Cone Vision Changes in the Enhanced S-Cone Syndrome Caused by NR2E3 Gene Mutations

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PURPOSE. To determine the progression of cone vision loss in patients with recessive disease from NR2E3 gene mutations.

METHODS. Patients with NR2E3 mutations (n = 37) were studied as a retrospective observational case series clinically and with chromatic static perimetry. Patients were investigated cross-sectionally, and a subset was followed longitudinally.

RESULTS. Patients showed a range of visual acuities; there was no clear relationship to age. With kinetic perimetry (V4e target), a full field could be retained over many years. Other patients showed progression from a full field, with or without pericentral scotomas, to a small central island. Three patterns of S-cone function were defined, based on percentage of hypersensitive S-cone loci in the field. From occupying most of the visual field, hyperfunctioning S-cone loci could diminish in percent, remaining largely in the periphery. Normal S-cone functioning then dominates, followed by the appearance of an annular region of abnormal S-cone loci approximately 10° to 40° from the fovea. Overall, S-cone sensitivity declined 2.6 times faster than L/M-cone sensitivity.

CONCLUSIONS. Murine proof-of-concept studies suggest that clinical trials of patients with NR2E3 mutations may be forthcoming. Patterns of S-cone hyperfunction across the field would serve as a means to categorize patients as entry criteria or cohort selection in clinical trials. S-cone perimetry can be measured in the clinic and would be the logical efficacy monitor for therapeutic strategies. Given further understanding of the natural history of the disease, targeting the annular region of S-cone dysfunction for a focal therapy or for monitoring in a retina-wide intervention warrants consideration.

Keywords: cones, visual fields, visual acuity

An autosomal recessive retinal disease that can be misdiagnosed as retinitis pigmentosa, congenital stationary night blindness, X-linked retinoschisis, and other retinopathies was recognized nearly 3 decades ago to be a unique entity.1 The disease had the unusual feature of manifesting a gain in visual function. The mechanism of this hyperfunction was investigated with spectral sensitivity measurements, and the unexpected conclusion was that the least common type of photoreceptor functioning in the retina, the short-wavelength sensitive (S-) cones, was the most hypersensitive across the retina while there was reduced long/middle-wavelength sensitive (L/M-) cone function and little or no rod function.1 We named the least common type of photoreceptor development, specifically a defect in cone differentiation.8 This hypothesis was buoyed by studies of cone differentiation in nonhuman primates10,11 and rodents.12

When the molecular cause of ESCS was determined, the notion of a disrupted photoreceptor developmental pathway leading to the disease manifestations gained support.13,14 The NR2E3 gene (also known as PNR, photoreceptor nuclear receptor) was identified inadvertently during a search for Bardet Biedl syndrome (BBS) genes on chromosome 15. When found not to be a BBS gene, NR2E3 was used to screen a large cohort of patients with inherited retinal degeneration (IRD), and a small group of these patients had plausible disease-causing NR2E3 mutations. These few patients all had ESCS. A pure sample of patients with enhanced or relatively enhanced S-cone function was then screened, and all of these patients showed NR2E3 mutations.15 NR2E3 is an orphan nuclear receptor of retinal photoreceptors; the disease may result from abnormal fate determination leading to excess S-cones at the expense of other photoreceptor subtypes.14 Further proof of the hypothesis of excess S-cones in the ESCS retina came from a postmortem donor retina with NR2E3 mutations showing an unexpectedly high number of residual S-cones relative to L/M-cones.15
Evidence from many studies of ESCS indicates that there is not only a unique photoreceptor developmental abnormality but also a spectrum of disease severity with definite retinal degeneration. Progress in understanding photoreceptor developmental pathways has proceeded (for example, see Refs. 16–20) and experiments now suggest that nuclear receptors can be modulators of disease and could play a role in therapy, including the retinal degeneration resulting from NR2E3 mutations.21,22 Given the possibility of therapy, the present study was performed using both cross-sectional and longitudinal studies of patients with NR2E3 mutations to begin to understand how this unique group of diseases could be monitored through a clinical trial intending to alter therapeutically the natural history of disease.

