Enhanced S-cone syndrome (ESCS) is a rare, autosomal recessive inherited retinal dystrophy first described more than 2 decades ago.1-3 Because of the variable clinical presentation, it is not unusual that patients with ESCS are misdiagnosed as having atypical retinitis pigmentosa, congenital stationary night blindness, or X-linked retinoschisis. The adult human retina typically consists of approximately 120 million rods and 6 million cones containing 3 cone subtypes: short-wavelength (S), medium-wavelength (M), and long-wavelength (L) cones. S-cones are the minority (about 10%)4 subset of cones in healthy human retinas whereas in ESCS, S-cones are the majority cone subtype.1-3,5,6 Electroretinography (ERG) and psychophysical testing play a key role in diagnosing patients with ESCS. Characteristic ERG findings are the presence of similar waveforms in the maximum dark-adapted response and single-flash light-adapted waveform. An associated ERG feature is disproportionately reduced 30-Hz cone flicker to the single-flash cone amplitude.7-9 The basis of these waveforms has been explored.10 All of these findings occur in the absence of rod function.1-3,7,8 S-cone stimuli can show supernormal responses, although the relative increase in S-cone function compared with L- and M-cone function is diagnostic and independent of degree of disease severity. Psychophysical testing demonstrating the abnormal ratio of S-cone to L- and M-cone function was especially helpful in proving that ESCS and the more severe expression Goldmann-Favre syndrome were part of the same disease spectrum.3

Importance New funduscopic findings in patients with enhanced S-cone syndrome (ESCS) may help clinicians in diagnosing this rare autosomal recessive retinal dystrophy.

Objective To expand the clinical spectrum of ESCS due to mutations in the NR2E3 gene.

Design Retrospective, noncomparative case series of 31 patients examined between 1983 and 2012.

Setting Academic and private ophthalmology practices specialized in retinal dystrophies.

Participants A cohort of patients diagnosed with ESCS and harboring known NR2E3 mutations.

Intervention Patients had ophthalmic examinations including visual function testing that led to the original diagnosis.

Main Outcomes and Measures New fundus features captured with imaging modalities.

Results New clinical observations in ESCS include (1) torpedo-like, deep atrophic lesions with a small hyperpigmented rim, variably sized and predominantly located along the arcades; (2) circumferential fibrotic scars in the posterior pole with a spared center and large fibrotic scars around the optic nerve head; and (3) yellow dots in areas of relatively normal-appearing retina.

Conclusions and Relevance Enhanced S-cone syndrome has more pleiotropy than previously appreciated. While the nummular type of pigmentation at the level of the retinal pigment epithelium and cystoid or schisis-like maculopathy with typical functional findings remain classic hallmarks of the disease, changes such as circumferential fibrosis of the macula or peripapillary area and “torpedo-like” lesions along the vascular arcades may also direct the clinical diagnosis and focus on screening the NR2E3 gene for a molecular diagnosis.
Postmortem donor retinas have been studied in rare reports in which the disease was identified as ESCS and these confirmed and extended the noninvasive observations. In the donor eye from a 77-year-old woman, there were no rods detectable, a 2-fold increase in cone density with an abnormally high number of S-cones and abnormally low number of L- and M-cones, and coexpression of S-cone opsin in some L- and M-cones. In another study, there were no detectable rods, and cones also coexpressed different opsins.

Enhanced S-cone syndrome is mainly caused by mutations in the NR2E3 gene that encodes the nuclear receptor class 2 subfamily E, member 3 protein (NR2E3, OMIM 604485). NR2E3 is uniquely expressed in the outer nuclear layer of the retina and there is evidence that it suppresses cone differentiation during embryogenesis. Multipotent retinal progenitor cells present in fetal eyes are directed to become rods or cones. Therefore, loss of NR2E3 results in retinas with a decreased number of rod photoreceptors but an increase in cones, predominantly expressing the S-cone opsin resulting in ESCS.

