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 Electrorretinography (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 pigmented 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, torpedolike 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 dilatation. Fundus photography was obtained with a fundus camera (FP450 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). 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 3.5 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. Thinning were noted in the outer retinal layers (interdigitation (PR–RPE) and choriocapillaris layers. Foci of irregularity and retinal surface. There was waviness of the photoreceptor–RPE segments of an epiretinal membrane and irregularity of the macula in both eyes (Fig. 2A). Fundus autofluorescence revealed hyperautofluorescent spots mainly along the vascular arcades and periphery, with milder diffuse hyperautofluorescence 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 sides (Fig. 1A, family B). Genetic testing proved that she was heterozygote for the same 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. Funduscopometry revealed no retinal abnormality in either sibling.

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 dysphonia, 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. Funduscopometry 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, and with milder diffuse hyperautofluorescence 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 (PR–RPE) and choriocapillaris layers. Foci of irregularity and thinning were noted in the outer retinal layers (interdigitation and ellipsoid zones), but no definite parafoveal thinning. Hyperreflective foci were evident in the retina, above the RPE, and in the choriocapillaris layer (Figs. 2D–F).

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 homozygote for this mutation, they are obligate heterozygotes. As expected, both were found to be heterozygotes 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 corrected 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. Ophthalmic 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. Funduscopometry demonstrated mild pallor 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 coherence 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
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 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).
A Recessive NRL Mutation Causes ESCS

The ERG 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 NR2E3, we sequenced the eight exons of NR2E3 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 NR2E3-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,9,14 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-center 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
fibrosis and retained ellipsoid zone subfoveally in that report. 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 Recessive NRL Mutation Causes ESCS.
autosomal recessive RP, but additional clinical data were not available. 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 pigmented 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. 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 Nrl23 have been found in 26 of them. One patient has been found to be heterozygous for an Nrl mutation (c.223insC, previously named c.224-225insC). A second Nrl mutation has not been detected in this patient. The patient has clumped pigment and yellow lesions in the vascular arcades and peripheral retina. Electroretinography 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 mutation (such as a large deletion or duplication; a deep intronic mutation; or a null mutation in Nrl), which was not detected (such as a large deletion or duplication; a deep intronic mutation; or a null mutation in Nrl). 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.

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.

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