**RDH12 Mutations Cause a Severe Retinal Degeneration With Relatively Spared Rod Function**

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**PURPOSE.** To describe the retinal phenotype of pediatric patients with mutations in the retinol dehydrogenase 12 (RDH12) gene.

**METHODS.** Twenty-one patients from 14 families (ages 2–17 years) with RDH12-associated inherited retinal degeneration (RDH12-IRD) underwent a complete ophthalmic exam and imaging with spectral domain optical coherence tomography (SD-OCT) and near infrared and short-wavelength fundus autofluorescence. Visual field extent was measured with Goldmann kinetic perimetry, visual thresholds with dark-adapted static perimetry or with dark-adapted chromatic full-field stimulus testing (FST) and transient pupillometry.

**RESULTS.** Visual acuity ranged from 20/40 to light perception. There was parafoveal depigmentation or atrophic maculopathies accompanied by midperipheral intraretinal pigment migration. SD-OCT revealed foveal thinning in all patients and detectable but thinned outer nuclear layer (ONL) at greater eccentricities from the fovea. Photoreceptor outer segment (POS) signals were only detectable in small pockets within the central retina. Measurable kinetic visual fields were limited to small (<5–10°) central islands of vision. Electroretinograms were reported as undetectable or severely reduced in amplitude. FST sensitivities to a 467 nm stimulus were rod-mediated and reduced on average by ~2.5 log units. A thinned central ONL colocalized with severely reduced to nondetectable cone-mediated sensitivities. Pupillometry confirmed the psychophysically measured abnormalities.

**CONCLUSIONS.** RDH12-IRD causes an early-onset, retina-wide disease with particularly severe central retinal abnormalities associated with relatively less severe rod photoreceptor dysfunction, a pattern consistent with an early-onset cone-rod dystrophy. Severely abnormal POS but detectable ONL in the pericentral and peripapillary retina suggest these regions may become targets for gene therapy.

Keywords: Leber congenital amaurosis, RDH12, optical coherence tomography, rods, cones, cone-rod dystrophy, photoreceptors

**Leber congenital amaurosis (LCA) and early onset retinal degenerations (EORD) describe a molecularly and clinically heterogeneous group of inherited retinal degenerations (IRDs) characterized by severe vision loss recognized during the first year of life or early in infancy.**1–3 The molecular cause of LCA/EORD has been elucidated in a large proportion of patients and there are 25 causative genes identified to date (https://sph.uth.edu/retnet/disease.htm, available in the public domain).4–7 Mutations in the retinol dehydrogenase 12 gene (RDH12), which maps to a locus on chromosome 14 (14q23.3-24.1) known as LCA13, accounts for about 4% of all autosomal recessive LCA, a disease that affects about 20% of all children that attend schools for the blind.1,4,8,9 The gene encodes a protein member of the family of retinol dehydrogenases that localizes to the photoreceptor inner segment of rods and cones. RDH12 and RDH8 provide most of the reductase activity in the inner and outer segment and reduce all-trans-retinal released after photoactivation, a step needed for photopigment regeneration.8,9 More importantly, there is evidence that by reducing free all-trans-retinal, RDH12 protects the inner segment against retinaldehyde-induced cytotoxicity.8–12

The most frequent retinal phenotype in patients with RDH12 mutations is an autosomal recessive inherited retinal degeneration (IRD) within the spectrum of severity of LCA, although relatively milder phenotypes have been categorized as forms of EORD or juvenile-onset retinitis pigmentosa (RP). Rarely, RDH12 can segregate with a milder phenotype in an autosomal dominant fashion.6 Independent of the age at presentation of the recessive disease, the phenotypic expression includes a progressive, retina-wide pigmentary retinopathy with peripapillary sparing and prominent central changes that result in early visual acuity (VA) loss.13

RDH12-associated EORDs (RDH12-EORD) or LCA (RDH12-LCA) do not appear to show the structural functional dissociation observed in other forms of LCA, particularly LCA associated with mutations in RPE65 (RPE65-LCA), a disease caused by loss-of-function of another visual cycle protein (RPE65) that has successfully been treated with gene therapy.14

Despite potential differences in disease mechanisms and
expression, there is reasonable hope that other genetic forms of IRD within the spectrum of EORD may be similarly treatable with gene augmentation. To increase our understanding of the retinal structural changes and associated visual dysfunction resulting from RDH12 mutations, we studied a relatively large group of pediatric patients with RDH12-LCA/EORD using psychophysics, measures of the transient pupillary light reflex (TPLR), and multimodal retinal imaging. The ultimate goal was to determine the treatment potential and targets as well as eligibility of patients for future gene augmentation clinical trials for this condition.

