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Reading Performance in Blue Cone Monochromacy: Defining an Outcome Measure for a Clinical Trial

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Purpose: Blue cone monochromacy (BCM), a congenital X-linked retinal disease caused by mutations in the *OPN1LW/OPN1MW* gene cluster, is under consideration for intravitreal gene therapy. Difficulties with near vision tasks experienced by these patients prompted this study of reading performance as a potential outcome measure for a future clinical trial.

Methods: Clinically and molecularly diagnosed patients with BCM ($n = 17$; ages 15–63 years) and subjects with normal vision ($n = 22$; ages 18–72 years) were examined with the MNREAD acuity chart for both uniocular and binocular conditions. Parameters derived from the measurements in patients were compared with normal data and also within the group of patients. Intersession, interocular and between-subject variabilities were determined. The frequent complaint of light sensitivity in BCM was examined by comparing results from black text on a white background (regular polarity) versus white on black (reverse polarity) conditions.

Results: MNREAD curves of print size versus reading speed were right-shifted compared with normal in all patients with BCM. All parameters in patients with BCM indicated abnormal reading performance. Intersession variability was slightly higher in BCM than in normal, but comparable with results previously reported for other patients with maculopathies. There was a high degree of disease symmetry in reading performance in this BCM cohort. Reverse polarity showed better reading parameters than regular polarity in 82% of the patients.

Conclusions: MNREAD measures of reading performance in patients with BCM would be a worthy and robust secondary outcome in a clinical trial protocol, given its dual purpose of quantifying macular vision and addressing an important quality of life issue.

Translational Relevance: Assessment of an outcome for a clinical trial.

Introduction

The human macula is defined anatomically as the retinal region of approximately 6 mm (about 18°) in diameter centered on the fovea.[1,2] The cone-rich fovea (1.5 mm or 5° diameter) is surrounded by para- and perifoveal retina with an increasing number of rod photoreceptors. At the edge of the macula, the ratio reaches about 25 rods for every cone.[3] The macula supports high spatial vision and the central visual field; macular disease typically causes symptoms of blurred and distorted vision, both at distance and near, and some increase in sensitivity to glare. Diseases of the macula can be acquired, multifactorial, or inherited.[4]

Major progress in the diagnosis and treatment of macular diseases has led to clinical trials that seek to test if proposed therapies have both safety and efficacy (e.g., Reference[5]). A key outcome in such trials has traditionally been best-corrected visual acuity (BCVA) using Early Treatment Diabetic Retinopathy Study (ETDRS) methodology to evaluate central vision.[6–8] Evaluation of the macula has become more sophisticated with the advent of newer psychophysical modalities[9–11] and imaging,[12–16] and recent trials have added such secondary and exploratory outcomes to

protocols. Further, there is an emphasis on assays of quality of life with instruments such as National Eye Institute Visual Function Questionnaire,[17] and these are commonly used in protocols (e.g., Reference[18]).

The most frequent target of research and treatment initiatives for macular disease continues to be age-related macular degeneration (AMD), the leading cause of central vision loss in later life.[19] The less common "juvenile macular degenerations" have now entered the era of gene discovery and many of these inherited retinal degenerations (IRDs) have molecular diagnoses and disease mechanisms that are much better understood. This progress has led to gene-based therapies in these previously incurable IRDs, with early manifestation of macular dysfunction or degeneration. For example, Stargardt disease caused by biallelic mutations in the *ABCA4* gene is one of the more common IRDs with pronounced maculopathy. A multitude of non–gene- and gene-based therapies have been attempted to date.[19] Recently, two genotypes (*CNGA3*, *CNGB3*) of achromatopsia (ACHM), a heterogeneous group of autosomal recessive IRDs with congenital retina-wide cone photoreceptor disease and maculopathy, have received or are undergoing treatment with subretinal gene augmentation therapy (NCT02610582, NCT03758404, and NCT04124185).[20,21]

Another IRD with retina-wide cone photoreceptor disease that causes congenital macular dysfunction and can lead to macular degeneration is being considered for gene augmentation therapy. Blue cone monochromacy (BCM) is an X-linked disease caused by mutations in the *OPN1LW*/*OPN1MW* gene cluster, resulting in impaired long (L) and middle (M) wavelength-sensitive cone photoreceptor function.[22–26] Among the visual complaints of patients with BCM are impaired visual acuity, color vision abnormalities, and sensitivity to light.[27] In an early phase clinical trial, the primary goal would be to assess safety of the therapy and the clinical eye examination with imaging of the retina would be a primary outcome. The conventional outcome measure of macular function, BCVA, would serve as both a safety assay and an early gauge of efficacy. We sought a further efficacy measure to quantify the visual symptoms of BCM and also serve as a method to understand the impact of the disease and therapy on quality of life. Because difficulty in reading is a notable patient complaint in BCM, we explored in detail how reading vision performance is impaired, what parameters would be most helpful as outcomes in a clinical trial, and their variability characteristics.

Methods

Human Subjects

The study included 17 patients with BCM (median age, 34 years; range, 15–63 years) and 22 subjects with normal vision (median age, 30 years; range, 18–72 years). Patients had a clinical and molecular diagnosis of BCM (Table 1). Molecular testing of the patients and their families has been previously reported.[25,27,28] No study participants reported cognitive impairment or reading disability (dyslexia) and all participants were native or fluent English speakers. Normal subjects had 20/25 or better BCVA in each eye. Procedures followed the Declaration of Helsinki and the study was approved by the University of Pennsylvania Institutional Review Board. Informed consent was obtained from adults, and assent with parental permission from children. All patients previously had a complete eye examination (median time since previous visit, 3 years; range, 2–7 years).

Instrumentation and Testing Procedures

Reading performance was measured using the MNREAD iPad application (Calabrese A, et al. *IOVS*. 2014;55:ARVO E-Abstract 5601).[29] Testing was performed using an iPad Pro with 12.9" Liquid Retina Display (2732 × 2048 pixels, 264 pixels per inch resolution). Screen luminance was set to 75%[29] and kept constant for all testing conditions. Under this setting, average measured luminance was 277 phot-cd.m^{-2}. For all reading tests, the iPad was positioned in landscape orientation at a fixed distance of 40 cm between the subject's eyes and the middle of the iPad screen[29–31] using a combination of an adjustable height tablet stand and an attached fixed-distance chin rest. The height of the iPad stand was adjusted for each subject so the center of the iPad screen was at eye level to ensure accurate sentence presentation.[29]

Subjects were tested in their homes using a testing kit mailed to their residence after they agreed to participate and signed a consent and/or assent. The testing package included an iPad, an iPad stand, a chin rest, and an instruction manual. All subjects were instructed to complete testing in a dimly lit environment away from potential sources of screen reflections and glare and they were given time to adjust to room lighting. Each test session was directed and supervised in its entirety by one author (E.S. or R.S.) over the telephone. A practice test was conducted before the first session using the practice chart feature of the application after verbal confirmation that the ambient conditions were