METHODS

Human Subjects

The study, a retrospective observational case series (between 1990 and 2017), was approved by the institutional review board at the University of Pennsylvania. Informed consent was obtained, and all procedures adhered to the tenets of the Declaration of Helsinki. There were 37 patients, representing 30 families, with ESCS caused by NR2E3 mutations, diagnosed and evaluated clinically and by molecular genetics (Supplementary Table S1). ERGs were not analyzed as a part of this study.

Kinetic and Static Perimetry

Goldmann kinetic visual fields were performed using V4e and 14e test targets. In brief, the solid angle subtended by each visual field isopter was calculated using the perimeter chart and converted to percentage of the corresponding normal mean. This value, the percentage of normal extent, was plotted on a log10 scale.23 Chromatic static threshold perimetry was performed with a modified automated perimeter (Humphrey Field Analyzer, HFA-750i analyzer; Zeiss-Humphrey, Dublin, CA, USA) to provide measures of L/M-cone and S-cone function across the field.1,3,8,12,15,18,24 For L/M-cones, the stimuli used were 650 nm in the dark-adapted state, and for S-cones, 440 nm on a 170 cd/m2 yellow background (targets, 1.7° in diameter; presentation time, 200 ms; 70 loci on a 12° grid). Data from healthy subjects and more complete details of the perimetric method used in this specific group of subjects have been published.5

Data Analysis

Each locus measured by chromatic static perimetry was classified according to function. Loci with nonzero sensitivities that were greater than mean ± 2 SD of normal were classified as super-normal (S); those less than mean ± 2 SD were abnormally reduced (A). Those within the ± 2 SD interval were classified as normal (N). There were locations at which the lower limit of normal was below the range of testing of the automated perimeter. In those cases, when the measured sensitivity is zero (floor effect), the discrimination between N and A was not possible and the location was labeled “U” (unclassifiable).

For greater understanding of the spatial and temporal distribution of the S, N, A, and U loci for S-cone function and the N and A loci for L/M-cone function, maps describing cone sensitivity over the visual field were generated. Additionally, plots relating cone function to eccentricity (distance from fixation in degrees) were created; cone sensitivities were subtracted from mean normal (labeled as differences from normal) and plotted against eccentricity. Longitudinal data for S-cone and L/M-cone function were studied in a subset of patients. The rates of change of sensitivity with time were calculated with a mixed-effects model using time, condition (cone sensitivity loss), and their interaction as fixed effects and subject and location as nested random effects using R statistical computing software (version 3.4.4; Vienna, Austria) with the lme4 package (version 1.1–15).

Patterns of S-cone function in the visual field were identified using S-cone sensitivity maps. The maps were grouped into three patterns based on the percentage of field showing hyperfunctioning S-cone loci. Pattern 1 maps show ≥50% super-normal loci; pattern 2 maps have <50% (but ≥20%) of the field with super-normal loci; and pattern 3 maps contain <20% of super-normal loci.

To understand the relation of abnormal S-cone function to abnormalities in ophthalmoscopic appearance, we used fundus photographs available from six of the patients. The photos were scaled to fit a sketch with fundus landmarks using a polar coordinate system centered on the fovea. Pigment abnormalities were outlined and shaded on each fundus photo using CorelDRAW X6 software (version 16; Ottawa, Canada), and a composite map was generated. A plot summarizing the percent loci classified as abnormal varying with eccentricity was created using a generalized additive model with R software (version 3.4.4; Vienna, Austria) and the mgcv package (version 1.8–25).

