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Twelve-month Natural History Study of Centrosomal Protein 290 (CEP290)-associated Inherited Retinal Degeneration

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Purpose: To define the clinical characteristics of centrosomal protein 290 (*CEP290*)-associated inherited retinal degeneration (IRD) and determine which assessments may provide reliable endpoints in future interventional trials.

Design: Participants in this natural history study were enrolled into 2 best-corrected visual acuity (BCVA) cohorts: light perception to $>$ 1.0 logarithm of the minimum angle of resolution (logMAR) and 1.0 logMAR to 0.4 logMAR. Each comprised 4 age cohorts (3–5, 6–11, 12–17, and $\geq$ 18 years).

Participants: Patients with *CEP290*-associated IRD caused by the intron 26 c.2991+1655A>G mutation and BCVA ranging from light perception to 0.4 logMAR.

Methods: Best-corrected visual acuity, full-field stimulus threshold (FST) sensitivity, Ora–Visual Navigation Challenge (Ora–VNC) composite score, and OCT–outer nuclear layer (OCT–ONL) average thickness were assessed at screening, baseline, 3 months, 6 months, and 12 months.

Main Outcome Measures: Best-corrected visual acuity, FST sensitivity, Ora–VNC composite score, and OCT–ONL average thickness.

Results: Twenty-six participants were included in this analysis. Nineteen were female. All participants were White and 4 reported Hispanic ethnicity. At screening, 13 of 16 adult and 9 of 10 pediatric participants had BCVA $>$ 1.0 logMAR. Baseline BCVA was variable (median [range] = 2.0 [0.5, 3.9] logMAR) and was uncorrelated with age, as were VNC composite score, FST sensitivity, and OCT–ONL average thickness. Mean (95% confidence interval [CI]) test-retest variability was −0.04 (−0.09, 0.01) logMAR for BCVA (n = 25); 0.6 (−0.1, 1.3) for VNC composite score (n = 18); and 0.10 (−0.07, 0.27) log cd.s/m^2 for red FST (n = 14). A greater than expected test-retest variability (5 [0, 10] μm, n = 14) was observed for OCT–ONL average thickness as nystagmus impacted ability to repeat measures at the same retinal location. Functional assessments were stable over 12 months. Mean (95% CI) change from baseline was 0.06 (−0.17, 0.29) logMAR for BCVA (n = 23); −0.1 (−1.2, 1.0) for VNC composite score (n = 21); and −0.15 (−0.43, 0.14) log cd.s/m^2 for red FST (n = 16).

Conclusions: Vision was stable over 12 months. Best-corrected visual acuity, FST, and VNC composite score are potentially viable endpoints for future studies in CEP290-associated IRD. Repeatability of OCT measures poses challenges for quantifying anatomical changes in this population.

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Inherited retinal degenerations (IRDs) are a leading cause of visual impairment, affecting approximately 1 in 2000 people worldwide.[1] Centrosomal protein 290 (*CEP290*)-associated IRD is a severe, early-onset IRD caused by biallelic mutations in the gene encoding cCEP290. Centrosomal protein 290-associated IRD is characterized by early rod and cone photoreceptor degeneration and severe visual impairment within the first decade of life.[2–4] Among mutations known to cause *CEP290*-associated IRD, the single A>G nucleotide change within intron 26 (c.2991+1655A>G mutation) is the most common and can be found in up to 77% of cases in the United States.[5] This mutation leads to the introduction of a cryptic exon and premature stop codon (p.Cys998X) that disrupts expression of full-length CEP290.[6] As the CEP290 protein localizes to the transition zone between photoreceptor inner and outer segments,[7,8] insufficient levels result in disorganized outer segments and early photoreceptor death in the midperipheral retina.[9]

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There is no currently available treatment for *CEP290*-associated IRD.[10,11] The standard of care is supportive, and includes glasses, magnifiers, high-contrast reading materials, canes, home modifications, and braille.[12] However, research indicates that even patients with no functional vision can retain an island of cone photoreceptors in the central retina.[13,14] Furthermore, patients' optic nerves and occipital cortices appear to be intact despite reduced photoreceptor input.[15] These findings suggest that there may be an opportunity to deliver gene-based treatments to restore vision.[15,16] However, intervention necessitates a clear understanding of the clinical features of *CEP290*-associated IRD, and several questions regarding its clinical course have not been fully elucidated, including the extent to which both eyes follow the same disease trajectory.[14]

Among previous clinical studies in patients with *CEP290*-associated IRD, only 1 was composed exclusively of patients with the common intron 26 c.2991+1655A>G mutation.[14] In that retrospective observational case series, 35% of participants had no light perception at baseline. Moreover, variable lengths of follow-up and nonuniform testing equipment likely limit investigators' ability to fully define the clinical characteristics of *CEP290*-associated IRD. As such, this prospective, 12-month natural history study with systematic assessments and uniform follow-up was undertaken to define the clinical characteristics of *CEP290*-associated IRD and to determine which assessments may provide reliable endpoints in future interventional trials.

Methods

Study Design and Population

This natural history study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. The study was designed to enroll 40 participants evenly distributed across 8 cohorts spanning 4 age ranges (3−5, 6−11, 12−17, and ≥ 18 years) and 2 visual acuity ranges (light perception to > 1.0 logarithm of the minimum angle of resolution [logMAR] and 1.0 logMAR−0.4 logMAR). However, flexibility in the target number of participants was permitted in order to accommodate participant availability. Recruitment occurred from December 2017 to May 2022 at 7 sites across the United States (Bascom Palmer Eye Institute, Massachusetts Eye and Ear Infirmary, W.K. Kellogg Eye Center, and Casey Eye Institute), Germany (Justus Liebig University Giessen and University Hospital of Giessen), France (Pierre and Marie Curie University), and the Netherlands (Radboud University Medical Center). Institutional Review Board approval was obtained prior to initiation of the study at each site. Participants were enrolled after the study procedure was explained and a consent form was signed. In the case of pediatric participants (age < 18 years), assent was sought and a legal representative signed the consent form. Participants attended a screening visit followed by 2 baseline visits within 3 weeks of each other (test-retest) and 3 follow-up visits at months 3, 6, and 12 (Fig 1).