A recent clinical study of ESCS reported an association with helicoid subretinal fibrosis, thereby adding another phenotypic expression to ESCS. The purpose of the current study was to review a cohort of established ESCS cases with mutations in NR2E3 to further determine the clinical spectrum of ESCS. New clinical findings may facilitate earlier diagnosis in patients presenting with unusual phenotypes and may increase the understanding of disease mechanisms due to NR2E3 gene causation.

Methods

All patients were evaluated by the coauthors S.G.J., S.H.T., I.B., M.J.vS., and L.A.Y. The procedures were approved by the ethics committees of involved sites and adhered to the tenets set out in the Declaration of Helsinki.

On identification of mutations in NR2E3 in patient 1, who showed several unusual and previously undescribed findings in ESCS, we obtained clinical data from 30 other patients with established ESCS, all but 1 having proven NR2E3 mutations. The patients had been examined between 1983 and 2012. All patients underwent a standard ophthalmic examination, and most had ERGs. Color fundus photographs were obtained by camera systems available at the time of examination and were mainly of the central retina; there were no montages or attempts to cover wide retinal areas. Fundus autofluorescence was obtained with a Topcon TRC 50 IX DX fundus camera or Heidelberg Spectralis (Heidelberg Engineering Inc). Optical coherence tomography was obtained using the Spectralis or Stratus (Zeiss Meditec Inc) in a subset of patients.

Results

Cohort of Patients With ESCS and NR2E3 Mutations

Thirty-one patients with ESCS (30 with proven NR2E3 mutations) were studied retrospectively. Patients ranged in age from 4 to 72 years (18 female and 13 male) and had different ethnic and geographic origins. They all had symptoms of night blindness from an early age. Best-corrected visual acuity (Snellen) ranged from 20/20 to 20/200. Many patients showed the well-known fundus features of ESCS such as macular pigment clumping at the level of the retinal pigment epithelium (RPE) along the vascular arcades and in the midperiphery; cystoid disturbances in the macula (retinoschisis), and chorioretinal atrophic changes. The patients who underwent ERG testing showed no detectable waveform to a dim flash of light intended to stimulate dark-adapted rods. A bright white flash, dark adapted, elicited a waveform that could be similar in shape to a flash of the same intensity on a white background. Flicker ERGs tended to be smaller in amplitude than the light-adapted single-flash responses. When S-cone ERGs were performed, responses were abnormally increased in amplitude.

New Funduscopic Observations

The new fundus features are described next and representative images are shown in Figures 1, 2, 3, 4, 5, and 6. The patients are numbered in order of appearance in the Figures, and the corresponding molecular genetic data as well as the patients’ age at time of examination are listed in the legends.

Circumferential Subretinal Fibrosis

A 25-year-old woman (patient 1) with a history of night blindness since early childhood presented with bilateral, large circumferential fibrosis in the macula, with hyperpigmentation and sparing of the fovea (Figure 1). Based on high-resolution optical coherence tomography images of the central area, the fibroses appeared to be located subretinally (Figure 2). There was loss of normal outer retinal architecture across most of the scanned region except at the fovea, which had a normal-appearing outer nuclear layer and a retained inner segment ellipsoid band of the photoreceptors. An epiretinal membrane, some disorganization of the retinal layers, and outer retinal tubulations (circular pattern of degenerate photoreceptors) were also present. Fundus autofluorescence images of both eyes showed a large hypoautofluorescent ring with a hyperautofluorescent center. The hypoautofluorescent ring corresponded to the area of subretinal fibrosis seen on color images. The midperiphery and far periphery showed areas of normal autofluorescence and areas of hypoautofluorescence. Within the normal and hypoautofluorescent areas, there were multiple small hyperautofluorescent and hypoautofluorescent dots (Figure 3).

Subretinal fibrosis was subsequently noted in 6 other patients (23%) (Figure 4). These fibrotic lesions were typically found in the posterior pole. Two patients showed peripapillary subretinal fibrosis. The subretinal fibrosis in patients presented herein showed different patterns. Most patients had bilateral and symmetrical subretinal fibrosis. Subretinal fibrosis was found as early as 4 years of age (patients 2 and 7). Unilateral subretinal fibrosis was seen only in 1 patient (patient 3).