RESULTS

Best-corrected visual acuity (BCVA) was available for all 37 patients (Supplementary Table S1). BCVA was 20/40 or better in at least one eye at first visit in 17 of the patients (age range, 12–33; median age, 31). The remaining 20 patients had 20/50 or worse at first visit (age range, 11–73; median age, 34). The annual rate of decline of BCVA was calculated in 20 patients with serial measurements over an interval of at least 3 years (median interval 12.3 years, range 3–34; median age at first visit, 29.4; age range, 11–65) and it was found to be 1.88% per year (0.00824 logMAR per year). The eye with the best BCVA at the baseline visit was used for the rate calculation. A plot of the change in BCVA as a function of time from first visit in these 20 patients is shown (Supplementary Fig. S1).

Longitudinal kinetic visual fields for seven patients with NR2E3 mutations are shown (Fig. 1A). Columns are arranged to suggest patterns of disease progression. P17, over a decade, still has a central scotoma and unchanged peripheral extent of field (with V4e target). The field to 14e, however, is further reduced. P15 has serial kinetic fields spanning 28 years. At age 27, the pattern of loss is similar to that of P17 at age 41. The extent to V4e diminishes to a pericentral temporal island by age 40 and is further reduced at age 55.

P18 retains V4e extent over a 12-year interval, but there is reduction to 14e. P34 at ages 66 and 70 has nearly full fields to V4e; an absolute scotoma in the infero-nasal field is detectable at age 70. With 14e, there is only a central island at age 66 and no detection at the later age.

A sequence of fields for P22 (covering 18 years) shows pericentral scotomas extending to an incomplete ring around the central field and then nasal and inferior fields becoming further diminished. P26 at age 40 has a central island and a far temporal peripheral island. By age 54, only the small central island remains. P24, over a 27-year interval, shows a full field to V4e at age 39 becoming reduced to a small central island by age 66. Overall, light-adapted kinetic fields show progression of dysfunction from a full field (with or without a central
scotoma) to only a residual central island, reminiscent of common patterns in other IRDs.

For each patient at each visit, kinetic field extent to the V4e target was plotted as a percentage of normal mean (Fig. 1B). Twenty patients had longitudinal data of \( \geq 3 \) years. Eleven of those patients (ages at first visit, 11–33 years; median, 16 years) were within normal limits over the period of observation (intervals observed, 3–12 years; median, 9 years). To calculate progression of field loss, we decided not to average in the data from the subgroup with normal and unchanging fields. We used the nine patients who were abnormal at first visit or declined from normal during the time of observation. Individual rates of loss to V4e (slopes of the gray dashed lines in the graph) were calculated and then averaged. Taken as a group, the overall rate of loss of field extent for the nine patients was 5.37% per year. Tempted to further dissect this small sample, we noted that four of these patients (ages at first visit, 13–64 years; median, 42.5 years) had relatively short follow-up duration (intervals, 3–6 years; median, 4 years) and an average loss of 0.91% of visual field extent per year. The other five patients (ages at first visit, 27–66 years; median, 40 years) had longer follow-up durations (intervals, 11–28 years; median, 19 years) and a faster average rate of loss, 8.8% of field extent per year. Genotype was not obviously related to field loss rates. When we considered those patients who showed change and those who did not, there was no combination of alleles or single specific mutations that occurred solely in one group or the other.

**What Is the Regional Variation of S-Cone Sensitivity in Patients With NR2E3 Mutations?**

The progression of light-adapted kinetic fields in patients with NR2E3 mutations, albeit complex, may serve as a rate of cone vision loss to compare with similarly analyzed and collected data from other IRDs (for example, see Refs. 26–28). The cone photoreceptor composition of retinas with NR2E3 mutations, however, differs from those of other IRDs because of the unique increase in S-cone numbers and function.1,3,8,15 We next separated the types of cone dysfunction across the visual field into S-cone and L/M-cone results using chromatic static perimetry; the goal was to determine whether the rates were the same, different, or interdependent.

