Inherited retinal degenerations: clinical characterization on the road to therapy

Talib, M.

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Defining inclusion criteria and endpoints for clinical trials: a prospective cross-sectional study in *CRB1*-associated retinal dystrophies

Mays Talib, MD[1], Mary J. van Schooneveld, MD, PhD[2,3], Jan Wijnholds, PhD[1], Maria M. van Genderen, MD, PhD[3], Nicoline E. Schalij-Delfos, MD, PhD[1], Herman E. Talsma[1,3], Ralph J. Florijn, PhD[4], Jacoline B. ten Brink, BAS[4], Frans P.M. Cremers, PhD[5], Alberta A.H.J. Thiadens, MD, PhD[6], L. Ingeborgh van den Born, MD, PhD[7], Carel B. Hoyng, MD, PhD[8], Magda A. Meester-Smoor[6], Arthur A. Bergen, PhD[4,9], Camiel J.F. Boon, MD, PhD[1,2]

Acta Ophthalmol 2021;99(3):e403-e414

1 Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands.
2 Department of Ophthalmology, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
3 Bartiméus, Diagnostic Centre for complex visual disorders, Zeist, The Netherlands.
4 Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
5 Department of Human Genetics and Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands.
6 Department of Ophthalmology, Erasmus Medical Center, Rotterdam, The Netherlands.
7 Rotterdam Eye Hospital, Rotterdam, The Netherlands.
8 Department of Ophthalmology, Radboud University Medical Center, Nijmegen, The Netherlands.
9 The Netherlands Institute for Neuroscience (NIN-KNAW), Amsterdam, The Netherlands.

ABSTRACT

Purpose: To investigate the retinal structure and function in patients with *CRB1*-associated retinal dystrophies (RD) and to explore potential clinical endpoints.

Methods: In this prospective cross-sectional study, 22 patients with genetically confirmed *CRB1*-RD (aged 6-74 years), and who had a decimal best-corrected visual acuity (BCVA) ≥ 0.05 at the last visit, were studied clinically with ETDRS BCVA, corneal topography, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence, Goldmann visual field (VF), microperimetry, full-field electroretinography (ERG) and full-field stimulus testing (FST). Ten patients were from a genetic isolate (GI).

Results: Patients had retinitis pigmentosa (n = 19; GI and non-GI), cone-rod dystrophy (n = 2; GI), or macular dystrophy (n = 1; non-GI). Median age at first symptom onset was 3 years (range 0.8-49). Median decimal BCVA in the better and worse-seeing eye was 0.18 (range 0.05-0.83) and 0.08 (range light perception-0.72), respectively. Spectral-domain optical coherence tomography (SD-OCT) showed cystoid maculopathy in 8 subjects; inner retinal thickening (n = 20), a well-preserved (para)foveal outer retina (n = 7) or severe para(foveal) outer retinal atrophy (n = 14). All retinal layers were discernible in 13/21 patients (62%), with mild to moderate laminar disorganization in the others. Nanophthalmos was observed in 8 patients (36%). Full-field stimulus testing (FST) provided a subjective outcome measure for retinal sensitivity in eyes with (nearly) extinguished ERG amplitudes.

Conclusions: Despite the generally severe course of *CRB1*-RDs, symptom onset and central visual function are variable, even at advanced ages. Phenotypes may vary within the same family. Imaging and functional studies in a prospective longitudinal setting should clarify which endpoints may be most appropriate in a clinical trial.

INTRODUCTION

Pathogenic variants in the *CRB1* gene are associated with a spectrum of retinal dystrophies (IRD). Each IRD can be distinguished clinically depending on the age at symptom onset, electrophysiological findings and other phenotypic features. *CRB1* mutations cause up to 17% of cases of Leber congenital amaurosis (LCA), and up to 9% of cases of autosomal recessive non-syndromic retinitis pigmentosa (RP), [1-3] as well as rare cases of isolated maculopathy.[4, 5] *CRB1*-associated LCA causes severe visual impairment or blindness from infancy, and *CRB1*-RP usually has an early onset of symptoms such as nyctalopia and visual field restriction, although cases of a later symptom onset and/or relative preservation of visual acuity in *CRB1*-RP have also been described.[6, 7] While most clinical studies of the human *CRB1*-associated phenotype have been case series or small cohorts,[4, 8-12] recent studies in larger populations have shown that half of *CRB1*-RP patients are expected to reach low vision or blindness by the ages of 18 and 44, respectively.[6, 7] Reports on associated features in *CRB1*-RP or LCA, such as keratoconus, have shown a variable prevalence.[9, 13-18] As protein CRB1 is crucial for the integrity of the retinal structure, *CRB1*-associated IRDs have shown an association with variable degrees of disorganization of the retinal laminar structure.[19] While no effective and approved treatment for *CRB1*-associated retinopathies is currently available, subretinal adeno-associated virus (AAV)-mediated *CRB1-CRB2* gene augmentation therapy has shown functional and structural rescue in murine models of *CRB1*-associated LCA and RP.[20] As human *CRB1* gene therapy is under development,[21] defining an optimal window of therapeutic opportunity and clinical endpoints for a *CRB1* gene therapy trial is essential. In such trials, measures that are time-dependent and can still remain stable in certain decades of life, such as visual acuity,[6] may need to be complemented with other, potentially more sensitive functional or structural outcome measures. These additional outcome measures have not yet been elucidated, as an inevitable limitation of retrospective studies is the limited availability of extensive imaging and functional examinations, as well as the unstandardized design. The aim of the present study was to extensively investigate the retinal structure and function in patients with *CRB1*-RDs in a clinical study of prospectively enrolled patients and to study the correlation between structural and functional parameters in order to assess potentially sensitive efficacy endpoints for a future gene therapy trial.

MATERIALS AND METHODS

Human subjects

This is a nationwide collaborative study, based on the Delleman archive for hereditary eye diseases at the Amsterdam UMC (University of Amsterdam) and the RD5000 consortium, the Dutch national consortium for the registry of patients with retinal dystrophies. Inclusion criteria for this prospective, cross-sectional study were (1) the presence of two confirmed pathogenic variants in

the *CRB1* gene (class 4 or 5), (2) a decimal BCVA of ≥0.05 in the better-seeing eye at the last available clinical examination, which (3) should not have been >20 years prior to enrolment in this study. Of the 63 identified *CRB1*-RD patients in the Netherlands, 22 could be included based on these inclusion criteria (Figure S1). In one patient (ID-9), the worse-seeing eye had light perception vision, and not all tests could be performed in the worse-seeing eye. The cohort included patients from a Dutch genetic isolate, that is a consanguineous pedigree described earlier.[22] Patients and/or their parents signed informed consent. This study was approved by the Medical Ethics Committee of the Erasmus Medical Center, as it was performed in the framework of the RD5000 consortium,[23] and by the local review board of the Leiden University Medical Center (LUMC), and complied with the tenets of the Declarations of Helsinki.

Ophthalmological assessment

A detailed medical and ophthalmological history was recorded in all subjects. Patients were surveyed on the history of their symptoms, the presence of photopsia, and in the case of photopsia, where in the visual field these were located (full field, periphery, or central areas). Participants underwent a complete ophthalmological examination, including best-corrected visual acuity (BCVA) measurements with the Early Treatment of Diabetic Retinopathy Study (ETDRS) ESV-3000 chart by Precision Vision,[24, 25] slit-lamp biomicroscopy, fundoscopy and Goldmann kinetic perimetry. Corneal topography was evaluated using the 4-map refractive report and the Belin/Ambrósio Enhanced Ectasia report (Oculus Optikgerate GmbH, Wetzlar, Germany). Biometry was performed (EyeSuite™ IOL, Haag-Streit Diagnostics) to measure eye axis length and anterior chamber depth. Macular threshold sensitivities were measured under mesopic conditions with Macular Integrity Assessment microperimetry (MAIA, Centervue, Padova, Italy) in 20 patients, as the youngest two patients (aged 6 and 9 years) could not complete the procedure. To minimize a learning effect, each eye was tested using the '4-levels-fixed' protocol, which familiarizes the patient to the test, followed by testing with the '4-2 staircase strategy', projected on a 10-2 Cartesian grid (37 points covering the central 10°). The latter was used to evaluate the macular sensitivity (average threshold) and fixation stability (bivariate contour ellipse area; BCEA). The 95% or 65% BCEA indicates the ellipse area that comprises 95% or 65%, respectively, of the fixation points used by the patients during the test. Thus, a smaller area indicates a more stable fixation. Seven-field colour fundus photographs were obtained (Topcon TRC-50DX, Topcon Medical Systems, Inc. Oakland, NJ, USA). Spectral-domain optical coherence tomography (SD-OCT; Spectralis, Heidelberg Engineering, Heidelberg, Germany) of the macula and optic disc was performed in 21 patients, but could not be reliably performed in the 6-year-old patient. Segmentation of SD-OCT images was performed with the integrated automatic segmentation Spectralis software, and errors in the segmentation were manually corrected. Thickness of the photoreceptor-retinal pigment epithelium complex (PR + RPE) was measured as the distance from the external limiting membrane (ELM) to the basal membrane, based on methods used earlier [26]. The horizontal width of detectable and uninterrupted (even if attenuated) ellipsoid zone (EZ) was measured drawing a

line parallel to the retinal pigment epithelium (RPE), starting at the fovea. In patients where the EZ signal became indistinguishable from other hyperreflective outer retinal signals and could no longer be differentiated from the ELM or interdigitation zone, the horizontal width of detectable hyperreflective outer retinal band was measured. The laminar organization of the retina, that is the retinal alignment into distinct layers, was assessed and categorized (Figure S2). In 20 patients, 488 nm wavelength fundus autofluorescence (FAF; Spectralis, Heidelberg Engineering, Heidelberg) was performed, which could not be reliably performed in the two youngest patients (aged 6 and 9 years). The fovea was defined as the central 5°, the parafovea as the following circumferential 3° around the fovea, and the perifovea or peripheral macula as the circumferential 10° around the parafovea.

