

# Prospective Evaluation of Patients With X-Linked Retinoschisis During 18 Months

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**PURPOSE.** Prospective evaluation of patients with X-linked retinoschisis (XLRs).

**METHODS.** Fifty-six males XLRs patients, age  $\geq 7$  years, had retinal structure and function tests performed every 6 months during an 18-month period.

**RESULTS.** Best corrected visual acuity (BCVA) was abnormal (mean  $\pm$  SD logMAR  $0.57 \pm 0.32$  OD and  $0.50 \pm 0.27$  OS), with weak correlation between visual acuity and age ( $R = -0.24$ ,  $P = 0.0095$ ). Mean cyst cavity volume (CCV) determined on optical coherence tomography showed weak correlation with age ( $R = -0.33$ ,  $P = 0.0009$ ) and no correlation with visual acuity. Subjects had modest reduction in mean kinetic and static perimetry results, reduced b-wave amplitude on electroretinography, abnormal reading speed results, and decreased visual function quality of life scores. Contrast sensitivity results were normal in 85 of 99 eyes tested. Most subjects had no meaningful change in BCVA during follow-up. Subjects who started carbonic anhydrase inhibitor (CAI) treatment at enrollment had improved BCVA (mean  $\pm$  SD change  $3.15 \pm 7.8$  ETDRS letters, with increase of  $\geq 15$  ETDRS letters at 8 of 110 visits [in 3 subjects]). There were no significant changes in other parameters tested.

**CONCLUSIONS.** Structural and functional results were stable during the 18-month follow-up period. Some patients starting CAI treatment at the baseline visit showed improvement in BCVA that was not correlated with changes in CCV. Natural history data such as these will be important for comparisons to the changes in measures of retinal structure and function following gene replacement therapy in patients with XLRs.

Keywords: retinoschisis, XLRs, cyst cavity volume

X-linked retinoschisis (XLRs), also known as X-linked juvenile retinoschisis, is an early-onset retinal degenerative disease and is one of the most common forms of juvenile-onset inherited retinal disease, having an estimated prevalence of 1 in 5,000 to 1 in 20,000,<sup>1</sup> with the highest rates in Scandinavia.<sup>2</sup> Characteristic features include mild to severe loss in central vision, radial streaks arising from foveal schisis, splitting of retinal layers in the peripheral retina, and a negative waveform electroretinogram (ERG) arising from a marked reduction in b-wave amplitude.<sup>3-8</sup> Affected males typically have vision of 20/60 to 20/120 on first presentation.<sup>7</sup> Disease progression and severity is highly variable even within families. During the course of the disease, secondary complications such as retinal tears, retinal detachment, and vitreous hemorrhage can occur, leading to a poor outcome. Female carriers are asymptomatic although detailed clinical examination can reveal subtle retinal abnormalities.<sup>9</sup>

XLRs is caused by sequence variations in the gene that encodes retinoschisin (RS1), a cell-surface adhesion protein expressed by photoreceptor and bipolar cells of the retina.<sup>1</sup>

The 24-kDa protein has two conserved sequence motifs: an initial signal sequence that targets the protein for secretion and the larger discoidin domain that is implicated in cell adhesion. RS1 helps to maintain the structural organization of the retinal cell layers and promotes visual signal transduction.

Mice deficient in RS1 have been developed and used to obtain insight into the role of RS1 in retinal structure, function, and pathology. Studies in these murine models of XLRs have shown that recombinant adeno-associated virus (rAAV) gene therapy vectors expressing normal RS1 can provide significant restoration of retinal structure and function in RS1-deficient mice.<sup>10-16</sup>

There is no specific treatment for XLRs. Previous reports suggest that administration of a topical or oral carbonic anhydrase inhibitor (CAI) may provide a reduction in the degree of schisis detected by optical coherence tomography (OCT) and improvement in visual acuity in some but not all patients,<sup>17-34</sup> but the use of CAIs in patients with XLRs is an off-label treatment.

As part of a prospective evaluation of individuals with XLR5, treatment with a topical CAI was initiated in 20 subjects who were not using a CAI before enrollment. We report here baseline values and changes over time in a variety of tests of retinal structure and function in these subjects compared to subjects using a CAI at enrollment who continued this treatment and subjects who were not treated with a CAI at enrollment or during the study. However, because this was not a randomized clinical trial, comparisons among groups should be interpreted with caution.

## METHODS

### Study Design

The study (NCT02331173) was approved by the Institutional Review Board at each site and adhered to the tenets of the Declaration of Helsinki. Male subjects at least 7 years of age with a clinical diagnosis of XLR5 and a confirmed disease-causing *RS1* gene variant were recruited into the study at three clinical sites in the United States. Subjects were not eligible for the study if they had other eye diseases that might affect the results, such as a history of retinal detachment, glaucoma or cataracts that interfered with imaging. After explanation of the nature and possible consequences of the study, each subject (and a parent of children <18 years of age) provided informed consent and was scheduled to be seen every 6 months during an 18-month period to obtain multiple tests of retinal structure and function. At the initial visit, each subject was questioned regarding their current and prior use of a CAI. Subjects who were not being treated with a CAI at the first visit were offered the option of participating in a substudy to evaluate the effect of CAI treatment. Subjects in the substudy were seen at additional visits 1 and 3 months after starting CAI treatment.

Demographic variables collected in this study include age, race, ethnicity, and genetic testing results. Applanation tonometry was used to measure intraocular pressure (IOP). Ophthalmic examinations included dilated slit-lamp examination and indirect ophthalmoscopy. Best-corrected visual acuity (BCVA) was measured using an electronic visual acuity (EVA) system<sup>35</sup> based on the Early Treatment of Diabetic Retinopathy Study (ETDRS) method.<sup>36,37</sup> The Octopus 900 perimeter (Haag-Streit USA, Mason, OH, USA) was used for both static and semi-automated kinetic perimetry.<sup>38</sup> Kinetic perimetry was performed with targets of size I4e, III4e, and V4e. Full-field static perimetry used the German Adaptive Thresholding Estimation strategy,<sup>39</sup> stimulus size III or V, and a radially designed, centrally condensed grid of 172 test locations that extend to 79° temporally, 67° inferiorly, and 54.8° degrees nasally and superiorly. Spectral domain optical coherence tomography (SD-OCT) was performed using a Spectralis SD-OCT imaging system (Heidelberg Engineering, Carlsbad, CA, USA). Fundus photography was performed using modified eight standard fields with a 30-degree lens. Full-field electroretinograms (ERG) were recorded with devices and protocols compliant with the International Society for Clinical Electrophysiology of Vision (ISCEV) standards<sup>40</sup> using Burian Allen contact lens electrodes, an Espion E<sup>3</sup> ERG system, and a standardized testing protocol at all sites that was defined in a study manual of procedures. Reading speed was measured binocularly using MNREAD charts (Precision Vision, LaSalle, IL, USA). Contrast sensitivity was determined using Pelli-Robson charts (Precision Vision). The charts were illuminated uniformly at a luminance between 75 and 125 foot-candles and testing was performed in each eye separately and then binocularly. Vision-related quality of life was measured using the National Eye Institute 25-item Visual Functioning Questionnaire (VFQ-25).<sup>41</sup>

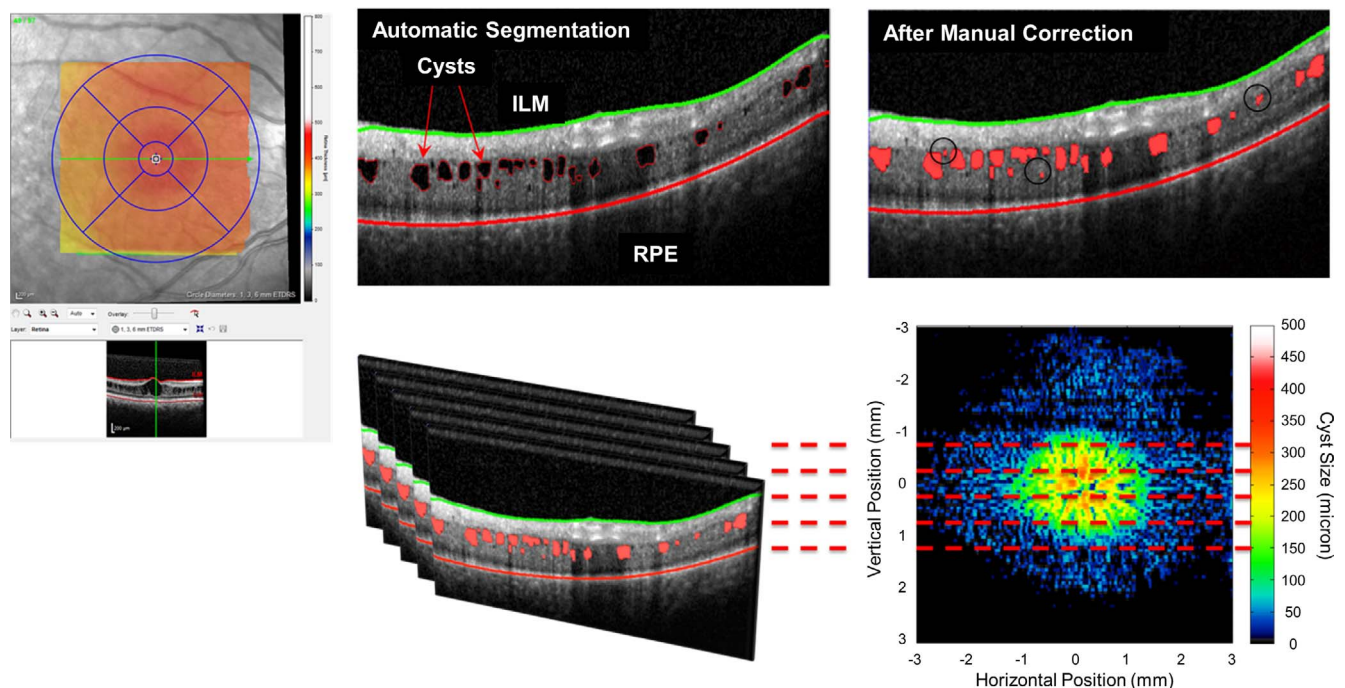
### Data Analysis

*RS1* variants were classified as missense, nonsense, exon deletion, or splice site variants. BCVA results were reported as the ETDRS letter score and log of the minimum angle of resolution (logMAR). For ophthalmic exams, abnormalities were listed for slit-lamp examinations (conjunctiva/lid, cornea, anterior chamber, lens, vitreous) and indirect ophthalmoscopy (vitreous, disc, macula, vessels, periphery). Fundus photographs were scored by the Casey Reading Center for the following parameters: features consistent with XLR5, foveal schisis visible, evidence of increased inflammation, hemorrhage, and retinal detachment.