First, we examined S-cone function and its variation across the visual field. Data from a representative sample of patients with different degrees of S-cone function are shown (Figs. 2A–
F); and then results from the entire cohort are displayed as maps and categorized into three patterns (Fig. 3A). In the six representative patients, S-cone sensitivity (expressed as difference from normal) is plotted as a function of eccentricity (left panels, Fig. 2) and as a visual field map (upper right panels, Fig. 2). The patients are ordered (A–F) by percentage of hypersensitive S-mediated loci. P12 at age 21 shows 66% of S-mediated loci to be hypersensitive; the remaining loci are within normal limits for S-cone sensitivity and there are no abnormally reduced S-cone loci. The hypersensitive S-cone loci are mainly in the peripheral field. P1 at age 15 also shows super-normal S-cone function in the peripheral field with normal function more centrally. P6 at age 13 has patches of S-cone hypersensitivity, largely found in the peripheral field. P20 at age 31 has patches of abnormally reduced loci between 17° and 40° from fixation, with up to 10 dB sensitivity loss relative to normal. Hyperfunctioning loci are in the peripheral field. P13 displays a ring of abnormally reduced loci between 17° and 40° from fixation, with 9 to 17 dB sensitivity loss relative to normal; a few hyperfunctioning loci are present peripherally. In P31, at age 48, no hypersensitive S-cone loci are present; most measurable loci are 10 dB less-sensitive than normal.

The variation in S-cone function across the visual field was studied in 32 patients at their earliest possible visit (Fig. 3A). Five patients were not included in this analysis (P26, P27, P35, P36, and P37) because their limited island of central vision was not well-represented on the 12° grid. Visual inspection suggested there were different patterns of S-cone hypersensitivity across the field. We grouped the data into distinct visual field changes.
three patterns by percentage of hyperfunctioning S-cone loci. Pattern 1 showed mainly super-normal S-cone loci (≥50%; range, 53%–91%) and some loci with normal sensitivity (range, 9%–46%); only one patient had abnormal loci. The hypersensitive S-cone loci were extracentral, whereas loci with normal sensitivities were more centrally located. Pattern 2 also showed hypersensitive loci in the periphery but less of the visual field was occupied by these loci (≥20% but <50%; range, 21%–49%). Normal loci in pattern 2 were greater in percent (range, 37%–74%) than in pattern 1 and occupied a wider region of the central and mid-peripheral field. There were abnormal loci in 10 of 13 patients. In pattern 3 patients, defined as having less than 20% hypersensitive loci, S-cone function was mostly normal (range, 3%–79%); hypersensitive loci were scarce (range, 0%–14%) and located in the far periphery. Abnormal loci (range, 7%–39%) are higher than in the other patterns. The patterns are less related to age than the year of disease stage. Patterns 1 and 2 are composed of cohorts of similarly aged patients; however, pattern 3, showing a more advanced stage, is composed of a slightly older patient group. Specifically, for pattern 1, mean age is 25, range 13–35, and median 30. For pattern 2, mean age is 24,
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range 12–46, and median 17. For pattern 3, mean age is 41, range 15–77, and median 38.

The loci with abnormally reduced S-cone function from all S-cone sensitivity maps (Fig. 3A) were plotted as a function of eccentricity from fixation (Fig. 3B). Most of these dysfunctional loci were in the retinal region between 10° and 40°. We then asked whether there was any relation of this area of dysfunction to ophthalmoscopically visible abnormalities.

The graph was plotted on a drawing of a fundus image to visualize where there may be coincidence of abnormal function and structure (Fig. 3B, inset). There were fundus images available from six patients in this study (P19, P20, P22, P26, P27, P34) and the pigmentary abnormalities observed were overlaid on the fundus drawing (Fig. 3C). In this subset of patients, the pigmentary disturbances were located most prominently in the annular region between approximately 10° and 40°. This localization of pigmentary retinopathy along the arcades has been shown or mentioned in many previous publications (for example, see Refs. 2, 4, 5, 7), although many other fundus features also have been reported.

