CERKL Mutations Cause an Autosomal Recessive Cone-Rod Dystrophy with Inner Retinopathy

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PURPOSE. To define the phenotype of the retinal degeneration associated with mutations in the CERKL gene.

METHODS. Six patients (ages, 26–54 years) from three unrelated families with CERKL mutations were studied clinically and by electroretinography, kinetic, and chromatic static perimetry, autofluorescence (AF) imaging, and optical coherence tomography (OCT).

RESULTS. Three siblings were homozygotes for p.R257X mutation; two siblings were compound heterozygotes for p.R257X and a novel p.C632X mutation; and one patient had only p.R257X mutation identified to date. There was a spectrum of severity: from mild visual acuity loss to light perception; from full kinetic fields with relative central scotomas to remnants of peripheral islands; from reduced ERGs (some with negative waveforms) to nondetectable signals. Maculopathy showed residual foveal islands or extensive central rod and cone scotomas. With AF imaging, there was evidence of hyperautofluorescence at earlier and hypofluorescence at later disease stages. Peripheral function was generally less affected than central function. With OCT there were small foveal islands of photoreceptors at earlier and hypoautofluorescence at later disease stages. Peripheral function was generally less affected than central function. With OCT there were small foveal islands of outer nuclear layer (ONL) in those with preserved acuity. Eccentric to an annular region with no discernible ONL, there could be ONL in the midperiphery. At early disease stages, ganglion cell layer thickness was less affected than ONL. Later disease stages were accompanied by inner nuclear layer and nerve fiber layer abnormalities.

CONCLUSIONS. CERKL mutations are associated with widespread retinal degeneration with prominent maculopathy. The clinical presentation is that of an autosomal recessive cone–rod dystrophy. Photoreceptor loss appears at all stages of disease and inner laminopathy complicates the phenotype at later stages.

Retinitis pigmentosa-26 (RP26), a previously uncharacterized locus on chromosome 2, was originally associated with nonsyndromic autosomal recessive retinitis pigmentosa (arRP) in families from Spain.1,2 A mutation in exon 5 of the gene encoding a ceramide kinase-like protein, CERKL, was more recently found to cosegregate with disease in these families.3–4 Additional mutations have been subsequently identified in retinal degeneration populations with Yemenite Jewish or Pakistani ancestry.5–6 CERKL encodes a novel ceramide kinase-like protein of unknown function.3 Ceramide is the metabolic product of sphingophospholipids, which are important constituents of the cell membrane, particularly of neurons, and have been associated with intracellular signaling, cell maintenance, and apoptotic mechanisms.7,8 Ceramide undergoes phosphorylation to ceramide-1-phosphate by ceramide kinase (CERK), a homologue of CERKL.9 Although the protein contains kinase domains, the ceramide kinase activity of CERKL remains to be demonstrated.10 In mouse retina, CERKL is predominantly expressed in the ganglion cell layer and to a lesser extent in the inner and outer nuclear cell layers.5 Recent research suggests that CERKL may act as an antiapoptosis agent within the retina,11 an attractive mechanistic possibility because apoptosis is the common pathway to photoreceptor death in retinal degenerations. The exact role of CERKL, however, remains uncertain and Cerk−/− mice have no retinal degeneration phenotype.12 To increase understanding of the retinal and visual dysfunction and determine the retinal anatomic abnormalities resulting from human CERKL mutations we studied a group of molecularly clarified patients with CERKL disease, by using psychophysics, electrophysiology, and retinal imaging, and compared our results with those previously published.2,4–6

MATERIALS AND METHODS

Subjects

There were six patients (ages, 26–54 years) with CERKL mutations, representing three families (Table 1). Subjects with normal vision (n = 28; ages 5–58 years) were also included. Informed consent was obtained from all subjects; procedures adhered to the Declaration of Helsinki, and the study was approved by the institutional review board.

Psychophysics and Electroretinography

Patients underwent a complete eye examination, electroretinography (ERG), Goldmann kinetic visual field testing, and light-adapted (600-nm
Clinical and Molecular Characteristics of the Study Patients with CERKL Disease

<table>
<thead>
<tr>
<th>Family (Mutation), Patient</th>
<th>Age(y)/Sex</th>
<th>Eye</th>
<th>Visual Acuity*</th>
<th>Refraction</th>
<th>Kinetic Visual Field Extent (V-4e/I-4e)†</th>
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ND, not detectable; NP, not performed; LP, light perception.
* Best corrected visual acuity.
† Expressed as a percentage of the normal mean response to the V-4e or I-4e target; 2SD below normal equals 90%.
‡ Codon numbering based on Ensembl transcript ID ENST00000410087 (ensembl http://www.ensembl.org developed by a consortium.
§ Only one allele identified. The patient shares the functional and structural phenotype with the other patients, but it remains possible that her true disease-causing mutation lies in some other gene and that she may be a carrier of the p.R257X change.