Pupil dilation protocol

Initially, all pupils were dilated using phenylephrine 2.5% and tropicamide 1%. After the occurrence of acute angle-closure glaucoma in one patient during mydriatic dark-adaptation with tropicamide only (patient-ID 15, the 9th consecutive patient who participated), the pupil dilation protocol was revised: only patients with an anterior chamber depth ≥ grade 2, as graded with the pen torch method and Van Herick's technique, received phenylephrine 2.5% and tropicamide 1%. In patients with an anterior chamber depth of grade 2, pupils were dilated using only tropicamide 1%. The pupils of one patient (patient-ID 4, the 10th consecutive patient) with an anterior chamber depth of grade 1, and a family history of acute angle-closure glaucoma in relatives with *CRB1*-RP, were not dilated.

Electrophysiological testing

Full-field electroretinography (ERG) was performed according to an extended protocol, which incorporated the International Society for Clinical Electrophysiology Standards [27]. All ERG responses were recorded using Dawson Trick Litzkow (DTL) fibre electrodes with the Espion ColorDome™ and console (Diagnosys LLC, Cambridge, UK). The set-up and reference values of the Rotterdam Eye Hospital (Rotterdam, The Netherlands) and Bartiméus (Zeist, The Netherlands) were used. For an RP diagnosis, attenuation of the dark-adapted responses had to be more severe than attenuation of the light-adapted responses. The reverse was the case for a cone-rod dystrophy (CORD) diagnosis. In the case of non-detectable responses, the diagnosis was made based on the clinical evidence (e.g. fundoscopy). For an isolated macular dystrophy diagnosis, the full-field ERG should display no panretinal dysfunction of dark- or light-adapted responses. Full-field stimulus testing (FST) was successfully performed in 15 subjects (28 eyes) with the Espion ColorDome™ LED full-field stimulator (Diagnosys LLC, Lowell, MA, USA) using methods described previously,[28-30] after dark-adaptation of ≥30 minutes, and using white, red, and blue stimuli, each lasting 4 milliseconds. Each eye was tested separately, and the fellow eye was patched. The reference luminance (0 dB) was set at 0.1 cd.s/m^2. Sensitivity thresholds were determined three times for each colour, and the three trials were averaged to determine the final thresholds.

General sensitivity thresholds were determined using the white stimuli. Chromatic sensitivities were used to determine whether these responses were rod-mediated (blue-red difference of >22 dB), cone-mediated (blue-red difference of <3 dB) or mixed rod- and-cone-mediated (blue-red difference between 3 and 22 dB). Based on earlier studies,[28, 31] and correcting for differences in reference luminance, the normal FST threshold for white stimuli was determined at -53 dB and should be rod-mediated.

Statistical analysis

Data were analysed using SPSS version 23.0 (IBM Corp, Armonk, NY, USA). Normality was tested using the normal probability plot and quantile-quantile plot, and the Shapiro-Wilk test. Normally distributed data were presented with means and standard deviations (SD). Non-normally distributed data were presented with medians and interquartile ranges (IQR). Goldmann visual field areas of the V4e target were digitized and converted to seeing retinal areas in mm^2 using a method described by Dagnelie.[32] Visual field areas were classified as large (>250 mm^2), intermediate (25-250 mm^2) or small (<25 mm^2), based on an earlier study.[33] Medians were compared using the Mann-Whitney *U*-test. The correlation between visual function parameters (BCVA, macular sensitivity on microperimetry, V4e and I4e visual field extent) and biomarkers on retinal imaging was tested with Spearman's correlation testing. Visual impairment, based on the BCVA, was categorized as defined by the World Health Organization: mild or no visual impairment (BCVA ≥ 0.3), low vision (BCVA < 0.3 and ≥ 0.1), severe visual impairment (BCVA < 0.1 and ≥ 0.05) and blindness (BCVA < 0.05). For statistical analysis, ETDRS BCVA was converted to the logarithm of the minimal angle of resolution (logMAR).

RESULTS

Twenty-two patients with *CRB1*-RD were included, from 12 families. Table 1 shows the baseline clinical characteristics. Ten patients (45%) were from the genetic isolate. The median age at examination was 25 years (IQR 20; range 6-74) and did not differ significantly between patients from within and from outside the genetic isolate ($p = 0.57$). Nine patients (41%) were male (aged 6-74), and 13 (59%) were female (aged 9-38). Based on current and previous ERG examinations, 19 patients (86%) were diagnosed with RP, 2 patients (9%; 1 from the genetic isolate) had a cone-rod dystrophy, and 1 patient (5%) had a macular dystrophy. Table S1 shows the genetic characteristics, including the novel *CRB1* variant p.(Phe978Ser) in a patient with severe RP (ID-16), and the homozygous p.(Ile167_Gly169del) variant in the macular dystrophy patient (ID-22). Nineteen patients (86%) were of Dutch Caucasian descent, 2 were of North-African origin (9%), and 1 patient was of Caribbean descent (Dutch Antilles; 5%).

Table 1. Demographics and baseline findings in ophthalmological examination in patients with *CRB1*-associated retinal dystrophies

		Age (y) at onset 1st symptom	BCVA (ETDRS letters [decimal Snellen])				Central horizontal VF diameter (°)		Total seeing retinal area V4e (mm^2)	
ID/Sex/ Family	Age (y)		OD	OS	SER (D)**	ffERG	OD	OS	OD	OS
1/M/GI*	29	6	34 (0.10)	61 (0.33)	+0.56	ND	27	30	154.6	158.1
2/F/GI	13	1.5	39 (0.12)	40 (0.13)	+3.06	ND‡	24	24	33.9	36.9
3/F/GI	16	1	52 (0.20)	56 (0.25)	+0.56	RCD	22	28	119.7	160.7
4/F/GI	38	<1	23 (0.06)	40 (0.13)	+1.00	ND‡	25	24	34.1	38.8
5/M/GI	41	2	40 (0.13)	13 (0.04)	+3.88	ND	19	19	90.5	74.9
6/F/GI	11	3	56 (0.26)	64 (0.38)	+3.19	MR	29	105	260.9	296.2
7/F/GI	9	2	19 (0.05)	19 (0.05)	+6.31		30	21	240.0	183.9
8/F/GI	10	3	31 (0.08)	22 (0.05)	+7.06	MR	150	45	624.4	450.4
9/F/GI	28	8	5 (0.03)	35 (0.10)	+5.75	ND	25	21	164.3	185.6
10/M/GI	39	34-35	78 (0.72)	78 (0.72)	+2.5	CORD	150	150	746.9	711.8
11/M	31	7-8	70 (0.50)	81 (0.83)	+1.00	MR	62	125	278.9	370.3
12/F	26	9	53 (0.23)	61 (0.33)	+1.44	ND	51	110	279.1	345.8
13/M	21	2	12 (0.03)	30 (0.08)	+2.56	ND‡	70	53	215.0	189.9
14/F	24	2	52 (0.22)	20 (0.05)	+5.25	ND‡	36	26	79.1	85.5
15/F	31	1	41 (0.13)	42 (0.14)	+4.75	ND‡	22	24	175.2	146.24
16/M	6	1-2	11 (0.03)	25 (0.06)	+3.38	NP	72	62	231.9	123.19
17/M	23	12	63 (0.36)	27 (0.07)	***	RCD	101	20	553.6	348.1
18/F	12	3	40 (0.13)	19 (0.05)	+5.00	ND	15	16	135.0	116.8
19/M	53	17	52 (0.22)	0 (NLP) †	***	ND	18	0	33.4	0
20/M	74	49	75 (0.63)	58 (0.29)	***	ND	20	19	17.9	22.4
21/F	31	4	30 (0.08)	31 (0.08)	+0.88	CORD	65	25	703.0	717.3
22/F	24	11-12	66 (0.41)	42 (0.14)	-5.75	Normal	148	148	723.7	754.8

BCVA = best-corrected visual acuity, CORD = cone-rod dystrophy, ETDRS = Early Treatment of Diabetic Retinopathy Study, ERG = full-field electroretinography, GI = genetic isolate, MR = minimal response, ND = no detectable responses, NP = not performed (patient did not tolerate the electrodes), SER = spherical equivalent of the refractive error, VF = (Goldmann) visual field.

*These patients were also included in the previous retrospective cohort. One patient (ID-1) underwent electrophysiological testing with a different device (MetroVision) prior to the acquisition of the Diagnosys device.

**Averaged between eyes.

***These patients had undergone cataract surgery in one (patient 17) or both (patients 19-20) eyes, and these refractive error measurements are postoperative. Preoperatively, the SER was -0.5 D and -1.875 D in patients 19 and 20, respectively.

†Patient 19 reported no light perception in OS during BCVA measurement, but after dark-adaptation in mydriasis in preparation for electrophysiological examination, he could perceive white, red, and blue stimuli with OS.

‡Scotopic and photopic responses had been nondetectable in ERG examination performed prior to this study.

Onset and visual acuity

The median self-reported or parent-reported age at symptom onset was 3 years in the RP group (IQR 6.5 years; range 9 months-49 years) and 11 years in patients with a cone-rod or macular dystrophy (range 4-34 years). In the RP group, the first-experienced symptoms, as noticed either by the patient or by the parents, were visual field loss (8/19; 42%), nyctalopia (5/19; 26%), central vision loss (4/19; 21%) or nystagmus (2/19; 11%). In RP patients ID-6 and ID-17, the initial erroneous diagnosis was intermediate uveitis, based on central vision loss, CME and vitreous cells, all of which preceded pigmentary fundus changes. After 1 and 4 years, respectively, the correct diagnosis of RP was established based on full-field ERG responses, and the course toward the correct diagnosis has been described previously.[34] In the three patients with cone-rod or macular dystrophy, the first symptom was subjective central vision loss. There was no statistically significant difference in median age at symptom onset between patients from within and from outside the genetic isolate ($p = 0.22$). Photopsia were reported by 13 patients (59%) in areas of decreased/no vision (n = 3), areas of good vision (n = 2), or in both (n = 8).