The total cyst cavity volume (CCV) was calculated using a custom software program written in MATLAB and applied to 97 sequential b-scans across the 20-degree OCT cube scan (Fig. 1). For each scan, an automated algorithm detected the inner limiting membrane (ILM) and retinal pigment epithelium (RPE) and found all dark regions between the ILM and RPE.<sup>42,43</sup> Twenty-seven features—related to intensity, shape, and position—were extracted from each dark region. Based on these features, a trained neural network was used to classify these dark regions to schisis cavity or non-cyst tissue. The accuracy of the automated algorithm is 92.0% in classifying the dark regions detected. Then, an experienced reader manually corrected each scan to eliminate artifacts, and the total cyst cavity area for each scan was summed across all 97 scans to calculate the total CCV. The reader also rejected scans with low image quality, such as shadowing, cropped retina, or low signal strength. Using this grading protocol, we achieved 6.79% intergrader variability and 7.27% intravisit variability, which is significantly less than the variability of CCV between visits. Foveal zone thickness was also tabulated from the automated output of the Spectralis SD-OCT. In addition, a line scan through the fovea for each eye was classified by two investigators (MEP and DGB) for the presence of atrophy, defined as disruption of the ellipsoid zone throughout the macula that was not accounted for by shadowing. In the initial independent evaluation, 10 of 112 eyes had discordant results between the two evaluators that were reconciled by discussion and consensus.

Kinetic perimetry results were reported as the total area in square degrees for each target size (V4e, III4e, and I4e). For static perimetry, the set of  $x, y, z$  examination data—where  $x, y$  are the Cartesian coordinates of the test location and  $z$  is the threshold level—was exported from the perimeter and the threshold values converted to differential luminous sensitivity values using a published transformation formula.<sup>44</sup> Results for eyes that had a false positive rate of >25% were excluded from analysis. The sensitivity values were imported into an application, Visual Field Modeling and Analysis, where the sensitivity data were fit in non-Euclidian space with a thin-plate spline, creating a 3-dimension model of the hill of vision.<sup>45</sup> The magnitude and extent of sensitivity was quantified in the unit decibel-steradians (dB-sr) by interpolating the volume beneath the surface of the thin-plate spline representation of the hill of vision. The total volume of the entire hill of vision ( $V_{TOT}$ ) was determined using a selection process that conformed to the external boundary of the test grid. The central 30° portion of the hill of vision ( $V_{30}$ ) was measured by a circle of 30° radius as a separate metric of the central portion of the visual field. Details of this type of analysis have been published previously.<sup>45</sup>

For full-field ERG, the peak a- and b-wave amplitude and implicit time for dark-adapted conditions with 0.01 and 3.0  $\text{cd}\cdot\text{s}\cdot\text{m}^{-2}$  stimuli and for light-adapted conditions with 3.0  $\text{cd}\cdot\text{s}\cdot\text{m}^{-2}$  stimuli, and the light-adapted 3.0  $\text{cd}\cdot\text{s}\cdot\text{m}^{-2}$  flicker amplitude were reported.



**FIGURE 1.** Calculation of total cyst cavity volume (CCV) by ocular coherence tomography. For each of 97 sequential b-scans across the 20-degree OCT volume scan, a custom software program identified and outlined each schisis cavity. An experienced reader manually corrected each scan to eliminate artifacts, and the total cyst cavity area for each scan was summed across all 97 scans to calculate the total CCV.

For reading speed, maximum reading speed and critical print size (CPS) were determined using a modification of the method of Patel et al.<sup>46</sup> The “maximum reading speed” was defined as the mean reading speed for print sizes larger than the CPS, and “CPS” was defined as the smallest print size supporting reading speed greater than 80% of the maximum reading speed. Reading acuity was calculated according to instructions provided with the MNREAD charts. The reading accessibility index was calculated using the method of Calabrese et al.<sup>47</sup>

Contrast sensitivity results were reported as the log of the minimum contrast at which letters on the Pelli-Robson chart could be correctly identified. Results were classified as normal ( $\geq 1.65$  log units), moderate loss (0.90–1.50 log units), and severe loss ( $< 0.90$  log unit).

VFQ-25 responses were scored according to the NEI VFQ-25 Scoring Algorithm.<sup>48</sup>

Descriptive statistics (median, mean, standard deviation, and range) were reported for all quantitative parameters. One-way analysis of variance was used to compare baseline values among groups. Linear regression was used to correlate age, visual acuity, CCV, and reading speed parameters. Unpaired *t*-test was used to compare BCVA, CCV,  $V_{TOT}$ , and ERG parameters between eyes with and without retinal atrophy detected on OCT images, and to compare VFQ-25 scores for XLR5 subjects with published scores for normal subjects.<sup>41</sup> Trends over time were evaluated using mixed-effects regression analysis (Version 9.4, SAS Proc Mixed, SAS Institute, Inc., Cary, NC, USA). Change from baseline measure was used as a response variable with time in months from baseline and baseline measure as covariates, group-by-time interaction as a fixed effect, and time-by-eye interaction as a random effect. The time-by-eye interaction effect accounts for the correlation between slopes of right eye and left eye within a subject. The model uses zero as an intercept. Group-by-time interaction was used to evaluate differential common slopes among groups. All

subjects with at least one change from baseline result were used in the analysis.

## RESULTS

Sixty-six subjects were screened for study eligibility and 56 were enrolled in the study and had at least one study visit. Subjects who did not meet the eligibility criteria ( $n = 8$ ) had a diagnosis other than XLR5 ( $n = 2$ ), additional retinal diseases that could interfere with interpretation of results (retinal detachment [ $n = 1$ ] or cone-rod dystrophy [ $n = 1$ ]), no detectable pathologic variant in the *RS1* gene ( $n = 1$ ), amblyopia ( $n = 1$ ), unexplained abnormal visual fields ( $n = 1$ ), or an inadequate understanding of protocol-related testing ( $n = 1$ ). Two additional subjects met enrollment criteria but withdrew consent before the first study visit. One subject had no light perception in one eye, and results for this eye were excluded from analyses of quantitative parameters.

The 56 enrolled subjects with at least one study visit had a mean  $\pm$  SD age of  $30.04 \pm 19.2$  years (range, 7.8–79; median 25.3). Forty-eight were Caucasian, four Asian, and four had other racial backgrounds.

Thirty-seven subjects completed the 18-month study. Twelve subjects discontinued participation in the study after the month 6 or month 12 visit, and seven subjects who enrolled in the study had only a single baseline study visit (Supplementary Table S1). Among the 51 enrolled subjects with at least one post-baseline study visit, 15 were using a CAI at enrollment (Group 1), 16 were not using a CAI at enrollment or during the study (Group 2), and 20 were not using a CAI before enrollment but started using a CAI at the first study visit (Group 3). Eighteen of the Group 3 subjects participated in the CAI substudy and had additional visits at 1 and 3 months after enrollment. The demographic and baseline characteristics of the three groups of subjects with at least one post-baseline study visit were generally similar (Table 1).

**TABLE 1.** Demographic and Baseline Characteristics of 51 XLR5 Subjects With at Least One Post-Baseline Study Visit

Variable	Group 1	Group 2	Group 3
Number of subjects	15	16	20
Age (years, mean $\pm$ SD)	25.0 $\pm$ 18.7	29.9 $\pm$ 18.4	32.3 $\pm$ 19.0
Race			
Caucasian	15	11	19
Black			
Asian	0	3	1
Other	0	2	0
Ethnicity			
Hispanic	1	0	1
Non-Hispanic	14	16	19
RS1 mutation			
Missense	11	13	15
Other (nonsense, exon deletion or splice site variant)	4	3	5
BCVA (ETDRS letter score, mean $\pm$ SD)			
OD	63.5 $\pm$ 8.0	56.7* $\pm$ 13.6	53.8 $\pm$ 17.1
OS	63.6 $\pm$ 9.8	65.9 $\pm$ 8.8	54.9 $\pm$ 15.0
Inter-eye difference >10 letters	1	6	3
Inter-eye difference >15 letters	0	1	3

\* Excludes one eye with no light perception. Between-group differences were not significant for age ( $P=0.5216$ ) and BCVA OD ( $P=0.1285$ ) but were significant for BCVA OS ( $P=0.0183$ ).

### RS1 Gene Variants

RS1 variants were classified as missense in 44 subjects, nonsense in 5, exon deletion in 4, and splice site variant in 3 (Supplementary Table S2). The most common variants were c.286T>C:p.Trp96Arg (6 subjects), c.214G>A:p.Glu72Lys and c.599G>A:p.Arg200His (5 subjects each), c.574C>T:p.Pro192Ser (4 subjects), and c.304C>T:p.Arg102Trp and c.329G>A:p.Cys110Tyr (3 subjects each).

### Ophthalmic Examinations

Abnormalities on slit-lamp examination were detected at the baseline visit in 27 of 56 subjects (44 eyes). The most common findings were cataracts in 17 subjects (29 eyes), posterior vitreous detachment in 6 subjects (8 eyes), syneresis in 4 subjects (7 eyes), and vitreous cells in 3 subjects (6 eyes; Supplementary Table S3). Subjects with trace or mild cataract were younger (age 32.2  $\pm$  19.6 years; range, 10–56) than subjects with 1+ cataract (age 50.7  $\pm$  19.2 years; range, 25–79) or subjects with grade 2+ or 3+ cataract (55.8  $\pm$  11.2 years; range, 40–67). Abnormalities not detected at the baseline visit were reported at one or more follow-up visits in 20 of 51 subjects (27 eyes). The most common new findings were posterior vitreous detachment in 5 subjects (5 eyes), rare anterior chamber cells in 4 subjects (5 eyes) and cataracts in 3 subjects (4 eyes), all of which were graded as trace (Supplementary Table S3).