Eligible participants were required to be > 3 years of age at screening, provide informed consent (parental/legal guardian consent if < 18 years of age), have best-corrected visual acuity (BCVA) ranging from light perception to 0.4 logMAR in each eye, have clear ocular media and adequate pupil dilation in ≥ 1 eye, and have an IRD caused by a compound heterozygous or homozygous intron 26 c.2991+1655A>G mutation confirmed by DNA sequencing that excluded other genetic causes. Participants were excluded if they had current gene therapy or oligonucleotide therapeutics, history or current evidence of a medical condition that could preclude safe participation or confound study assessments (systemic disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding), a passing score on the Ora−Visual Navigation Challenge (Ora−VNC) at maximum level of difficulty with each eye independently or both eyes together, history or current evidence of ocular disease in either eye that could confound study assessments or preclude adequate visualization of the fundus, or were enrolled in an interventional drug or device study within 30 days of screening. Full eligibility criteria are presented in Table S1.

Study Assessments

Study procedures and assessments are summarized in Table S2.

Complete ophthalmic examinations with dilated and nondilated components were performed. Nondilated components included a standard examination of the external eye and the anterior segment of the eye evaluating the cornea, anterior chamber, iris, and pupil. Dilated components included indirect ophthalmoscopy and retinal imaging. Intraocular pressure measurements were taken prior to pupil dilation.

Functional Assessments

Best-corrected visual acuity was assessed using the ETDRS chart. Additional instruments included the Berkley Rudimentary Visual Test if BCVA was worse than 1.6 logMAR[17,18] and the LEA Symbols 15 Line Pediatric Eye Chart if age was < 5 years. Best-corrected visual acuity of white field projection, black-white discrimination, and light perception were reassigned as 3.2, 3.5, and 3.9 logMAR, respectively. Best-corrected visual acuity testing, including refraction, was performed in each eye independently and both eyes together in the order of right eye, then left eye, then both eyes. The test examiners were trained, and the examination lanes were certified for the performance of the BCVA assessments to ensure reliability of the testing results and consistency across sites.

Dark-adapted full-field stimulus threshold (FST) sensitivity to blue, white, and red light[19−21] was assessed using the Espion Ganzfeld Profile E3 ERG machine V6.0 (Diagnosys LLC). Participants were dilated and dark-adapted for 45 minutes prior to testing with blue (448 nm peak wavelength), red (627 nm peak wavelength), and white light (white 6500 K using red, blue, and green light emitting diodes). Full-field stimulus threshold assessments were repeated 3 times per stimulus and were considered reliable when ≥ 1 assessment had a quality metric > 1. The sensitivity output was the mean of all reliable assessments.

The novel Ora-VNC was used to assess visual function navigation.[16] This challenge involves navigation of 4 multiluminance mobility courses, with each progressive course having an increasing level of difficulty based on the number of turns, type, and number of obstacles, contrast between the course path and borders, and illumination levels. The first course is the Backlit Room Exit, which contains 2 illumination levels scored from 1 to 2, the second is the High Contrast Room Exit—a straight course with high contrast obstacles and 3 illumination levels scored from 3 to 5. The penultimate course is the High Contrast VNC, which comprises several turns, high contrast obstacles, and 8 illumination levels scored from 6 to 13. The final course is the Low Contrast VNC, which contains several turns, low contrast obstacles, and 8 illumination levels scored from 14 to 21. Higher Ora-VNC composite scores represent better visual function navigation. A composite score of 0 is assigned when a patient failed all

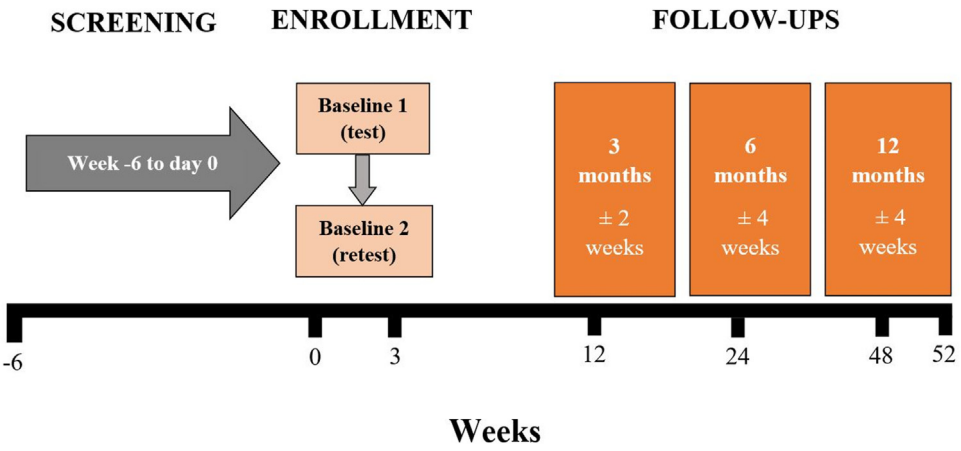

Figure 1. Natural history study design. Natural history study visits over 12 months. A 6-week screening period followed by 2 baseline visits (test-retest) and 3 follow-up visits at 3, 6, and 12 months.

mobility courses and lux levels. Participants were tested in each eye separately and in both eyes together. To reduce learning effects, the course pattern was changed for each assessment using manufacturer-defined patterns. Test scores were determined by participants' ability to remain on the course path, the number of obstacles hit, and the time to course completion. Night-mode video recordings were acquired and graded independently by 2 reviewers at a grading center.