The median decimal BCVA in the better-seeing eye was 0.18 (0.05-0.83), and 0.08 in the worse-seeing eye (range light perception vision-0.72), with a moderate symmetry between the right and left eye (Spearman's rho = 0.467; $p < 0.028$). Best-corrected visual acuity (BCVA) in the better-seeing eye did not significantly differ between patients from within or from outside the genetic isolate ($p = 0.53$), but was significantly better in older patients (Spearman's rho = 0.435; $p = 0.04$; Figure 1).

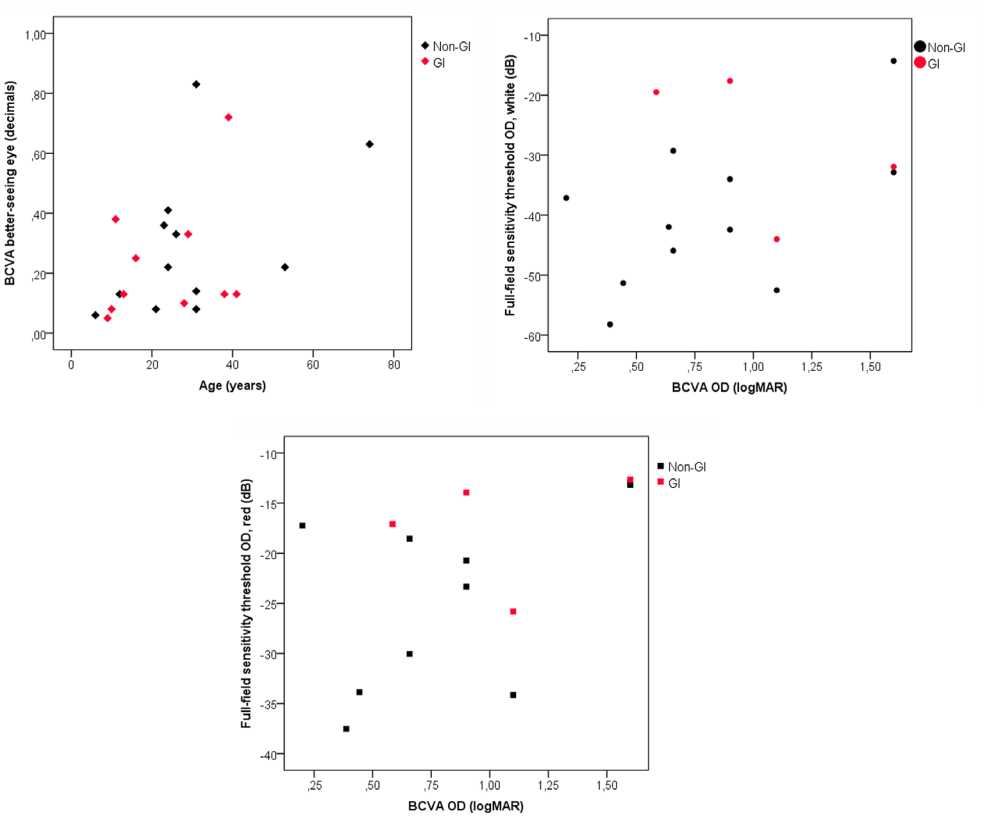

Figure 1. Visual function in patients with *CRB1*-associated retinal dystrophies. Patients from the genetic isolate (GI) are indicated in red, and the other patients are indicated in black. The top row, left panel shows the best-corrected visual acuity (BCVA) as plotted against age. A higher BCVA is seen in older patients (Spearman's rho = 0.435; $p = 0.043$), possibly because the inclusion criterion of a BCVA ≥ 0.05 allows only for the inclusion of either young patients with a severe phenotype, or patients with a mild phenotype, who have a wider window of opportunity for study inclusion. **The top row, right panel** shows the full-field sensitivity threshold to white light (in dB), plotted against BCVA. Although sensitivity thresholds to white stimuli appeared better (signified by lower dB) in patients with a better BCVA, this trend was not significant (Spearman's rho = 0.344; $p = 0.209$). **The second row** shows full-field sensitivity threshold to red light (dB), plotted against BCVA. Again, sensitivity thresholds to red stimuli appeared lower in those with a higher BCVA. This trend seemed more strongly apparent for the red stimuli than for the white stimuli, but was still not significant (Spearman's rho = 0.493; $p = 0.073$).

Ocular and fundus features

The median spherical equivalent of the refractive error, as averaged between both eyes, was +3.1D (IQR 4.0, range -5.75D - +7.06D). The median eye axis length was 20.7 mm, with a median anterior chamber depth of 2.8 mm. Nanophthalmos (axial length < 20.5 mm) was observed in eight patients (36%). Cataracts, defined as significant opacification of the lens, were observed in 11 eyes of six patients (27%), and 3 patients (14%) were pseudophakic in at least one eye. Corneal topography could be reliably performed in 20 patients and revealed a mild to moderate with-the-rule astigmatism in 15 patients (75%), a mild oblique astigmatism in 1 patient (5%), and a mild against-the-rule astigmatism in 1 patient (5%). One patient had a mild forme fruste keratoconus (patient ID-21), and 3 more had a corneal topography suspicious of a mild forme fruste keratoconus, which would need to be confirmed upon follow-up (patients ID-11; ID-15; ID-22). Vitreous abnormalities in 14 patients (63%) included cells (n = 12), veils (n = 3), and asteroid hyalosis (n = 1). Two patients had undergone a pars plana vitrectomy with inner limiting membrane peeling due to cystoid macular oedema (patients ID-17 and ID-19) or the presumably erroneous diagnosis of intermediate uveitis, which was unresponsive to lengthy immunosuppressive treatment. Fundus features were variable, even within the genetic isolate (Figures 2 and 3), but all patients had macular alterations of the retinal pigment epithelium, and 16/22 patients (73%), aged 10-53, had atrophic changes of the macula. Drusen within or hamartomas around the optic nerve head were seen in seven patients (32%), and were observed more frequently in patients from the genetic isolate (6/10 versus 1/12; $p = 0.02$). Fine yellow punctate spots in the peripheral retina were observed in eight patients (36%), all with RP, and were more prominent in the nasal periphery (Figure 2). Intraretinal pigment migrations were present in 21/22 patients with RP or CORD (95%; aged 6-74 years), and not in the patient with a macular dystrophy. These pigment migrations were bone-spicule-like (n = 5), nummular (n = 1) or a combination of both (n = 15). Preservation of the para-arteriolar retinal pigment epithelium (PPRPE) was observed in 3/22 patients (14%). Retinal vascular changes included a unilateral preretinal haemorrhage of unknown origin in patient ID-8, aged 10, and parapapillary hard exudates in patient ID-5 (Figure 2), whose affected younger sister (not included in the current study) had unilateral Coats-like exudates. Furthermore, vascular attenuation was featured in 18/22 patients (82%), but was absent in the macular dystrophy patient (age 24) and in the youngest RP patients (aged 6-10), considerably mild in the adolescent RP patients (aged 12-16), and was mild in the CORD patients (aged 31 and 39).