Table 1. Characteristics of Patients with BCM

Patient No.	Age at Test	*OPN1MW/OPN1LW* Gene Cluster Mutation	Visual Acuity*		Correction Worn for MNRead‡		IS/OS Defect Extent (°)¶	Patient No. in Previous Publications
			Right	Left	Right	Left		
P1	14	Deletion: LCR, *OPN1LW* (partial)	20/400†	20/200	−1.25 −2.00 × 167†	−7.50 −1.25 × 155	1.2	P7#,**, P1††
P2	14	Deletion: LCR, *OPN1LW* (partial)	20/100	20/100	−5.00 −1.75 × 020§	−4.00 −2.25 × 160§	0	P5#,**, P4††
P3	19	Deletion: LCR, *OPN1LW* (partial)	20/100	20/80	−7.00 −1.75 × 10§	−7.00 −1.75 × 10§	0.3	P6#,**, P7††
P4	19	Missense: C203R	20/80	20/80	−6.50 −2.25 × 20§	−6.00 −1.25 × 170§	0.34	P28**, P24††
P5	20	Deletion: LCR, *OPN1LW* (partial)	20/100	20/100	none	none	0.29	P15#,**, P8††
P6	26	Deletion: LCR, *OPN1LW* (partial)	20/100	20/125	−4.50§	−3.50§	0.61	P8#,**, P10††
P7	27	Missense: C203R	20/100	20/125	−5.25 −1.50 × 43	−5.25 −1.75 × 143	0	P26††
P8	32	Deletion: LCR, *OPN1LW*	20/250	20/250	−9.25 +1.00 × 126	−6.75 +1.25 × 60	0.33	P14††
P9	32	Deletion: LCR, *OPN1LW* (partial)	20/80	20/100	−8.25 −1.50 × 020	−10.25 −1.50 × 015	1.57	P9#,**, P12††
P10	35	Deletion: LCR, *OPN1LW* (partial)	20/125	20/100	−4.50§	−4.50 −1.75 × 070§	1.75	P10#,**, P13††
P11	37	Missense: C203R	20/100	20/100	−12.00§	−12.00§	0.21	P27††
P12	38	Missense: C203R	20/160	20/100	−7.25§	−7.25§	0.23	P28††
P13	41	Missense: C203R	20/80	20/80	−6.50 −2.00 × 35	−6.50 −2.50 × 150	0	P29**,††
P14	43	Deletion: LCR, *OPN1LW* (partial)	20/100	20/80	−4.50§,‖	−4.00§,‖	2.53	P18#,**, P17††
P15	54	Missense: C203R	20/80	20/80	−4.00‖	−3.25 −1.00 × 60‖	0.85	P31††
P16	62	Deletion: LCR, *OPN1LW* (partial)	20/63	20/80	−3.25§,‖	−3.25§,‖	1.44	P20**,††
P17	62	Deletion: LCR, *OPN1LW, OPN1MW*	20/160	20/200	−3.75‖	−3.75 −0.75 × 127‖	5.79	P21††

*BCVA measured at most recent evaluation.
†RE has macular coloboma and this eye was not used in reading performance analyses.
‡Current prescription worn for MNRead test was provided by the patient during the testing session.
§Contact lenses (clear, not tinted) used.
‖Correction for P14–P17 includes the patient's presbyopic correction.
¶Extent of defect in the IS/OS line (EZ line).
#Cideciyan et al., 2013.[25]
**Luo et al., 2015.[27]
††Sumaroka et al., 2018.[28]

met. Tests were performed sequentially under monocular and binocular conditions using two screen settings: a white background with black text (named regular polarity) and a black background with white text (named reverse polarity). Tests were first conducted in the regular polarity setting in the order of right eye, left eye, both eyes, and then repeated in the reverse polarity setting.

Testing sessions were scheduled requiring subjects to allow for 1 to 2 hours for the procedure; a normally sighted second person was present for the full session duration to aid with equipment setup and iPad operation. At the scheduled date and time of the session, the tester telephoned the subject and went through a checklist to verify proper setup. By listening to the patient reading over the phone, the tester recorded the reading timing and errors manually using a form and a stopwatch. This was done in addition to the automatic recording by the iPad and permitted verification of the data quality, and overcoming any inadvertent mistakes on the subject's side (e.g., button pressing errors, spontaneous dialogs while timing).

Each subject underwent two complete testing sessions, conducted on different days with at least a 12-hour interval. Tests were performed in the same sequence for both sessions. All 5 available sentence charts in the English version on the MNREAD iPad app were used in each testing session. One chart was always repeated for the sixth reading test. Subjects wore their prescription correction for reading during all tests (e.g. glasses, contacts, or both). The four standard MNREAD parameters were analyzed: maximum reading speed (MRS, the reading speed attainable with unrestricted print size), critical print size (CPS, the smallest size permitting reading at maximum speed), reading acuity (RA, the smallest size needed for reading without significant errors), and the reading accessibility index (ACC, an adimensional index representing visual access to commonly encountered printed material, ranging from 0 [no access] to 1 [normal access]).[29] Due to a range limitation of the instrument, values for RA or CPS may be affected by a ceiling effect[29] when testing subjects with normal vision (the scheme cannot measure RA or CPS lower than –0.1 logarithm of the minimum angle of resolution [logMAR] due to insufficient iPad resolution). Data for patients with BCM are not affected by this limitation.

In all patients, BCVA was available from previous visits to our clinic (Table 1). In addition, in one eye of all patients, fixation records had been obtained previously with a microperimeter (MP1, Nidek Technologies America, Inc, Greensboro, NC) using a 1° diameter red fixation target.[27,32] Fixation instability was quantified by calculating the bivariate contour ellipse area in log minarc^2 encompassing 68% of fixation locations recorded at 25 Hz over a 10-second recording epoch.[33] Optical coherence tomography (OCT) scans were not performed at the same visit as MNREAD testing (which was in the patients' homes), but data were available from a previous visit (median time since previous visit, 3 years; range, 2–7 years).[28] The extents of disruption of the inner segment/outer segment line (IS/OS) or EZ line (distance between edges of intact IS/OS) were obtained from horizontal OCT scans through the fovea (RTvue-100; Optovue, Inc., Fremont, CA); when multiple IS/OS disruptions were present, the longest one was used.

Data Analysis

Data collected via the MNREAD iPad app were analyzed as follows. The iPad app software performs automatic extraction of the four MNREAD parameters and provides information such as testing conditions (viewing distance and screen polarity), print sizes, timing, and errors. These data were emailed to the tester at the end of each session. On reception, the tester checked all collected data to ensure reported reading times and error counts were correct, and any data that necessitated change due to improper initial recording were corrected and reanalyzed using the mnreadR package[34] from the R statistical software.

Visual inspection of the plotted MNREAD curves (log reading speed as a function of print size) was further performed test by test. In a small number of cases in which the algorithms failed to estimate a parameter, the value was corrected manually. All parameter estimation by means of either iPad software, mnreadR R package, or manual correction followed previously reported methods and standard formulas.[29,35,36]

Statistical Analysis

Descriptive statistics were obtained for all MNREAD extracted parameters in patients with BCM and normal subjects. Intersession (session 2 minus session 1), interocular (eye 1 minus eye 2), polarity related (reverse minus regular), and between-subject variabilities were obtained from random effects standard deviation estimates (σ) of mixed effects models to account for the internal correlation structure of the data and small imbalances due to missing observations, which were not imputed. 95% confidence intervals for the differences were obtained as $\pm 1.96*\sigma$. The 95% confidence intervals for σ were obtained by parametric bootstrapping. We used *t*-tests on the

Table 2. BCM Patient Symptoms, Coping Mechanisms, and MNREAD Parameters

	Visual Symptoms			Coping Mechanisms			MNREAD Parameters			
Patient No.	Sensitivity to Light	Trouble Adjusting to Lighting Changes	Difficulty Reading Printed Materials	Difficulty Reading on Electronic Devices	Adjusting Electronic Device Screen Brightness	Adjusting Electronic Device Screen Polarity	MRS	CPS	RA	ACC
P1	Y	Y	Y	Y	Y	Y			+	
P2	Y		Y	Y				+	+	
P3	Y	Y		Y		Y			+	+
P4	Y	Y						+	+	
P5	Y	Y	Y	Y	Y	Y				
P6	Y	Y	Y	Y	Y	Y		+	+	
P7	Y	Y	Y	Y		Y			+	
P8	Y	Y	Y	Y		Y		+	+	+
P9	Y	Y	Y	Y	Y	Y				
P10	Y	Y	Y	Y		Y			+	
P11	Y	Y	Y	Y	Y	Y	+	+	+	+
P12	Y	Y	Y	Y		Y			+	
P13	Y	Y	Y	Y	Y					
P14	Y	Y	Y	Y		Y		+	+	
P15	Y	Y	Y	Y	Y	Y		+	+	+
P16	Y	Y	Y	Y	Y	Y			+	
P17	Y	Y	Y	Y	Y	Y			+	
Total = 17	17 (100%)	16 (94%)	15 (88%)	16 (94%)	9 (53%)	14 (82%)	1 (6%)	7 (41%)	14 (82%)	4 (24%)

Y, yes; MRS, maximum reading speed; CPS, critical print size; RA, reading acuity; ACC, accessibility index.