Autofluorescence Imaging

Reduced-illumiance autofluorescence imaging (RAFI) was performed to estimate the health of the retinal pigment epithelium (RPE). This method is intended to minimize absorption of imaging light by rod and cone opsins and lipofuscin, and thus reduces the likelihood of accelerating the natural history of the disease.17–21 A confocal scanning laser ophthalmoscope was used for imaging (HRA2; Heidelberg Engineering GmbH, Heidelberg, Germany). One of the RAFI methods used 790-nm near-infrared (NIR) excitation light and a band-pass blocking filter for >810 nm. This NIR-RAFI signal is believed to be dominated by the melanolipofuscin in RPE and melanin in the RPE and choroid.19,20–23 The other RAFI method used 488-nm short-wavelength (SW) excitation light and a band-pass blocking filter for >500 nm. This SW-RAFI signal is believed to be dominated by lipofuscin and melanolipofuscin in the RPE.17–19,21–23 All RAFI images were obtained with a sensitivity setting of 95% as 25-frame stacks at 4.7 frames/s for 30° × 30° (10 × 10 mm²) regions sampled at 1536 × 1536 pixels. The normalization and averaging features of the HRA software were turned off.17–21,23 SW-RAFI was obtained only for the central retina, whereas NIR-RAFI was used for overlapping regions extending to the midperipheral retina. Images were exported from the manufacturer’s software and analyzed as described.17–21 Images without visible distortion were selected in each stack, spatially registered, and averaged. Averaged images of the neighboring regions were digitally mosaiced by manually specifying retinal landmark pairs. Black levels were estimated from the minimum signal intensity at the optic nerve head region and subtracted out to define a relative AF signal intensity. Normal subjects (n = 18, ages 20–49 years) were analyzed with the same method. The relative AF signal was measured along a 30-pixel-wide (176 μm) horizontal profile passing through the foveola and compared quantitatively to normal limits (mean ± 2 SD).

Optical Coherence Tomography

Retinal cross-sections were obtained with OCT. Most data collection (4 patients; 10 normal subjects) used a Fourier-domain (FD) OCT system (RTVue-100; Optovue Inc., Fremont, CA). In two patients, data were acquired with a different OCT system (OCT1; Carl Zeiss Meditec Inc., Dublin, CA). Our recording and analysis techniques for both systems have been published.19,30–33 For this work the high-definition line protocol (HD line) of the FD-OCT system was used to obtain 4.5-mm-long scans composed of 4,091 longitudinal reflectivity profiles (LRPs) acquired at 26,000 LRPs per second. In all subjects, overlapping OCT scans of 4.5-mm length were used to cover the horizontal and vertical meridian up to 9 mm eccentricity from the fovea. A circular scan of 3.4-mm diameter centered on the optic nerve was also acquired.31–34 Postacquisition processing of OCT data was performed with custom programs (MatLab 6.5; MathWorks, Natick, MA). LRPs making up the OCT scans were aligned by straightening the major RPE reflection.32 In abnormal retinas, the presumed RPE peak was sometimes the only signal peak deep in the retina; in other cases, other major peaks were in apposition. In the latter case, the RPE peak was specified manually by considering the properties of the backscattering signal originating from layers vitread and scleral to it.30 In normal subjects, the signal corresponding to the RPE was assumed to be the most scleral peak within the multipeaked scattering signal complex deep in the retina.30 Lateral sampling density of the FD-OCT scans was reduced by averaging eight neighbors to increase the signal-to-noise ratio. Quantitative measurements of retinal laminae were performed after further reduction of lateral sampling density (sampling bins were 0.15 mm for OCT1, 0.07 mm for FD-OCT). Three nuclear layers, the outer photoreceptor laminae (ONL), the inner nuclear lamina (INL), and the ganglion cell layer (GCL), were defined in regions of scans showing three parallel stereotypical hyporeflective layers between the RPE and vitreoretinal interface.19,30–32 The boundaries of these hyporeflective layers were determined by the minima and maxima of the signal slopes and taking into account neighboring layers of higher reflectivity on LRPs: ONL; between outer limiting membrane (OLM) or RPE and the scleral boundary of the outer plexiform layer (OPL); INL; between the vitreal boundary of the OPL and the scleral boundary of the inner...
plexiform layer (IPL); GCL, between the vitreal boundary of the IPL and the scleral boundary of the retinal nerve fiber layer (NFL), the highly reflective layer at the vitreoretinal interface. Transition regions where there was change from the two major hyporeflective layers (ONL and INL) to one layer were present in patient scans. This single hyporeflective layer appeared to be laterally continuous with the INL. NFL thickness for circular scans around the optic nerve was calculated with the commercial OCT software, which uses the depth of the high-reflectivity signal near the vitreoretinal interface as a measure of the thickness of the NFL around the optic nerve.