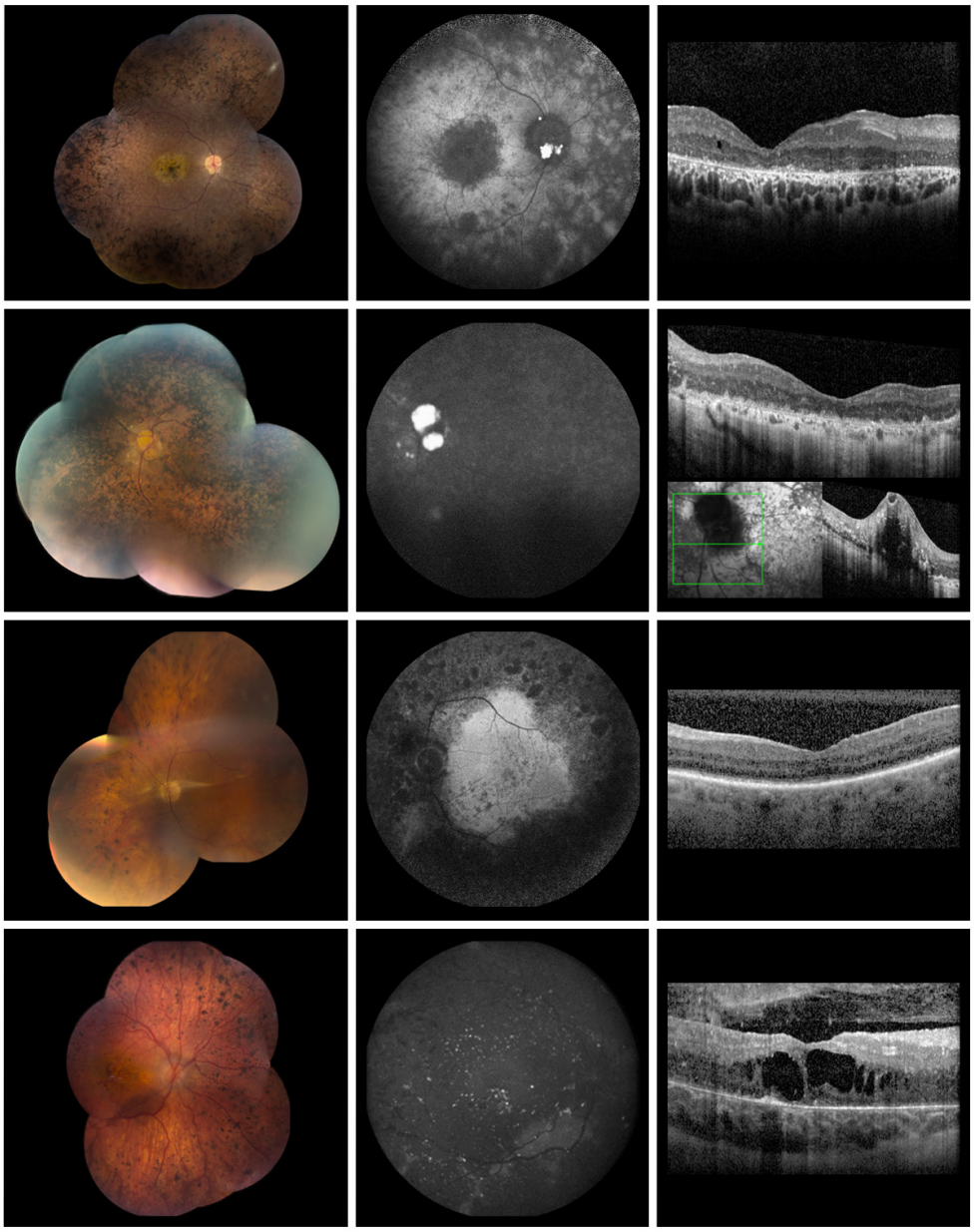

Figure 2. Multimodal imaging in patients with *CRB1*-associated retinitis pigmentosa (RP). The **top row** shows the right eye of patient ID-9, a 28-year-old female patient from the genetic isolate (GI) with a decimal best-corrected visual acuity (BCVA) of 0.03, whose fundus (left panel) showed the typical RP-associated changes of bone-spicule-like pigmentation in the (mid)periphery, vascular attenuation and optic disc palor, but also large parapapillary hamartomas, atrophy of the retina and retinal pigment epithelium (RPE) in the posterior pole, and white flecks resembling reticular pseudodrusen throughout the retina, associated with outer retinal atrophy. Fundus autofluorescence (FAF; middle panel) showed large meshwork-like areas of hypo-autofluorescence in the midperiphery and central macula,

with relatively spared perimacular autofluorescence. Spectral-domain optical coherence tomography (SD-OCT; right panel) showed inner retinal thickening, outer retinal atrophy, (para)foveal granular remnants of the hyperreflective outer retinal bands, but no laminar disorganization. The **second row** shows the left eye of patient ID-5, a 41-year-old male patient from the GI (BCVA 0.04). The fundus (left panel) was profoundly atrophic with dense intraretinal pigmentation, parapapillary hamartomas, fine drusenoid deposits in the nasal periphery, and parapapillary hard exudates that indicate a vascular disease component. FAF (middle panel) showed a near absence of autofluorescence. SD-OCT (right panel) showed mild laminar disorganization, a relatively thickened inner retina, outer retinal atrophy with a severely disrupted ellipsoid zone (EZ) and a barely identifiable external limiting membrane (ELM), and small hyperreflective foci mainly in the inner nuclear layer and ganglion cell layer. SD-OCT of the optic disc (right panel) showed the hamartoma, and dense hyperreflective foci colocalizing with the parapapillary exudates, but no source of the exudation. The **third row** shows the left eye of patient ID-20, a 74-year-old male patient from outside the GI (BCVA 0.29), which showed typical RP-associated changes (left panel), along with mild macular RPE alterations. FAF (middle panel) was relatively preserved in the posterior pole with scattered round hypo-autofluorescent lesions and severe hypo-autofluorescence outside the vascular arcades. SD-OCT (right panel) showed a relatively preserved outer nuclear layer, ELM, and EZ. The **fourth row** shows the right eye of patient ID-8, a 10-year-old female patient from the GI (BCVA 0.08), whose fundus (left panel) showed bone-spicule-like and round intraretinal pigmentation, atrophy in the posterior pole, a preretinal bleeding under the central macula, some preservation of the peri-arteriolar RPE in the inferior and superior periphery, and limited fine drusenoid deposits, mostly in the nasal midperiphery. FAF (middle panel) showed severely decreased autofluorescence, with some relative preservation along the inferior vascular arcade, and sharply circumscribed hyperautofluorescent speckles, which may be photoreceptor debris containing lipofuscin precursors. These speckles were also found in patient ID-18. SD-OCT (right panel) showed cystoid macular oedema, a thickened inner retina, and a severely atrophic ELM and EZ, with scattered para- and perifoveal remnants.

Perimetry

Visual field areas, averaged between right and left eyes, were large in 8/22 patients (36%), intermediate in 12/22 (55%) and small in 2/22 patients (9%). Visual field areas did not significantly correlate to age ($p = 0.25$ and $p = 0.61$ for the V4e and I4e isopter).

Microperimetry could not be reliably performed in the two youngest patients and was challenging to perform in patients with severe visual impairment: between the test run using the 4-levels-fixed strategy, and the examination using the 4-2 strategy, the preferred retinal locus had shifted in nine eyes of six patients (three eyes with low vision; five eyes with severe visual impairment; and one blind eye). The average threshold of the macular sensitivity on microperimetry was 8.0 dB (SD 6.9; range 0.2-25.3; normal range 26.0-36.0 dB; Table S2). Macular sensitivity was significantly lower in eyes with decreased fixation stability, as indicated by a higher BCEA 95%, averaged between right and left eyes (Spearman's rho = -0.478; $p = 0.045$). Inter-eye symmetry was very high for the average threshold of the macular sensitivity (Spearman's rho = 0.821; $p < 0.0001$) and V4e visual field area (Spearman's rho = 0.955; $p < 0.00001$; Figure S3). There was no significant difference between patients from within and from outside the genetic isolate in visual field areas for the V4e ($p = 0.74$) and I4e ($p = 0.60$), or macular sensitivity ($p = 0.66$).

Rod and cone function

Dark- and light-adapted full-field ERG responses were nondetectable in 12/20 patients (60%; aged 12-73; Table 1). Dark-adapted FST thresholds were obtained in 15 subjects (28 eyes for white stimulus; 27 eyes for red and blue stimuli), nine of whom had no detectable dark- or light-adapted responses on ERG, and two more with nearly non-detectable responses. General FST sensitivity for the white stimulus, averaged between eyes, ranged between -56.2 and -13.4 dB (mean -36.1; SD 12.7). Sensitivity thresholds were mediated by a mixed rod-cone response (17/27 eyes; 63%) or were rod-mediated (10/27 eyes; 37%, which included the four eyes of two patients with cone-rod or macular dystrophy). The FST thresholds for red stimuli, which are typically mostly cone-mediated, were not significantly associated with biomarkers on SD-OCT (Table 2). The median inter-eye difference in FST threshold was 4.0 dB (IQR 7.8; range 0.24-14.0). For each eye, each threshold was tested three times. The results of the three intra-ocular measurements were generally consistent (median largest within-visit difference between each pair of measurements of the white stimulus threshold was 2.07 dB; IQR 1.84; range 0.31-8.62).

Table 2. Structure and function correlations in *CRB1*-associated retinopathies

	Foveal EZ width		PR+RPE thickness		CRT**	
Visual function parameter	**Spearman's rho**	***p*-value**	**Spearman's rho**	***p*-value**	**Spearman's rho**	***p*-value**
BCVA (logMAR)	-0.581	0.007	0.656	**0.001**	0.424	0.070
Average threshold for MS (dB)	0.433	0.064	0.489	0.029	0.449	0.062
Central sensitivity* (dB)	0.506	0.027	0.502	0.024	0.424	0.080
BCEA 63%	-0.385	0.127	-0.453	0.059	-0.159	0.557
BCEA 95%	-0.385	0.127	-0.453	0.059	-0.159	0.557
Seeing retinal area V4e	-0.095	0.691	-0.226	0.324	-0.144	0.557
Seeing retinal area I4e	0.123	0.605	0.097	0.674	0.181	0.457
FST thresholds white light (dB)	0.319	0.289	0.319	0.267	0.140	0.665
FST thresholds red light (dB)	0.396	0.181	0.358	0.208	0.259	0.417

Values for all parameters were averaged between the right and left eye for each patient. When data were available for one eye only, the data for that eye were used. For measurement of the EZ width, values were measured from the central fovea. If the EZ band was interrupted and continued in the peripheral macula, this peripheral EZ was not included in the EZ width. Significance level was set at $p < 0.002$ following Bonferroni correction.

BCEA = bivariate contour ellipse area, BCVA = best-corrected visual acuity, CRT = central retinal thickness, EZ = ellipsoid zone, FST = full-field stimulus testing, MS = macular sensitivity on microperimetry, PR = photoreceptor layers, RPE = retinal pigment epithelium.

*Sensitivity at the central fixation point, as measured with microperimetry, which was the fovea in 17/39 eyes (44%), and an eccentric location in the other eyes.

**Patients with cystoid macular oedema, and therefore increased central retinal thickness, were not included in the analysis of correlations between central retinal thickness and visual function parameters.

Imaging

Spectral-domain optical coherence tomography (SD-OCT) showed bilateral cystoid macular oedema (CME) in the inner nuclear layer (INL) of 8/21 patients (38%), two of whom also had CME in the ONL. Patient ID-11 also had macular retinoschisis with splitting in the outer plexiform layer. Patients with CME seemed younger (mean age 21.1; SD 10.4; range 10-39) than those without CME (mean age 31.9; SD 17.1; range 9-74), although this difference was not statistically significant (p = 0.13). However, decimal BCVA was higher in patients with CME (median 0.25; IQR 0.43; range 0.07-0.72) than in those without CME (median 0.10; IQR 0.10; range 0.05-0.46; p = 0.03). CME was mostly refractory to treatment (Table S3). A mild epiretinal membrane was observed in nine patients (41%; bilateral in 6/9), and patient ID-11 had a lamellar pseudohole in one eye. The retinal laminar organization was relatively disorganized in 8/21 patients (38%), had a coarse aspect but no disorganization in 8/21 patients (38%) and was normal in 5/21 patients (24%; Figure S2). No outer retinal tubulations were observed.