+, Polarity difference (better performance with reverse polarity compared with normal, alpha = 0.05, with Bonferroni correction for multiple comparisons).

intercept term in the model to assess significance of mean departures from zero for intersession, interocular, and polarity-related differences. Comparison of intersession variabilities between normal and BCM cohorts were assessed by likelihood ratio using models with and without allowance for different variances per cohort. Association between MNREAD parameters and fixation instability as well as any association with IS/OS line disruption was studied using Kendall's rank-correlation. Statistical significance was defined as $P \leq 0.05$.

Results

Patients with BCM had ETDRS BCVA within the range from 20/63 to 20/250 (0.50–1.10 logMAR); they were all myopic (Table 1). All patients complained of sensitivity to light and all but one patient (94%) had difficulty adjusting to lighting changes (Table 2). Most patients described problems reading printed materials (88%) as well as electronic materials (94%) owing to print size, contrast, and/or light sensitivity. Coping mechanisms for reading on electronic devices were common and 53% of patients reported that they usually decrease screen brightness and 82% reverse the screen polarity (to white text on a black background) to read with less discomfort (Table 2).

Reading performance was evaluated from MNREAD curves displaying reading speed (words per minute) versus print size (logMAR) (Fig. 1). The standard four parameters were extracted from each session curve. Data from a representative BCM patient, P7, for the six sessions (two sessions each for the left eye, right eye, and both eyes) are shown (Fig. 1). His MNREAD curves are right shifted compared with normal (larger print size needed to read), indicating a decrease in the RA and CPS (ΔRA and ΔCPS), with near normal MRS (small ΔMRS). Between-subjects standard deviations for the normal cohort were 18 words per minute, 0.12 logMAR, 0.10 logMAR, and 0.10 for the MRS, CPS, RA, and ACC, respectively. The corresponding values for patients with BCM were 20 words per minute, 0.060 logMAR, 0.11 logMAR, and 0.092. There was a correlation between RA and visual acuity ($r^2 = 0.66$; $P = 0.004$). Other MNREAD parameters did not show significant correlations ($P = 0.19$, 0.46, and 0.07 for the MRS, CPS, and ACC respectively, Pearson's product-moment correlation).

Reading Performance under Regular Polarity (Black on White) Conditions

The four MNRead parameters (MRS, CPS, RA, and ACC) for normal subjects and patients with BCM are shown for data collected under regular polarity conditions (Figs. 2A–D; Supplementary Table S1). All parameters in patients with BCM indicated an

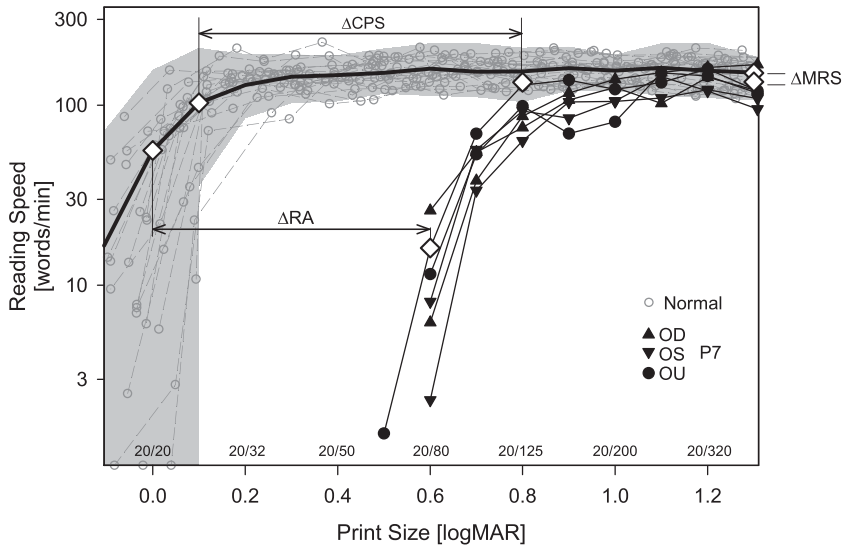

Figure 1. Reading performance in BCM. Standard MNREAD reading speed versus print size curve showing the median (*thick black line*) and range (*shaded grey area* limited by ±1.5 × interquartile range of observations at each print size) for the normal cohort. Individual curves for normal test sessions are shown as *grey open circles* joined by *dashed lines*. *Filled symbols* joined by *solid lines* show data for a representative BCM patient, P7. Data for his six test sessions (two sessions for the left eye [OS], right eye [OD], and both eyes [OU]) are shown. The MNREAD parameters are: MRS (*far right*), CPS (*middle*), and RA (*far left*). ΔCPS, ΔRA, and ΔMRS denote parameter differences between median normal and patient (marked by unfilled diamonds joined by *arrowed lines*). Only regular polarity (black text on white background) data are shown for simplicity. Labels on the horizontal axis are Snellen equivalents for each print size.

abnormal reading performance with similar departures from normal for monocular or binocular conditions. For monocular conditions, MRS data from patients with BCM partially overlapped the normal and the mean speed was lower by 45 words per minute (Fig. 2A). There was minimal or no overlap for CPS, RA, and ACC, with mean respective deficits of 0.82 logMAR, 0.77 logMAR, and 0.49, respectively (Figs. 2B–D). Corresponding deficits for binocular conditions were 43 words per minute, 0.86 logMAR, 0.73 logMAR, and 0.46. The logMAR differences between BCM and normal for CPS and RA correspond with approximately 7 to 8 ETDRS lines. Mean reading performance was higher by small amounts for the binocular compared with monocular condition (Supplementary Table S1).

Effect of Screen Polarity on Reading Performance

Prompted by the BCM patient complaints of light sensitivity, reading difficulty and how they cope with this symptom (Table 2), we studied reading performance with reverse (white on black) screen polarity and compared the results with those using the regular (black on white) screen polarity. The MNREAD curves from patients with BCM P8 and P15 illustrate an observable difference between the results of the two conditions (Fig. 3A, *top*). In contrast, results from patients with BCM P13 and P16 show little or no difference between reading performance with regular versus reverse polarity (Fig. 3A, *bottom*). Differences between the screen polarity conditions for each of the four parameters in normal subjects are shown (Fig. 3B). The polarity-related difference in reading performance for parameters CPS and RA was statistically significant, indicating a decrease in performance with reverse polarity. For the patients with BCM, a polarity-related difference was significant for CPS, RA, and ACC (Fig. 3C; Table 3). These parameters, in contrast to with those of the normal data, showed an increase of performance when reading with reverse polarity compared with the regular polarity. Mean differences were –0.053 logMAR, −0.082 logMAR, and 0.044 for CPS, RA, and ACC respectively. MRS did not show a difference (Table 3).