Pupillary light reflex characteristics were quantified using the PLR-3000 pupillometer (NeurOptics). In short, pupillary responses were measured after 40 minutes of dark adaptation followed by a "swinging flash test" and sequential stimulation in each eye with white light. The light stimulus was presented alternatively to the right and left eye and each test was repeated twice, the first time starting with the stimulation of the right eye, and the second time starting with the stimulation of the left eye. Each sequence of light stimuli was interleaved with a pause of $\geq$ 3 seconds to recover baseline pupil diameter. Video recordings of the pupillary responses were quantified and analyzed to assess the percent change in pupillary diameter from baseline, constriction velocity, latency, and recovery rate using previously reported methods.[22]

In participants with BCVA $\leq$ 1.0 logMAR, color vision, visual field sensitivity, and contrast assessments were performed. Color vision assessment was performed using the Farnsworth D15 test. Briefly, 15 caps were randomly arranged in an upright position on a black surface. Participants were instructed to locate the cap within the group of 15 that was closest in color to the starter cap. Once located, the first cap selected was placed in the position adjacent to the starter cap. Participants then selected the next cap, which was now closest in color to the one that was just put into position. This process was repeated until all the caps proceeded logically from left to right in terms of their spectral hue progression. The number of correctly placed caps was graded.

Microperimetry was assessed with the Macular Integrity Assessment Microperimeter (CenterVue) using the 10-2 grid and 4-2-1 strategy with a G-III white light stimuli on a 10cd/m^2 background. The mean sensitivity across all 68 perimetric grid loci was computed. Kinetic perimetry was performed using the Octopus 900 perimeter (Haag Streit) and visual field sensitivities were obtained using the V4e, III4e, and I4e stimuli. Contrast sensitivity was assessed using the Pelli-Robson chart and the LEA symbols low-contrast chart for participants $\leq$ 5 years of age following standard clinical procedures and the manufacturer's instruction manuals.

Anatomical Assessments

Anatomical features including outer nuclear layer (ONL) average thickness, ONL center point thickness, photoreceptor layer thickness, and ellipsoid zone width from horizontal cross-sectional B-scans through the fovea were assessed using spectral-domain OCT (Heidelberg Spectralis OCT or HRA+OCT; Heidelberg Engineering) following standard clinical procedures. For all anatomical assessments, images were independently assessed and graded at a reading center to ensure uniformity.

Patient-Reported Outcomes

The most consistent patient-reported outcome used throughout the duration of the study was the Clinical Global Impressions-Severity scale if age $\geq$ 18 years[23] or the Caregiver Global Impressions-Severity scale if age $<$ 18 years.

Data Reliability and Standardization of Assessments

Given that this was a multisite study, efforts were made to standardize testing across sites. Specifically, all BCVA lanes were standardized for luminance levels, and protocols were implemented to ensure that luminance levels were maintained throughout the study period. Study personnel were also trained and certified for all functional and structural assessments, and OCT images were pooled and read at a single reading center. In addition, mobility courses were arranged by the vendor across sites and luminance levels were calibrated and standardized. The path, obstacles, and guide for arranging the course patterns were also similar across sites, and the examiners were trained and certified by the vendor. The VNC testing videos were graded at the reading center, with graders blinded to the luminance levels of the assessments.

Statistical Analysis

As this was a natural history study, no formal power analyses were performed to determine the sample size, which was based on the availability of a clinical population with *CEP290*-associated IRD. Assessments were summarized based on worse- and better-seeing eyes, with worse eyes defined as eyes with worse BCVA at the baseline retest visit. If the worse eye could not be determined at baseline retest, the baseline test visit was used. If BCVA was equal at baseline retest and baseline test, the right eye was assigned as the

worse eye. Student *t* test was used to quantify test-retest variability and detect a significant change from baseline. Analyses were performed at the 0.05 significance level (2-sided) in SAS 9.4.

Results

Participant Disposition and Characteristics

Of 41 screened participants, 29 were enrolled (Fig 2). Three enrolled participants were excluded from the analysis population owing to the study site's failure to complete the screening process according to procedures outlined in the protocol. The remaining 26 participants included in the analysis were distributed 4:2:4:16 across the 4 age cohorts (3−5; 6−11; 12−17; and ≥ 18 years) and 22:4 across the 2 BCVA cohorts (light perception to > 1.0 logMAR; 1.0 logMAR−0.4 logMAR). Of the 26 participants, 23 completed the 12-month visit, yielding a dropout rate of approximately 11%. Table 3 presents the demographic characteristics of participants included in the analysis population. Mean (standard deviation [SD]) [range] age was 27.9 (18.04) [3−60] years. All participants were White and 15% identified as Hispanic. Approximately 73% of participants were females. There was notable heterogeneity in the retinal, structural, and functional findings across the study participants; data from 2 representative participants are shown in Figure 3.

BCVA

At baseline, mean (SD) BCVA was 2.18 (1.17) logMAR in the worse eye and 2.05 (1.27) logMAR in the better eye. Best-corrected visual acuity between the study and nonstudy eyes was similar (Table 4 and Table S5). Approximately 73% of participants (19/26) had a between-eye difference ≤ 0.1 logMAR at baseline (Fig 4 and Table S5). Of the remaining 7, 3 had a between-eye difference ≤ 0.2 logMAR and 4 had a between-eye difference > 0.2 logMAR. Of note, the 2 participants with the largest between-eye differences (> 0.4 logMAR) had a diagnosis of keratoconus. Exclusion of all 3 participants with keratoconus yielded a mean (95% confidence interval [CI]) between-eye difference of 0.07 (0.02, 0.12) logMAR. Best-corrected visual acuity demonstrated moderate variability from baseline test to retest (test-retest variability) and demonstrated stability at 12 months. In the worse eye, mean (95% CI) change from baseline test to retest was −0.04 (−0.09, 0.01) logMAR and mean change from baseline to 12 months was 0.06 (−0.17, 0.29) logMAR (Table 4 and Fig 5). Two participants had significant worsening of BCVA (> 0.3 logMAR) over the 12-month follow-up period. Retinal cysts on OCT were