Torpedo-like Lesions

Unusual torpedo-like lesions along the superior arcades were observed in patient 1. She had 1 large choroidal-RPE-retinal atrophic lesion along the superior arcades in the right and left eyes. These were located in relatively healthy-appearing retina and

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showed a hyperpigmented rim. They were oval and showed hypoautofluorescence on fundus autofluorescence. The presence of torpedo-like lesions was subsequently observed in 9 additional patients (32%) (5 of whom are shown in Figure 5). These lesions were variable in size. The exact location varied but all torpedo-like lesions were observed in a ring along the superior and/or inferior arcade. Typically, they consisted of central depigmentation or chorioretinal atrophy with a hyperpigmented border.

In patient 11, the torpedo-like lesions appeared to be non-progressive given that they had not changed over a 10-year follow-up period starting at age 24 years (Figure 5). The patients with torpedo-like lesions in this study were 24 to 55 years of age at the time of documentation of the lesions.

**Yellow Dots in the Periphery**

Patient 1 had yellow dots in peripheral areas that also showed small nummular hyperpigmentation. The dots were mainly seen nasal to the optic disc and below the inferior arcade. The location, size, and distribution of these yellow dots were variable among patients (Figure 6). The dots were not perfectly round and were...
also not of a uniform size within retinas of individual patients studied. Hyperpigmented nummular dots were present in areas of yellow dots in most patients except for patients 2 and 7. The dots in patients 2 and 7 were mainly found temporal to the fovea, whereas in the other patients, they were primarily located nasal to the disc. The yellow dots were noted in areas of relatively normal-appearing retina. All patients in whom subretinal fibrosis was observed also had yellow dots.

**Discussion**

Our review of funduscopic abnormalities in a cohort of patients with ESCS and NR2E3 mutations allowed us to identify additional, previously underappreciated features of this autosomal recessive retinal degeneration and thereby expand the clinical spectrum. A novel observation in ESCS is the presence of hyperautofluorescent dots which we refer to as "yellow dots." These are present in areas of relatively normal-appearing retina. All patients in whom subretinal fibrosis was observed also had yellow dots.
ence of torpedo-like lesions. These lesions are variable in size, oval, and show a central area of RPE depigmentation or choroidal atrophy with a hyperpigmented margin. Most of these lesions were located along the arcades. The hyperpigmented rim in torpedo-like lesions was not surrounded by normal RPE and the lesions were located in retinal areas with many other retinal and RPE abnormalities (Figure 5). The torpedo-like lesions differed from the torpedo lesions described in the literature. Torpedo lesions tend to be solitary and relatively large with a sharp narrowed end that points to the foveola and are located temporal to or under the fovea. Surrounding the hyperpigmented rim is normal RPE. The torpedo lesions are congenital and have been thought to be due to abnormal choroidal or ciliary vasculature development or a fetal RPE defect.19,20

Subretinal fibrosis as seen in our patient 1 was previously observed in 2 patients with ESCS and NR2E3 mutations.13 The basis for the distribution of these lesions, in the region of the arcades in some patients and in the peripapillary or submacular areas in others, remains unexplained. A recent study showed subretinal hemorrhage and subretinal fibrosis in a 14-month-old boy with ESCS.21 Subretinal hemorrhages early in the course of the disease have been documented and may be the cause of the subretinal fibrosis.22,23 It remains unclear why subretinal hemorrhages occur in ESCS. A submacular vascular abnormality was suggested to cause the fibrosis and macular hemorrhage in the 14-month-old child.21