On an individual-by-individual basis, we identified patients with BCM who showed polarity-related performance differences exceeding the normal range (Table 2). From the mean data, the MRS was not expected to show a difference and did not in all but one patient. Only a limited number of patients had significant differences in CPS and ACC versus normal using reverse polarity. RA, in contrast, was the most telling with the greatest percentage

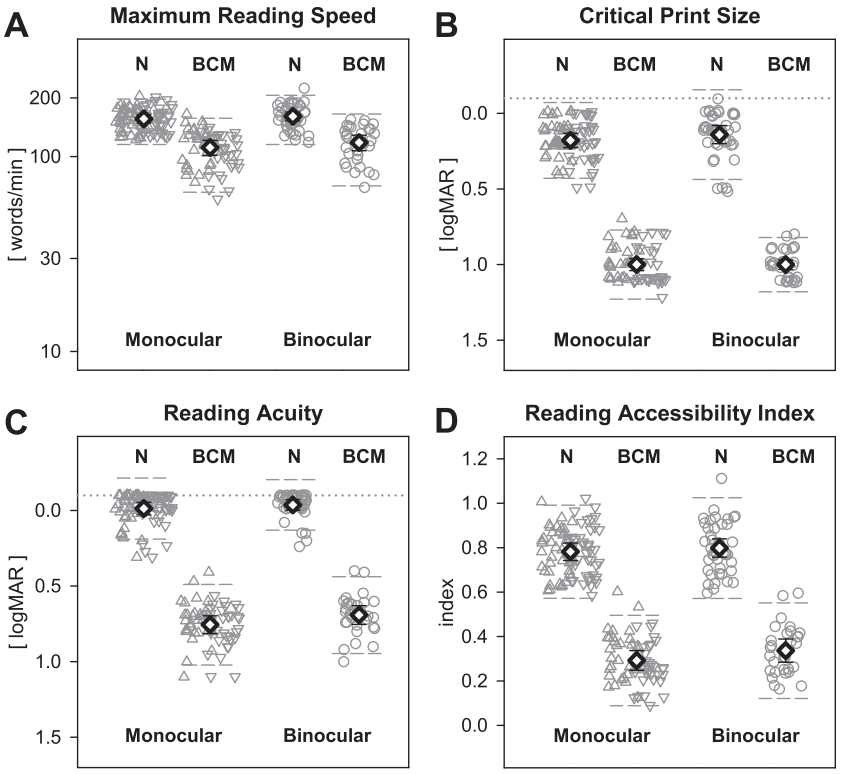

Figure 2. MNREAD parameters for patients with BCM and normal subjects. Monocular and binocular data are shown for the two test sessions performed on each subject using regular (black on white) polarity presentation. (**A**) MRS; (**B**) CPS; (**C**) RA; (**D**) ACC. *Grey open symbols* are the measurements for normal subjects (N) and patients with BCM (*up-triangles*, right eye [OD]; *down-triangles*, left eye [OS]). *Dashed lines* correspond with the mean and ±2 standard deviations of the population. *White-filled diamonds* (and associated error bars) indicate means and ±2 standard error of the mean. *Dotted lines* in (B) and (C) indicate test ceiling limits. Individual data points were horizontally jittered for visibility.

Table 3. Difference Between Regular and Reverse Screen Polarity in BCM Reading

	Normal Subjects		Patients with BCM	
Parameter	Mean† (SEM)	*P* Value	Mean† (SEM)	*P* Value
MRS (wpm)	1.2 (1.5)	0.42	2.4 (2.4)	0.34
CPS (logMAR)	0.079 (0.014)*	0.00	−0.054 (0.015)*	0.0020
RA (logMAR)	0.037 (0.0060)*	0.00	−0.084 (0.014)*	0.00
ACC	0.00 (0.0070)	0.99	0.044 (0.010)*	0.0010

MRS, maximum reading speed; CPS, critical print size; RA, reading acuity; ACC, accessibility index; SEM, standard error of the mean; wpm, words per minute.

*Difference is significantly different than zero.

†Mean difference, reverse minus regular polarity. Positive differences in MRS and ACC, and negative differences in CPS and RA indicate better reading performance for reverse polarity (white on black).

of patients showing differences to normal. Of the 14 patients who by history reported adjusting their electronic devices to reverse polarity, there were 12 with abnormal RA polarity-related differences. There were no patients showing a worse performance difference than normal when using reverse polarity.

Effect of Fixation Instability and IS/OS Defect Extents on Reading Performance

We asked whether fixation instability in patients with BCM played a detectable role in the reading abnormalities. Fixation instability as quantified by the bivariate contour ellipse area (BCEA) in patients

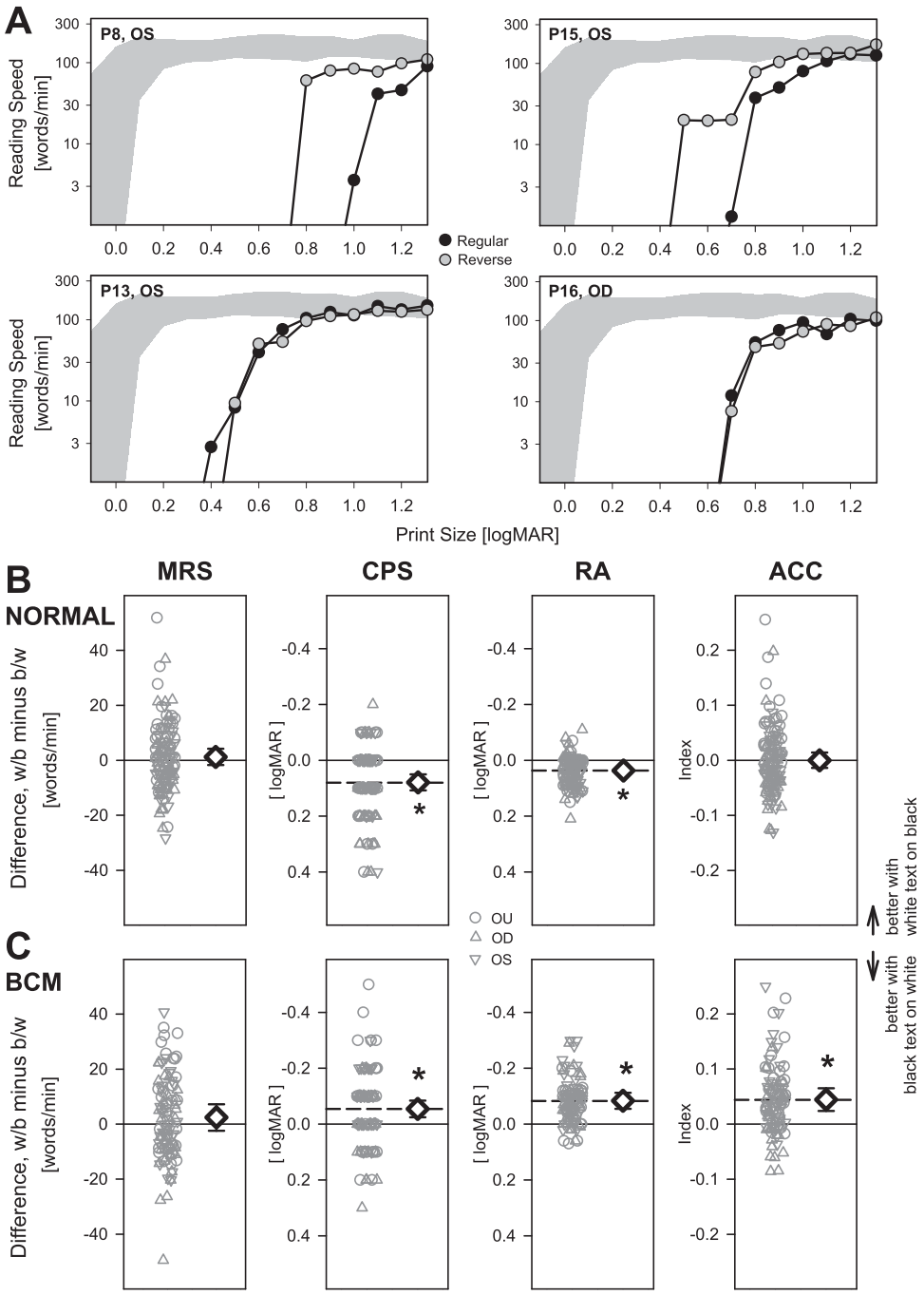

Figure 3. Impact of screen polarity and light sensitivity on reading performance. **(A)** Reading speed versus print size curves for four representative patients with BCM that showed a difference (P8, P15; *top*) or did not show a difference (P13,P16; *bottom*) in the standard MNREAD curves for the two screen polarities: regular, black text on white background (b/w), and reverse, white text on black background (w/b). The effect of screen polarity for each MNREAD parameter in normal subjects (**B**) and patients with BCM (**C**) expressed as the difference in performance for regular minus reverse polarities. Values above zero (solid line) indicate better performance when w/b is presented. *White-filled diamonds* (and associated error bars) indicate means ±2 standard error of the mean. Individual data points were horizontally jittered for visibility in (**B**) and (**C**).