Abnormalities were detected by indirect ophthalmoscopy in all subjects (112 eyes) examined at the initial visit. The most common findings were macular schisis in 42 subjects (80 eyes), peripheral schisis in 14 subjects (26 eyes) and pigmentary changes in the macula (15 eyes in 10 subjects) or periphery (13 eyes in 10 subjects; Supplementary Table S4). Abnormalities not detected at the baseline visit were reported at one or more follow-up visits in 23 of 50 subjects (33 eyes). The most common new findings were disc pallor in 3 subjects (4 eyes) and peripheral retinal pigmentary changes in 2 subjects (3 eyes; Supplementary Table S4).

### CAI Use

CAI use in subjects with at least one post-baseline study visit is summarized in Table 2. Topical dorzolamide or brinzolamide was used in 14 of 15 subjects in Group 1 and all 20 subjects in Group 3. One subject in Group 1 was taking oral acetazolamide at enrollment and continued this during the study. Three subjects in Group 3 were treated with topical dorzolamide for 6 months, at which point treatment was changed to oral acetazolamide for the remainder of the study.

Nineteen subjects discontinued use of a CAI during the study because of reported lack of clinical response (6), side

**TABLE 2.** Carbonic Anhydrase Inhibitor (CAI) Use in 51 XLR5 Subjects With at Least One Post-Baseline Study Visit

Variable	Group 1	Group 2	Group 3
Number of subjects	15	16	20
Dorzolamide topical	12	1*	19
Brinzolamide topical	2	0	1
Acetazolamide oral	1	0	3†
Stopped using CAI during study	10	1*	8
No response	3	0	3
Wanted to enroll in gene therapy study	5	0	2
Side effects	1	1	2
Too much trouble	1	0	1
Duration of CAI use (months)‡			
Mean $\pm$ SD (min, max)	12.6 $\pm$ 6.5 (0.4, 18)	0.014 $\pm$ 0.058 (0, 0.2)	14.0 $\pm$ 5.2 (1.2, 18)
$\geq 1$	13	0	18
$\geq 6$	11	0	16
$\geq 12$	10	0	12
18	7	0	10

\* One subject in Group 2 received topical dorzolamide at study month 9 that was stopped after 1 week because of side effects (burning when drops administered).

† Three subjects in Group 3 were treated with topical dorzolamide for 6 months at which point treatment was changed to oral acetazolamide for the remainder of the study.

‡ For subjects who did not attend the month 12 or month 18 visit, the duration of CAI use was assumed to end at their last study visit.

**TABLE 3.** Baseline Visual Acuity and Cyst Cavity Volume Results in 56 Subjects With XLRS

Variable	ETDRS Letter Score (logMAR)		Cyst Cavity Volume (mm <sup>3</sup> )	
	OD	OS	OD	OS
N	55*	56	46†	51†
Mean	56.6 (0.57)	59.9 (0.50)	0.852	1.031
SD	15.9 (0.32)	13.3 (0.27)	1.143	1.346
Median	62 (0.46)	62.5 (0.45)	0.654	0.726
Minimum	13 (0.06)	16 (0.10)	0	0
Maximum	82 (1.44)	80 (1.38)	6.987	6.722

An ETDRS letter score of 85 (logMAR of 0) is equivalent to Snellen value of 20/20; an ETDRS letter score of 55 (logMAR of 0.6) is equivalent to Snellen value of 20/80; and an ETDRS letter score of 35 (logMAR of 1.0) is equivalent to Snellen value of 20/200.

\* Does not include results for one subject with no light perception in the right eye.

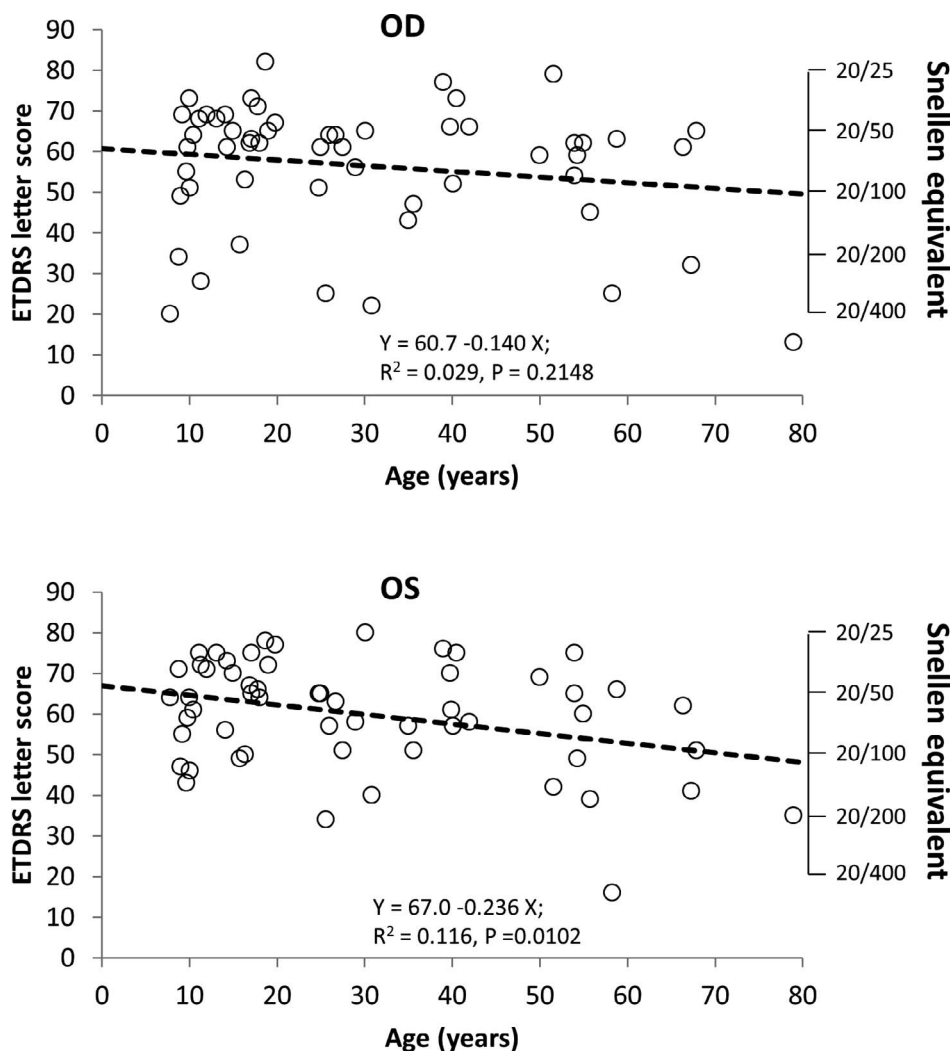
† OCT images for 15 eyes were not of sufficient quality to allow calculation of cyst cavity volume.

effects (4), inconvenience (2), or a desire to participate in a gene therapy study (7). The mean ± SD duration of CAI use during the trial was 12.6 ± 6.5 months in Group 1 and 14.0 ± 5.2 months in Group 3.

**Visual Acuity**

Mean ± SD BCVA for 56 subjects at the initial visit was 56.6 ± 15.9 ETDRS letters in the right eye and 59.9 ± 13.3 ETDRS letters in the left eye (Table 3). There was a weak correlation between visual acuity and age that was statistically significant for the left eye but not the right eye (Fig. 2).

Baseline visual acuity was highly variable among subjects but was generally similar between the two eyes (Supplementary Fig. S1). The interocular difference in visual acuity was ≤10 ETDRS letters in 69% of subjects and ≤15 ETDRS letters in 89% of subjects (Supplementary Fig. S2). One subject (Group 2 subject 13 [RFS-318]) had NLP OD and ETDRS letter score of 61 OS. Six other subjects had an inter-eye difference of more than 15 ETDRS letters (Group 2 subjects [CEI-004, KEC-018 and RFS-326, respectively], 20 OD vs. 64 OS; KEC-018, 13 OD vs. 35 OS; and RFS-326, 22 OD vs. 40 OS as well as Group 3 subjects [CEI-013, CEI-018, and RFS-303, respectively], 34 OD vs. 71 OS; 28 OD vs. 72 OS; 79 OD vs. 42 OS). Two of these subjects (CEI-004 and RFS-303) had retinal atrophy in the eye



**FIGURE 2.** Correlation of visual acuity with age at baseline. There was a weak correlation between visual acuity and age that was statistically significant for the left eye (OS) but not the right eye (OD).