The outer retinal layers at the (para) fovea, that is, outer nuclear layer and hyperreflective outer retinal bands (external limiting membrane and ellipsoid), showed moderate to severe disintegration in 14/21 patients (67%) aged 13-53, with a near absence of these layers in three of these patients, but only mild thinning in 7/21 patients (33%) aged 9-74. In the more peripheral macula, the outer retinal layers were nearly absent in 5/21 (24%), markedly attenuated in 10/21 patients (48%) and only mild thinning in 6/21 patients (29%). The general pattern consisted of more severe outer retinal attenuation in the peripheral macula with relative foveal remnants of the outer retina, with the exception of patients ID-10, ID-21 and ID-22, who had more intact outer retina in the peripheral macula. Figure S4 shows the horizontal width of detectable uninterrupted ellipsoid zone band for each patient. No significant correlations were found between foveal retinal thickness or the horizontal width of visible ellipsoid zone and visual function parameters after correction for multiple testing, but the PR + RPE thickness correlated significantly with BCVA (p = 0.001; Table 2). Mild to severe thickening of the inner retinal layers was observed in 20/21 patients (95%), which was not correlated to thinning of the outer retina and RPE (Figure S4). Fundus autofluorescence (FAF) images showed intra- and interfamilial variability in the degree of retinal pigment epithelium preservation (Figures 2 and 3), ranging from generalized severely decreased or (nearly) absent autofluorescence (n = 4; aged 13-41 years), to relative preservation of autofluorescence in the posterior pole or around the vascular arcade (n = 10; aged 11-74 years), to a generally normal or mildly hypo-autofluorescent retina with zones of granular or bone-spicule-like hypo-autofluorescence (n = 3; aged 16-31 years). Patients with CORD had different patterns of macular hypo-autofluorescence. A hyperautofluorescent ring was only observed in patient ID-22 with a macular dystrophy.

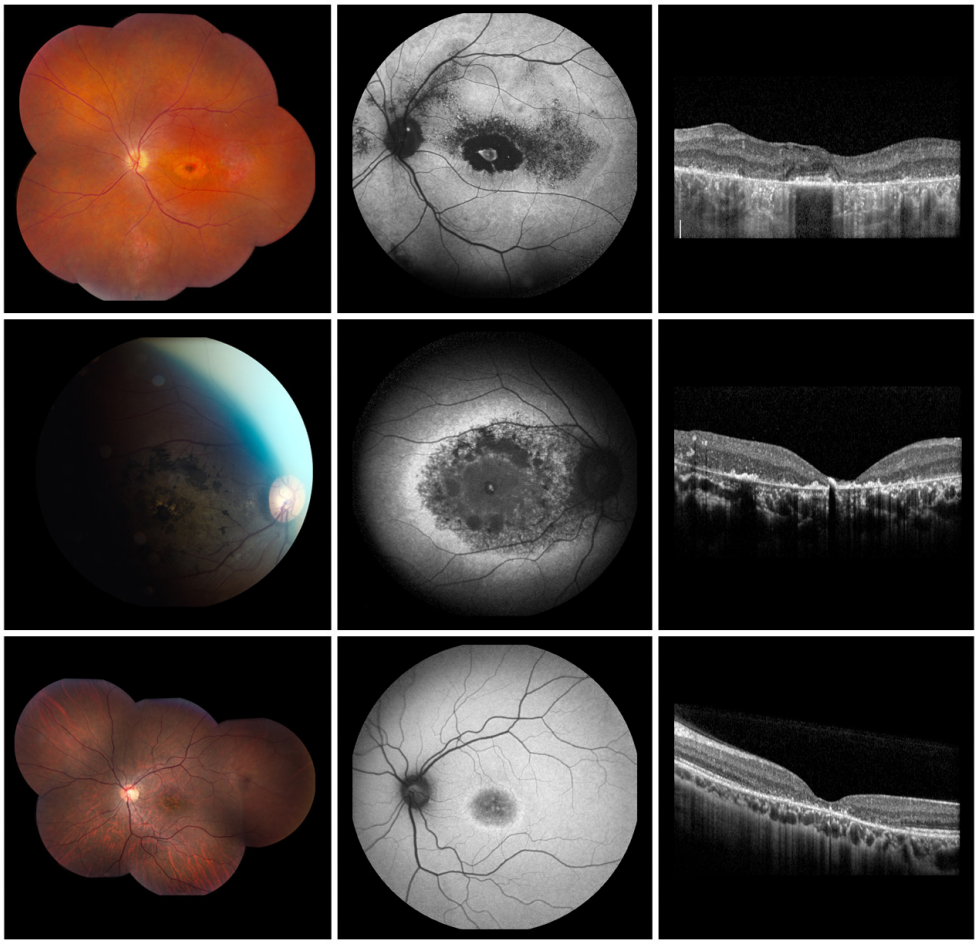

Figure 3. Multimodal imaging in patients with *CRB1*-associated cone-rod or macular dystrophy. The **top row** shows the left eye of patient ID-10, a 39-year-old male patient from the genetic isolate with cone-rod dystrophy (CORD) and a decimal best-corrected visual acuity (BCVA) of 0.72, whose fundus (left panel) showed mild parapapillary atrophy, mild vascular attenuation and stretching in the periphery, atrophy of the retina and retinal pigment epithelium (RPE) in a parafoveal ring around a hyperpigmented fovea, along with RPE alterations in the temporal posterior pole and regional chorioretinal atrophy with limited bone-spicule-like pigmentation in the inferior periphery. Fundus autofluorescence (FAF; middle panel) showed a small optic disc druse, a parafoveal ring of absent autofluorescence, surrounded by dense granular hypo-autofluorescence with scattered hyperautofluorescent lesions mainly in the superior and temporal posterior pole. These granular changes are also visible along the superior vascular arcade, and in the nasal and inferior midperiphery. Spectral-domain optical coherence tomography (SD-OCT) (right panel) showed a thickened inner retina, a severely attenuated outer nuclear layer (ONL) and relative sparing of the hyperreflective outer retinal bands in the fovea and to a lesser extent in the peripheral macula, with severe disruption in the parafoveal region. The **second row** shows the right eye of patient ID-21, a 31-year-old female patient of Caribbean origin (Dutch Antilles) with CORD (BCVA 0.08), which showed (left panel) relative preservation of the optic disc colour and vascular calibre, but atrophy of the retina and RPE in the posterior pole, with large round and bone-spicule-like pigmentations that partially fused, and some bright RPE alterations in the

central macula. The (mid)peripheral retina (not shown here) was well-preserved. FAF (middle panel) showed a large parafoveal area of even hypo-autofluorescence around a foveal hyperautofluorescent lesion, surrounded by patches of absent autofluorescence and granular hypo-autofluorescence. A broad and mildly hyperautofluorescent ring encircled this region of hypo-autofluorescent changes. SD-OCT (right panel) showed a mildly thickened inner retina and a profoundly atrophic outer retina, and a relatively preserved laminar structure. The **third row** shows the left eye of patient ID-22, a 24-year-old female patient of North-African origin with macular dystrophy (BCVA 0.14), whose fundus (left panel) showed a mild temporal pallor of the optic disc, atrophic macular RPE alterations with visible choroid, and an otherwise normal fundus. FAF (middle panel) showed hypo-autofluorescent changes in the central macula, encircled by a hyperautofluorescent ring, surrounded by normo-autofluorescent retina. The granular hypo-autofluorescent changes corresponded with severe ONL atrophy, and disruptions of the external limiting membrane and the ellipsoid zone on SD-OCT (right panel).

DISCUSSION

In this prospective cross-sectional study, we performed detailed clinical examinations in patients with *CRB1*-associated IRDs, demonstrating a wide clinical spectrum. We define parameters that are potentially the most appropriate clinical and surrogate endpoints for a future *CRB1*-gene therapy trial, as well as risk factors for potential adverse treatment reactions. Complementary to time-dependant endpoints, which may remain stable for years and which we investigated earlier in the largest retrospective longitudinal study to date, potential surrogate endpoints were evaluated in this cross-sectional study through careful structure-function correlation analysis. Diagnoses included RP (86%), CORD (9%) and macular dystrophy (5%). Interfamilial variability and a degree of intrafamilial variability were observed on ophthalmoscopy and retinal imaging, with both adulthood-onset CORD and infancy-onset RP present in the same genetic isolate, in patients carrying the same homozygous mutation. Generally, interfamilial variability was greater than intrafamilial variability. There was a wide range of age at initial symptom onset, although half of RP patients reported this onset to be either in infancy or first years of life. Patients with a cone-rod or macular dystrophy had a later symptom onset, which follows some reports of *CRB1*-associated macular dystrophies that have a decidedly milder phenotype than *CRB1*-RP.[35]

Contrasting the progressive nature of *CRB1*-associated disease in individual patients,[6, 7] in this cross-sectional study we found lower visual acuities in younger patients. This counterintuitive finding may be a result of the inclusion criterion of a BCVA ≥ 0.05, which could result in a narrow and particularly early-in-life window for study inclusion in patients with severe early-onset RP, while posing a broader window for inclusion in patients with mild RP or CORD, who maintain a better BCVA into mid-adulthood. Our results show that despite the previously demonstrated intra-individual progressive visual deterioration,[6, 7] at a cross-sectional population level no simple relationship is found between age and visual function parameters, such as BCVA and visual field area.