RESULTS

Affected members of three unrelated families were identified with mutations in CERKL (Table 1). Family 1 was of German and English/Irish ancestry, and the affected members were homozygotes for the p.R257X mutation. This mutation has been previously reported in seven unrelated Spanish families with arRP.4–10 Family 2 also had German and English/Irish ancestry; affected members were compound heterozygous for the p.R257X mutation and a 2-bp deletion in exon 9 (c.60_61delGT; p.C362X) of CERKL. The novel nonsense CERKL mutation segregated with disease in the family. F3,P1 was of English/Irish/Welsh ancestry, and the p.R257X mutation was identified in one allele; no other CERKL variant has been detected to date (Table 1). Her unaffected sister did not share the same two parental alleles of CERKL, which is compatible but not definitive evidence in support of a causative role.

Additional screening for common molecular causes of autosomal recessive cone rod dystrophy (arCRD; ABCA4) and arRP (USH2A) in all six patients did not reveal disease-causing mutations in these genes.

Presenting visual symptoms were mainly related to reading difficulties. There were also night vision disturbances. Visual acuity ranged from 20/25 to light perception (Table 1). Fundus examination in the mildest disease expression showed regions of macular RPE depigmentation or atrophy, and a diffuse granular appearance of the more peripheral retina. In the more severely affected patients, there was extensive central chorioretinal atrophy and peripheral pigmentary retinopathy. F1,P2 had many scolloped areas of chorioretinal atrophy in the mid-periphery.5

Retina-Wide Cone- and Rod-Mediated Dysfunction

Electroretinography (ERG) indicated retina-wide dysfunction (Fig. 1). ERGs were abnormally reduced in the five patients with detectable signals (Fig. 1A); F1,P2 had nondetectable ERGs (not shown). Patients with sizeable rod and cone ERGs (F1,P1 and F1,P3) showed similar loss of b-wave amplitude of rod (11.6–30.7%) and cone (21.4–54.5%) mediated responses (Figs. 1A, 1B). Three patients showed reduced b to a-wave ratios to a mixed cone and rod response (F2,P2 = 0.7; F2,P1 = 0.4; and F3,P1 = 0.5) compared with normal (normal mean ± 2 SD = 1.6 ± 0.4; Fig. 1A). To study the pattern of retinal-wide rod versus cone dysfunction, we determined the relationship between rod and cone ERG amplitudes in the four patients with both signals measurable (F1,P1, F1,P3, F2,P2, and F2,P1). The data were analyzed and plotted according to our published methodology that defines different patterns of dysfunction in ungenotyped patients with CRD.16 Normalized amplitudes (expressed in log units) for rod and cone ERGs fall near the line that predicts equal reduction of rod and cone amplitudes in two patients (F1,P1 and F1,P3), and below that line in two others (F2,P2 and F2,P1), indicating greater cone than rod dysfunction. These ERG results in CERKL are comparable to those in CRD.16,15

The regional variation in retinal disease underlying the abnormal ERGs was examined by kinetic and static perimetry. Kinetic field abnormalities varied in severity, but central visual field defects were a shared feature. Fixation, as documented by OCT, was foveal in all patients except those with the most severe central retinal disease (F1,P2 and F3,P1; Table 1). Mild disease expression was exemplified by F2,P2, who had a small incomplete annular relative scotoma surrounding fixation and a normal peripheral kinetic field extent in response to a large bright target (V-4e), but reduced extent in response to the smaller (I-4e) target (Fig. 2A). F1,P1 and F1,P3 had a small central island of function surrounded by an absolute scotoma in response to both target sizes extending peripherally to ~20° of eccentricity. Peripheral field extent with V-4e was normal or near normal and there was reduced extent with I-4e. F2,P1 had more advanced disease and showed an altitudinal and pericentral scotoma; residual islands (detected only with the V-4e target) were present at fixation and inferiorly (Fig. 2A).