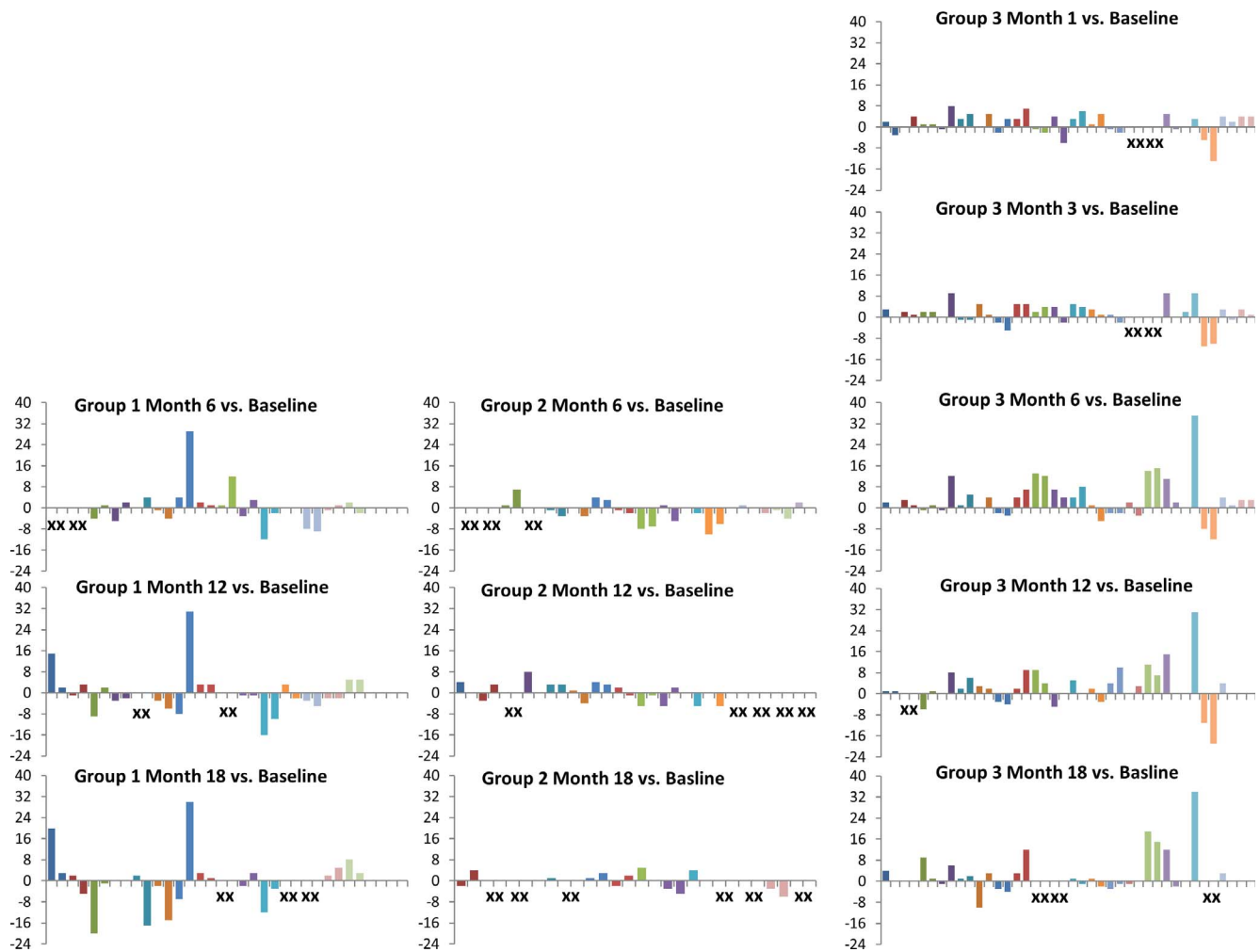


FIGURE 3. Changes from baseline best corrected visual acuity (ETDRS letter score) at study month 1, 3, 6, 12, or 18. Subjects are arranged from left to right within each group, with the value for right eye plotted first and the value for left eye plotted second. Subjects who did not have a study visit at a given time point are indicated by an X.

with worse vision, and two subjects (KEC-018 and RFS-326) had retinal atrophy in both eyes. The other two did not have retinal atrophy in either eye.

Subjects who were not using a CAI during the study (Group 2) had little change in their BCVA over time (Fig. 3). Bland-Altman analysis of visual acuity data from Group 2 subjects determined that the coefficient of repeatability was 8.7 ETDRS letters in the right eye and 7.4 ETDRS letters in the left eye. For the 67 eyes evaluated at months 6, 12, or 18, the mean  $\pm$  SD change from baseline was  $-0.4 \pm 3.7$  ETDRS letters, and none of the changes were  $>10$  ETDRS letters.

For subjects who were using a CAI at enrollment and continued its use during the study (Group 1), changes in BCVA over time were more common (Fig. 3). For the 76 eyes evaluated at months 6, 12, or 18, the mean  $\pm$  SD change from baseline was  $0.33 \pm 8.6$  ETDRS letters, with an increase of  $\geq 15$  ETDRS letters on five occasions and a decrease of  $\geq 15$  ETDRS letters on three occasions. Group 1 Subject KEC-004 had an increase of 29, 31, and 30 letters OS at months 6, 12, and 18. Group 1 Subject CEI-006 had an increase of 15 and 20 letters at months 12 and 18 (with no month 6 visit). Group 1 Subject RFS-304 had a decrease of 16 letters at month 12 (and a decrease of 12 letters each at months 6 and 18). Group 1 Subject CEI-017 had a decrease of 20 letters at month 18 (and a decrease of 4 and 9 letters at months 6 and 12). Group 1

Subject KEC-003 had a decrease of 15 letters at month 18 (and a decrease of 4 and 6 letters at months 6 and 12).

Changes in BCVA over time were also more common for subjects who began using a CAI at the first study visit (Group 3). For the 110 eyes evaluated at months 6, 12, or 18, the mean  $\pm$  SD change from baseline was  $3.15 \pm 7.8$  ETDRS letters, with an increase of  $\geq 15$  ETDRS letters on seven occasions and a decrease of  $\geq 15$  ETDRS letters on one occasion. Group 3 Subject RFS-303 had no change over time OD but had an increase of 35, 31, and 34 letters OS at months 6, 12, and 18. Group 3 Subject KEC-015 had an increase of 14, 11, and 19 letters OD and an increase of 15, 7, and 15 letters OS at months 6, 12, and 18. Group 3 Subject KEC-017 had no change over time OS but had an increase of 11, 15, and 12 letters OD at months 6, 12, and 18. Group 3 Subject RFS-309 had a decrease of 8 and 11 letters OD and a decrease of 12 and 19 letters OS at months 6 and 12, with no visit at month 18. Results in Supplementary Table S5 show each visit for subjects with an increase of at least 15 letters at one or more visits.

Changes from baseline visual acuity were not clinically significant for subjects in Group 1 or Group 2. Subjects in Group 3 had a modest increase in BCVA (mean of 2.6 to 3.5 ETDRS letters at months 6, 12, and 18) with smaller changes at months 1 and 3, but mixed-effects regression analysis found no significant differences among the three groups (Table 4).

TABLE 4. Summary of Changes From Baseline Visual Acuity

Group	Month 1	Month 3	Month 6	Month 12	Month 18
1					
N			26	26	24
mean			0.4	-0.1	0.7
SD			7.5	8.7	10.0
2					
N			25	22	20
mean			-1.4	0.2	0.2
SD			3.8	3.6	3.4
3					
N	36	36	40	38	32
mean	1.3	1.4	3.5	2.6	3.4
SD	4.1	4.3	7.7	7.7	8.1

Values are the difference in best corrected visual acuity (ETDRS letter score) at each time point compared to the baseline value for left and right eyes combined. N = number of eyes with results at each time point. Mixed-effects regression analysis demonstrated that slope (letter score/month) over time was not significant for any group (P = 0.3463).

OCT

Cyst cavity volume results at baseline were available for 51 subjects (97 eyes). Poor OCT image quality precluded measuring CCV in the other 15 eyes. Baseline CCV was highly variable among subjects but was generally similar

between the two eyes (Supplementary Fig. S3). The mean ± SD CCV at the initial visit was 0.852 ± 1.143 mm<sup>3</sup> in the right eye and 1.031 ± 1.346 mm<sup>3</sup> in the left eye (Table 3). Five eyes in four subjects had no cysts detected in the CCV analysis. Three of these subjects (ages 59, 68, and 79) had retinal atrophy in both eyes with CCV values of 0 in one eye and 0, 0.001, or 0.003 mm<sup>3</sup> in the other eye. The other subject was age 27 with no retinal atrophy in either eye and had CCV values of 0 in one eye and 0.031 mm<sup>3</sup> in the other eye.

For each eye, there was a significant correlation between CCV and age but no significant correlation between CCV and visual acuity (Fig. 4). There was generally good interocular symmetry (Supplementary Fig. S4). The mean ± SD difference in CCV was 0.436 ± 0.728 mm<sup>3</sup> (range, 0-4.637; median 0.145).

Retinal atrophy was detected on OCT images in 21 (37.5%) of 56 subjects and 39 (34.2%) of 111 eyes. Eighteen of these subjects had retinal atrophy in both eyes, and three had atrophy in only one eye. Subjects with atrophy were older (mean age 44.9 ± 19.6 vs. 22.4 ± 14.1 years). Eyes with atrophy had worse BCVA and lower CCV values than eyes without atrophy (Table 5).

Changes in CCV over time were quite variable, with increases or decreases for subjects in each group (Fig. 5), and statistical analysis did not show any consistent pattern (Table 6).