Spectral-domain optical coherence tomography (SD-OCT) revealed CME in 38% of patients in this cohort, which is a comparable number to earlier reports of CME in genetically diverse or undifferentiated RP cohorts.[36, 37] Patients with CME in this cohort were on average 10 years younger and had a significantly better BCVA than those without CME, despite the trend for a lower BCVA in younger patients at a cross-sectional level. This suggests that CME is an early disease feature in *CRB1* retinopathy. Follow-up SD-OCTs in the same patients should reveal whether the CME reduces after an extended period of time and whether the macula is more strongly degenerated in areas of previous cystoid maculopathy. The aetiology for CME in RP is not fully clear. Müller cell dysfunction and oedema has been posed as a potential cause of CME,[38] which is supported by the location of the CME in the INL. Müller cells have already been shown to play an important role in the etiology of *CRB1*-associated vascular abnormalities in mice and rats.[39, 40] Earlier fluorescein angiography in three cases of *CRB1*-retinopathy has shown no leakage in patients with CME,[10, 41] in contrast with extensive leakage starting from the early phases in Coats-like exudative vasculopathy,[42] which is a recurring finding in *CRB1*-retinopathies.[22, 43] Our current findings of parapapillary hard exudates in a 41-year old patient and a preretinal haemorrhage of unknown origin in a 10-year-old patient further point to a vascular component of *CRB1* retinopathies. The parapapillary hard exudates may be a precursor of Coats-like exudative vasculopathy, which the older and blind sibling of this patient had been diagnosed with, but SD-OCT of the optic disc revealed no source of the exudation. A preretinal haemorrhage as in the 10-year-old has been described in *CRB1* retinopathies once before in two affected sisters (aged 3 and 5) undergoing interventions in the alternative circuit involving the Valsalva manoeuvre and repeated intense pressure on the soft palate, where these haemorrhages were bilateral and self-limiting.[44] Other findings on SD-OCT included thickening of the inner retina (95%), and coarsening (38%) or disorganization (38%) of the retinal laminar structure. Inner retinal thickening has been observed in human patients with *CRB1* retinopathy,[45] and has also been demonstrated in mice lacking CRB1 and CRB2 in retinal progenitor cells, in which the thickening was caused by a proliferation of retinal progenitors which resulted in an increase in the number of rod photoreceptors, Müller cells and bipolar cells.[46] A recent mouse study showed a role for Crb2 in the thickening of the ganglion cell layer due to ectopic photoreceptors.[47] Some studies have shown a milder degree of inner retinal thickening in association with ONL thinning, for example in *RHO*- and *RPGR*-associated retinopathies.[48, 49] These studies have suggested that this inner retinal thickening may be due to a remodelling process, and may precede eventual inner retinal atrophy.[48, 49] Retinal remodelling could possibly interfere with a functional (gene) therapeutic effect. However, in this cohort, inner retinal thickness was not associated with thinning of the outer retina and RPE, indicating that the cause for the inner retinal thickening is more likely the presence of ectopic photoreceptors, or Müller cell or bipolar cell proliferation, rather than remodelling.

In some cases, the *CRB1*-associated phenotype appears associated with the genotype. For instance, optic nerve head drusen or hamartomas were more prevalent in patients from the genetic isolate

in our cohort (p = 0.02), confirming the findings in our previous retrospective study.[6] Patients from the genetic isolate did not have significant differences in visual function with patients from outside the genetic isolate. Another genotype-phenotype correlation in this study involved the p.(Ile167_Gly169del) mutation, found in homozygous form in patient ID-22, who had an isolated maculopathy, and which has been reported before in most cases of isolated maculopathy, even in compound heterozygosity.[1, 5, 35, 50] *CRB1*-associated maculopathy has also been reported in association with other mutations.[4, 51] It should be noted that in our study, the p.(Tyr631Cys) mutation, which to our knowledge has not been described outside of this cohort, was observed in two patients with an unusually mild phenotype, characterized by preserved BCVA, a normal retinal laminar organization and preservation of outer retinal structures into the 4th and 8th decade of life. The p.(Pro836Thr) mutation was found in association with CORD in this study (patient 21; originally from the Caribbean), but has been reported before in association with early-onset retinal dystrophy in a Malinese patient.[52] As genotype-phenotype correlations do not explain the contrasting phenotypic findings in this cohort or the intrafamilial phenotypic variability, there may be a role for genetic and/or environmental modifiers. Disruption of one allele or two alleles of *Crb2* has recently been shown to aggravate the *Crb1*-associated phenotype in mice,[47] and may be an avenue for further investigation.

Implications of this study for therapeutic interventions such as gene therapy include the assessment of the phenotype's amenability to treatment, identification of potential risk factors and the evaluation of structure function correlations. The amenability of the *CRB1*-associated phenotype to treatment is supported by the variable degrees of EZ preservation on SD-OCT, and the (near-) normal laminar structure in 24% of patients, and laminar coarsening without disorganization in another 38% of patients. However, the visual deterioration in young patients, and the universal presence of macular RPE alterations or atrophy appears to suggest that intervention is desirable before the 3rd or 4th decades of life. Some retinas may have been weakened by the presence of CME, as is illustrated by the presence of lamellar pseudohole in one patient, and this may be a contra-indication for intervention using subretinal injection due to the risk of for instance macular hole formation. Generally, intra-ocular surgery in nanophthalmic eyes has been associated with a higher intra-operative and postoperative complication rate,[53, 54] although no data have been published on the technical challenges in subretinal gene therapy surgery in nanophthalmic eyes. Appropriate preoperative assessment and careful intra-operative measures should be taken in these high-risk eyes. A risk factor for complications in a future gene therapy trial is the narrow anterior chamber angle, as the associated risk of acute angle-closure glaucoma may be increased by repeated mydriasis and dark adaptation during a trial, as happened with patient ID-15 in this study. In clinical practice, biometry and assessment of the anterior chamber angle and intra-ocular pressure are useful in patients with *CRB1* retinopathies, and at-risk patients should be instructed on alarm features. In the case of acute angle-closure glaucoma, prophylactic peripheral iridotomy in the contralateral eye may be indicated.

An important assumption in gene therapy trials where one eye is treated in each patient, and the nontreated eye is used as a control, is the symmetry and thus the comparability in visual function between eyes. We have found statistically significant intra-individual between-eye symmetry in all measures of visual function in our study, with a moderate degree of symmetry in BCVA, and very high degrees of symmetry in sensitivity thresholds on full-field stimulus testing and microperimetry, and in seeing retinal areas on Goldmann visual fields. Therefore, the nontreated contralateral eye is a suitable control in a future gene therapy trial for *CRB1*-associated IRDs.

The most robust structure-function correlation, and the only statistically significant one after stringent correction for multiple testing, was the foveal PR + RPE thickness, as measured from the ELM to the basal membrane, to BCVA. The granular aspect of the hyperreflective outer retinal bands, including the EZ, complicated the evaluation of the EZ diameter and may explain why EZ diameter proved a less robust correlation with function parameters, such as BCVA and macular sensitivity. In other forms of RP, EZ diameter has been sensitive in detecting disease progression,[55, 56] and further prospective follow-up measurements are necessary to test this sensitivity in *CRB1* retinopathies. On the other hand, substantial photoreceptor populations have been demonstrated with adaptive optics in areas of low or no EZ reflectivity, indicating that biomarkers on SD-OCT do not always accurately represent photoreceptor cytology.[57] Prospective longitudinal measurements of the biomarkers on SD-OCT need to be correlated to visual function decline in *CRB1* retinopathies, in order to assess their potential as a surrogate endpoint in a future clinical gene therapy trial. Performing microperimetry was challenging in patients with severe visual impairment and could not be reliably performed in the two youngest patients. Inconveniently, these patients groups are of particular interest for microperimetric evaluation, as severely visually impaired patients (over the age of 18) will be the most likely to be included in a phase I trial. The shifting of the preferred retinal locus between the test run and the examination, which occurred in six patients, might pose a challenge in the second measurement for these patients. Prospective longitudinal measurements will decide whether this is correct and whether microperimetry offers a reliable functional outcome parameter. In this study, FST was successful in determining retinal sensitivity thresholds in patients with a wide range of vision loss and loss of electrophysiological responses. FST response to red stimuli at a lower intensity is mostly cone-mediated and would thus provide a method for measuring changes in cone sensitivity. A limitation of FST is its inability to localize the retinal area mediating the sensitivity threshold. In future subretinal gene therapy trials, this area may not co-localize with the location of the retinal area that was treated with subretinal injection of the treatment vector. Nonetheless, it has been sensitive in detecting sensitivity changes in gene therapy trials, while BCVA proved less sensitive.[58] Based on our findings, changes in FST would have to exceed the variability threshold of 4 dB in order to be reliably attributed to a therapeutic effect.

In conclusion, this prospective cross-sectional study provides extensive phenotypic characterization of *CRB1*-associated retinopathies, which are a candidate for gene therapy. Longitudinal prospective measurements of the same parameters are necessary in order to assess which outcome measures are the most sensitive in detecting the rate of progression and potential treatment effect in a future gene therapy trial.

Acknowledgements

Funding/Support: This work was supported by the Curing Retinal Blindness Foundation (Ivyland, PA, USA), Stichting Blindenhulp (The Netherlands), Janivo Stichting (The Netherlands), and Bayer Ophthalmology Research Award (The Netherlands).

Financial Disclosures: The Leiden University Medical Center (LUMC) is the holder of patent application PCT/NL2014/050549, which describes the potential clinical use of CRB2; JW is listed as inventor on this patent, and JW is an employee of the LUMC.

Other Acknowledgements: The authors wish to acknowledge dr. Y.Y. Cheng (LUMC) for reviewing the corneal topography results.

All authors attest that they meet the current ICMJE criteria for authorship.