Methods

Patients

Twenty-one patients, ages 2–17 years, representing 14 families diagnosed with RDH12-LCA and RDH12-EORD were included in this prospective, cross-sectional study. Genotyping was undertaken using saliva samples when not already determined. Informed consent and assent were obtained after explanation of the nature of the study; procedures complied with the Declaration of Helsinki and were approved by the institutional review board (IRB #808828, 815348). All patients underwent a comprehensive eye examination. Fixation pattern and tracking was documented in the youngest patient (Patient 1, P1); the rest of the patients had VAs measured with Snellen visual acuity charts. Testing was done for each eye independently; for clarity, imaging results illustrated in figures correspond to the eye with better acuity or favored by the patient.

Retinal Imaging

Imaging was performed with spectral domain optical coherence tomography (SD-OCT; Spectralis; Heidelberg Engineering, Carlsbad, CA, USA) with 9 mm-long horizontal sections crossing the anatomical fovea, extending when possible into the midperipheral retina (n = 14 patients). Our SD-OCT scanning protocol in young patients, analyses, and normative data have been published.15–17 In brief, segmentation of SD-OCT images was performed with the built-in segmentation software of the Spectralis OCT system. A separate imaging analysis software (http://image.nih.gov/ij/links.html, available in the public domain) was used to generate longitudinal reflectivity profiles from exported images and help supervise and correct automatic segmentation errors.18–20 Retinal thickness was defined as the distance between the signal peak at the vitreoretinal interface (the internal limiting membrane [ILM]) and the posterior boundary of the major signal peak that corresponds to the basal retinal pigment epithelium/Bruch’s membrane complex (RPE/BrM). In normal subjects, the RPE/BrM is the last reflectivity within the 4–6 signals that are identifiable in the outer retina.20 In patients, the presumed RPE signal was sometimes the only signal in the outer retina and often merged with signals from the anterior choroid. The RPE/BrM peak intensity was then specified manually by considering the properties of the backscattering signal originating from layers vitread and sclerad to it.19 The outer nuclear layer (ONL) thickness was defined as the major intraretinal hyporeflective signal bracketed between the outer plexiform layer (OPL) and the external limiting membrane (ELM) or the BrM/RPE signal when the ELM was not detectable. To avoid ambiguities in the determination of the ELM in severely degenerated regions, the term “outer retinal thickness” was adopted to define the thickness between the OPL and RPE/BrM. The “inner retinal thickness” was defined as the distance between the ILM and the OPL.15,17,19

near infrared reflectance (NIR-REF) and autofluorescence (NIR-FAF) images were obtained during the acquisition of the OCTs with overlapping 30’ and/or 55’ fields. In a subset of patients (n = 5) short-wavelength autofluorescence (SW-FAF) imaging was performed in a similar manner.

Pupillometry

The direct transient pupillary light reflex (TPLR) was elicited by full-field, short duration (100 msec), chromatic stimuli, delivered using the stimulator of the system used for full field stimulus testing (FST; Espion 3; Diagnosys, Lowell, MA, USA).21 The pupil was illuminated and imaged with a head-mounted near infrared LED and infrared-sensitive video camera, respectively, and the images of the pupil were recorded and processed by an eye-track (EyeFrame; Arrington Research, Scottsdale, AZ, USA). A separate program (VoltagePoint; Arrington Research) synchronized both instruments through a signal input delivered by the software of the stimulator system.22 TPLR luminance response functions were recorded with increasing intensities of scotopically matched blue (peak 467 nm; from –4.25 to +4.25 log scot-cd.s.m−2) and red (peak 637 nm; –4.25 to +3.25 log scot-cd.s.m−2) lights presented at 0.5 log unit increments in the dark-adapted (>45 minutes) state.22 The recording window was 4 seconds long. Interstimulus intervals were long enough to allow the pupil to return to its baseline diameter and varied from ~15 seconds at lower intensities to ~30 seconds at the higher end. In patients, the starting intensity was arbitrarily selected at ~2 log units above normal dark-adapted TPLR thresholds.22 TPLR amplitudes were measured as the difference in horizontal pupil diameter between baseline and a fixed 0.6-second time after stimulus presentation; TPLR thresholds were defined as pupil contraction amplitudes reaching a 0.3 mm criterion response as defined previously.22 A video clip was recorded by the pupillometry, which was used to perform manual measures in patients with wandering eye movements or large-amplitude nystagmus in which the automated software was unable to calculate pupil diameter.