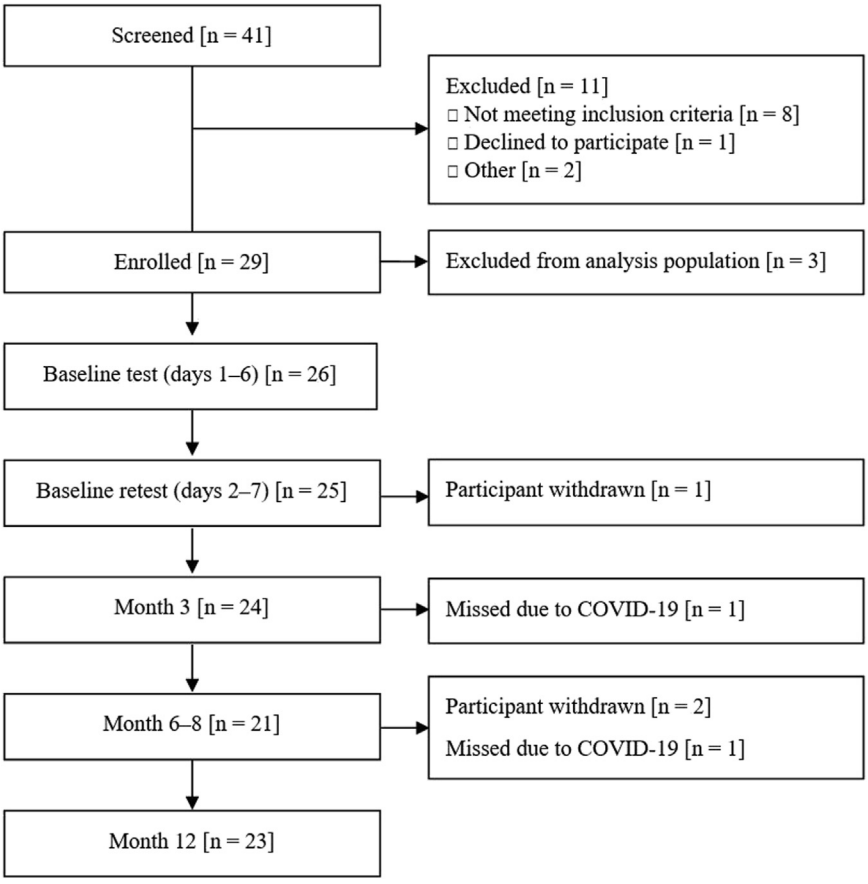

Figure 2. Participant disposition. Participant disposition throughout the natural history study. COVID-19 = coronavirus disease 2019.

Table 3. Participant Demographics and Baseline Characteristics

Characteristic	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6	Cohort 7	Cohort 8	Overall
Cohort size	4	0	2	0	3	1	13	3	26
Age (years)									
Mean (SD)	3.5 (0.58)	-	9.5 (2.12)	-	15.0 (1.73)	16.0	38.9 (12.09)	42.0 (15.72)	27.9 (18.04)
Median (IQR) [range]	3.5 (3–4) [3–4]		9.5 (8–11) [8–11]		14.0 (14–17) [14–17]	16.0 (16–16) [16–16]	37.0 (31–49) [18–56]	35.0 (31–60) [31–60]	28.5 (14–44) [3–60]
Sex (n, %)									
Male	1 (25)	-	1 (50)	-	1 (33.3)	-	4 (30.8)	0 (0)	7 (26.9)
Female	3 (75)	-	1 (50)	-	2 (66.7)	1 (100)	9 (69.2)	3 (100)	19 (73.1)
Ethnicity (n, %)									
Hispanic	1 (25)	-	1 (50)	-	-	-	2 (15.4)	-	4 (15.4)
Non-Hispanic	3 (75)	-	1 (50)	-	3 (100)	1 (100)	11 (84.6)	3 (100)	22 (84.6)
Not reported or unknown	-	-	-	-	-	-	-	-	-
Race (n, %)									
White	4 (100)	-	2 (100)	-	3 (100)	1 (100)	13 (100)	3 (100)	26 (100)

IQR = interquartile range; SD = standard deviation.
Demographics and baseline characteristics of study participants.

noted in 1 case while the other (a pediatric participant) was unexplained. Best-corrected visual acuity was not correlated with age or zygosity (Table 6 and Fig S6).

FST Sensitivity

At baseline, mean (SD) FST sensitivity to blue light was −2.41 (1.42) log cd.s/m^2 in the worse eye and −2.63 (1.55) log cd.s/m^2 in the better eye. Mean sensitivity to red light was −2.15 (1.13) log cd.s/m^2 in the worse eye and −2.28 (0.96) log cd.s/m^2 in the better eye. Mean sensitivity to white light was −2.22 (1.03) log cd.s/m^2 in the worse eye and −2.56 (1.24) log cd.s/m^2 in the better eye (Table 4 and Table S5). Full-field stimulus threshold demonstrated moderate test-retest variability and stability. In the worse eye, mean (95% CI) change from baseline test to retest was 0.17 (−0.38, 0.73) log cd.s/m^2 for blue light FST and mean change from baseline to 12 months was −0.33 (−0.88, 0.21) log cd.s/m^2. For red light FST, mean change from baseline test to retest was 0.10 (−0.07, 0.27) log cd.s/m^2 and mean change from baseline to 12 months was −0.15 (−0.43, 0.14) log cd.s/m^2. For white light FST, mean change from baseline test to retest was 0.16 (−0.24, 0.56) log cd.s/m^2 and mean change from baseline to 12 months was −0.39 (−1.02, 0.24) log cd.s/m^2. There were no significant between-eye differences in FST test-retest variability or stability.

VNC Composite Score

At baseline, mean (SD) VNC composite score was 9.3 (6.95) in the worse eye, 9.4 (7.19) in the better eye, and 9.3 (7.40) in both eyes. Visual Navigation Challenge composite score demonstrated moderate test-retest variability, with 95% of all scores at the baseline retest visit falling within 3 points of the composite score at the baseline test visit. In the worse eye, mean (95% CI) change from baseline test to retest was 0.6 (−0.1, 1.3) points and mean change from baseline to 12 months was −0.1 (−1.2, 1.0) points. No significant between-eye differences were observed in VNC composite score test-retest variability or stability (Table 4 and Table S5). Visual Navigation Challenge composite score was correlated with BCVA in both eyes, white light FST in both eyes, red light FST in both eyes, and blue light FST in the better eye ($P < 0.05$) (Table 6 and Fig S6).