The more severely affected patients (F3,P1 and F1,P2) showed only remnants of vision in the mid or far periphery or no perception of the targets (Table 1).

Maps of sensitivity loss from light-adapted static perimetry complemented the kinetic perimetry results (Fig. 2B). F2,P2 and F1,P1 had relatively mild and diffuse retina-wide loss of sensitivity (Fig. 2B). F1,P3 showed greater sensitivity loss around a retained small island of central function and peripheral sensitivity losses were increased compared with those in F2,P2 and F1,P1. F2,P1 had even more prominent sensitivity losses surrounding fixation and extending into the superior periphery, whereas the inferior peripheral field was relatively spared, albeit abnormal in sensitivity. F1,P2, the most severely affected patient, could not perceive these chromatic stimuli. Dark-adapted perimetry with an achromatic (white) target demonstrated a relatively large inferotemporal peripheral island with at least 5 log units of sensitivity loss (data not shown).15–36

Sensitivity profiles along the horizontal meridian in select patients were used to define rod- and cone-mediated function within the macula. Both photoreceptor systems were affected in the macula by the disease. Small central islands that account for better visual acuity in some of the patients (e.g., F1,P1 and F1,P3), became undetectable in other patients at later disease stages (e.g., F3,P1). Rod- and cone-mediated function was detected eccentric to the trough of visual loss surrounding any residual central islands (Fig. 2C).

RPE Defects and Retinal Laminopathy

Spatial topography of RPE health in CERKL disease was defined with NIR-RAFI and SW-RAFI dominated by the natural RPE fluorophores melanin and lipofuscin, respectively (Fig. 3). Normal NIR-RAFI showed a circular region (~2 mm diameter) of higher brightness centered at the fovea surrounded by a lower brightness signal across most of the retina. Retinal blood vessels and the optic nerve head (ONH) appeared darker (Fig. 3A, top). Normal SW-RAFI showed (Fig. 3A, bottom) a small (~0.6 mm diameter), dark region at the fovea dominated by absorption of the SW excitation light by macular pigments.17,37 An annular region (extending to ~2 mm diameter) of relatively low AF intensity surrounded the dark center. Highest AF intensities corresponded to retinal regions 3 to 4 mm eccentric from the fovea.17,38 Darker blood vessels were contrasted against a brighter background and there was a dark ONH.

Patient F2,P2 showed a central elliptical region of relatively low signal with both NIR-RAFI and SW-RAFI (Fig. 3B). Lowest values within this region were still higher than the ONH signal and corresponded to the lower and higher limits of normal for NIR-RAFI and SW-RAFI, respectively (Fig. 3B, overlays). The
highest signal levels were on a perifoveal ring of 2 to 3 mm eccentricity. NIR-RAFI signals showed a minor elevation above the higher limit of normal, whereas the SW-RAFI signals demonstrated significant hyperautofluorescence, most likely corresponding to hyperaccumulation of RPE lipofuscin. Spatial homogeneity of the NIR-RAFI extending to the midperipheral retina (Fig. 3B, inset) supported a relatively healthy-appearing RPE outside the central region. F2,P1 had a larger central area of signal loss on NIR-RAFI and a signal with choroidal appearance that was probably unmasked due to loss of melanin in the RPE. SW-RAFI showed a parafoveal ring of RPE atrophy surrounded by a perifoveal ring with definite hyperautofluorescence corresponding to a better preserved RPE (Fig. 3C).

Cross-sectional OCT images along the vertical meridian in patients with CERKL mutations were compared with a normal scan (Fig. 4A). The normal retina showed a foveal depression that was surrounded by thicker retina and decreasing thickness with distance from the center. Normal retinal organization has hypo- and hyperreflective laminae that correspond to the nuclear (INL, ONL, and GCL) and synaptic (IPL and OPL) layers. A band of high reflectivity near the retinal surface corresponds to the NFL. Highly reflective signals deep in the retina correspond to the OLM, the photoreceptor inner seg-