with BCM ranged from 3.10 to 4.67 log minarc^2 (normal range, 2.33–3.21 log minarc^2; $n = 6$). We studied whether there were monotonic associations between the BCEA fixation instability metric and each of the MNREAD parameters in the BCM cohort, for regular and reversed polarities.[37] There was no evidence of monotonic relationships between instability of fixation and any of the MNREAD

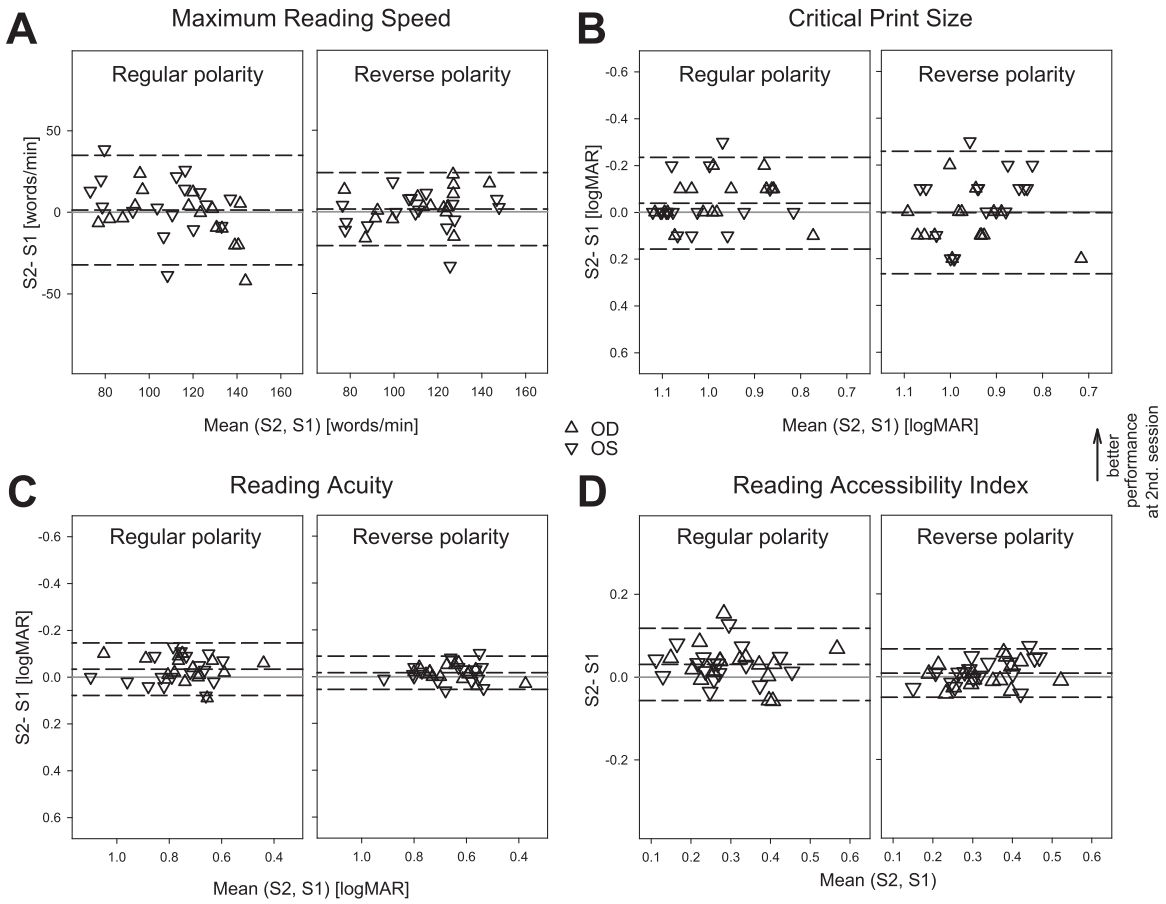

Figure 4. Intersession variability in reading performance. Bland-Altman plots of intersession differences (session 2 minus session 1) versus the mean of both sessions in the BCM cohort. *Dashed lines* represent mean and 95% confidence intervals for those differences. S1: Session 1; S2: Session 2. Intersession variabilities are shown for monocular data of the two presentation polarities, regular (black on white) and reverse (white on black). Individual data points in (**B**) were horizontally jittered for visibility.

parameters. Rank coefficients (τ) were not significantly different than zero for both regular and reversed polarity ($P > 0.17$ for all parameters, Kendall's rank-correlation). Likewise, we studied the relation between the extent of IS/OS defect extents and MNREAD parameters. There was evidence of monotonic relations between the extent of MRS, RA and ACC but not for CPS ($P = 0.016$, 0.27, 0.029, and 0.0025 for MRS, CPS, RA, and ACC, respectively). Wider extents corresponded with worse MNREAD parameters.

Intersession Variability

MNREAD data were examined for intersession variability in the BCM patient cohort and in the normal subjects. Considering the differences in performance with reverse versus regular screen polarity, the two conditions were analyzed separately (Table 4). Bland-Altman plots for MRS, RA, CPS, and ACC are shown for the monocular condition in the patients with BCM (Fig. 4). If a measurement is obtained at a certain point in time, a second measurement in the future will show a difference from the first. If these pairs of measurements were performed multiple times, for 95% of those times the values of the difference between measurements are expected to be within the limits shown in the plots. The limits therefore inform the design of treatment trials where multiple measurements are taken across time. There were some differences in the width of the intervals when considering regular and reverse polarities, with the latter showing narrower intervals, except in CPS. Most variabilities of patients with BCM were slightly higher than those from normal. Those for CPS, RA, and ACC for regular polarity, and RA for reverse polarity were statistically significant. Table 4 includes 95% confidence intervals for the limits themselves to enable comparisons across cohorts and indicate the precision attained in the intersession variability estimates. Small departures from zero for the means were present for some parameters (asterisks