OCT Assessments

At baseline, mean (SD) ONL average thickness in the worse eye was 76 (19) μm with a center point thickness of 127 (26) μm compared with a mean ONL average thickness of 75 (17) μm with a center point thickness of 132 (30) μm in the better eye. No significant between-eye differences in ONL average or central point thickness were noted (Table 4 and Table S5). In the worse eye, mean (95% CI) change from baseline test to retest was 5 (0, 10) μm for ONL average thickness and 2 (−4, 9) μm for center point thickness. Mean change from baseline to 12 months was −2 (−10, 6) μm for ONL average thickness and −8 (−17, 2) μm for center point thickness, suggesting stability. Outer nuclear layer thickness was not correlated with any

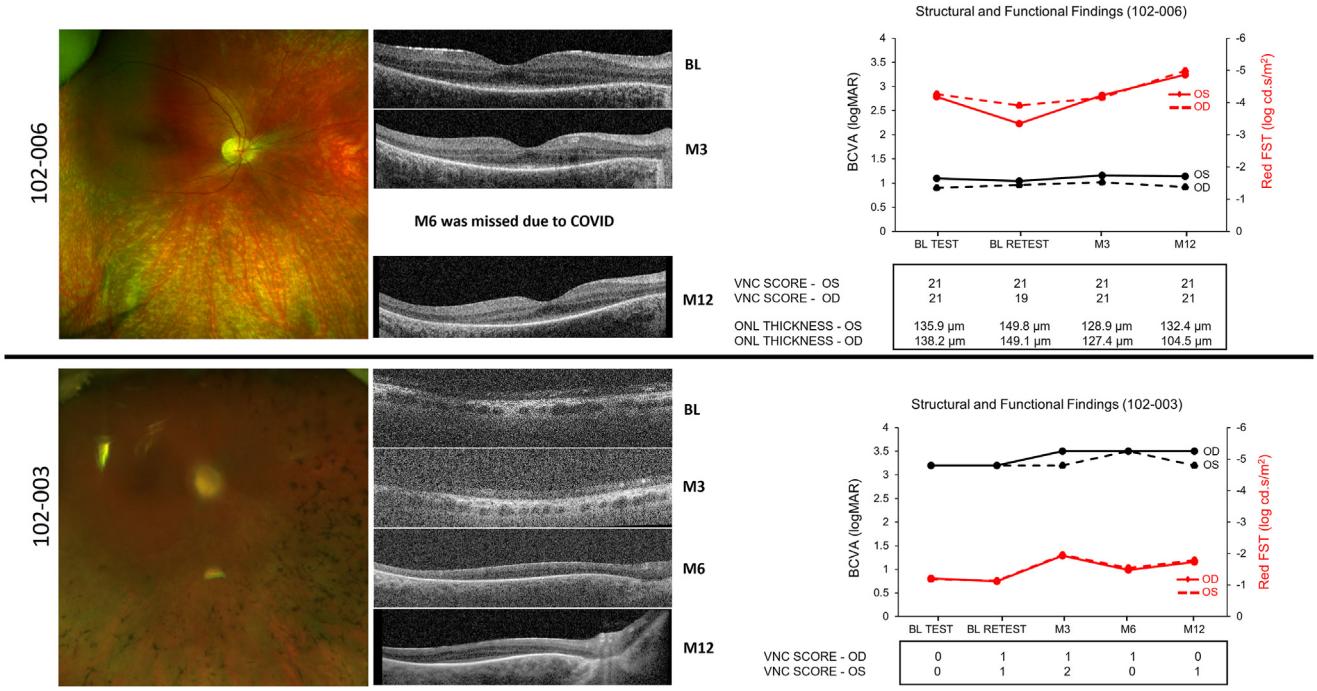

Figure 3. Heterogeneity of structural and functional findings in 2 representative participants with centrosomal protein 290 intron 26 c.2991+1655A>G mutation-associated inherited retinal disease. Participant 102-006 is male and is 8 years old, participant 102-003 is female and is 49 years old. The figure shows colored fundus images, OCT cross-sectional images acquired through the center of the fovea, and functional findings. Despite the heterogeneity in structural and functional findings between participants, the between-eyes comparison was similar. For outer nuclear layer (ONL), center point thickness is reported. For Visual Navigation Challenge (VNC), vendor composite score is reported. BCVA = best-corrected visual acuity; BL = baseline; COVID = coronavirus disease; FST = full-field stimulus threshold; logMAR = logarithm of the minimum angle of resolution; M = month; OD = right eye; OS = left eye.

functional measures (BCVA, FST sensitivity, or VNC composite score) (Table 6 and Fig S6).

Pupillometry

At baseline, mean (SD) percent change in pupillary diameter was 15.9% (16.6) in the worse eye and 18.3% (17.5) in the better eye (Table S5). Pupillometry findings between eyes were similar. In the worse eye, mean (95% CI) change from baseline test to retest was 1.4% (−5.0, 7.9) and mean change from baseline to 12 months was 4.4% (−6.4, 15.2), indicating stability. Percent change in pupillary diameter was not correlated with any functional or structural measures except in the worse eye, where it correlated with white FST sensitivity (r = 0.51, $P = 0.03$) and blue FST sensitivity (r = 0.54, $P = 0.03$) (Table 6 and Fig S6).

Patient-Reported Severity of Visual Impairment

Ninety percent of participants perceived their visual impairment to be stable from baseline to 12 months.

Discussion

This natural history study aimed to define the clinical characteristics of *CEP290*-associated IRD in patients with the common intron 26 c.2991+1655A>G mutation and to determine reliable assessments for future interventional trials in this population. Key study findings include the similarity of functional and structural outcomes between eyes and the repeatability and stability of visual functional measures over 12 months, particularly BCVA, FST sensitivity, and VNC composite score.