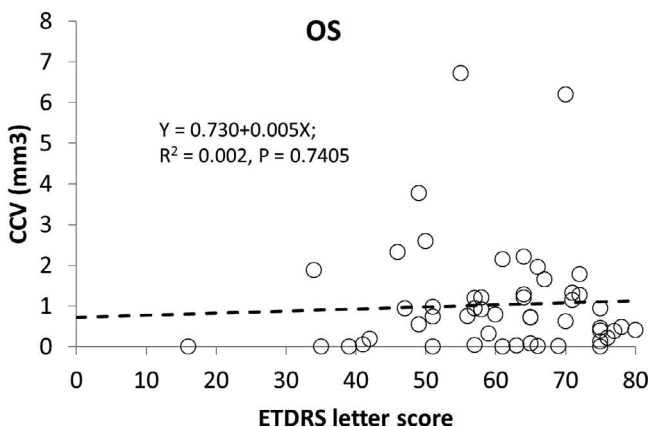
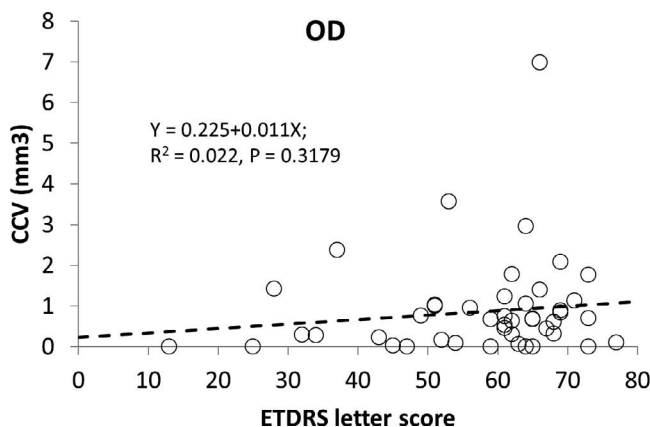
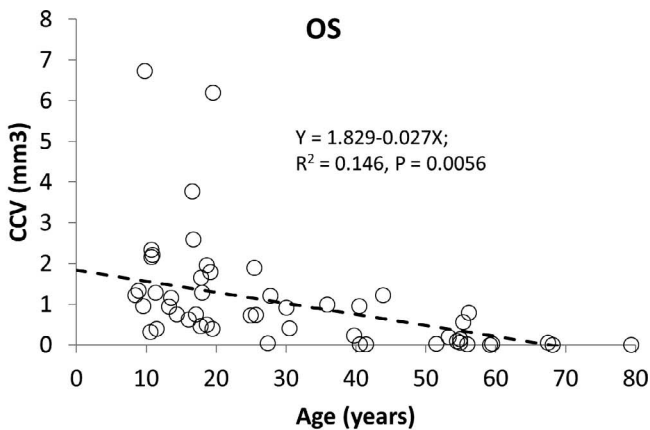
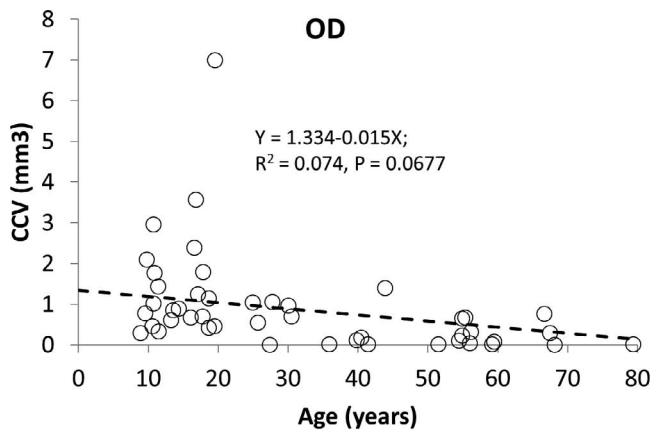


FIGURE 4. Correlation of retinal cyst cavity volume (CCV) with age and visual acuity at baseline. There was a significant correlation between CCV and age but no significant correlation between CCV and visual acuity.

TABLE 5. Baseline Parameters in Subjects With and Without Retinal Atrophy Detected by OCT

Variable	BCVA (ETDRS Letter Score)	CCV (mm <sup>3</sup> )	V <sub>TOT</sub> Size V (dB-sr)	V <sub>TOT</sub> Size III (dB-sr)	ERG 3.0 a-Wave Amplitude (µv)	ERG 3.0 b-Wave Amplitude (µv)	ERG 3.0 b/a Ratio	Log CS
Eyes without retinal atrophy								
N	73	65	17	41	71	70	70	68
Mean	63.6	1.1962	88.839	67.257	243.101	261.1	1.091	1.826
SD	9.9	1.3483	14.808	12.227	62.741	106.2	0.335	0.264
Median	64	0.8390	84.1623	69.275	235.000	243.5	1.099	1.95
Min	28	0	63.717	35.667	72	104	0.47	0
Max	82	6.987	111.9672	82.016	397	773	2.09	1.95
Eyes with retinal atrophy								
N	38	32	11	26	33	29	29	29
Mean	48.0	0.4386	72.0323	51.8047	182.545	134.041	0.745	1.567
SD	17.0	0.8339	18.7702	12.5570	66.276	58.479	0.243	0.291
Median	50	0.0875	77.0967	52.9170	168.500	120.000	0.710	1.65
Min	13	0	36.5257	26.8270	63.9	54.8	0.40	0.75
Max	77	3.77	94.3435	71.2235	338	296.6	1.35	1.95
t-test P	<0.0001	0.0010	0.0220	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
Delta mean	-15.6	-0.7576	-16.807	-15.4525	-60.5560	-127.1	-0.3461	-0.259
Delta %	-24%	-62%	-18%	-27%	-24%	-49%	-32%	-14%

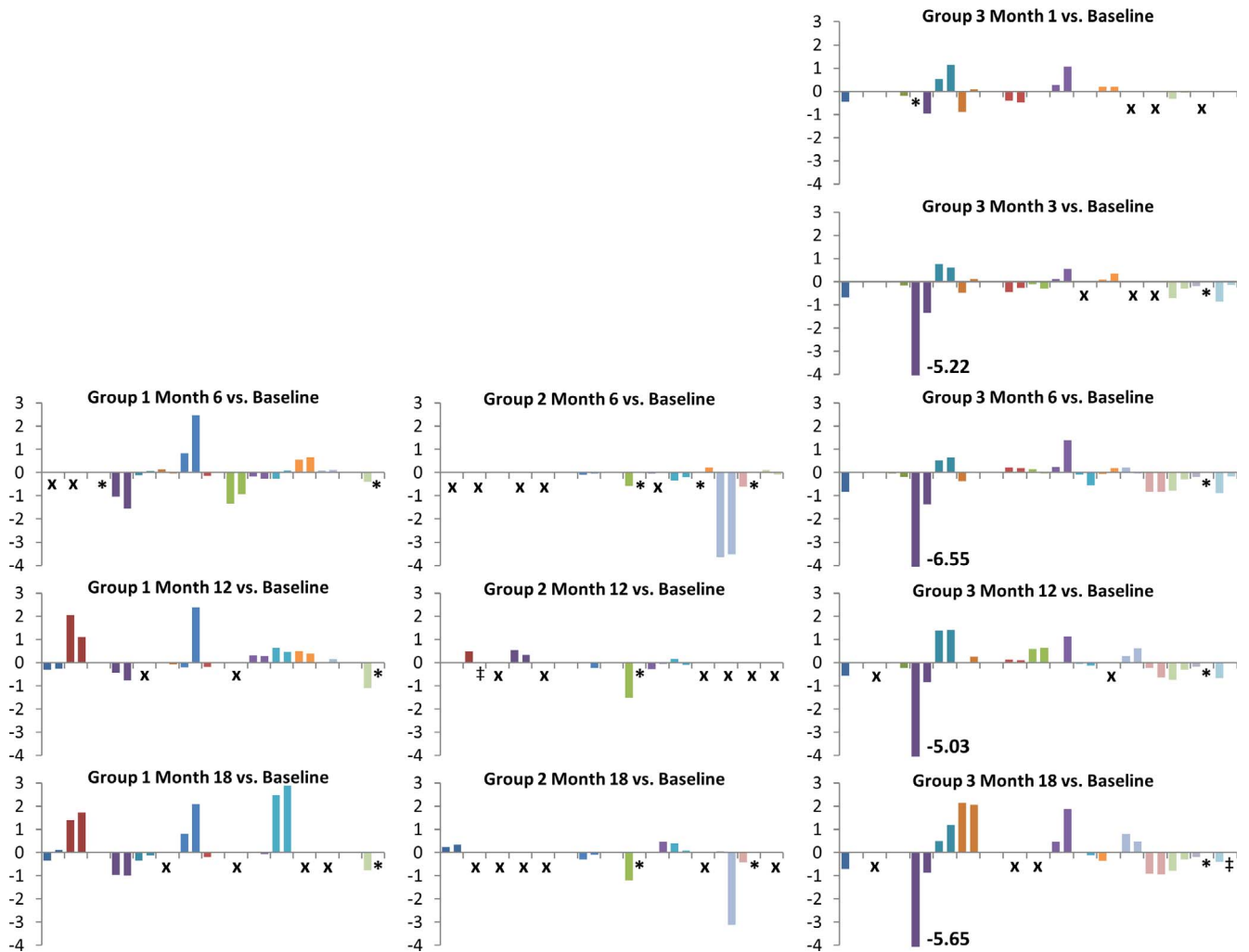


FIGURE 5. Changes from baseline cyst cavity volume (mm<sup>3</sup>) at study month 1, 3, 6, 12, or 18. Subjects are arranged from left to right within each group, with the value for right eye plotted first and the value for left eye plotted second. Subjects who did not have a study visit at a given time point are indicated by an X. \* = Eyes with no baseline value. ‡ = Time points when OCT images were not of sufficient quality to allow CCV to be calculated. Values for one subject with CCV decreases of more than 4 mm<sup>3</sup> are listed.



TABLE 6. Summary of Changes From Baseline Cyst Cavity Volume

Group	Month 1	Month 3	Month 6	Month 12	Month 18
1					
N			24	25	21
mean			-0.051	0.198	0.371
SD			0.781	0.750	1.107
2					
N			19	16	14
mean			-0.462	-0.045	-0.248
SD			1.121	0.454	0.926
3					
N	27	27	33	29	26
mean	-0.017	-0.321	-0.313	-0.107	-0.068
SD	0.454	1.078	1.231	1.103	1.428

Values are the difference in cyst cavity volume (mm<sup>3</sup>) at each time point compared to the baseline value for left and right eyes combined. Mixed-effects regression analysis demonstrated that slope (CCV/month) over time was not significant for any group ( $P = 0.8016$ ).

### Correlation of Changes in Visual Acuity and Cyst Cavity Volume

There was no significant correlation between changes in visual acuity and changes in cyst cavity volume in any group (Fig. 6). The correlation for the five subjects with an increase in visual acuity of at least 15 letters at one or more time points was inconsistent (Supplementary Table S5). Subject KEC-004 had an increase of 29 to 31 letters in his left eye at months 6, 12, and 18, but his cyst cavity volume was increased at two of these visits and decreased only slightly at the other visit. Subject CEI-006 had an increase of 15 and 20 letters in his right eye at months 12 and 18 with little change in cyst cavity volume. Subject RFS-303 had an increase of 31 to 35 letters at months 6, 12, and 18 that was associated with a marked decrease in cyst cavity volume at each of these visits. Subject KEC-015 had an increase of 11 to 19 letters in his right eye and 7 to 15 letters in his left eye at months 6, 12, and 18, and the changes in BCVA appeared to correlate with changes in cyst cavity volume. Subject KEC-017 had a progressive decrease in cyst cavity volume in both eyes at months 1, 3, 6, 12, and 18 and improved BCVA at these visits in the right eye but not the left eye.