Visual Psychophysics

Visual field extent was determined with Goldmann kinetic perimetry. Visual sensitivity was measured in dark-adapted (>45 minutes) patients using FST.21,23,24 Mydriatics were delivered after pupillometry and additional dark-adaptation (15–20 minutes) occurred while pupils dilated. Dark-adapted psychophysic thresholds were determined with chromatic (blue, peak 467 nm and red, peak 637 nm) stimuli using a thresholding algorithm built-in into a computer-driven electroretinography (ERG) system.22 Two to three separate determinations of the sensitivity were performed for each stimulus type. Sensitivity differences between each of the determinations or runs (run1 minus run2, run1-run3) were used to estimate the variability of the measurements. Estimates in patients (~1.9 ± 3.8 dB) were similar to values determined in young normal subjects (n = –1.6 ± 4.1 dB, P = 0.89) from this study, and were comparable to previous reports.21,22 One patient was too variable (differences >30 dB), so her values were not included in the analyses. Spectral sensitivity differences were used to determine photoreceptor mediation of each stimulus condition.21 All patients, except Patient 1 (P1) and P2 were able to complete the FST test in each eye; results in P5 were too variable, were considered not reliable, and were excluded from analyses. Goldmann kinetic perimetry was performed in all but the youngest patient (Table). Five patients (P12-P14, P17, P20) were able to complete light-adapted achromatic and dark-adapted chromatic (500 nm and 650 nm)
### Table. Clinical and Molecular Characteristic of the Patients

<table>
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<tr>
<th>Pt ID</th>
<th>Age*/Sex</th>
<th>Age at Diagnosis</th>
<th>Ancestry</th>
<th>RDH12 Variants Allele1/Allele2†</th>
<th>Mutation Type</th>
<th>Visual Acuity‡</th>
<th>Refraction§</th>
<th>Kinetic Perimetry#</th>
<th>Foveal Thickness (µm)</th>
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<td>Ala269del/Val233Leu Null/MS</td>
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<td>10+T</td>
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<td>German, Irish, English, Dutch</td>
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<td>Arg295Stop/Asp101Gly Null/Null</td>
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<td>Ala269del/Ala126Glu Null/MS</td>
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<td>Ala269del/Val233Leu Null/MS</td>
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<td>-5.12</td>
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np, not performed; na, not available; nl, normal; abn., severely reduced ERG amplitudes; nd, nondetectable; a, well-delimited chorioretinal atrophy; m, maculopathy; p-coloboma, bilateral pseudo-coloboma.

* Age in years.
† Predicted amino acid change; MS, missense mutation.
‡ FF, fixates and follows; LP, light perception.
§ Spherical equivalent.
|| Patients are siblings: P1, P6, P17, & P20; P2 & P3; P4 & P15; P8 & P14; P16 & P19. All families originate from the United States; closest ancestral origin tabulated.
# Extent in eccentricity to the largest extent of the central isopter (Goldmann V-4e target); +T denotes small temporal island of vision separated from the central residual island.
automated static perimetry using 200 ms duration, 1.7° diameter stimuli, in a modified Humphrey Field Analyzer (HFA II-i; Carl Zeiss Meditec, Dublin, CA, USA), following published methodology.25–27 Thresholds were measured along the horizontal meridian at 2° intervals, extending to 30° of eccentricity, corresponding to the retinal region scanned with SD-OCT.27 Photoreceptor mediation was assessed at each locus using the sensitivity difference between the two colors.25

**RESULTS**

Twenty-one pediatric patients from 14 families carrying homozygous or compound heterozygous mutations in *RDH12* were included in this study (Table). These included frameshift mutations leading to premature stop codons, null mutations, and missense mutations. All patients presented within the first 2 years of life with poor vision, nystagmus, or for screening due to history of an IRD in one or more siblings (Table). Visual acuity ranged from 20/40 to light perception (LP) with several examples of marked interocular asymmetry (Table). Refractive errors were <6D spherical, <-2D astigmatic as measured with retinoscopy in the youngest patients (P1 and P2) and with manifest refraction in the rest. There were no cataracts. Kinetic perimetry with a large (Goldmann size V-4e) target performed in 19 patients demonstrated oval-shaped or circular fields that extended to 5–10° of eccentricity along the meridian with the largest extent; a smaller target (I-4e) was not detectable (Table). Full-field ERGs when recorded at the initial evaluation were reported as undetectable or severely reduced in amplitude in both eyes (Table).

Fundus findings confirmed previous reports. There were midperipheral pigmentary changes in all patients ranging from localized, often paravascular, depigmentation or sparing of the RPE with minimal intraretinal pigment migration (Fig. 1, P9; Supplementary Fig. S1), to an overt pigmentary retinopathy with bone spicule pigmentation within the macula (Fig. 1, P17). Waxy optic nerve pallor and various degrees of vascular attenuation were seen in all patients. There were macular changes in all patients. In milder disease, there was obvious central depigmentation that contrasted with a better appearing RPE in midperipheral retina (Fig. 1, P9, arrows). Peripheral RPE changes or obvious intraretinal pigment migration are not confluent but appear to be surrounded by better appearing retina with brownish RPE (Fig. 1, P17; Supplementary Fig. S1, P8). In some patients without macular atrophy the foveal center appeared more yellowish than the coloration expected for the macular pigment, and there was RPE mottling (Fig. 1, P9; Supplementary Fig. S1). Patients with more advanced macular disease showed parafoveal areas of depigmentation in a bullseye configuration or overt atrophy and pigmentation (Fig. 1A, P17). In some, there was posterior displacement of the atrophic macula in a pseudo-collodamaticus configuration (Table).