Our finding of similar functional outcomes between eyes was independent of zygosity, as well as age, and is consistent with previous reports.[14] These observations open up the possibility that the contralateral eye may be used as a within-subject control to the study eye in future interventional trials. Within-subject controls may also help to minimize clinical heterogeneity, thereby reducing sample sizes—an important benefit in this ultrarare disease population in which recruitment may be a challenge. Despite the appeal of using the contralateral eye as a control, it is worth noting that other limitations such as inability to mask participants and the crossover effect from treatment remain a challenge and warrant further investigation.[24,25]

In this study, similar FST thresholds for blue and red stimuli were observed, suggesting that retinal sensitivity was primarily mediated by cone photoreceptors. Thus, therapeutic trials looking to improve retinal sensitivity with a mechanism of action aimed at boosting the expression of normal CEP290 protein may have a better opportunity in patients with greater photoreceptor layer thickness as posited by Boye et al.[26] We also observed that VNC composite score was correlated with BCVA and FST sensitivity. Visual Navigation Challenge

Table 4. Assessment Test-Retest Variability and Stability in the Worse Eye

Assessment	Mean (SD) [n] of Worse Eye at Baseline	Test-Retest	Mean (95% CI) [n] Change from Baseline in Worse Eye		
			M3	*M6*	*M12*
BCVA (logMAR)	2.18 (1.17) [26]	−0.04 (−0.09, 0.01) [25]	0.09 (−0.02, 0.20) [24]	0.04 (−0.08, 0.15) [20]	0.06 (−0.17, 0.29) [23]
Blue light FST (log cd.s/m^2)	−2.41 (1.42) [20]	0.17 (−0.38, 0.73) [16]	−0.43 (−1.00, 0.13) [14]	−0.05 (−0.23, 0.13) [14]	−0.33 (−0.88, 0.21) [17]
Red light FST (log cd.s/m^2)	−2.15 (1.13) [20]	0.10 (−0.07, 0.27) [14]	−0.15 (−0.37, 0.07) [13]	−0.10 (−0.30, 0.10) [13]	−0.15 (−0.43, 0.14) [16]
White light FST (log cd.s/m^2)	−2.22 (1.03) [22]	0.16 (−0.24, 0.56) [18]	−0.32 (−0.91, 0.27) [15]	−0.16 (−0.48, 0.15) [16]	−0.39 (−1.02, 0.24) [19]
VNC score	9.3 (6.95) [23]	0.6 (−0.1, 1.3) [18]	0.1 (−0.4, 0.6) [21]	0.7 (−0.3, 1.6) [18]	−0.1 (−1.2, 1.0) [21]
Minimum diameter (mm)	4.4 (1.40) [20]	0.09 (−0.55, 0.73) [17]	−0.37 (−0.76, 0.03) [18]	−0.24 (−0.79, 0.32) [16]	−0.18 (−0.65, 0.29) [16]
Latency (s)	0.35 (0.10) [20]	0.02 (−0.05, 0.10) [17]	0.00 (−0.03, 0.04) [17]	0.04 (−0.01, 0.10) [16]	0.03 (−0.04, 0.11) [16]
75% recovery rate (mm/s)	0.85 (0.62) [17]	−0.06 (−0.48, 0.37) [9]	−0.07 (−0.37, 0.22) [8]	−0.11 (−0.48, 0.27) [12]	0.03 (−0.69, 0.74) [11]
Percent pupillary change (%)	15.91 (16.63) [20]	1.43 (−4.98, 7.85) [17]	8.98 (1.18, 16.78) [18]	1.90 (−10.89, 14.69) [16]	4.41 (−6.35, 15.16) [16]
Constriction velocity (mm/s)	−2.70 (4.41) [20]	−0.62 (−2.24, 0.99) [17]	−3.60 (−7.09, −0.11) [18]	−0.10 (−3.03, 2.83) [16]	0.55 (−0.64, 1.75) [16]
EZ width (mm)	2.2 (0.83) [14]	0.08 (−0.25, 0.41) [14]	−0.06 (−0.24, 0.13) [13]	−0.10 (−0.32, 0.13) [11]	−0.01 (−0.32, 0.30) [13]
ONL average thickness (μm)	76 (19) [15]	5 (0, 10) [14]	−1 (−5, 3) [13]	6 (−15, 4) [10]	−2 (−10, 6) [13]
ONL center point thickness (μm)	127 (26) [15]	2 (−4, 9) [14]	0 (−4, 5) [13]	−1 (−9, 8) [10]	−8 (−17, 2) [13]
Average photoreceptor layer thickness (μm)	88 (24) [16]	4 (0, 8) [15]	−1 (−5, 2) [13]	−6 (−15, 3) [12]	−1 (−10, 9) [14]
Microperimetry mean sensitivity	2.40 (Limited data) [1]	Limited data	0 (Limited data) [1]	Limited data	Limited data
Color vision	9.0 (5.37) [6]	Limited data	−1.2 (−4.2, 1.9) [6]	−0.8 (−3.5, 2.0) [4]	0.6 (−3.2, 4.4) [5]
Contrast sensitivity	1.33 (0.58) [6]	Limited data	−0.05 (−0.18, 0.08) [6]	−0.04 (−0.27, 0.19) [4]	−0.06 (−0.31, 0.19) [5]

BCVA = best-corrected visual acuity; CI = confidence interval; EZ = ellipsoid zone; FST = full-field stimulus threshold; logMAR = logarithm of the minimum angle of resolution; MX = month x; ONL = outer nuclear layer; SD = standard deviation; VNC = Visual Navigation Challenge.
Test-retest variability and stability of assessments performed in the worse eye.