### Kinetic Perimetry

Kinetic perimetry results at baseline were available for 53 subjects (Table 7). There was a modest reduction in the visual field area with V4e and III4e targets and a more marked reduction with the I4e target. Visual field defects were usually in the superior field, consistent with more pronounced schisis seen inferiorly on OCT testing.

Changes in kinetic perimetry over time were variable, with increases or decreases for subjects in each group (Supplementary Fig. S5), and statistical analysis did not show any consistent pattern (Supplementary Table S6).

### Static Perimetry

Static perimetry results at baseline were available for 36 subjects (Table 8). Static perimetry testing early in the study was performed using spot size V and showed a modest reduction in the retinal sensitivity hill of vision compared to normal subjects. Because some subjects had a “ceiling effect,” with results in the normal range, testing was changed to use spot size III, which also showed a modest reduction in the retinal sensitivity hill of vision compared to normal subjects,

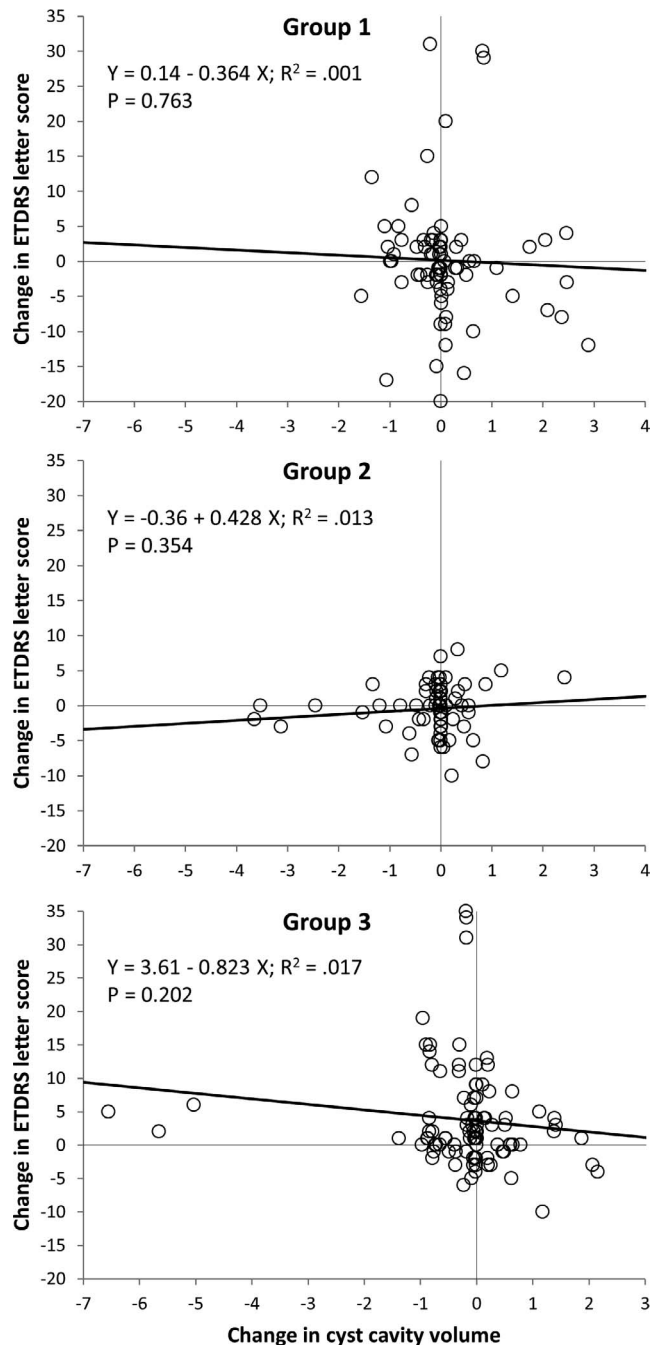


FIGURE 6. Correlation of changes in visual acuity and cyst cavity volume at study months 6, 12, and 18.

although some subjects also had results in the normal range with spot size III testing. For both spot sizes, eyes with atrophy had lower static perimetry hill of vision values than eyes without atrophy (Table 5).

Changes in static perimetry over time were variable, with increases or decreases for subjects in each group (Supplementary Fig. S6), and statistical analysis did not show any consistent pattern (Supplementary Table S7).

### Electroretinography

Full-field ERG results at baseline were available for 53 subjects (Table 9). Under dark-adapted conditions, XLRs subjects had

TABLE 7. Kinetic Perimetry Results at Baseline

Target Eye	V4e		III4e		I4e	
	OD	OS	OD	OS	OD	OS
Results for subjects with XLRs						
N	53	53	53	53	53	53
Mean (% of normal)	12,322 (86.6%)	12,229 (87.7%)	10,945 (83.5%)	10,781 (84.4%)	8,015 (76.5%)	7,942 (74.5%)
SD	2,422	2,044	2,620	2,384	2,570	2,435
Median	12,872	12,578	11,335	10,969	8,472	8,114
Minimum	4,702	6,116	3,299	2,810	0	564
Maximum	15,347	15,608	14,547	14,496	12,124	12,841
Number (%) <LLN	22 (42%)	19 (36%)	21 (40%)	23 (43%)	23 (43%)	27 (51%)
Results for normal adults						
N	15	13	15	13	15	13
Mean	14,235	13,948	13,104	12,781	10,478	10,666
SD	1,090	993	1,102	1,289	1,162	1,364
LLN	12,089		10,666		8,147	

Values are the results, in square degrees, for the kinetic visual field determined during testing with the indicated target size stimulus. Normative data are from Weleber et al.<sup>61</sup> LLN = lower limit of normal, calculated for data from left and right eyes combined.

reduced mean b-wave amplitude (~30% of normal) and prolonged implicit time (~120% of normal) in response to the 0.01 cd-s-m<sup>-2</sup> stimulus. To the dark-adapted 3.0 stimulus, responses were near-normal for the a-wave (amplitude and implicit times ~85 to 88% of normal) but were markedly abnormal for b-wave responses (amplitude ~45% of normal) and implicit time (~112% of normal). The b/a ratio was abnormal (<1.2) in 72 of 99 eyes tested, and in only two eyes (one subject) was the b/a ratio >2.0. Eyes with atrophy had lower a- and b-wave amplitudes and a lower b/a ratio than eyes without atrophy (Table 5). There was a significant negative correlation of dark-adapted 3.0 b-wave amplitude and b/a ratio with age for missense variants, but the correlation was not significant for other (nonsense, splice site, or exon deletion) variants (Fig. 7).

With a light-adapted 3.0 stimulus, XLRs subjects had reduced mean amplitudes for a-waves (~50% of normal) and b-waves (~40% of normal) and increased mean implicit times (~124% of normal for a-waves and ~110% of normal for b-waves). There was also a reduction in mean flicker amplitude and an increase in mean flicker implicit time (Table 9).

Changes in ERG responses over time were variable, with increases or decreases for subjects in each group (Supplementary Fig. S7), and statistical analysis did not show any consistent pattern (Supplementary Table S8).

### Reading Speed

Results of reading speed testing at baseline were available for 49 subjects (Supplementary Table S9). Compared to published results in normal subjects,<sup>49</sup> there was a moderate reduction in maximum reading speed and reading accessibility index, and a marked increase in critical print size and reading acuity values (Fig. 8). The mean ± SD reading accessibility index (ACC) was 0.64 ± 0.22 (range, 0.02–1.04). There was a strong correlation of maximum reading speed and reading accessibility index with BCVA but no significant correlation with age (Supplementary Fig. S8).

Changes in reading speed parameters over time were variable, with increases or decreases for subjects in each group (Supplementary Fig. S9), and statistical analysis did not show any consistent pattern (Supplementary Table S10).

TABLE 8. Static Perimetry Results at Baseline

Target Parameter Eye	Size V				Size III			
	V <sub>TOT</sub>		V <sub>30</sub>		V <sub>TOT</sub>		V <sub>30</sub>	
	OD	OS	OD	OS	OD	OS	OD	OS
Results for subjects with XLRs								
N	13	15	14	16	34	33	36	34
Mean (% of normal)	81.30 (77%)	83.1 (80%)	23.54 (85%)	24.62 (89%)	60.07 (75%)	62.49 (80%)	18.55 (78%)	18.97 (84%)
SD	18.77	18.27	4.79	4.37	15.89	12.86	3.82	3.55
Median	84.05	82.32	23.39	25.37	65.80	65.84	19.38	20.11
Minimum	36.5	48.8	11.4	15.7	26.83	37.59	10.4	12.5
Maximum	110.3	112.0	31.3	30.8	81.0	82.0	23.2	23.6
Number (%) <LLN	9 (69%)	11 (73%)	10 (71%)	9 (56%)	17 (52%)	17 (53%)	20 (57%)	22 (67%)
Results for normal subjects								
N	18	18	18	18	18	18	18	18
Mean	105.75	104.40	27.79	27.62	80.12	78.39	23.41	23.18
SD	7.59	6.00	1.06	1.12	5.80	5.97	1.22	1.36
LLN	89.73		25.46		66.39		20.69	

Values are the results, in decibel-steradians, for the total hill of vision (V<sub>TOT</sub>) or the central 30° hill of vision (V<sub>30</sub>) determined during testing with stimuli of the indicated target size. Results of testing in which the false positive rate was >25% were excluded from analyses. Normative data are from R. Weleber, 2013 (unpublished). The normal subjects had a mean age of 36.2 years (SD 10.7; range, 18.4–56.1).