NIR-FAF imaging proved to be a comfortable way of examining the topography of the central retinal abnormalities in this young group of patients. A NIR-FAF image in a normal subject shows the expected gradient of normal autofluorescence with greatest signal intensity near the foveal center due to the greater melanin density and taller RPE cells at the fovea, and a decline in intensity to a homogenous grayish background with increasing eccentricity (Fig. 1, NIR-FAF, inset). NIR-FAF in the patients showed generalized central hypofluorescence, surrounded by numerous hyperautofluorescent lesions that colocalized with bone spicules and with hyperautofluorescent lesions within that corresponded with parafoveal areas of hyperpigmentation (Fig. 1, P17, arrowheads). In most patients (except P1, P2, P3, P15), an area of relative preservation of the NIR-FAF signal was observed in the peripapillary region confirming previous reports (Fig. 1; Table).8,13,28,29 There was no obvious association between the degree of the abnormalities on ophthalmoscopy and the patients’ VA, with the exception of patients with obvious foveal chorioretinal atrophy with associated poor VA (Table).

**Severe Central Retinal Abnormalities**

The central retinal structure was always abnormal as exemplified by representative patients in Figure 2. The youngest subject in this cohort showed severe central retinal abnormalities with marked ONL thinning (Fig. 2, P1). The ONL is nearly undetectable within a thinned foveal center but becomes visible in the parafovea as a dark band riddled with punctate hyperreflectivities having a more normal appearance in peripapillary retina. The ellipsoid zone (EZ) and the interdigitation (IZ) bands are not visible for most of the scan with the exception of faint discontinuous signals viritread to the RPE (Fig. 2, arrows) that may represent remnants of the EZ and/or IZ in nasal pericentral and peripapillary retina. The external limiting membrane (ELM) is not clearly visible. Similar patterns are seen in other patients without an obvious relationship to age. RPE depigmentation was associated with well-defined, round...
Peripapillary retina with preservation of the NIR-REF signal showed similarly shaped areas of NIR- and SW-FAF as exemplified in P14 (Fig. 3A). There is an abrupt transition zone (TZ) where the FAF for both excitation lights vanishes corresponding to the location where the IZ signal on SD-OCT becomes undetectable (Fig. 3A, yellow arrow). A small island of foveal autofluorescence, most noticeable on NIR-FAF, colocalizes with remnants of the EZ/IZ (Fig. 3A, white arrow) and supports 20/40 acuity, despite the severe structural disorganization of the fovea. Foveal thickness was not related to the level of visual acuity ($r = 0.05$). Quantitation of retinal thickness parameters from horizontal cross-sections from all patients confirmed overall retinal thinning, which was most pronounced within the central 2 mm of eccentricity and less severe at greater eccentricities from the foveal center, especially in peripapillary retina (Fig. 3B, left panel). The inner retina was remarkably thick, likely a consequence of inner retinal remodeling documented in other IRD (Fig. 3B, middle panel). There was detectable but severely thinned outer retina in all patients (Fig. 3B, right panel). Outer retinal thinning was most severe at the most central locations, as illustrated by a greater separation of the patients’ average outer retinal thickness (Fig. 3B, right panel, dark thick trace) from the normal range within 2 mm of eccentricity than at more peripheral locations. There was no obvious relationship between the patients’ age and the extent and severity of the abnormalities (Fig. 3B). Central disease severity as judged by their VA and foveal thickness also did not relate well with the specific genotype. Patients with missense mutations ($P7, P9, P10$) did not differ from slightly older patients ($P11, P12, P16, P19$) with bi-allelic null mutations (Fig. 3; Table). Longitudinal reflectivity profiles (LRPs) generated from the transitional zone of structural change in peripapillary retina of patients with less severe disease ($P14$ and $P9$) served to identify the sequence of abnormalities in the outer retina. All outer retinal signal peaks are present in the locations near the optic nerve, most obvious in $P14$ (Fig. 3C, LRP ‘a’). A short distance from this location (closer to the fovea), the IZ becomes undetectable and there is only a faint EZ in both patients (Fig. 3C, LRPs ‘b’ and ‘c’). Of note, there is minor thickening of the INL as the ONL thins with increased distance from the nerve toward the fovea and no obvious change in thickness of the retinal nerve fiber layer (RNFL) in this small region. LRPs from all patients were examined to determine the location and extent of any detectable segment of the EZ band. All but the oldest patient in this series (P21, age 17) showed signals in the outer retina that were superficial to the RPE/BrM band but deeper than the ONL consistent with EZ and/or IZ, suggesting the possible existence of abnormal but detectable photoreceptor inner and possibly outer segments (POS) within the central retina of these patients (Fig. 3D).