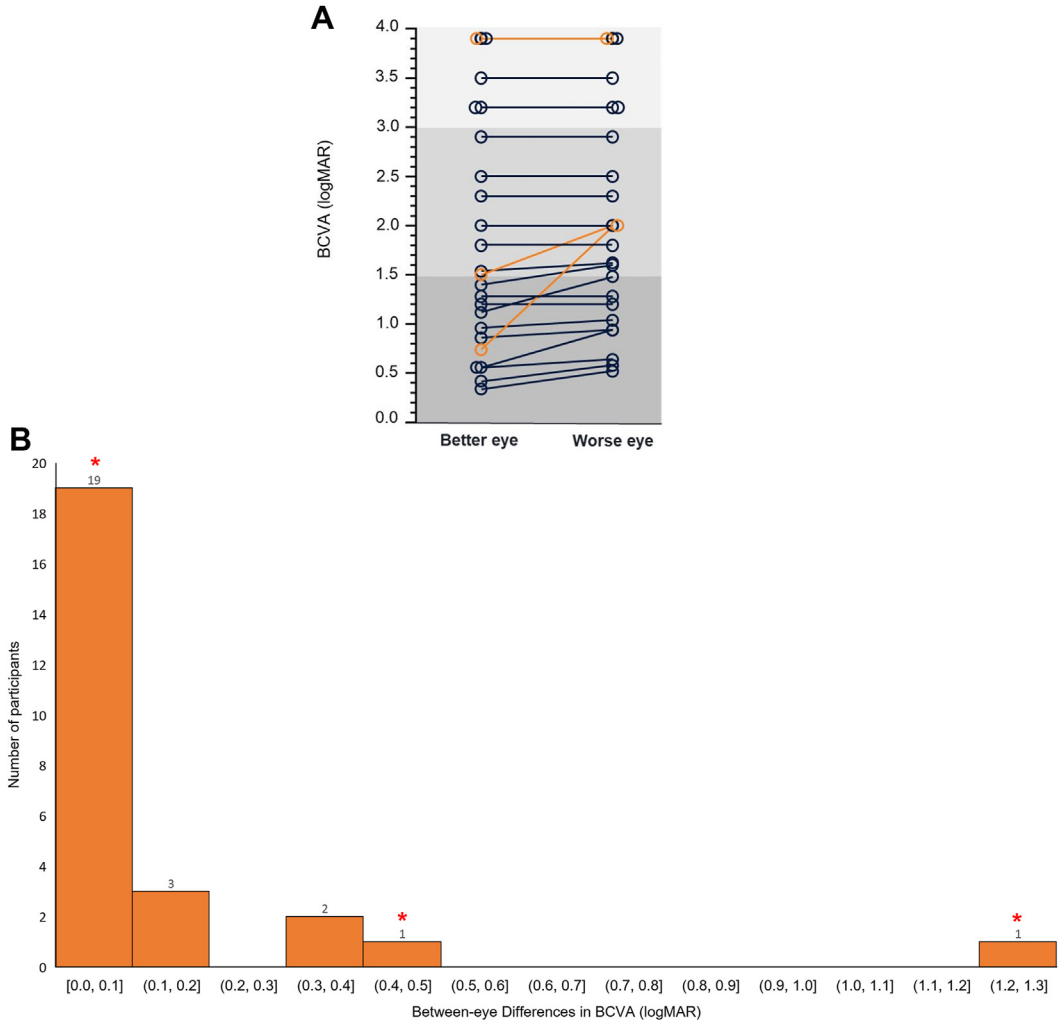

Figure 4. Between-eye differences in best-corrected visual acuity (BCVA) at baseline. Between-eye differences in BCVA at baseline. **A,** Black trace indicates participants with comparable BCVA between eyes. Orange trace indicates participants with keratoconus. **B,** Asterisks indicate groups containing a participant with keratoconus. The x-axis presents between-eye BCVA differences in logarithm of the minimum angle of resolution (logMAR) intervals.

composite score may be a potential metric for assessing the impact of treatment on visual function through its indirect assessment of psychometric properties such as contrast sensitivity, which are not always measurable in this clinical population. Notably, visual function navigation, as assessed with the multiluminance mobility test, was used as a study endpoint in the United States Food and Drug Administration approval of Luxturna (Voretigene neparvovec) for treatment of retinal pigment epithelium *65*-associated IRD.[11] In that study, visual function navigation was also correlated with FST sensitivity and BCVA.

Outer nuclear layer average and center point thickness did not significantly differ from baseline to 12 months. However, the assessment of OCT features was largely limited by scan quality and investigators' inability to repeat measures at the same retinal location. As such, repeatability of OCT measures poses a potential challenge for quantifying anatomical changes in this population. A similar observation was noted by Chung et al,[27] who did not detect corresponding ONL changes despite observing age-related changes in BCVA in patients with retinal pigment epithelium *65*-associated IRD. In the present study, collection of reliable measurements for microperimetry, kinetic perimetry, color vision, and contrast sensitivity were limited by nystagmus, low BCVA, and restricted visual fields in participants. Consequently, these assessments may have limited utility in quantifying visual changes in patients with CEP290-related retinal degeneration.

This study has several strengths, including being a multicenter study. This facilitated recruitment of a clinically diverse study population and assessment of clinical characteristics across a range of visual ability and disease severity. To the best of our knowledge, this is the most comprehensive natural history study of IRD caused by the *CEP290* intron 26 c.2991+1655A>G mutation. This is notable as there have been calls for greater inclusion of

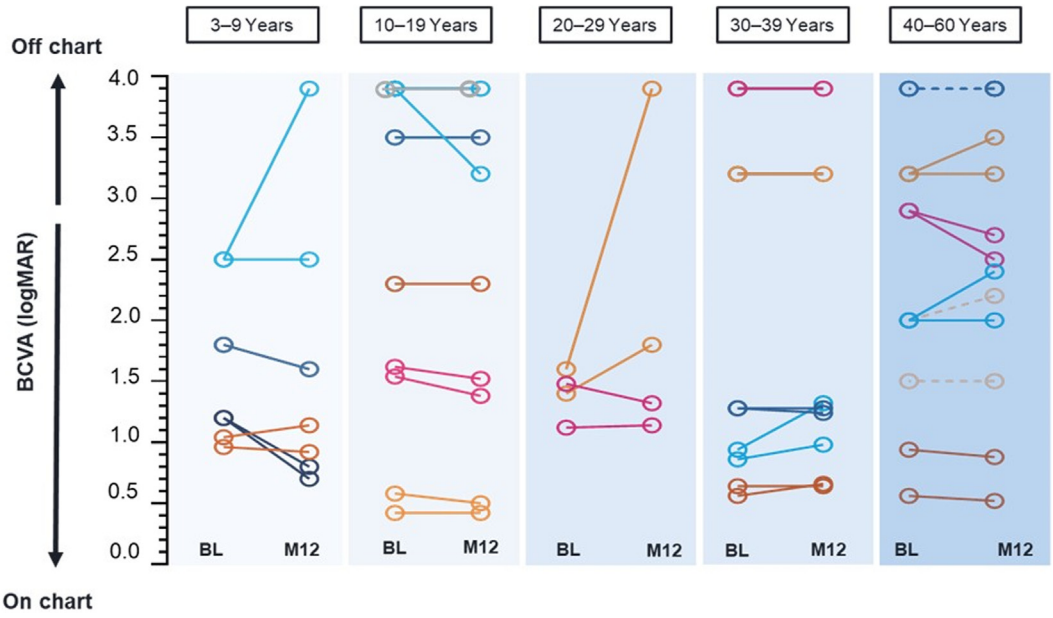

Figure 5. Change in best-corrected visual acuity (BCVA) from baseline (BL) to 12 months (M12) by age group. Each pair of lines bounded by circles of the same color represents the worse and better eyes at BL and M12 in a single participant. Unpaired lines are superimposed and represent participants with a similar change in both eyes from BL to M12. Dashed lines represent participants with keratoconus. On-chart assessment: BCVA or Berkeley Rudimentary Vision Test; off-chart assessment: light perception (3.9), black-white discrimination (3.5), and white field projection (3.2).