We postulate that the patterns represent disease stages and may be of value in categorizing patients with NR2E3 mutations in future treatment trials. In summary, S-cone hypersensitivity can be present across most of the visual field but is eventually lost except for small peripheral islands. At later disease stages, there is normal sensitivity across the visual field and an annular ring of S-cone dysfunction that can coincide with pigmentary changes in a region extending from approximately 10° to 40°, a region that has been described as pericentral or near peripheral.50 These data confirm the impression from the sample of six patients (Figs. 2A–F).

**S- and L/M-Cone Sensitivity Changes, Measured Longitudinally**

Having sampled S-cone function in individual patients and then across a large group of patients in this study, we then asked what changes occur in longitudinal data over 4 to 6 years (Fig. 4) and over 10 to 27 years (Fig. 5). Format for these presentations resembles that in Figure 2 but now we have added L/M-cone sensitivity graphs and maps adjacent to S-cone results. Data were available for an interval of 4 to 6 years in four patients with NR2E3 mutations, ranging in age at first visit from 16 to 65 years. P2, at age 16 years, would be considered within S-cone pattern 2: hyperfunctioning loci encompass 49% of the field, largely in the periphery, and there are a few abnormal loci (7%), appearing within the near periphery (17°–24° from fovea). L/M-cone sensitivity, only possible to be categorized as normal and subnormal, shows normal sensitivity in the periphery (38% of loci) and subnormal results in mid-peripheral and central loci (62%) (Fig. 4A). Four years later, at age 20, P2 shows no major change in degree of function for S- and L/M-cones and in pattern. In contrast, there are changes over a comparable interval in the other three patients illustrated (Figs. 4B–D). P8 shows a decrease in hyperfunctioning S-cone loci, from 16% to none between ages 18 and 24. There is also a decrease in values within normal limits (56% to 36%) and a small increase in subnormal S-cone loci (11% to 16%). L/M-cone function appears relatively unchanged over the interval with only a few normally functioning loci at both ages (3% at age 18; 7% at age 24). A 4-year interval (ages 41–45) in P24 shows loss of the few hyperfunctioning S-cone loci in the periphery (10% to zero) and an increase in subnormal loci from 14% to 30% in the peri-central and near-peripheral regions. L/M-cone sensitivity is not obviously changed in these 4 years. P34, between ages 65 and 70 years, shows loss of peripheral hyperfunctioning S-cones and normal L/M-cone loci. Hyperfunctioning S-cones decreased from 7% to zero, while normal-functioning S-cones decreased from 79% to 54%. Subnormal S-cones increased from 7% to 20%. Normal L/M loci decreased from 51% to 1%.

Longitudinal results with intervals ranging from 10 to 27 years were available in three patients; with such data, it can be inquired further whether the patterns of progression suggested by the grouped results occur in individual cases (Figs. 5A–C). P18, over an interval of 12 years (ages 33–45), loses 56% of the S-cone hypersensitive loci; from an initial 66%, only 10% remain after 8 and 12 years. Over time, regions of S-cone hypersensitivity are located in only the far periphery. Most remaining loci are within normal limits with rare subnormal loci, first appearing at age 41. This resembles the changes from pattern 1 to pattern 3. L/M-cone loci with normal sensitivity across regions of the field (42% at age 33) are also lost in this interval, and only abnormally reduced loci remain (Fig. 5A). P10 over a decade (ages 17–27) also shows loss of peripheral hyper-S-cone function, from 41% to 4% of loci. After this interval, most loci are within normal limits or abnormally reduced, and the region of abnormal sensitivity has become an annulus in the mid-periphery; this can be considered similar to pattern 2 to pattern 3 progression. L/M-cone function is already reduced to a few normal loci in the periphery (13%) at age 17 and there is further progression to mainly abnormal loci by age 27 (Fig. 5B). P15 over 13 years shows progression in S-cone dysfunction. At age 28, S-cone results would be categorized as pattern 2, with 21% of the field composed of hyperfunctioning loci and some abnormal loci present. After further decades, all hyperfunctioning S-cone loci are lost and the results would fit pattern 3. The few normal L/M-cone results at age 28 (8%) are lost, and by ages 41 and 55, there are only abnormally reduced L/M-cone sensitivities (Fig. 5C).