**Severe Central Retinal Dysfunction but Relatively Spared Extramacular Rod Function**

Fixation stability was adequate in at least one eye of five patients ($P12–P14, P17, P20$) to perform automated static perimetry. A horizontal light-adapted sensitivity profile in P20 exemplifies the results (Fig. 4A). There was detectable ONL on SD-OCT throughout the central cross-section although the laminination of the outer retina was very abnormal. As in the majority of the patients, faint, interrupted linear signals above the RPE suggested the possible presence of inner and outer segments, although a clear outer laminar with intact ELM, EZ, or IZ was not observed. Locations near the foveal center and peripapillary retina had better organization (Fig. 4A, green arrows). Light-adapted sensitivity to an achromatic, large (Goldmann, size V) stimulus near the foveal center was reduced by 0.7 log units compared to the normal mean (mean $= 2.38$ ± 4 dB) and becomes nondetectable within 2 mm of the foveal center; sensitivity was again measurable near the optic nerve in nasal retina coinciding with a return to a better laminated retina (Fig. 4A, green arrow). The remaining patients ($P12–P14, P17$) had measurable sensitivities only near the foveal center with maximal sensitivities ranging from 18 to 31 dB (mean = 24 dB). Photoreceptor mediation was assessed with dark-adapted, two-color, automated perimetry, which
confirmed cone mediation and no detectable rod function within the central retina to this stimulus (>6 log units of rod sensitivity loss). In contrast, dark-adapted FST sensitivity estimates to two color stimuli in 18/21 patients who were old enough to perform the test (i.e., except P1 and P2), or reliably complete the test (P5 was unreliable; interest variability >30 dB), showed rod-mediated sensitivities that were reduced on average by ~2 log units (mean ± SD = 6.2 ± 3.3 log scot-cd.s.m²) compared to young normal subjects (n = 8, mean age = 15 years; range, 2-24 years). Only one eye for each patient is shown for clarity. Thick black line is the average thickness for the parameter from all patients. Gray bands: normal limits (mean ± 2 SD) obtained from a group of young normal subjects (n = 32; mean age = 15 years; range, 2-24 years). (C) Magnified 1 mm SD-OCT horizontal cross-section that straddles a TZ in peripapillary retina of two patients; the direction of the scans is from nasal (N) (close to the nerve) to temporal (T) retina (closer to the fovea). Overlaid traces are three LRPs positioned every ~300 μm and located on the nasal side of the TZ (‘a’), at the TZ where outer photoreceptor laminae change (‘b’), and temporal to this region (‘c’) where there is thinning and loss of outer retinal laminae. Colored segments denote the extent of the signal trough that corresponds to the ONL (blue) and the distance from the EZ or ELM signal peak to the apical RPE/BrM. (D) Lateral extent (horizontal green bars) and location of sections of the central retina with detectable albeit interrupted EZ band for each patient (vertical axis). Patients’ ID are sorted by age.