**Figure 1.** ERGs in patients with CERKL disease. (A) The five of six patients with detectable ERGs are illustrated. All patients showed both rod and cone ERG abnormalities; three (F2,P2; F2,P1; and F3,P1) had negative waveforms (greater b- than a-wave amplitude reduction) to the mixed cone–rod response. Vertical gray bars on the traces mark stimulus onset; calibration bars are at right and below the waveforms. (B) Summary parameters for ERGs in the patients compared with normal limits (rectangle: ±2 SD from the mean). (C) Relationship of rod b-wave amplitude to cone flicker amplitude in the 4 patients with detectable cone and rod responses, compared to 11 normal subjects (small unfilled symbols; normal data are from Ref. 16). Data are analyzed and plotted as described in that study. Amplitudes are normalized to mean normal values and expressed in log units. Dashed line: equal reduction of rod and cone amplitudes; C<R, greater cone than rod amplitude reduction (gray region).
ment (IS), cilial and outer segment (OS) complex, RPE, and anterior choroid.30,39

Patient F2,P2 (age 28) had a thinned fovea (130 μm) (normal mean, 207 μm; SD, 16 μm; n = 26, ages 5–58). There was discernible but reduced foveal ONL. Deep to the ONL was hyperreflectivity probably representing IS, cilium, and OS signals that extended only a short distance (~0.5 mm) from the foveal center; associated with these structures was reduced cone function. From 0.5 mm to ~2 mm eccentricity into the superior retina there was loss of the IS-cilial-OS signals and little or no measurable vision. More normally laminated outer retina was present at approximately 2 mm, and there was measurable cone- and rod-mediated sensitivity. ONL thickness increased with eccentricity, reaching approximately 34% of normal thickness at ~6 mm superior (18 μm; normal mean, 53 μm; SD, 8 μm). There was identifiable GCL and NFL across this region. The NFL appeared abnormally thickened at ~3–4 mm superior but with increasing eccentricity there was less abnormality of the inner retina. F2,P1 (age 26) also showed a small central island of retained but abnormal retinal structure (foveal ONL thickness, 13 μm) with reduced cone sensitivity. Remnants of ONL and deep patches of hyperreflectivity (presumed abnormal IS-cilial-OS) were detectable within 2 mm of the fovea. From 2 to 5 mm eccentric to the fovea, only a single thick hyporeflective layer was definable. Patchy deep hyperreflectivity was also evident in this region. Two of the patients (F2,P1

**FIGURE 2.** Kinetic and static threshold perimetry in patients with CERKL disease. (A) Kinetic perimetry from the right eye of representative patients using two targets (V-4e and I-4e). Isopters for each target are shown; shaded regions within isopters represent relative (gray) and absolute (black) scotomas. (B) Maps of sensitivity loss in the same four patients measured by static perimetry (600 nm, light-adapted). The grayscale has 16 levels, representing 0- to 30-dB losses. Black square: physiologic blind spot represented at 12° in the temporal field. N, nasal; T, temporal; I, inferior; S, superior visual field. (C) Dark-adapted (500 nm, top) and light-adapted (600 nm, bottom) horizontal sensitivity profiles in three patients (symbols connected by lines) compared with normal data (dashed lines, shaded area, mean ± 2 SD). For dark-adapted perimetry, mediation based on two-color (500 nm, 650 nm) testing, is shown above the results: R, rod-mediated; M, mixed rod- and cone-mediated; C, cone-mediated. Hatched bar: physiologic blind spot.
and F3,P1) had correlative data available, and these OCT hyperreflectivities corresponded to regions of AF signal heterogeneity. A GCL was visible and there was a thick vitreal hyperreflective layer, normally corresponding to the NFL, which thinned at approximately 5 mm eccentric. Vision became measurable at ~4 to 5 mm superior, where there was limited rod sensitivity and the ONL was discernible at approximately 5-mm eccentricity, with return of a laminar pattern. A later disease stage is represented by F3,P1 (age 40). ONL was visible in small patches, the GCL was detectable, and the hyperreflective band normally corresponding to the NFL was extremely thick. Limited rod-mediated vision was present in the superior retina where there was coincident retinal thinning, a reappearance of ONL, but persistent inner laminopathy. End-stage disease in F1,P2 (age 45) showed a thinner and less organized retina with epiretinal membrane formation, intraretinal hyperreflectivities, and no detectable vision.