The progression rates for S- and L/M-cones were calculated and plotted (Fig. 5D). S-cones decreased by 0.56 dB per year and L/M-cones by 0.22 dB per year. The two rates were significantly different (P < 0.0001); the S-cone metric decays approximately 2.6 times faster than the L/M metric. The limited number of subjects able to be followed for many years prompts the need for prospective natural history studies involving larger numbers of patients.

**Discussion**

**Distribution of S-Cone Function With Progression in Patients With NR2E3 Mutations**

The funduscopic findings associated with NR2E3 mutations have been well described.4,7,29,31 Some of the fundus pathology also can be seen in other IRDs, hence leading to early confusion about clinical diagnosis before the unique functional mechanism was reported and later availability of molecular diagnostics.1,13,24 Specific fundus abnormalities have not been clearly associated with specific NR2E3 genotypes.5 The unifying feature of the disease caused by NR2E3 mutations, despite variation in severity and the many different fundus changes, is the fact that S-cone function is greater than L/M-cone function: a uniquely abnormal relationship of these photoreceptors.1,3 To begin thinking about NR2E3 as a candidate for any type of therapy, it would seem sensible to consider it as a disease group sharing a mechanism of abnormal photoreceptor development and having a spectrum of disease severity based on the common feature of retinal degeneration.

How do the data from this study fit with those of past investigations of patients with NR2E3 mutations? Previous studies have not determined disease progression by functional criteria. If treatment trials are in the future of NR2E3, however, there is a need to decide what parameters would be sensible.
FIGURE 4. S- and L/M-cone sensitivity changes measured longitudinally over intervals of 4 to 6 years in four patients with NR2E3 mutations. Results of the difference from normal sensitivity for each test locus are plotted against eccentricity for patients for both S-cones (left in each panel) and L/M-cones (right). Visual field maps for each age are also given. S-cone loci are categorized as S, N, A, or U (as in Figs. 2, 3). L/M-cone loci are categorized as normal (N, red) or subnormal (A, light gray). The blind spot is represented as a black square at 12° in the temporal field. (A) P2 shows little difference in both S- and L/M-cone function over a 4-year interval. (B) P8 shows a reduction in super-normal S-cone loci over time, along with an increase in number of abnormal S-cone loci. L/M-cone function remains largely unchanged over this interval. (C) P24 also shows a reduction in hypersensitive S-cone loci with an increase in abnormal S-cone loci over time. For abnormal L/M-cone loci, the degree of abnormality increases over this interval, particularly in the near-peripheral visual field. (D) In P34, S-cone sensitivity shows progressive losses over the 5-year interval. Normal L/M-cone loci decrease in number as abnormal loci increase in number and show a general reduction in sensitivity relative to normal.
and feasible to measure not only for safety but also to
determine efficacy. It would be an unrealistic goal of therapy
to rescue the phenotype of \textit{NR2E3} by changing the
photoreceptor composition of these developmentally altered
retinas. To slow or halt progression of the retinal degenerative
component of the disease, however, would be worth
considering. The conventional measure of visual acuity would
be an unlikely outcome to rely on entirely because of
the various maculopathies associated with the disease and our
longitudinal data suggesting a slow rate of decline. Light-
adapted kinetic fields are another possible functional out-
come, but rates of change may be too slow; the rates of
loss are among the slowest previously reported for
\textit{IRDs}. Studies to date recognize that patients with \textit{NR2E3}
mutations have essentially an all-cone retina, so we focused
on the two cone subtypes, S-cones and L/M-cones, both able to
be measured across the visual field in a clinical setting. The
predominant function in these patients is from S-cones and
there were different patterns of regional variation in this
function. Hyperfunction of S-cones in disease from \textit{NR2E3}
mutations is not a constant feature of the central field, where
there can be normal or abnormally reduced S-cone sensitivity.
This was an early observation that led to confusion about the
basis of the disease. Hypersensitivity of S-cones at early
disease stages is extracentral and extends into the far
peripheral field. What is the cellular basis of the hypersensi-
tivity of S-cones? The finding of large S-cone–driven ERG
photoreceptor responses, a-waves, in patients with
\textit{NR2E3} mutations led to our conclusion that a greater number of S-
cone photoreceptors was the source; in other words, S-cone
hyperfunction was not due to some unknown amplification of
inner retinal function. Postmortem histopathology and in
vivo measures of retinal micro-anatomy with optical coherence
tomography (OCT) have not been helpful to answer the
question about the type of photoreceptors in the peripheral
retina of early stages of \textit{NR2E3}-associated retinopathy. The
only two postmortem donor retinas have been in patients at
late disease stages with severe retinal degeneration of the