The retinal region with many of the observed abnormalities in ESCS is the perimacular elliptical ring near the vascular arcades that also extends into the nasal retina. The NR2E3 mutations in ESCS lead to transcriptional misregulation and abnormal photoreceptor development with an increase in the number of S-cone-type photoreceptors at the expense of rods that fail to differentiate.24,25 In normal retinas, the perimacular elliptical ring is a rod-rich zone.4 In ESCS, the perimacular region is thought
to be filled with S-cone photoreceptors instead of rods. Studies of retinal organization in patients with ESCS have shown disturbed structure in this region; optical coherence tomography indicated thick and bulging retina in the ring with abnormal laminar architecture. It has been speculated that larger cone cells in place of rods could cause abnormal packing of photoreceptors, secondary impairment in phagocytosis by the RPE, and resulting degeneration. This mismatch between photoreceptor cell type and underlying RPE would be expected to be exaggerated in the usually dense rod ring filled with S-cones. Patchy loss of RPE cells or their pigment or increased density of melanin granules in remaining RPE cells could be the funduscopically visible result of a chronically abnormal interface of photoreceptors and RPE. Histopathological analysis of an ungenotyped eye donor with clumped pigmentary retinopathy showed such findings. There were patches of more densely pigmented RPE near areas of less pigment, accounting for the clumping appearance. Such images in donor eyes of patients with ESCS from NR2E3 mutations have also been published (Milam et al Figure 2E and Bonilha et al Figure 1C). The basis of the torpedo-like configuration of some of these RPE disturbances is unknown. There is the possibility that patches of retina with more abnormal photoreceptor and RPE approximation, possibly caused by retinal folds or pseudorosettes, as seen in Nr2e3rd7 mouse models and in ESCS histopathological analysis, could lead to round or oval regions of RPE cell loss.

We found intraretinal yellow dots in several patients (Figures 1, 2, 3, 4, 5, and 6). Although this has been described before in ESCS and other retinal dystrophies, it is of interest that all patients with subretinal scarring had intraretinal yellow dots. In another study of patients with NR2E3 mutations, hyperautofluorescent loci were noted and colocalized cross-sectional optical coherence tomography images showed these features to be intraretinal and associated with dysmorphology of the photoreceptor layer. The Nr2e3rd7 mutant mouse also has white-yellow dots visible ophthalmoscopically at certain ages; autofluorescent dots were studied in these mice and attributed to material within macrophages, which were associated with retinal pseudorosettes.

Were there any clear genotype-phenotype relationships? The question was asked only of those patients with common NR2E3 mutations: p.R311Q and c.119-2A>C (IVS1-2A>C). In the present cohort, these 2 mutations were present in the homozygous state in 19 patients: 14 patients for R311Q and 5 patients for IVS1-2A>C. We asked whether the torpedo-like lesions and subretinal fibrosis were present in all or most of the patients with one or the other mutation. Among the 14 R311Q homozygotes, 5 of 14 (36%) showed torpedo-like lesions, 4 of 14 (29%) showed subretinal fibrosis in the posterior pole, and 1 patient showed both. None of the 5 homozygotes for IVS1-2A>C showed torpedo-like lesions, and 1 had subretinal fibrosis surrounding the optic nerves. Yellow dots mainly were seen in association with the subretinal...
fibrosis. The torpedo-like lesions, however, were not exclusive to the R311Q homozygotes and subretinal fibrosis was not exclusive to the 2 genotypes. Other genotypes in our cohort (but only represented anatomically by 1 patient each) showed these fundus features. We therefore could not establish a clear genotype-phenotype relationship.

In conclusion, patients with torpedo-like lesions along the vascular arcades or circumferential macular fibrosis should have ESCS considered among the differential diagnoses. While fibrosis and torpedo-like lesions may be the most striking funduscopic abnormalities in some individuals, careful clinical observation usually reveals other characteristics such as nummular pigmentary changes, interspersed with yellow dots; cystoid maculopathy; and macular or peripheral schisis. Regardless of the clinical presentation, the diagnosis of ESCS will need to be confirmed by ERG, psychophysical testing, and/or mutation analysis of the NR2E3 gene. Phenotype recognition and improved knowledge of disease causation should aid in developing treatment strategies to prevent progressive vision loss in ESCS in the future. Such insight is also valuable for understanding normal retinal and photoreceptor development.

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