Extrafoveal outer retinal abnormalities in CERKL disease are illustrated in magnified views (Fig. 4B). Deep to normal ONL are multiple laminae representing the IS/OS, RPE, and anterior choroid. Retinal vessels can block reflections from deeper retinal layers, and this was evident in the more superiorly located normal section. F2,P2 shows a thinned ONL and also has discernible but thinned deeper laminae that may represent reduced IS/OS, a well-described feature in the histopathology of human retinal degenerations.40 F2,P1 showed no discernible ONL and a thickened INL, or possibly a composite (INL and ONL) hyperreflective layer continuous with the INL.18,19 Patchy deep hyperreflectivities extended into the hyporeflective layer (highlighted with yellow in magnified views, Fig. 4B); blocked deep reflections were noted in the more superiorly located section and may have been due to a retinal vessel, and/or possibly RPE cells that had detached from Bruch’s membrane and were migrating to inner retinal perivascular sites.40,41 The RPE layer appeared thin, consistent with the concept of RPE cell detachment and intraretinal migration; Bruch’s membrane may be the site of the residual hyperreflectivity.42 F3,P1 had a comparable constellation of outer retinal findings. F1,P2 showed more extreme retinal disorganization with deep hyperreflectivities, thinned RPE, and coarse intraretinal hyperreflectivities with blocked deeper reflections.

Quantitation of laminae from the OCT cross sections along the horizontal and vertical meridians revealed major thinning to nonmeasurable ONL in all patients in a region around the fovea extending from 1.5 mm to 5 to 6 mm of eccentricity (Fig. 5A). At the foveal center, all patients except F1,P2 had a detectable but reduced ONL (42%–24% of normal mean). At eccentricity greater than ~6 mm, ONL thickness was detectable and could approach the lower limits of normal in those patients with milder disease. The GCL (measured only in the patients studied with FD-OCT: F2,P1; F2,P2; F3,P1, and F1,P2) was detectable in most of the patients, and thickness was within normal limits in the extrafoveal retina in some of them (Fig. 5B). Other patients had GCL thickness losses at ~1.5 mm eccentricity, a region where ganglion cells receive input from foveal cones.43,44 F1,P2 had abnormally reduced GCL thickness in the central 4 mm, and no GCL was detectable at greater eccentricities.

The relationship between photoreceptor (ONL) loss and GCL integrity in patients with CERKL disease examined with FD-OCT was explored by plotting change from normal for cone and rod locations (Fig. 5C). Specifically, average foveal cone ONL thickness (at eccentricities <0.5 mm45) plotted against average GCL at corresponding loci (displaced by 0.6 mm45) showed that the more prominent abnormality in these patients was cone photoreceptor layer loss. GCL loss was observed in locations with the most advanced disease. ONL and GCL measurements at the rod hotspot or peak rod density (4–6 mm eccentricity46) showed a similar relationship as that found for cones.

Notable thickening of the hyperreflective layer closest to the vitreoretinal surface in the superior retina of the patients in
cross-sectional imaging (Fig. 4) prompted quantitation of this presumed NFL reflectivity (Fig. 6). Along the horizontal meridian, there was no evident thickening. None was expected in the temporal retina along the horizontal raphe, but there was also no thickening nasally. The reflectivity was abnormally thickened along the vertical meridian between 2- and 5-mm eccentricity in four patients (F2,P2; F2,P1; F3,P1; and F1,P2).

The extent and degree of thickening appeared to relate to stage of disease. F2,P2 with the mildest disease expression showed minor thickening and over a limited extent; F2,P1 had greater thickening; and F3,P1 showed dramatic thickening over most of the extent of the cross section. F1,P2 with end-stage disease had minimal extent of thickening and a relatively thinned retina. We next asked if the presumed NFL thickening observed along the vertical meridian was localized to that region or if it was also detectable at the ONH, where the NFL is conventionally measured.57 Polar plots of NFL thickness were derived from circular OCT cross-sections performed at a 1.7 mm radius around the ONH (Fig. 6C). NFL thickness was within normal limits at nearly all loci in all patients (Fig. 6C, gray region). F3,P1 showed borderline thickening at loci within the superior, inferior, and nasal sectors. F1,P2 with end-stage disease was at the lower limit of normal. Of note, NFL thickness in the temporal sector of the ONH, where axons from the fovea converge,48 tended toward the lower limit of normal in some patients, a finding that may be related to the loss of foveal GCL after central cone loss (Figs. 5A, 5B).