TABLE 9. Full-Field Electroretinogram Results at Baseline

Variable	Amplitude ( $\mu\text{V}$ )			Implicit Time (ms)		
	OD	OS	Normal	OD	OS	Normal
Dark-adapted responses						
0.01 b-wave	104.9 $\pm$ 64.4 (0-348) N = 41	106.6 $\pm$ 73.3 (20-420) N = 41	Mean 349	102.8 $\pm$ 22.1 (0-148) N = 41	105.4 $\pm$ 15.1 (67-134) N = 41	Mean 86.0
3.0 a-wave	221.4 $\pm$ 72.9 (0-397) N = 53	222.2 $\pm$ 73.0 (72-370) N = 52	Mean 252 LLN 188	18.5 $\pm$ 3.3 (0-25) N = 53	18.7 $\pm$ 2.0 (16-25) N = 52	Mean 21.8
3.0 b-wave	223.5 $\pm$ 109.3 (0-627) N = 51	219.8 $\pm$ 116.8 (55-773) N = 49	Mean 499 LLN 370	55.5 $\pm$ 13.2 (0-74) N = 51	56.6 $\pm$ 11.1 (33-79) N = 49	Mean 49.4
3.0 b/a ratio	0.99 $\pm$ 0.34 (0.40-2.05) N = 50	0.99 $\pm$ 0.36 (0.47-2.09) N = 49	2.11 $\pm$ 0.50 (1.46-3.39) N = 18 LLN 1.2			
Light-adapted responses						
3.0 a-wave	32.4 $\pm$ 13.3 (0-63) N = 52	33.3 $\pm$ 14.3 (14-74) N = 51	Mean 62	16.9 $\pm$ 3.0 (0-26) N = 52	17.1 $\pm$ 1.6 (14-24) N = 51	Mean 13.6
3.0 b-wave	64.2 $\pm$ 31.3 (0-172) N = 52	64.4 $\pm$ 35.4 (18-223) N = 51	Mean 156	35.0 $\pm$ 6.0 (0-48) N = 52	35.5 $\pm$ 3.0 (30-43) N = 51	Mean 32.2
30 Hz flicker response	50.1 $\pm$ 27.0 (0-130) N = 51	53.0 $\pm$ 32.8 (10-188) N = 48	Median 100	33.5 $\pm$ 5.6 (0-40) N = 51	33.8 $\pm$ 2.8 (28-39) N = 48	Range, 26.2-32.4

Values are mean  $\pm$  SD (with range in parentheses). Normal values for b/a ratio based on D. Birch (unpublished data). Normal values for flicker amplitude based on Paravesh et al.<sup>62</sup> Normal values for flicker implicit time based on Ponjavic et al.<sup>63</sup> Other mean normal values based on Table 38.1 in Heckenlively and Arden.<sup>64</sup> Values for LLN are from Bowles et al.<sup>57</sup>

## Contrast Sensitivity

Results of contrast sensitivity testing at baseline were available for 49 subjects (Supplementary Table S11). Eighty-five of 101 eyes tested had normal contrast sensitivity. Eleven eyes had moderate loss of contrast sensitivity (log CS 0.9-1.5 log units). Two eyes had severe loss of contrast sensitivity (log CS 0 or 0.75 log units) and each of these eyes had severe loss of BCVA (28 and 13 ETDRS letter score, respectively). Contrast sensitivity was lower in eyes with atrophy than in eyes without atrophy (Table 5).

Changes in contrast sensitivity over time were variable, with increases or decreases for subjects in each group (Supplementary Fig. S10), and statistical analysis did not show any consistent pattern (Supplementary Table S12).

## Visual Function Quality of Life

Results for the VFQ-25 questionnaire at baseline were available for 47 subjects (Supplementary Table S13). The composite score was significantly lower (mean  $\pm$  SD 75.1  $\pm$  0.90) than in a group of 122 normal subjects<sup>41</sup> (93.0  $\pm$  0.33,  $t = 4.2501$ ,  $df = 2054$ ,  $P = 0.0001$ ). The subscale scores for general health and ocular pain were not significantly different from normal subjects, but results for the other 10 subscales were all significantly different from normal subjects (Supplementary Table S13).

Changes in VFQ-25 scores over time were variable, with increases or decreases for subjects in each group (Supplementary Fig. S14), and statistical analysis did not show any consistent pattern (Supplementary Table S14).

## Fundus Photographs

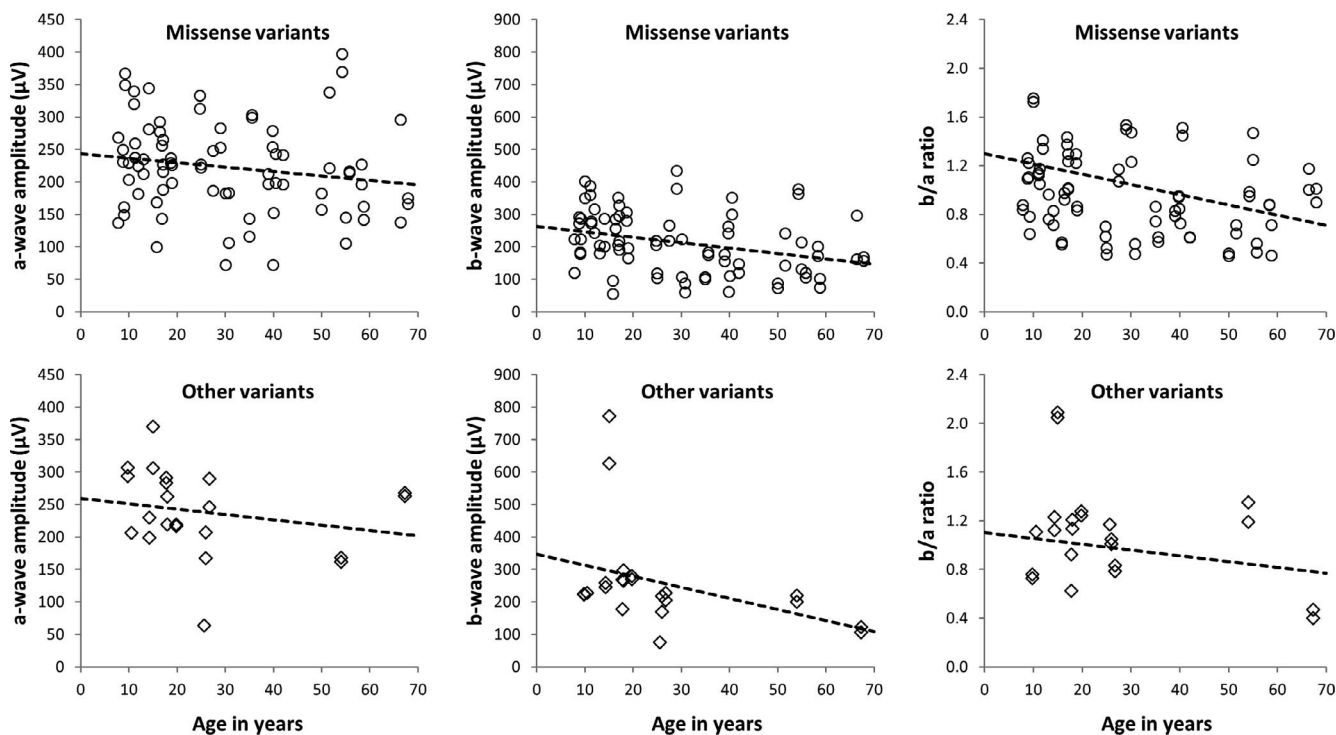
Fundus photographs in 112 eyes from 56 subjects at baseline showed no evidence of retinal detachment or inflammation. Features consistent with XLRS were observed in 50 subjects (89.3%), visible foveal schisis in 40 subjects (71.4%), and hemorrhage in 1 subject (1.8%).

During follow-up visits, changes from the baseline were reported for 90 of 258 fundus photograph. The most common changes were from "Yes" to "No" for the presence of features of XLRS ( $N = 38$ ) or foveal schisis visible ( $N = 39$ ) or from "No" to "Yes" for the presence of features of XLRS ( $N = 22$ ) or foveal schisis visible ( $N = 16$ ). Fundus photos could switch back and forth between "Yes" and "No" for both these parameters in the same patient at sequential visits. One subject had a retinal detachment noted in both eyes at the month 6 visit but not at visits at months 0, 12, or 18.

## DISCUSSION

We recruited 56 subjects with XLRS at three academic referral centers in the United States. The clinical characteristics of the subjects were generally similar to those in other published studies, which reported macular schisis in ~75% of patients and peripheral schisis in 32% to 71% of subjects (Supplementary Table S15). We observed a higher incidence of retinal atrophy in our study (38% of subjects and 34% of eyes) than in two previous studies (8% of subjects or 10% of eyes), which is likely due to differences in the methods used to detect retinal atrophy (OCT criteria in our study vs. ophthalmoscopic examination in previous studies).

Visual acuity results have been reported in a variety of ways in previous cross-sectional and longitudinal studies of XLRS. Forsius et al.<sup>4</sup> reported visual acuity as a decimal fraction (numerator divided by denominator of the Snellen value) for 1026 examinations in 183 patients in Finland. George et al.<sup>5</sup>



	a-wave amplitude		b-wave amplitude		b/a ratio	
	Missense	Other	Missense	Other	Missense	Other
N	78	22	78	22	78	22
Slope	-0.6885	-0.8302	-1.6634	-3.4086	-0.0047	-0.0084
Pearson r	-0.1758	-0.2245	-0.3205	-0.3891	-0.2631	-0.3584
p-value	0.1241	0.3163	0.0043	0.0736	0.0200	0.1019

FIGURE 7. ERG parameters vs. age for different types of *RS1* variants. Dark-adapted 3.0 a-wave, b-wave and b/a ratio data for 78 subjects with missense variants (circles) and 22 subjects with other variants (nonsense, splice site or exon deletion; diamonds). Summary statistics are provided in the table below the figure.

reported Snellen visual acuity at a single visit for 55 subjects in Great Britain. Apushkin et al.<sup>3</sup> reported Snellen visual acuity and logMAR equivalent for 152 examinations in 38 patients in the United States, with each eye examined at two visits with the second visit occurring between 1 and 28 years (mean 10.2 year) after the first. In the current study, visual acuity was measured in each eye using the ETDRS method, and letter

TABLE 10. Age-Related Visual Acuity Findings in the Current Study Compared to Previous Studies

Visual Acuity Impairment Category†	% of Patients >40 Years of Age by Study*				Median Age (Years) by Study*			
	A	B	C	D	A	B	C	D
Little or none	8	15	23	17	14	15	22	17
Moderate	15	17	23	31	18	15	21	21
Severe	54	50	58	38	43	45	40	31