The TPLR was used to objectively explore this finding. In a normal subject, the TPLR can be detected at very low light levels (mean ± 2 SD = -3.35 ± 0.18 log scot-cd.s.m²). Increasing stimulus intensity leads to an acceleration of the constriction phase of the response, an increase in peak response amplitude, and a retardation of the dilation phase (Fig. 4D). At low intensities, the pupil rapidly redilates, whereas at the highest intensities there is a “tonic” contraction that lasts for a few seconds. TPLR responses elicited by a pair of dim, scotopically matched blue and red stimuli elicit TPLR responses that are matched in waveform (Fig. 4D, red and blue traces). In a representative patient, TPLR threshold responses were first detected with a brighter stimulus near ~0.5 scot-cd.s.m⁻². As in the normal subject, increasing intensity of lights leads to an increase in the amplitude of the responses and a similar change in morphology (Fig. 4D). TPLR responses to
FIGURE 4. Structural–functional relationships in RDH12-associated retinal degeneration. (A) 9 mm-long, non-straightened, SD-OCT cross-sections along the horizontal meridian through the fovea in a patient. Bar above the scan show psychophysically determined cone (light-adapted, white stimulus) sensitivities. Dotted line above bar defines lower limit (mean –2 SD) of sensitivity for normal subjects. A sensitivity scale is at the top left of the panel. Cone photoreceptor mediation of the stimulus was confirmed with two color dark-adapted perimetry.25 T, temporal retina; N, nasal retina. Calibration bar to the bottom left. Horizontal green bars denote sections of the scan where a line or band can be seen above the apical RPE signal which may correspond to remnants of outer segments. Arrows point to locations where outer photoreceptor laminae (ELM, EZ, IZ) are less disorganized and are detectable as pockets that bear a resemblance to the normal lamination pattern. Asterisks denote intraretinal hyperreflectivities with posterior blocking of the SD-OCT signal consistent with central intraretinal pigment migration. (B) FST sensitivity estimates to dark-adapted spectral stimuli (blue, 467 nm; red, 637 nm) in 18/21 patients that were able to perform the test. Dashed line is the lower limit (mean –2 SD) of the sensitivity to the short wavelength 467 nm stimulus in normal subjects, dotted line is the lower limit for the 637 nm stimulus. Patients' ID are sorted by age. Only right eye shown for clarity. (C) Interocular difference of the dark-adapted FST estimates for each stimulus condition (blue, 467 nm; red, 637 nm). Diagonal line is the equality line; dashed lines represent the limits (± 2 SD) of the interocular differences. (D) Families of dark-adapted TPLR waveforms elicited with brief (100 ms), blue (467 nm) stimuli delivered over the intensity range of −3.75 to +4.25 log scot-cd.s.m⁻² (0.5 log unit steps) on the same stimulator unit used for FST measurements. Traces plot the horizontal pupil diameter as a function of time in a normal subject compared to a representative patient. Overlapping thick red traces near TPLR threshold are responses elicited by red (637 nm) stimuli scotopically matched to the blue stimuli (blue traces). Stimulus monitor shown as a vertical bar at bottom left. (E) FST sensitivity loss plotted against TPLR sensitivity loss for each eye of patient who were able to complete both tests (n = 18). Symbols with black outline = right eye; gray = left eye. Diagonal line is the equality line.
Retinal Structure and Function in RDH12-LCA

scotopically matched blue and red stimuli were grossly matched in waveform, suggesting rod mediation of the pupillary light reflex as in the normal subjects (Fig. 4D). The waveform shape at threshold is similar to that of the normal subject at threshold, suggesting an insensitive, rod-driven TPLR with normal kinetics.22 TPLR thresholds in the patients were elevated by an average 2 log units (~1.1 ± 0.5 log scot-cd.s.m⁻²) compared to normal subjects (~3.4 ± 0.2 log scot-cd.s.m⁻²). The difference in TPLR thresholds between these two spectral stimuli in the patients (~0.2 ± 0.7 log scot-cd.s.m⁻²) fell well within the range expected for rod photoreceptor mediated responses in normal subjects (~0.5 ± 0.4 log scot-cd.s.m⁻²). Sensitivity losses estimated with TPLR related well with losses determined with FST (r = 0.83; Fig. 4E).

DISCUSSION

Homozygous mutations in RDH12 have been associated with LCA, EORD, or autosomal recessive retinitis pigmentosa, and rarely with cone rod dystrophy (CRD).3,5,8,15,30,32,36–44 There are examples of segregation of RDH12 mutations in an autosomal dominant fashion and several heterozygous mutations have been reported in patients with RP.6,8,32,36,37,39,42 Instead of the phenotypic diversity implied by the different diagnoses used to describe the autosomal recessive disease, review of the literature reveals a consistent phenotype characterized by a midperipherally pigmented retinopathy of early onset.3,5,8,15,28,30,33,36–41 That includes macular pseudo-colobomas.15,16,30–33 Whereas there is a wealth of information on the retinal structure in the retinal degeneration associated with RDH12 mutations, details of the associated retinal dysfunction are comparatively scarce.15,14,50,51,36,44 Previous studies that have used electroretinography have reported severely abnormal or undetectable responses and functional data other than visual acuity is limited.14,30–32 In this study, we used multimodal imaging, visual psychophysics, and dark-adapted chromatic pupillometry to describe the retinal structure and function of pediatric patients with homozygous or compound heterozygous mutations in RDH12. We found severe and early macular disease in all our patients, confirming previous observations.5,8,32,36–40,42,43 The changes were obvious by SD-OCT in our youngest patient (P1, age 2) who had an otherwise benign fundus appearance and was asymptomatic.