**DISCUSSION**

**CERKL Clinical Disease Category: Cone–Rod Dystrophy**

There are now five studies of the retinal disease associated with CERKL mutations. Two studies of families of Spanish origin have shown all affected individuals to be homozygous for the
p.R257X mutation. Of the 14 Spanish patients (ages 24 – 55 years) with clinical details tabulated, two patients in the third decade of life (ages 24 and 28 years) had visual acuity better than 20/200; the remainder, including a 23-year-old, had 20/200 or worse. Maculopathy and retina-wide pigmentary changes were noted on funduscopic examination in most patients. All but two patients had nondetectable ERGs: the 28-year-old patient had reduced cone and rod ERGs and the 24-year-old patient with near normal acuity also had near normal ERG b-waves. The original RP26 designation from linkage studies was used in this description of patients with CERKL mutations. CERKL remains listed among the causes of arRP in compilations of genes and retinal diseases (RetNet; http://www.sph.uth.tmc.edu/retnet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX).

Yemenite Jewish patients with a recessive retinal degeneration harbored a homozygous splice site (c.238+1G>A) CERKL mutation. The disease was defined in a relatively younger age group (n = 12; ages 14 – 42 years). Three of the 12 patients had visual acuity better than 20/200; the remainder, including a 23-year-old, had 20/200 or worse. Maculopathy and retina-wide pigmentary changes were noted on funduscopic examination in most patients. All but two patients had nondetectable ERGs: the 28-year-old patient had reduced cone and rod ERGs and the 24-year-old patient with near normal acuity also had near normal ERG b-waves. The original RP26 designation from linkage studies was used in this description of patients with CERKL mutations. CERKL remains listed among the causes of arRP in compilations of genes and retinal diseases (RetNet; http://www.sph.uth.tmc.edu/retnet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX).

A consensus about clinical diagnosis could expedite the identification of further patients with CERKL mutations. The current results taken together with those of previous reports strongly suggest that autosomal recessive cone rod dystrophy (arCRD), rather than arRP, is the clinical disease category associated with CERKL mutations. ERG data from patients with CERKL disease fell among data from patients with CRD previously reported. Unlike catalogues of arRP genes, there are currently relatively few identified genes known to cause arCRD (RetNet; http://www.sph.uth.tmc.edu/retnet/). CERKL and the common ABCA4 retinopathy would be worth distinguishing, considering progress in understanding ABCA4-associated disease and current thoughts about either treatment or avoidance of accelerating risk factors.

Features of the Maculopathy in CERKL

The maculopathy of CERKL is likely to occur early in the time course of disease, antedating detectable change in visual acuity. The best acuities recorded in our patients (e.g., F2,P2, 20/25; F2,P1, 20/40; F1,P3, 20/50) represented only small central islands of ONL and function surrounded by large regions of rod- and cone-mediated macular dysfunction and abnormal structure. The extensive maculopathy with islands of residual central function surrounded by abnormality and then better function and structure at greater eccentricities resem-
bles stages of age-related macular degeneration, Stargardt disease with foveal sparing, and a subgroup of patients with ungenotyped cone–rod dystrophies. Early CERKL disease stages with subtle maculopathy in which the clinical picture may be dominated by pericentral scotomas may resemble stages of pericentral retinal degenerations. Screening for CERKL mutations in pericentral retinopathies seems warranted.

The longevity of the small islands of preserved function and structure is uncertain at the current stage of understanding of CERKL retinopathy. It is important to determine whether any therapeutic interventions, such as those suggested to prevent foveal photoreceptor loss in other maculopathies, are applicable to this disease. Of interest, oxidative stress has recently been suggested as a pathologic mechanism contributing to photoreceptor apoptosis in CERKL retinopathy, and precursors of RPE lipofuscin may be generated in photoreceptors as a natural detoxification process. Consistent with such a speculation was hyperautofluorescence found at earlier stages of disease, implying overaccumulation of RPE lipofuscin. Alternative or additional causes of increased lipofuscin accumulation may include modification of the visual cycle or lysosomal function of the RPE.

Loss of melanin in the RPE by AF-imaging and thinning of the OCT layer usually ascribed to the NFL in superior and inferior retinal scans. There was marked photoreceptor disease in patients with CERKL disease by OCT imaging of the ONL and by ERG a-wave amplitudes, but there were also signs of postreceptoral dysfunction. Some full-field ERGs showed greater b- than a-wave loss of signal and this occurred mainly in patients with more severe disease. A negative waveform response to the maximum white stimulus was not present in two of the more mildly affected patients in this study (F1,P1 and F1,P3). The likelihood is that the negative ERG signal is a stage of CERKL disease, as has been postulated to explain a similar observation in the Bardet-Biedl syndrome. The Yemenite Jewish patients with CERKL disease, most in the second and third decades of life, did not have negative waveforms. Of interest, negative ERGs have been observed, not only in RP, but also in forms of CRD.