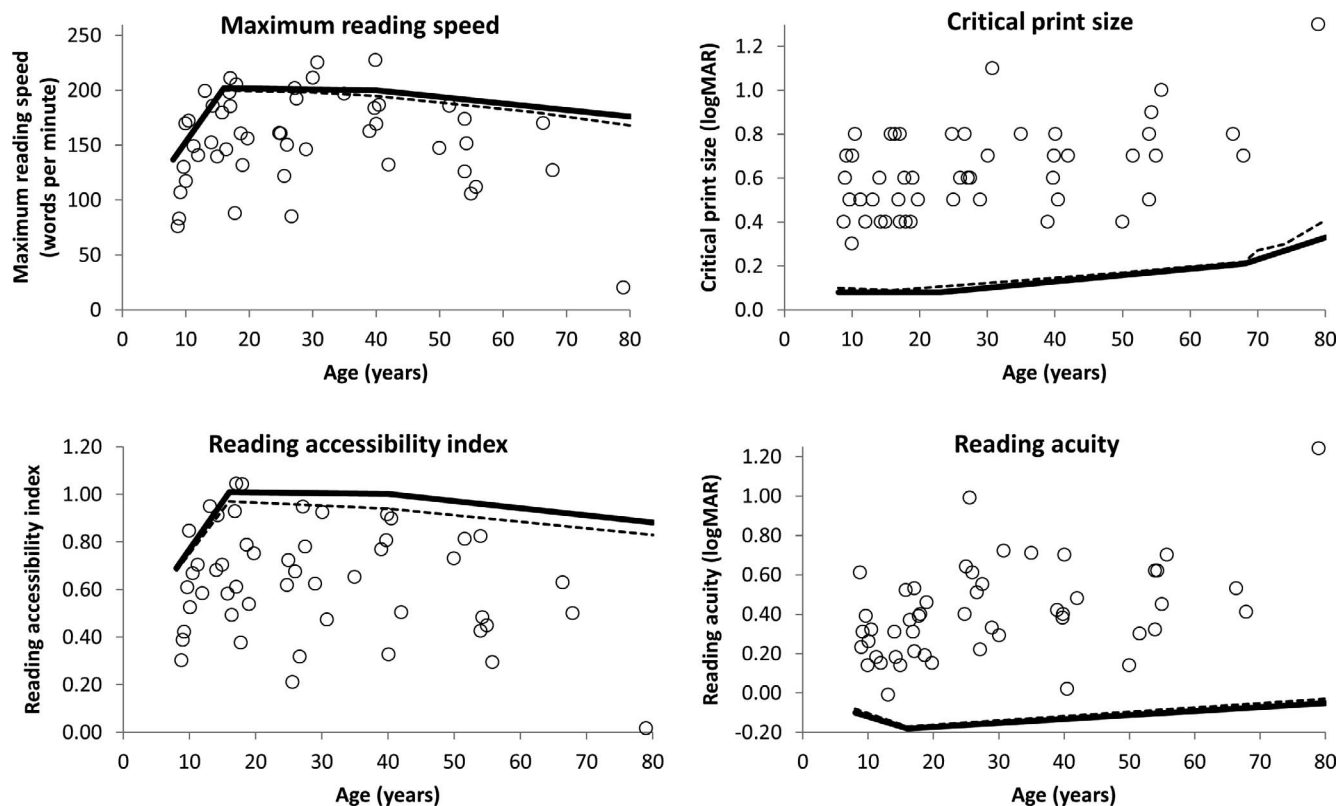
\* Study A is by Forsius et al.,<sup>4</sup> study B is by George,<sup>5</sup> study C is by Apushkin et al.,<sup>3</sup> and study D is the present study.

† Little or none is a Snellen equivalent of 20/40 or better, severe is a Snellen equivalent of worse than 20/200 in study A and 20/200 or worse in the other three studies, and moderate is a Snellen equivalent worse than little or none but better than severe.

scores for each of the 112 values in 56 subjects were converted to a Snellen equivalent. Cross-sectional results from all four studies are consistent with the hypothesis that XLRS is a slowly progressive disease, with severe impairment of visual acuity generally observed in a minority of patients <40 years of age (Table 10, Supplementary Fig. S12).

Previous OCT studies of XLRS have focused on total retinal thickness.<sup>50,51</sup> However, total retinal thickness encompasses two components: thickening due to the presence of cystic cavities and thinning due to outer retinal atrophy. When total retinal thickness decreases, this could result from photoreceptor loss or a decrease in cyst cavity volume. We have developed a novel method for directly measuring cystic cavity volume in XLRS patients. Interestingly, we did not see a relationship between visual acuity and cystic cavity volume. This is consistent with a previous report showing that acuity was highly correlated with macular outer segment thickness but was not correlated with total retinal thickness.<sup>52</sup>

Changes in visual acuity and schisis evaluated by OCT in response to CAI administration in the 51 subjects with more than one study visit in our study were consistent with previously reported studies in smaller numbers of patients. Our review of the literature found seven reports of between 4 and 36 patients each.<sup>17,20–22,25,29,32</sup> Most patients had little or no change in visual acuity (Supplementary Table S16;



**FIGURE 8.** Comparison of MNREAD results in XLRs subjects with published results for normal subjects. The *solid line* represents mean values and the *dashed line* represents the 95% confidence interval estimated by bi- or trilinear regression modeling as described by Calebrèse et al.<sup>49</sup> *Open circles* are results in XLRs subjects.

Supplementary Fig. S13) or foveal zone thickness (Supplementary Table S17; Supplementary Fig. S14). This contrasts with reports indicating that CAIs are effective for reducing central macular thickness in patients with retinitis pigmentosa and cystoid macular edema, although effects on visual acuity are variable across studies.<sup>53,54</sup>

We also found 11 case reports of one patient each,<sup>18,19,23,24,26-28,30,31,33,34</sup> most of which noted a reduction in the size of macular cysts detected by OCT after treatment with a topical or oral CAI. One reported a paradoxical worsening of schisis after treatment with topical dorzolamide with a return to baseline after treatment was stopped,<sup>34</sup> and a 63-year-old woman showed reduction of foveal cyst cavities after treatment but no reduction in foveal cysts after CAI treatment in her son.<sup>24</sup> The preponderance of case reports in which a positive response was noted may reflect reporting bias, as it is generally easier to publish a report of a favorable response than to publish a report of no response to a novel treatment. However, because some XLRs patients appear to have a favorable response, it would seem reasonable to consider a trial of CAI treatment in patients who have not previously used a CAI.

There is limited published information about visual field testing in XLRs patients. Apushkin et al.<sup>5</sup> reported superior nasal or superior peripheral restriction of Goldmann visual fields in 11 of 15 patients with a normal appearing retina. Kjellström et al.<sup>55</sup> reported normal Goldmann visual fields with a V4 target and various degrees of constriction in the visual field with a I4e target in 7 patients. Duncan et al.<sup>56</sup> reported constriction of Goldmann visual fields and reduced foveal sensitivity by Humphrey static perimetry in 2 patients. Our subjects had abnormal kinetic perimetry testing in 34%, 42%, and 47% of eyes with the size V, III, and I targets, respectively,

and abnormal static perimetry in 28% and 30% of eyes for  $V_{TOT}$  and  $V_{30}$ , respectively, with a size V target, confirming that abnormal visual fields occur commonly in XLRs.

ERG responses in our subjects were generally consistent with results reported in the literature.<sup>8,57-59</sup> Dark-adapted 0.01 and 3.0 ERG testing demonstrated reduced b-wave amplitudes. The dark-adapted 3.0 ERG also demonstrated normal or near-normal a-wave amplitude and a reduced b/a ratio in most subjects, with a significant age-related effect in our study (Fig. 7) and in the study by Bowles et al.<sup>57</sup> The light-adapted 3.0 ERG and 30Hz flicker ERG also demonstrated reduced amplitudes and prolonged implicit times.

Reading difficulty is a major challenge for many individuals with low vision, and the MNREAD test is a useful tool for assessing reading difficulties but, to our knowledge, has not previously been evaluated in XLRs patients. Subjects in our study showed marked abnormalities in all key parameters for the MNREAD for all age groups (Fig. 8). This test could be useful for evaluating outcomes in future therapeutic trials.

Using Pelli-Robson charts, we identified normal contrast sensitivity in 41 of 50 subjects (85 of 99 eyes tested), indicating this test is unlikely to be useful for evaluating responses to treatment in future therapeutic trials. Alexander et al.<sup>60</sup> reported mildly reduced contrast sensitivity in 4 of 6 XLRs patients using Pelli-Robson charts, but more detailed testing demonstrated pronounced loss of contrast sensitivity at high spatial frequencies as well as reduced contrast sensitivity and contrast discrimination at low spatial frequencies. Testing at multiple spatial frequencies might be useful in evaluating responses to treatment.

VFQ-25 quality of life questionnaire results were abnormal for all domains except general health and ocular pain (Supplementary Table S13).

It is noteworthy that changes over time for reading speed parameters, contrast sensitivity, and VFQ-25 scores were variable, with increases or decreases for subjects in each group and no consistent pattern. This variability will need to be considered when determining sample sizes and the types of parameters to be evaluated in future studies to evaluate new therapies.

The color vision question ("Because of your eyesight, how much difficulty do you have picking out and matching your own clothes?") identified abnormal color vision in 14 of 52 subjects. Abnormal color vision is not generally considered a manifestation of XLRs, although George<sup>5</sup> reported results of color vision testing with City University cards in 50 XLRs patients and identified 11 with tritan defects, 2 with protan defects, and 4 who saw none of the cards.

This multicenter natural history study of subjects with XLRs due to *RS1* pathogenic variants (tested using a standardized battery of methods) documents the variable visual burden and retinal functional losses in patients from the first to the eighth decade of life. Results in subjects across this age spectrum generally indicate a slow decline in both visual function and retinal structural parameters, with more severe impairment in patients who progress to retinal atrophy, although some older subjects retained good function and some younger subjects had poor function. Baseline characteristics and changes over an 18-month follow-up period in our study can be useful in clinical practice for patient counseling and in a research setting for design of clinical trials evaluating response to investigational treatments.

### Acknowledgments

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## APPENDIX

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