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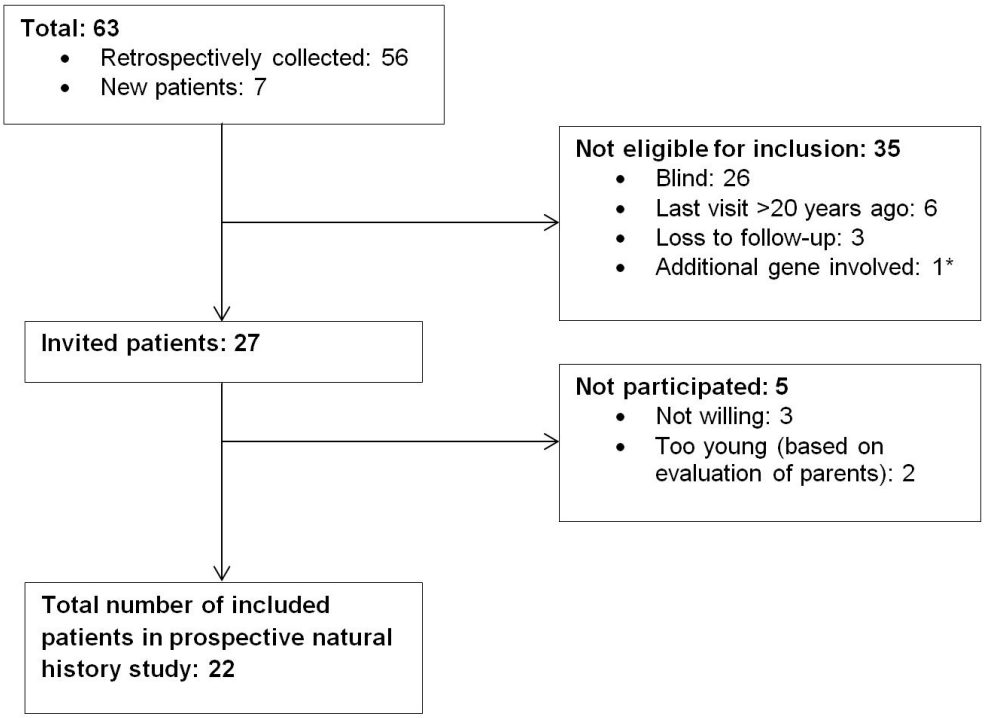
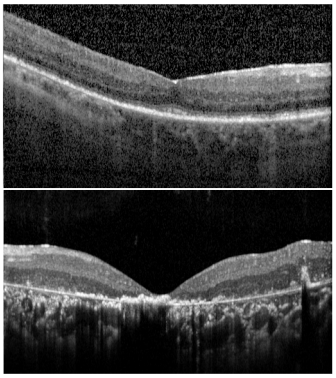
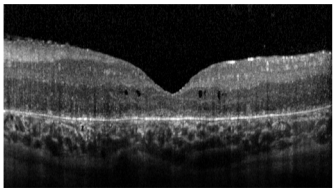
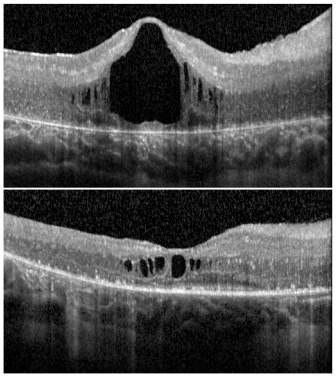
SUPPLEMENTAL MATERIAL



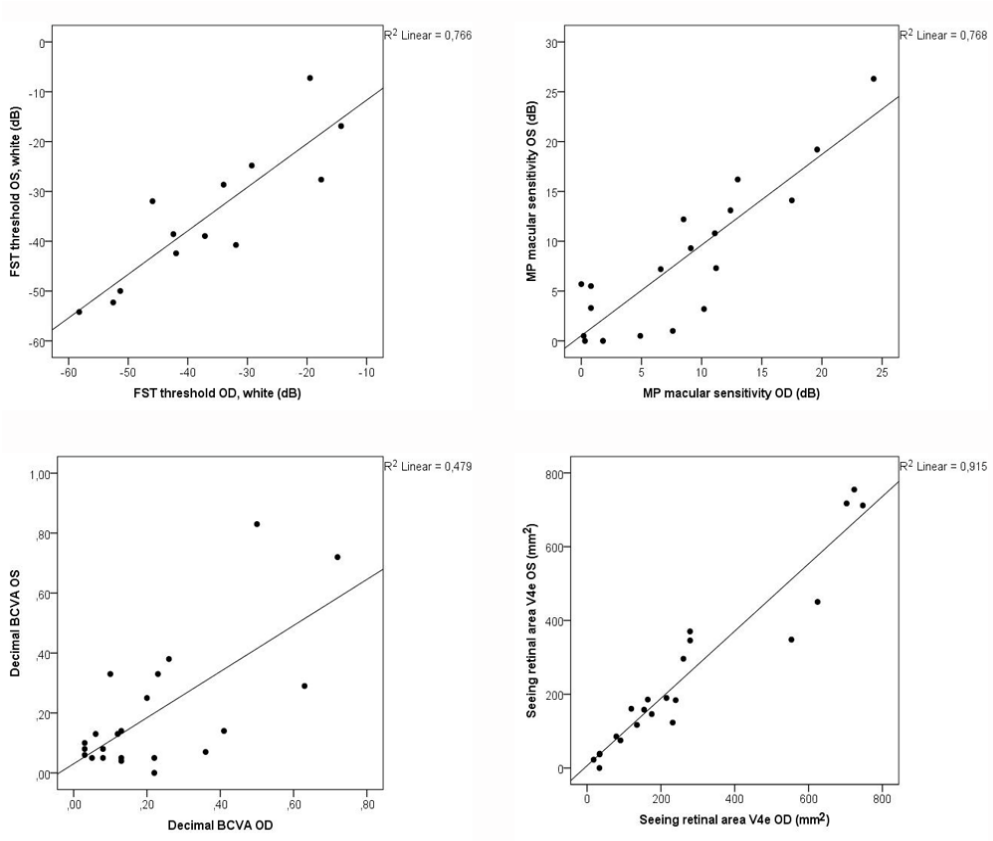
Supplemental Figure 1. Flowchart showing the patient outreach and inclusion process.

*One patient, the brother of patient 12, had a pathogenic variant in the *RS1* gene, as well as the same pathogenic *CRB1* variants as patient 12.

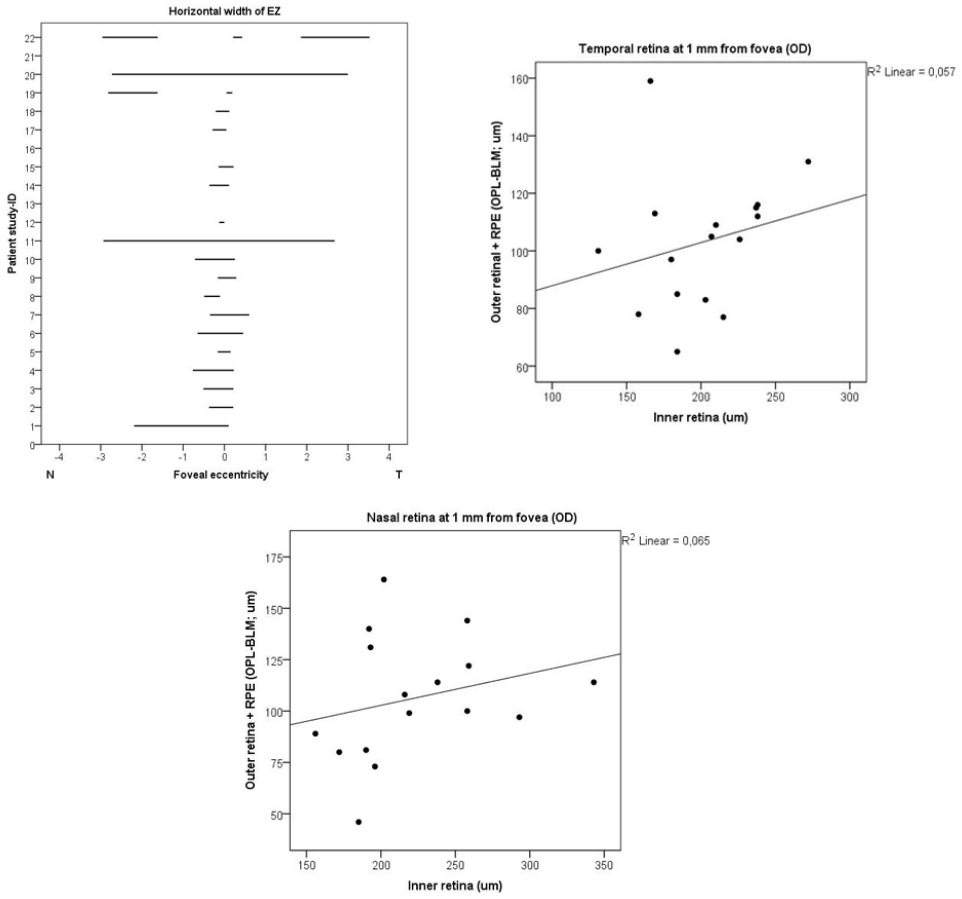
Laminar organization of retinal layers

Category	Examples	N (%); ages
1. Normal lamination. Outer retinal layers and the hyperreflective outer retinal bands may still be atrophic. Shown in the upper row: ID-20; second row: ID-21.		5 (24%); ages 24-74 years
2. No disorganization, all separate layers are identifiable. However, coarse aspect of layers. Attenuation of the OPL may locally obscure distinction between INL and ONL. Shown in the third row: ID-3.		8 (38%); ages 12-53 years
3. Mild to moderate disorganization: not each layer is fully discernible throughout the macula,with the impression of coalescence of the inner retinal layers. Hypo-reflective layers have increased reflectivity. Shown in the fourth row: ID-2. Shown in the fifth row: ID-6.		8 (38%); ages 9-41 years

Supplemental Figure 2. Evaluation of laminar organization and retinal architecture on spectral domain optical coherence tomography (SD-OCT) in patients with *CRB1*-associated retinal dystrophy. INL = inner nuclear layer. ONL = outer nuclear layer. OPL = outer plexiform layer. Patients with normal laminar architecture were not overall younger than those with relative disorganization, as disorganization was seen across age groups.



