-445insGCTGCGGG recessive mutation was diagnosed as...
A recessive NRL mutation causes ESCS

**Table.** NRL mutations reported in patients with inherited retinal dystrophy

<table>
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Unclear inheritance pattern

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* Base positions refer to GeneBank accession number NM_006177.3.
† Found in compound heterozygosity with p.L160P.
‡ Found in compound heterozygosity with c.224-225insC.

Autosomal recessive RP, but additional clinical data were not available.39 In a second report, two siblings, who were compound heterozygotes for two recessive NRL mutations (c.224-225insC and p.L160P), were diagnosed with a clumped pigmentary retinal degeneration. The affected patients have suffered from night blindness since early childhood, but color vision is normal, suggesting the presence of the three spectral types of cones. The ERG responses are severely reduced in amplitude, and S-cone function is evaluated only by chromatic Humphrey static perimetry. A comparison of central visual fields using white-on-white and blue-on-yellow light stimuli reveals a relatively enhanced function of short-wavelength-sensitive cones in the macula.45 In an additional study, 27 patients with confirmed ESCS by ERG recording were subjected to genetic analysis. Homozygous (N = 13), compound heterozygous (N = 11), or heterozygous (N = 2) mutations in NR2E3 have been found in 26 of them. One patient has been found to be heterozygous for an NRL mutation (c.223insC), previously named c.353insC. A second NRL mutation has not been detected in this patient.36 The patient has clumped pigment and yellow lesions in the vascular arcades and peripheral retina. Electoretinography is characteristic of ESCS, but chromatic perimetry reveals peripheral rod-mediated vision. As the same NRL mutation has been found heterozygously in unaffected family members, the authors suspect a digenic mechanism with another unknown gene. It is also possible that this patient may have a second heterozygous null mutation in NRL, which was not detected (such as a large deletion or duplication; a deep intronic mutation; or a mutation in a regulatory site).

The two patients described previously45 and the two patients described here have two recessive, loss-of-function alleles of the NRL gene. Their genotypes are homologous to those of previously reported Nrl-knockout mice, which have a complete loss of rod function and a supernormal cone function, mediated by S-cones.7 The photoreceptors in the Nrl−/− mice retina have cone-like nuclear morphology and short sparse outer segments with abnormal discs. Analysis of retinal gene expression has confirmed the functional transformation of rods into S-cones, consistent with the assumption that in normal development NRL modulates rod-specific genes, while inhibiting S-cone pathway through the activation of NR2E3.7

In summary, this report expands the spectrum of NRL recessive mutations, as well as the genetic spectrum of ESCS, and indicates that recessive mutations in NRL can present an ESCS-like phenotype. The cases presented here indicate a new syndrome of OPMD with ESCS.

**Acknowledgements**

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**References**

12. Schorderet DF, Escher P. NR2E3 mutations in enhanced S-cone sensitivity syndrome (ESCS), Goldmann-Favre syndrome (GPS),...