We confirmed severe central cone and rod dysfunction accompanying the severe central structural abnormalities in association with comparatively milder, presumably extramacular rod dysfunction as assessed with FST and two-color static perimetry, and confirmed by dark-adapted chromatic TPLR.22 The TPLR responses to relatively dim and brief (100 ms) stimuli near threshold in the patients are inconsistent with nonclassic photoreceptor mediation of the pupillary light reflex, known to co-exist or replace classical photoreception in cases of EORD.40 We could not confirm, however, if the level of peripheral function was proportionally reduced for cone and rod function as the FST sensitivities for both short- and long-wavelength stimuli were mediated by rods in all patients.21 The use of alternative methods of cone isolation will help answer this important question.47–49 The reliability of the sensitivity estimates in this group of young patients was also considered. Normal age-related changes in rod sensitivity may not be expected to occur in children within the age range represented in this study.50–52 FST and TPLR thresholds related well, but the relationship was not perfect with examples of TPLR showing less sensitivity losses compared with FST measures. FST thresholds were considered reproducible. Within-visit variability estimates in the patients were similar to reports in older subjects and not significantly different to estimates from a small group of normal children from this study.21,53 It remains possible that young subjects may respond conservatively, albeit reproducibly, to the FST task and overestimate the sensitivity loss. A topic that deserves further study. The TPLR as measured in this study was initially described in cohorts of patients and normal subjects that included children,22 but there is still a need to further define the reliability of the methodology in pediatric populations, a factor that may help explain the minor discrepancies in the sensitivity estimates between the two methodologies.

FST does not provide information about the topography of the visual field, which we could document only in a small group of patients with static perimetry. There were sectors in the periphery of some of our patients that showed a better appearance on en-face imaging. It remains to be determined where in the extramacular retina the residual rod function originates and whether these better-appearing regions mediate the residual rod function as has been done in other EORD.53 There is also the possibility of rod-mediation within the central retina in regions not explored by the horizontal sensitivity profile used in this work. While the overall topography of the retinal dysfunction awaits confirmation in larger groups of patients with RDH12-IRDs, the pattern observed in this work, taken together with those of previous reports, strongly suggests that autosomal recessive mutations in RDH12 cause a predominantly severe central retinal degeneration that is consistent with an early CRD phenotype.32,45,54–57 Reports of CRD phenotypes in milder forms of RDH12-IRD supports a common disease expression with a wide spectrum of severity in RDH12-IRD.52,54,55

The disease mechanisms in RDH12-IRD are not fully understood, but the severity and early presentation of the phenotype that results suggest a critical role in human retinal physiology. RDH12 localizes to the rod and cone photoreceptor inner segment and is able to reduce all-trans- and all-cis retinoids.11,56,59 Murine Rdb12 knockouts, however, do not show an overt retinal degeneration or major slowing of the visual cycle.58,60,61 Redundancy of retinal dehydrogenases, specifically Rdh8, has been offered as an explanation for this discrepancy or a different role of RDH12 in humans compared to the murine model.60 An alternative role in clearing all-trans-retinal from rod and cone inner segments, in the movement of retinoids, or in the detoxification of lipid peroxidation products, has been suggested. Removal of all-trans retinal would prevent this photosensitizer from damaging photoreceptors.62 The pattern of disease expression documented in this work suggests vulnerability of both rods and cones within the central retina and relative peripheral rod preservation.28 Similar patterns have been reported in at least two other forms of LCA/EORD, AIPL1-LCA, and CRB1-LCA.53,63–65 Coincidentally, in these three molecular subtypes, a role of the encoded proteins in photoreceptor maintenance has been suggested with light exposure being a possible common pathogenic factor.53,63,64 It has been suggested that the preservation of peripheral rod function in LCA-CRB1, for example, may be related to the survival of peripheral, rod-dominated retina, in the shadow of the iris and ciliary body in this and other light-sensitive retinopathies.55

The peculiar preservation of the peripapillary retina in RDH12-LCA, PRPH2-IRD, and ABCA4-IRD has been suggested to result from light protection conferred by the “shadow” of an overlying thick retina.5,57–60 However, in this study, we did not find a relationship between the thickness of the overlying RNFL or inner retina and the preservation of the outer retina in peripapillary retina, arguing against this mechanism. The area of peripapillary preservation tended to extend just outside of
the vascular arcades and did not match exactly the expected direction of the RNFL bundles, which correspond to the thickest inner retina, also standing against this hypothesis.34,76

Mapping the topography of each of the individual retinal layers should help answer this question, a task that may be complicated by unstable fixation in severe RDH12-IRD. In retinal differences in expression of protective factors, photoreceptor densities, photoreceptor/RPE ratios, or in the movements of retinoids between glia and/or the vascular compartment and the retina may also be at play.71–73 RPE cells deliver retinosomes, retinyl esters-containing lipid droplets thought to be involved in the storage and/or trafficking of retinoids between the RPE and photoreceptors. These organelles may ensure a steady supply of 11-cis retinal to photoreceptors despite fluctuations in dietary vitamin A.72 In vitro site-directed mutagenesis that abolishes the oxidoreductase activity of ENV9, an ortholog of human RDH12, suggests a role for the enzyme in the formation of lipid droplets.3 It is possible that the central retina may be particularly susceptible to degeneration due to higher demands for retinoids, particularly by cone photoreceptors.16,76