adolescents and adults in future interventional trials in this population.[14] An additional strength of this study is the use of state-of-the-art endpoint measures and systematic measurement of anatomical and functional changes over time. Many retrospective studies would not have included assessments such as FST or pupillometry as these are not routine clinical assessments.

However, this study was limited by the short follow-up period. Notably, this duration was sufficient for demonstrating a treatment effect on visual function after

Table 6. Correlation between Study Endpoints

Correlation Analysis	Worse Eye (N = 26)			Better Eye (N = 26)		
	N	*Coefficient*	P *Value*	*N*	*Coefficient*	P *Value*
Age, BCVA	26	−0.0199	0.9230	26	−0.0971	0.6371
Age, blue light FST	20	0.3749	0.1034	20	0.3363	0.1471
Age, red light FST	20	0.0999	0.6751	20	0.0023	0.9924
Age, white light FST	22	0.1521	0.4993	21	0.2494	0.2757
Age, VNC score	23	−0.1294	0.5563	23	−0.1155	0.5997
VNC score, BCVA	**23**	**−0.8935**	**< 0.0001**	**23**	**−0.8922**	**< 0.0001**
VNC score, blue light FST	19	−0.3474	0.1451	**19**	**−0.5865**	**0.0083**
VNC score, red light FST	**19**	**−0.7214**	**0.0005**	**19**	**−0.7900**	**< 0.0001**
VNC score, white light FST	**21**	**−0.5398**	**0.0115**	**20**	**−0.6048**	**0.0047**
Percent change in pupillary diameter, BCVA*	20	−0.1899	0.4226	22	0.2155	0.3355
Percent change in pupillary diameter, blue light FST*	**16**	**−0.5353**	**0.0326**	18	−0.2714	0.2760
Percent change in pupillary diameter, Red light FST*	16	−0.3000	0.2589	18	0.0114	0.9643
Percent change in pupillary diameter, white light FST*	**18**	**−0.5108**	**0.0303**	19	−0.2667	0.2698
OCT-ONL horizontal thickness, BCVA	15	−0.0547	0.8466	14	0.0963	0.7434
OCT-ONL horizontal thickness, blue light FST	12	0.0415	0.8981	12	0.0681	0.8335
OCT-ONL horizontal thickness, red light FST	12	0.1233	0.7027	12	−0.0275	0.9324
OCT-ONL horizontal thickness, white light FST	13	−0.0224	0.9420	13	0.0549	0.8587
BCVA, zygosity*	26	0.1142	0.5785	26	0.0999	0.6274

BCVA = best-corrected visual acuity; FST = full-field stimulus threshold; ONL = outer nuclear layer; VNC = Visual Navigation Challenge.
Correlation between study endpoints in worse and better eyes.
All correlations that are bolded were significant at $P < 0.05$. The *P*-values are reported in the adjacent columns. The values for VNC score X blue light FST (worse eye), Percent change in pupillary diameter X blue light FST (better eye), and Percent change in pupillary diameter X white light FST (better eye) were bolded in error.
*Spearman correlation coefficient is reported vs. the Pearson correlation coefficient.

therapeutic intervention in other *CEP290*-associated IRD trials.[16] Another limitation is that nonuniform instruments were used for some assessments (ETDRS charts, LEA symbols cards, or Berkeley Rudimentary Vision Test charts for BCVA assessment). However, this was necessary given the range in participants' cognitive ability, age, and disease severity. Additionally, the conoravirus disease 2019 pandemic likely affected this study as some participants missed scheduled visits. Lastly, our inability to reach the target sample size limited our group-based analyses and highlights the recruitment challenge in this population, which should be considered in future trials.

In conclusion, this natural history study aimed to define the clinical characteristics of *CEP290*-associated IRD in patients with the common intron 26 c.2991+1655A>G mutation and to determine reliable assessments for future interventional trials in this population. Given the possibility of new therapies in *CEP290*-associated IRD, conventional outcomes such as BCVA may need to be supplemented. In the present study, we found that BCVA, FST sensitivity, and VNC score were relatively stable and comparable between worse and better eyes, indicating their potential viability as endpoints for future clinical studies in CEP290-associated IRD. Nystagmus limited the repeatability of OCT measures and poses potential challenges for quantifying anatomical changes in this patient population. The findings of this study will inform the design of a phase I/II single ascending dose study of EDIT-101, an in vivo clustered regularly interspaced short palindromic repeat (CRISPR) gene editing therapy under development for treatment of *CEP290*-associated IRD.[28]

Footnotes and Disclosures

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No animal subjects were included in this study.

Author Contributions:
All authors were responsible for reviewing and revising this manuscript.

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Data collection: Pierce, Jayasundera, Hoyng, Lam, Lorenz, Pennesi

Analysis and interpretation: Pierce, Ashimatey, Kim, Pennesi

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Overall responsibility: Pierce, Ashimatey, Jayasundera, Hoyng, Lam, Lorenz, Kim, Rashid, Myers, Pennesi

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Abbreviations and Acronyms:
BCVA = best-corrected visual acuity; **CEP290** = centrosomal protein 290; **CI** = confidence interval; **FST** = full-field stimulus threshold; **IRD** = inherited retinal disease; **logMAR** = logarithm of the minimum angle of resolution; **ONL** = outer nuclear layer; **SD** = standard deviation; **VNC** = Visual Navigation Challenge.

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