Table 4. Intersession Variability in patients with BCM and Normal Subjects

	Normal Subjects						Patients with BCM					
	Monocular			Binocular			Monocular			Binocular		
Parameter	Intersession Variability[†]	Mean Difference S2 – S1 (SEM)	*P* Value	Intersession Variability[†]	Mean Difference S2 – S1 (SEM)	*P* Value	Intersession Variability[†]	Mean Difference S2 – S1 (SEM)	*P* Value	Intersession Variability[†]	Mean Difference S2 – S1 (SEM)	*P* Value
Regular (b/w)												
MRS (wpm)	± 21 [16–25]	3.3 (2.3)	0.18	± 22 [15–29]	3.4 (2.5)	0.20	± 33 [25–42][‡]	1.4 (3.4)	0.69	± 39 [26–53][‡]	5.6 (4.8)	0.27
CPS (logMAR)	± 0.16 [0.12–0.19]	0.0030 (0.013)	0.84	± 0.2 [0.13–0.25]	−0.033 (0.022)	0.16	± 0.21 [0.15–0.26]	−0.039 (0.017)[*]	0.042	± 0.25 [0.17–0.31]	0.063 (0.036)	0.11
RA (logMAR)	± 0.094 [0.072–0.108]	−0.029 (0.0070)[*]	0.0010	± 0.049 [0.035–0.066]	−0.013 (0.0050)[*]	0.027	± 0.129 [0.098–0.157][‡]	−0.034 (0.012)[*]	0.014	± 0.107 [0.07–0.145][‡]	−0.018 (0.013)	0.20
ACC	± 0.11 [0.08–0.13]	0.018 (0.012)	0.16	± 0.1 [0.07–0.13]	0.022 (0.011)	0.065	± 0.11 [0.08–0.13]	0.031 (0.0090)[*]	0.0030	± 0.1 [0.06–0.13]	0.025 (0.011)[*]	0.041
Reverse (w/b)												
MRS (wpm)	± 23 [17–26]	2.8 (2.6)	0.32	± 32 [22–42]	6.2 (3.6)	0.11	± 22 [17–27]	1.7 (2.0)	0.42	± 34 [22–45]	7.1 (4.0)	0.11
CPS (logMAR)	± 0.26 [0.2–0.31]	−0.026 (0.027)	0.35	± 0.25 [0.17–0.33]	−0.024 (0.031)	0.47	± 0.26 [0.2–0.31]	0.0030 (0.023)	0.90	± 0.29 [0.19–0.38]	0.031 (0.037)	0.42
RA (logMAR)	± 0.091 [0.07–0.113]	−0.015 (0.0080)	0.097	± 0.059 [0.038–0.08]	−0.016 (0.0060)[*]	0.025	± 0.077 [0.06–0.095]	−0.016 (0.0070)[*]	0.029	± 0.101 [0.067–0.134][‡]	−0.026 (0.011)[*]	0.043
ACC	± 0.1 [0.08–0.12]	0.017 (0.012)	0.18	± 0.16 [0.10–0.22]	0.033 (0.018)	0.091	± 0.06 [0.05–0.08][‡]	0.0090 (0.0050)	0.10	± 0.1 [0.07–0.14]	0.028 (0.012)[*]	0.032

MRS, maximum reading speed; CPS, critical print size; RA, reading acuity; ACC, accessibility index; SEM, standard error of the mean; wpm, words per minute.

[*]Mean difference between sessions is significantly different than zero.

[†]The 95% confidence limits for the difference, session 2 (S2) minus session 1 (S1). Ranges between square brackets are 95% confidence intervals for these limits.

[‡]Variability is statistically different than in the normal group.

Table 5. Interocular Differences in Patients with BCM and Normal Subjects

	Normal Subjects			Patients with BCM		
Parameter	Interocular Variability*	Mean Difference (SEM)	*P* Value	Interocular Variability*	Mean Difference (SEM)	*P* Value
Regular (b/w)						
MRS (wpm)	± 17	0.24 (1.4)	0.86	± 36	–3.1 (3.8)	0.43
CPS (logMAR)	± 0.22	–0.0090 (0.020)	0.67	± 0.28	0.028 (0.031)	0.38
RA (logMAR)	± 0.080	–0.011 (0.0070)	0.13	± 0.21	–0.0070 (0.025)	0.78
ACC	± 0.091	0.0080 (0.0070)	0.29	± 0.16	–0.015 (0.018)	0.44
Reverse (w/b)						
MRS (wpm)	± 17	2.8 (1.4)	0.055	± 27	1.8 (2.8)	0.54
CPS (logMAR)	± 0.24	–0.0080 (0.019)	0.70	± 0.28	–0.022 (0.026)	0.42
RA (logMAR)	± 0.13	0.0070 (0.013)	0.59	± 0.18	–0.029 (0.022)	0.21
ACC	± 0.079	0.010 (0.0070)	0.18	± 0.10	0.016 (0.012)	0.20

MRS, maximum reading speed; CPS, critical print size; RA, reading acuity; ACC, accessibility index; SEM, standard error of the mean; wpm, words per minute.

*The 95% confidence interval for interocular differences.

in Table 4), with signs indicating slightly better performance in the second session, and could be the manifestation of a learning effect. This finding would favor the inclusion of more than one session at baseline in treatment trial design.

Interocular Variability

The 95% confidence limits for the difference between eyes were consistent with and slightly higher than the intersession variability (Table 5 and Fig. 5, *left*). This is to be expected, as the interocular variability can be partitioned as a component due to testing two sessions (left and right eyes) plus another to account for the actual difference between eyes. The small difference between the two variabilities indicates that the between-eyes contribution is less substantial. Interocular differences were studied using the eye with better acuity minus the fellow eye, as this is a usual criterion to select the eye in many uniocular treatment trials. This choice lets us assess the degree of asymmetry that could be expected on average in those situations. Mean differences between the eye with better VA (Eye 1) and the fellow eye (Eye 2), however, were not different than zero for all parameters. There was therefore no suggestion of significant asymmetry for BCM in terms of reading performance parameters. The square boxes to the right of each panel in Fig. 5 illustrate the actual departures from perfect symmetry indicated by the 45° diagonal.

Discussion

Difficulties with near vision are a key complaint of patients with maculopathy.[7,19,38,39] The most common outcome measure in clinical trials involving maculopathies has historically been distance visual acuity; reading performance assays have been less consistently used.[19,40] Further, the recent wealth of new structural and functional endpoints has attracted considerable academic interest in summary documents that list outcomes for clinical trials of macular and retina-wide diseases; quantifying reading ability is less a topic of discussion.[8] To our knowledge, there have been no previous studies of reading performance in BCM, a retina-wide cone dysfunction with maculopathy being discussed as a candidate for a gene therapy.[25,27] Patients with BCM cite reading difficulties as one of their key visual problems (Table 2). In the current work, we studied a cohort of patients with BCM using the MNREAD method, developed about three decades ago[30] and since refined and translated in several languages as well as moved to a digital platform.[29,35,41,42] We asked whether these reading parameters could be of use as an outcome for a gene therapy clinical trial in BCM.

When reading performance has been measured, it has usually been in AMD.[39,40,42] In general, AMD protocols have found that MRS was slower and RA was lower than in controls (e.g., References[39,40]). The abnormalities depended on AMD disease stage and severity. There are patients with early AMD (neither neovascular abnormalities nor geographic atrophy)[43] whose reading curve has only slightly slower MRS and reduced RA (reviewed in[42]). Then, there are patients with late AMD and absolute central scotomas. Their reading curves show considerably reduced MRS as well as RA and CPS reductions. Illustrating the complexity of the AMD macular pathology, there are studies of geographic atrophy with relatively preserved distance visual acuity due to a small fovea-spared region which, however, may not allow for reading continuous text.[44,45] The size of the atrophic lesion and its growth have been related to decline in MRS and