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\caption{S- and L/M-cone sensitivity changes measured longitudinally over 10- to 27-year intervals in three patients with \textit{NR2E3} mutations. Differences from normal sensitivity for S-cones and L/M-cones are plotted against eccentricity at the different ages. Visual field maps corresponding to each age are also shown; S-cone loci are categorized as S, N, A, or U, and L/M-cone loci are categorized as N or A. (A) P18 shows a gradual decrease in hypersensitive S-cone loci over a 12-year interval. L/M-cone sensitivity also shows a reduction in normal loci. (B) Over a 10-year interval, the S-cone sensitivity of P10 shows negative change. The number of hypersensitive S-cone loci decreases and abnormal loci increase. L/M-cone sensitivity also decreases over time. (C) P15 shows progression between ages 28 and 41, but less so in the subsequent 14 years to age 55. L/M-cone sensitivity shows the greatest decreases in normal loci between ages 28 and 41 and then remains stable between 41 and 55. (D) Plots for S-cones (left) and L/M-cones (right) show the follow-up time and partial residuals from a mixed-effects model allowing for interaction between follow-up time and condition.}
\end{figure}
peripheral retina; OCTs beyond the central retina are not possible with current technology.15,57

Cross-sectional and longitudinal data indicated that the S-cone hypersensitivity can be present throughout most of the visual field. First, there is a diminution in the mid-periphery, and with further change, only a few hyper- sensitive loci are detectable in the far peripheral field. The remaining S-cone function is within normal limits. There is eventual abnormal S-cone sensitivity surrounding the central field. The distribution of this elliptical zone of S-cone dysfunction is reminiscent of the distribution (by fundus photography) of pigmentary changes (mainly clumps) and torpedo-like lesions at and eccentric to the vascular arcades.7,25 We previously described eventual loss of vision and retinal thickening in this area.38

Other than these signs of retinal degeneration in a similar distribution as the S-cone dysfunction in patients with NR2E3 mutations, it is of interest that this is also the distribution of peak rod photoreceptor density.35 Assuming that photoreceptor fate is disturbed by the NR2E3 mutations and rod photoreceptor differentiation does not occur,7 we speculate that an increased S-cone-like photoreceptor cell population fills the topography intended for rods but the differences between the two photoreceptor types and the relationship to adjacent RPE cells may contribute to retinal degeneration. Many studies of murine models with an abnormal Nr2e3 transcriptional network have provided hypotheses about the pathogenesis of degeneration. For example, it could result from a disturbed photoreceptor maintenance function,40 impairment of phagocytosis due to the aberrant photoreceptors,41 microglial proliferation,42 and the complex interaction with modifier genes.7,22