Most variants reported in the current work are predicted to cause lack-of-function proteins. The most common mutation was Ala269del (＞60%; 13/21 of our patients), which is a frameshift mutation leading to a premature stop codon in exon 6 and an expected truncation of TRD12 protein.13,14,37,77 The mutation likely leads to nonsense-mediated RNA decay and either a nonfunctional or absent protein. The third most common variant was Val233Leu (24%; 5/21 patients), a potentially milder missense mutation—may cause sufficient dysfunction to be expressed clinically in a uniformly severe phenotype, as characterized in this work.

Limited longitudinal studies and multiple cross-sectional observations in patients with RDH12-associated IRD indicate visual acuity may be relatively preserved early in the course of the disease, while a somewhat steep decline occurs in the second decade of life, reaching count finger or LP level of vision by age 20,5,8,14,24–26,37,52–56,41,78 Relatively preserved visual acuity and even color vision, may be documented in RDH12-IRD despite severe central retinal disease and is still in keeping with a diagnosis of CRD.52,37 Such pattern of early relative preservation of visual acuity and then rapid loss is reminiscent of numerous maculopathies that show preservation of visual acuity despite severe central retinal disease.79–84 Thus, visual acuity in this condition, as in most IRDs, is not a reliable indicator of the severity of the central retinal phenotype, although it is critical to the patients' quality of life.27,78 Once the central island is compromised a rapid decline of the pericentral retina’s VA may be expected.35 In RDH12-IRD the surrounding pericentral retina, however, may not be able to sustain usable or rehabilitable central vision as is the case in other macular diseases, such as Stargardt's disease. The hope is that residual peripheral rod vision at that stage may be enough for ambulation, a pattern observed in patients with comparatively less severe CRDs and consistent with residual peripheral visual fields for island of vision by age 20.13,28,36–38,52–54,56–59 This concern is that severe peripheral rod dysfunction at that stage in RDH12-IRD may not serve patients well due to the depth of the peripheral dysfunction and that total blindness will result.

EORDs remain excellent platforms where the risk/benefit ratio for vision change after gene therapy may be stacked in favor of visual gain. Today, targets of treatments in the EORD group are somewhat biased toward genetic subtypes that show structural-functional dissociations (for example RPE65, CEP290, GUCY2D).85–86 These conditions may be simpler to treat as the expectation is that treatments of relatively preserved retina with a disproportional dysfunction should theoretically lead to measurable gains in vision in the short-term supporting efficacy, as it did in RPE65-LCA trials. However, such structural-functional dissociation phenotype constitutes a small fraction if the entire field of inherited retinal degenerations is viewed as a whole. There is a need for a different approach to the alternative scenario where the retinal degeneration does not follow clear functional–structural dissociations, where the depth of the disease is more severe as it occurs in RDH12-IRD. Treatments of forms of LCA/EORD that are caused by defects in photoreceptor proteins such as RDH12-LCA also promise to move the target of interventions from the retinal pigment epithelium (RPE)—the primary site of diseases such as RPE65-LCA—into the photoreceptor but, more importantly, from simpler mechanisms of disease to hopefully offer therapeutic solutions of complex causes of vision loss where epigenetic modifiers of disease expression, such as light exposure, may play a role.10,91–94 In this work, we present evidence of retained photoreceptors within the central retina in all patients examined, which raises hope that correction of the genetic defect may lead to arrest of the degeneration and hopefully improve the visual acuity. The absence of clear SD-OCT signals that correspond to preserved POS within the central retina of even our youngest patient is concerning, and gene augmentation may lead to restoration of the structure of the POS and vision as is known to occur.

Studies in larger groups of patients representing a broader age range are required to better determine the relationships between the phenotype and genotype and between disease severity and age. Alternatively, RDH12 mutations—potentially milder missense mutations—may cause sufficient
following correction of various insults to this important structure.35–39

Although there may be examples of milder phenotypes, the majority of patients with RDH12-DRD in this and previous reports share a severe, early disease, which suggests we may have no choice but to move the window for potential treatments to younger ages when there is still treatable retina, raising new risk/benefit concerns. Early clinical trials that focus on safety are expected to include patients with the most severe abnormalities, and it can be anticipated that results in terms of efficacy may be modest. Careful consideration of the outcomes chosen for future clinical trials and careful interpretation of early results of those interventions will be needed to prevent that a treatment, such as gene augmentation, is prematurely deemed ineffective depriving less severely affected patients with better treatment potential the opportunity of a vision-saving treatment.

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