Supplemental Figure 3. Scatterplots of between-eye symmetry of visual function in patients with *CRB1*-associated retinal dystrophies. Top row, left panel. Sensitivity thresholds on full-field stimulus testing (FST) of white stimuli show a very high degree of between-eye symmetry (Spearman's rho = 0.857; p = 0.0002). **Top row, right panel.** Average thresholds for the macular sensitivity on microperimetry (MP) show very high between-eye symmetry. **Second row, left panel.** Best-corrected visual acuity (BCVA) show a moderate between-eye symmetry (Spearman's rho = 0.467; p 0.028). **Second row, right panel.** Seeing retinal areas in mm² for the V4e isopter on Goldmann visual fields show a very high degree of between-eye symmetry.



Supplemental Figure 4. Quantitative analysis of biomarkers on spectral-domain optical coherence tomography (SD-OCT) in *CRB1*-retinopathies. The left panel shows the horizontal extent of the ellipsoid zone (EZ) plotted as a function of foveal eccentricity in mm for each patient. The right eye was used for analysis. Negative values indicate the nasal (N) extent of EZ. In patient 13, no EZ-band was detected in the central scan of the macula. Patient 16 did not undergo SD-OCT analysis, and in patient 21 the hyperreflective outer retinal bands including the EZ were indistinguishable from other hyperreflective structures, such as retinal pigment epithelium and intraretinal pigment migrations (but the EZ was intact in the peripheral macula). Aside from the (para)foveal EZ, all patients had isolated regions of intact EZ, separated from the (para)foveal EZ by areas of absent hyperreflective outer retinal bands, except for patient 11, who had a fully intact EZ. The middle and right panel show the relation between the inner retina, as measured from the vitreoretinal interface to the inner border of the outer plexiform layer (OPL), and the outer retina and retinal pigment epithelium (RPE) complex, as measured from the OPL to the basal membrane (BM), as measured at 1 mm from the fovea in the temporal (middle panel) and nasal (right panel) retina. Thickness of the outer retina and RPE complex and the inner retinal thickness were not correlated at 1 mm temporally (Spearman's rho = 0.367; p = 0.162) or nasally (Spearman's rho = 0.424; p = 0.102) from the fovea. Patients who had cystoid macular edema lesions at 1 mm from the fovea were not included in this analysis.

Supplemental Table 1. *CRB1* mutations in this study

	Allele 1		Allele 2		
ID	**Mutation**	**Effect**	**Mutation**	**Effect**	**Phenotype**
1-10	c.3122T>C	p.(Met1041Thr)	c.3122T>C	p.(Met1041Thr)	RP, CORD
11*	c.2983G>T	p.(Glu995*)	c.1892A>G	p.(Tyr631Cys)	Mild RP
12	c.2693A>C	p.(Asn898Thr)	c.2693A>C	p.(Asn898Thr)	RP
13	c.2843G>A	p.(Cys948Tyr)	c.3122T>C	p.(Met1041Thr)	RP
14, 15	c.2290C>T	p.(Arg764Cys)	c.2983G>T	p.(Glu995*)	RP
16**	c.929G>A	p.(Cys310Tyr)	c.2933T>C	p.(Phe978Ser)	Severe RP
17	c.2843G>A	p.(Cys948Tyr)	c.1892A>G	p.(Tyr631Cys)	RP
18	c.2234C>T	p.(Thr745Met)	c.2842+5G>A	p.(?)	RP
19	c.2234C>T	p.(Thr745Met)	c.1602G>T	p.(Lys534Asn)	RP with severe early macular involvement
20	c.2945C>A	p.(Thr982Lys)	c.1892A>G	p.(Tyr631Cys)	Mild RP
21***	c.2506C>A	p.(Pro836Thr)	c.2842T>A	p.(Cys948Ser)	CORD
22	c.498_506del	p.(Ile167_Gly169del)	c.498_506del	p.(Ile167_Gly169del)	Macular dystrophy

The mutation notation is based on the NM_201253.2 nomenclature.

*This patient also had the variant c.1892A>G (p.(His631Arg)) in the *RPGRIP1* gene, which was classified as a variant of unknown significance.

**This patient also carried the heterozygous variant c.2991+1655A>G (p.(Cys988*)) in *CEP290*.

***This patient was originally from the Caribbean (Dutch Antilles).

Supplemental Table 2. Retinal sensitivity measurements in *CRB1*-associated retinal dystrophies

		Average macular sensitivity (dB)		Foveal sensitivity		Fixation		FST threshold for white stimulus (dB)	
ID/Sex	**Age**	**OD**	**OS**	**OD**	**OS**	**OD**	**OS**	**OD**	**OS**
1/M	29	1.8	0.0	5.0		Unstable	Unstable	NP	NP
2/F	13	9.1	9.3	14.0	14.0	Stable	Stable	NP	NP
3/F	16	12.4	13.1	17.0	21.0	Stable	Stable	NP	NP
4/F	38	0.0	5.7		16.0	Unstable	Unstable	NP	NP
5/M	41	4.9	0.5	14.0		Stable	Rel. unstable	-17.6 (MM)	-27.6 (RM)
6/F	11	17.5	14.1	20.0	24.0	Stable	Stable	-19.5 (MM)	-7.2 (MM)
7/F	9	NA	NA					*	
8/F	10	10.2	3.2	22.0		Stable	Unstable	-44.0 (RM)	NP
9/F	28	0.8	5.5	0.0	5.0	Unstable	Unstable	-31.9 (MM)	-40.8 (MM)
10/M	39	11.1	10.8	24.0	24.0	Stable	Stable		
11/M	31	24.3	26.3	22.0	26.0	Stable	Stable		
12/F	26	8.5	12.2	15.0	21.0	Stable	Rel. unstable	-42.0**	-42.4 (RM)
13/M	21	0.8	3.3		20.0	Unstable	Unstable	-14.3 (MM)	-16.9 (MM)
14/F	24	11.2	7.3	27.0	0.0	Stable	Stable	-29.3 (MM)	-24.8 (MM)
15/F	31	6.6	7.2	12.0	14.0	Unstable	Stable	-34.0 (MM)	-28.7 (MM)
16/M	6	NA	NA					-32.9 (MM)	NP
17/M	23	7.6	1.0	19.0	0.0	Stable	Unstable	-51.3 (MM)	-50.0 (MM)
18/F	12	0.2	0.5	8.0		Unstable	Rel. unstable	-42.4 (RM)	-38.6 (MM)
19/M	53	4.4	NA	13.0		Rel. unstable		-45.9 (RM)	-32.0 (MM)
20/M	74	13.0	16.2	18.0	14.0	Stable	Stable	-37.1 (MM)	-39.0 (MM)
21/F	31	0.3	0.0	6.0	0.0	Stable	Rel. unstable	-52.5 (RM)	-52.3 (RM)
22/F	24	19.6	19.2	19.0	17.0	Stable	Unstable	-58.2 (RM)	-54.2 (RM)

The range of normal values for the average threshold of the macular sensitivity (dB) on microperimetry is 26 to 36 dB. Sensitivity thresholds to red and blue FST stimuli were used to determine whether these responses were rod-mediated (blue-red difference of >22 dB), cone-mediated (blue-red difference of <3 dB), or mixed rod-and-cone-mediated (blue-red difference between 3 and 22 dB). The normal FST thresholds for white stimuli was considered -56 dB, and should be rod-mediated.

FST = full-field stimulus testing. MM = mixed rod-cone mediation. NP = not performed. RM = rod-mediated. General sensitivity was obtained with white stimuli.

*FST was attempted in this young patient, but could not be reliably performed due to exhaustion.

**The white, red, and blue FST stimuli were tested for the left eye (the better-seeing eye), but not for the right eye, as the patient was exhausted. Therefore, it cannot be determined for the right eye whether the responses were predominantly rod-mediated, cone-mediated, or mediated by a mix of rods and cones.

Supplemental Table 3. Treatment histories for cystoid macular edema in *CRB1*-associated retinopathies

ID	Treatment	Age at treatment initiation	Treatment duration	Treatment response
2	Initially topical brinzolamide. After several months, switch to oral acetazolamide, due to irritation to the eyes.	8 years	5 years and ongoing	Refractory; Patient still has severe CME.
3	None; CME is mild	-	-	-
6	Currently none. Previously methotrexate, due to an initial diagnosis of panuveitis.	7 years	3-4 months	Mild to moderate, but insufficient. Temporary serum aminotransferase elevations.
8	Initial CME discovery during current study. Referral to own ophthalmologist, with the advice to start with topical brinzolamide (due to age), followed by acetazolamide in the case of non-response.	10 years	Ongoing	To be evaluated.
10	None; CME is mild	-	-	-
11	Oral acetazolamide, after insufficient response to topical brinzolamide.	30 years (acetazolamide since age 31)	1 year and ongoing	Refractory; Patient still has severe CME.
12	None; CME is moderate	-	-	-
17	Combination treatment of methotrexate with prednisone and oral acetazolamide, due to an initial diagnosis of intermediate uveitis. Due to non-response, intravenous methylprednisolone was administered, later followed by bilateral intravitreal triamcinolone depot, which resulted in glaucoma in the left eye. Glaucoma was treated and triamcinolone depots were removed. Eventually, bilateral pars plana vitrectomy and inner limiting membrane peeling was performed, and CME persisted nonetheless. Combination treatment of timolol and dorzolamide did not have a sufficient effect. Oral acetazolamide and oral prednisolone did not have a sufficient effect. Treatment was tapered to acetazolamide only, which did not have a sufficient effect on the CME.	13 years	9 years (patient quit acetazolamide use on his own initiative due to perceived inefficacy, followed by a shared decision with his ophthalmologist)	Refractory, but the patient currently has very mild remaining CME, consisting mostly of degenerative cystoid spaces.
20	Bilateral pars plana vitrectomy and inner limiting membrane peeling following insufficient response to intravitreal triamcinolone injection.	37 years (right eye); 39 years (left eye)	Several months	No remaining CME; only degenerative cystoid spaces.

CME = cystoid macular edema.