**Approaching a Clinical Trial for Patients With NR2E3 Mutations**

Given the considerable S-cone function detectable until later disease stages, the cellular target of therapy would be the S-cone. Even though both S-cone and L/M-cone measures of sensitivity changed rather slowly over time in our sample of longitudinal data, there was a difference in rate: S-cone function was lost faster than L/M-cone function. Monitoring S-cone function should be feasible using automated perimeters that have algorithms designed for “blue-on-yellow” perimeter, mainly used for glaucoma testing.43 There would be no need, as in other IRDs, to dark-adapt the patients and measure rod function,44–46 thereby making S-cone perimetry far more convenient and time-saving. Further studies would need to be performed in anticipation of a trial, depending on the intended delivery of treatment, whether focal (e.g., subretinal injection), intravitreal, or by some other method. A subretinal injection could be delivered in the pericentral retina; for example, when there is normal but not yet abnormal S-cone function. A patient having patterns 1 and 2 may be a candidate for this approach. A transition region between abnormal and normal S-cone function could be another potential retinal location. A pattern 2 or pattern 3 phenotype would have a transition zone that would become more clearly delineated. Considering the cystoid changes and other forms of maculopathy in NR2E3, the very central retina would be ill-advised as a target location. The predicted effect of the treatment on cone types also should be considered. Patients at advanced stages of degeneration, such as in some of the patients depicted in Figure 1 with central islands only, tend to have only L/M-cones in these remnant fields. If there was evidence that the therapy would affect all cones, rather than S-cones only, then these patients’ candidacy could be considered. An obstacle for a therapeutic trial to slow degeneration in patients with NR2E3 mutations would be the time to determine efficacy, assuming the rates we measured in the present work represent what will be determined in future natural history studies. Determining the rates of change in a vulnerable region, such as the pericentral area with dysfunction, may be faster than other retinal regions. A protocol with higher sampling rate in the pericentral retina in patients known to already have measurable S-cone dysfunction in that region may expedite a natural history and reduce the time to determine efficacy of therapy.

**How Does the Human Disease Due to NR2E3 Mutations Compare With the Murine Model Used for Therapeutic Strategies?**

The rd7 mouse carries a homozygous deletion mutation in the mouse ortholog of NR2E3 gene.47,48 and has been considered to share features with NR2E3 patients. Of relevance, these mice have been used in recent attempts at therapeutic interventions.22 Before the present work, the diagnoses of patients, such as ESCS, Goldmann-Favre syndrome, clumped retinalinopathy, and retinitis pigmentosa, have usually been listed as the human disease expressions comparable to the mouse model.16,17,49 Based on the results of this study, more specific comparisons about disease progression can now be made between rd7 mice and patients with NR2E3 mutations. Already noted as a difference between rd7 and human patients is that young rd7 mice have rod ERG function and normal (not hypernormal) S-cone ERGs, although how long these signals persist is debated.47,50 There is a 2- to 3-fold increase in the number of S-opsin-expressing photoreceptors in rd7 mice at 1 month of age.51 There are photoreceptors with normal rhodopsin expression, but dysfunctional, probably because they have some cone-like features.48,52 Another feature of young rd7 mice (among other mouse models53) is the readily visible dysplasia across the entire retina; the white spots represent whorls and rosettes and are well-explored.47,51,54 Although noninvasive imaging and postmortem histopathology have noted such changes in human NR2E3 disease,15,55 it is less prominent a feature than in the mice. Retinal dysplasia in the mice is followed by retinal degeneration.47,56 The progressive retinal degeneration in rd7 mice is associated with reduction in rod ERG b-wave over 1 to 3 months of age and cone ERG reduction from 3 to 12 months of age.52 This time-course of progressive dysfunction could be related to several decades of human life by allometric scaling57; the patterns of progressive S-cone dysfunction in the current study would be generally comparable. As has been found in other animal models used at early ages for proof-of-concept therapeutic studies,58 it would seem judicious to use rd7 mice for such experiments at later ages when there is definite progressive reduction of function and structure and not only dysplasia.

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**References**