Adult murine retinas express Cerkl mainly in the GCL. There was marked photoreceptor disease in patients with CERKL disease by OCT imaging of the ONL and by ERG a-wave amplitudes, but there were also signs of postreceptoral dysfunction. Some full-field ERGs showed greater b- than a-wave loss of signal and this occurred mainly in patients with more severe disease. A negative waveform response to the maximum white stimulus was not present in two of the more mildly affected patients in this study (F1,P1 and F1,P3). The likelihood is that the negative ERG signal is a stage of CERKL disease, as has been postulated to explain a similar observation in the Bardet-Biedl syndrome. The Yemenite Jewish patients with CERKL disease, most in the second and third decades of life, did not have negative waveforms. Of interest, negative ERGs have been observed, not only in RP, but also in forms of CRD.

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**Inner Retinal Dysfunction and Laminopathy**

There was marked photoreceptor disease in patients with CERKL disease by OCT imaging of the ONL and by ERG a-wave amplitudes, but there were also signs of postreceptoral dysfunction. Some full-field ERGs showed greater b- than a-wave loss of signal and this occurred mainly in patients with more severe disease. A negative waveform response to the maximum white stimulus was not present in two of the more mildly affected patients in this study (F1,P1 and F1,P3). The likelihood is that the negative ERG signal is a stage of CERKL disease, as has been postulated to explain a similar observation in the Bardet-Biedl syndrome. The Yemenite Jewish patients with CERKL disease, most in the second and third decades of life, did not have negative waveforms. Of interest, negative ERGs have been observed, not only in RP, but also in forms of CRD.

Adult murine retinas express Cerkl mainly in the GCL. This observation and the proposed role of Cerkl in protection against apoptosis led to our analysis of whether the GCL was abnormally thinned in CERKL patients. Measurements of GCL thickness by OCT in a wide expanse of central retina did not reveal evidence of early stage thinning. A comparison of ONL and GCL thickness for cone- and rod-dominated retinal loci indicated greater photoreceptor than ganglion cell disease at both loci. The GCL in CERKL declined in thickness in association with more severe photoreceptor degeneration.

What were the results of NFL thickness measurements, the standard surrogate for GCL integrity by OCT? NFL thickness has been evaluated recently in cohorts of patients with retinitis pigmentosa and cone–rod dystrophy. The clinical interest in such measurement is to develop sound inclusion and exclusion criteria in future or ongoing clinical trials, such as with electronic retinal prostheses that would depend on intact GCL and NFL for efficacy. In patients with CERKL disease, we noted thickening of the superficial hyperreflective layer normally ascribed to the NFL in superior and inferior retinal scans. At this location, the NFL would involve nerve fibers from the
temporal retina that arch around the central retina en route to the superior and inferior sectors of the ONH. The coarse thickening of this presumed NFL in superior and inferior retina was not evident in similar measurements nasal to the fovea and in corresponding sectors in the peripapillary region. The normal or thinned peripapillary NFL measurements in CERKL are consistent with recently published data in arCRD.

The most parsimonious interpretation of these results is that the thick hyperreflectivity in superior and inferior locations between 2 to 6 mm from the fovea is a composite layer with nerve fibers as well as thickened glial processes, aberrant neurites, reactive hyperplasia of astrocytes, possible vascular elements and other inner retinal constituents. The thickening would then be considered a regional retinal phenomenon, given the lack of thickening at the ONH and the evidence of GC losses. It is also possible that this thick structure is complicating measurements of the GCL in this region. In general, this becomes another feature of remodeling of the human retina in response to photoreceptor degeneration, as detected by OCT imaging in many retinal degenerative diseases. Such observations began with choroideremia11 and have proceeded more recently to human genetic retinopathies that have animal models permitting histopathologic comparisons and confirmation of the details of the laminar disorganization. More subtle and early features of laminopathy have become possible with higher resolution optical scanning methods.

In summary, CERKL mutations are associated with widespread retinal degeneration that resembles a form of cone–rod dystrophy rather than RP, as originally designated. Photoreceptor loss appears at all stages of disease and inner laminopathy complicates the phenotype at later stages. Although adult murine retinas express Cerk1 mainly in the GCL, our analysis of the human disease did not reveal evidence of a predilection for early-stage GCL thinning.

References