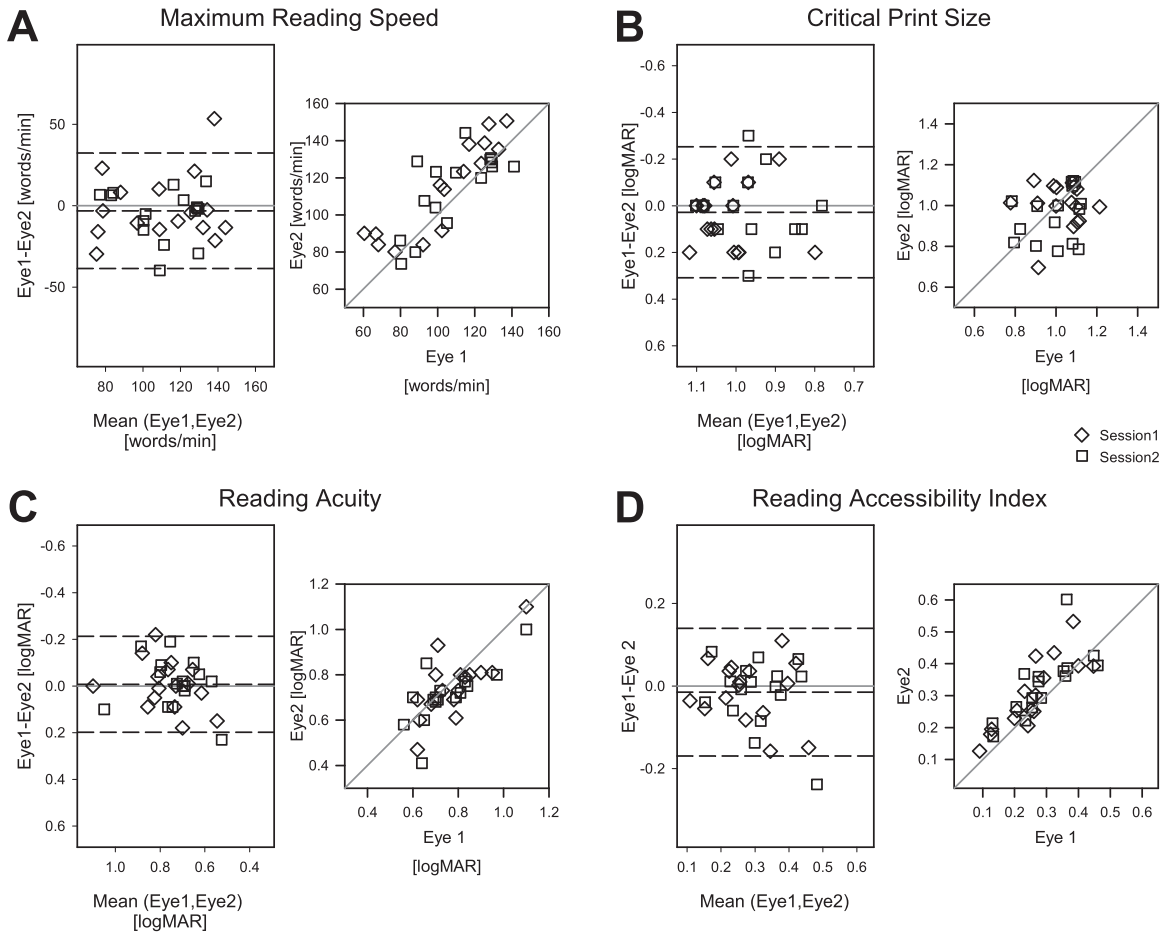

Figure 5. Interocular variability and symmetry in reading performance. (**A**, **B**, **C**, and **D** for MRS, CPS, RA, and ACC, respectively). (*Left*) Bland-Altman plots of interocular differences (Eye 1 minus Eye 2) versus the mean of both eyes in the BCM cohort. Dashed lines represent mean and 95% confidence intervals for those differences. Interocular variabilities are shown for regular presentation polarity (black on white) and data from all available sessions. Individual data points in (**B**) were horizontally jittered for visibility. (*Right*) Plots of values from Eye 1 versus Eye 2. Data points having equal values in both eyes would lie on the solid line of slope 1, which indicates perfect symmetry.

this led to suggesting that MRS be used as a functional vision outcome in AMD trials.[46] In a low vision clinical trial that included many different maculopathies, MRS and RA were informative variables while CPS was not.[47] Another study of reading parameters in rehabilitation of subjects with central vision loss (mostly from AMD) emphasized the value of the ACC parameter.[48]

What is the basis of the reduced MRS in AMD? There is a history of trying to explain the MRS abnormalities in patients with AMD with central scotomas as simply the result of using more peripheral (actually paracentral) retina for the task.[49] The basis of the reduced MRS in AMD, however, is likely to be more complex and multifactorial, with contributors being impaired oculomotor control, poor fixation stability, shrinkage in the size of the visual span, and slower temporal processing of letter information (reviewed in[50]). Patients with AMD with central field loss also can have a preferred retinal locus outside the macular lesion and its location and stability can influence reading performance.[49]

Less often, there has been assessment of reading performance in juvenile macular degenerations. For example, in *ABCA4*-STGD, MRS and RA were strongly related to quality-of-life measures.[51] Results of recent gene therapy trials in *CNGA3*- and *CNGB3*-ACHM (achromatopsia) could be a useful comparison to those in BCM, considering the retina-wide cone dysfunction. However, a phase I/II study of subretinal gene augmentation in the *CNGA3* molecular form of ACHM listed on a clinical trial protocol a number of secondary end points, including the spatial vision measures of visual acuity and contrast sensitivity, but not a reading test.[20,21] Other ACHM trials have not reported results to date.

The BCM results in the current work showed abnormal reading performance in all four parameters. BCM curves were readily distinguishable from normal with clearly right-shifted data (Fig. 1). The MRS in some of the patients could fall within the lower limits of normal. Of the four parameters, MRS was the least profoundly affected by BCM; CPS, RA, and ACC were notably different from normal. Given the purpose of this study to identify parameters that would be sufficiently abnormal to reveal a change in a clinical trial, all parameters would qualify ,but especially the CPS, RA, and ACC. Further, variability around the respective means in these parameters was comparable with or less than in the normal data.

What contributes to the pathophysiology of the reading difficulty in BCM? As a group, the reading performance results in patients with BCM were relatively similar in pattern, which is unlike the spectrum of changes reported for patients with AMD.[39,40,42] The near normal MRS in BCM may be due to a lack of a discrete central scotoma. There is a relatively homogeneous central L/M cone dysfunction (with residual and normally functioning S-cones and rods) that extends into the periphery.[27] Given the reduced distance visual acuity, it is not surprising that RA and CPS could also be reduced. A decrease in MNREAD performance by three metrics (MRS, RA, and ACC) was associated with an increased IS/OS defect extent. There was a lack of association, however, between fixation instability and reading abnormalities. Future studies could evaluate the fixation stability during the reading test to remove the possibility of different photoreceptor systems (rods vs S-cones) dominating perception[27] in two tests performed separately under different ambient conditions.

Intersession variability of an outcome measure is important to quantify before initiating a clinical trial. Previous studies of variability with MNREAD charts have used different methods and populations, but such data offer opportunities for comparison with the current results.[40,41,52] MNREAD intersession variability has been reported for cohorts of normal subjects (e.g., References[40,52]). For a cohort of subjects with impaired vision mainly due to AMD, the results were ±24 words per minute, ±0.20 logMAR, and ±0.10 logMAR for MRS, CPS, and RA, respectively.[41] For another AMD cohort deemed to have stable disease, the corresponding coefficients were ±66 words per minute, ±0.55 logMAR, and ±0.30 logMAR.[40] In the present study, the intersession variability in BCM for regular polarity (±34 words per minute, ±0.20 logMAR, and ±0.11 logMAR, correspondingly; Table 4) lie between or near the data from the two groups of patients with AMD, that is, not remarkably different from the findings with MNREAD methods in other maculopathy studies.

As we document in Table 2, most patients with BCM complain about increased light sensitivity and seek a means to ameliorate the symptom. Patients tend to use reverse polarity (white on black background) in their reading material (e.g., cell phones, tablets; Table 2). We examined whether screen polarity made a difference in the reading parameters in patients with BCM and whether those that manifested a difference also were the patients that had greater symptoms from light sensitivity. The conclusion from these results was that reverse polarity conditions would be worth measuring at baseline in addition to the regular polarity considering the length of MNREAD testing is not onerous. A change in this behavior as a result of treatment could serve as an additional outcome parameter in a clinical trial.

For orphan retinal diseases being considered for a clinical trial, the question of disease symmetry should be raised to decide if there is similar therapeutic potential in the two eyes and whether an untreated eye can serve as a control. The relatively limited number of patients available for such trials makes a case for this approach. The results in the present study indicated there was no significant asymmetry for BCM in terms of reading performance parameters.

What have we learned that would help in the design of a future clinical trial? From the standpoint of feasibility, MNREAD would be a worthy, quantifiable outcome for a clinical trial in BCM. It serves as both a spatial vision task and has implications for the quality of life of the patients, given a positive change in reading performance. Administration of the test is feasible for the examiner and the patient, and the electronic version was convenient and even portable, making it possible to perform this outcome measure on remote visits. There may be some learning effects in patients (and normal subjects) because the mean performance on the second session was slightly better than on the first for some parameters in the intersession analyses. It would thus be judicious to have a reading performance measure on screening and baseline visits and to determine individual intersession variabilities before the onset of the clinical trial.

Given the symptoms of patients about their reading difficulties and their coping mechanism of reversing the polarity on electronic devices to avoid negative effects of brightness, we examined if there were reading parameters that were quantifiably impaired more with regular versus reverse polarity in the patients. Three of the four parameters were negatively affected by the regular polarity and on intersession variability, there was less variability with the reverse polarity than with

regular polarity. The condition with white letters on a black background (reverse polarity) would seem to be advisable to include in a BCM clinical trial.

Although the immediate purpose of the current studies in BCM was to determine whether a reading performance outcome would be a worthy addition to a protocol in a gene therapy clinical trial, there was also another finding in the data that confirms and extends previous studies in S-cone psychophysics. There has been a longstanding interest in determining features of the S-cone pathways and comparing those features with those of the L/M cone system. Isolating the S-cone mechanism from that of L/M cones in trichromats has been undertaken with a variety of methods, such as two-color increment thresholds, silent substitution, and chromatic adaptation (reviewed in[53]). S-cone acuity has been previously determined with such techniques in normal observers.[54] There are also rare studies that have tested patients with BCM for this purpose (e.g., References[55–57]). In a 17-year-old patient with BCM, grating acuity was 4 to 8 cycles/degree (20/80–20/140 Snellen) and in two 15-year-old patients with BCM, grating acuity was approximately 6 to 9 cycles per degree (20/67–20/150 Snellen). These results compare favorably with the distance acuities in the current cohort of patients with BCM of 20/63 to 20/250 and the average RA of 0.69 logMAR (or 20/100). The estimates from S-cone topography in donor retinas were also consistent.[58–60] Another way to look at the current study, therefore, is that it may represent a glimpse at the reading performance (under these specific conditions) of the S-cone system as compared with that of the L/M-cone system. Whether there was intrusion of the light-adapted rod system in the results needs further study.[27,61,62]

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References

1. Hogan M, Alvaredo JA, Weddell JE. *Histology of the Human Eye: An Atlas and Textbook*. Philadelphia, PA: Saunders; 1971.
2. Spaide RF, Curcio CA. Anatomical correlates to the bands seen in the outer retina by optical coherence tomography: literature review and model. *Retina*. 2011;31:1609–1619.
3. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol*. 1990;292:497–523.
4. Jonas JB, Bourne RRA, White RA, et al. Visual impairment and blindness due to macular diseases globally: a systematic review and meta-analysis. *Am J Ophthalmol*. 2014;158:808–815.
5. Sacconi R, Giuffrè C, Corbelli E, Borrelli E, Querques G, Bandello F. Emerging therapies in the management of macular edema: a review. *F1000Research*. 2019;8:F1000.
6. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. *Optom Vis Sci*. 1976;53:740–745.
7. Csaky KG, Richman EA, Ferris FL. Report from the NEI/FDA ophthalmic clinical trial design and endpoints symposium. *Invest Ophthalmol Vis Sci*. 2008;49:479–489.
8. Csaky K, Ferris F, Chew EY, Nair P, Cheetham JK, Duncan JL. Report from the NEI/FDA endpoints workshop on age- related macular degeneration and inherited retinal diseases. *Invest Ophthalmol Vis Sci*. 2017;58:3456–3463.
9. Cideciyan AV, Swider M, Aleman TS, et al. Macular function in macular degenerations: repeatability of microperimetry as a potential outcome measure for ABCA4-associated retinopathy trials. *Invest Ophthalmol Vis Sci*. 2012;53:841–852.
10. Pfau M, Lindner M, Müller PL, et al. Effective dynamic range and retest reliability of dark-adapted two-color fundus-controlled perimetry in patients with macular diseases. *Invest Ophthalmol Vis Sci*. 2017;58:BIO158–BIO167.
11. Csaky KG, Patel PJ, Sepah YJ, et al. Microperimetry for geographic atrophy secondary to age-related macular degeneration. *Surv Ophthalmol*. 2019;64:353–364.
12. Carroll J, Dubra A, Gardner JC, et al. The effect of cone opsin mutations on retinal structure and the integrity of the photoreceptor mosaic. *Invest Ophthalmol Vis Sci*. 2012;53:8006–8015.
13. Carroll J, Scoles DH, Langlo S, et al. Imaging cone structure in patients with *OPN1LW* and *OPN1MW* mutations. *Invest Ophthalmol Vis Sci*. 2014;55:4542.
14. Patterson EJ, Kalitzeos A, Kasilian M, et al. Residual cone structure in patients with X-linked

cone opsin mutations. *Invest Ophthalmol Vis Sci*. 2018;59:4238–4248.
15. Burns SA, Elsner AE, Sapoznik KA, Warner RL, Gast TJ. Adaptive optics imaging of the human retina. *Prog Retin Eye Res*. 2019;68:1–30.
16. Müller P, Wolf S, Dolz-Marco R, Tafreshi A, Schmitz-Valckenberg S, Holz F. Ophthalmic diagnostic imaging: retina. In: Bille JF, ed. *High Resolution Imaging in Microscopy and Ophthalmology: New Frontiers in Biomedical Optics*. New York: Springer International Publishing; 2019:87–106.
17. Mangione CM, Lee PP, Pitts J, Gutierrez P, Berry S, Hays RD. Psychometric properties of the National Eye Institute Visual Function Questionnaire (NEI-VFQ). *Arch Ophthalmol*. 1998;116:1496–1504.
18. Jelin E, Wisløff T, Moe MC, Heiberg T. Psychometric properties of the National Eye Institute Visual Function Questionnaire (NEI-VFQ 25) in a Norwegian population of patients with neovascular age-related macular degeneration compared to a control population. *Health Qual Life Outcomes*. 2019;17:140.
19. Waugh N, Loveman E, Colquitt J, et al. Treatments for dry age-related macular degeneration and Stargardt disease: a systematic review. *Health Technol Assess*. 2018;22:1–167.
20. Kahle NA, Peters T, Zobor D, et al. Development of methodology and study protocol: safety and efficacy of a single subretinal injection of rAAV.hCNGA3 in patients with CNGA3-linked achromatopsia investigated in an exploratory dose-escalation trial. *Hum Gene Ther Clin Dev*. 2018;29:121–131.
21. Fischer MD, Michalakis S, Wilhelm B, et al. Safety and vision outcomes of subretinal gene therapy targeting cone photoreceptors in achromatopsia. *JAMA Ophthalmol*. 2020;138(6):643–651.
22. Nathans J, Davenport CM, Maumenee IH, et al. Molecular genetics of human blue cone monochromacy. *Science*. 1989;245:831–838.
23. Nathans J, Maumenee IH, Zrenner E, et al. Genetic heterogeneity among blue-cone monochromats. *Am J Hum Genet*. 1993;53:987–1000.
24. Mizrahi-Meissonnier L, Merin S, Banin E, Sharon D. Variable retinal phenotypes caused by mutations in the X-linked photopigment gene array. *Invest Ophthalmol Vis Sci*. 2010;51:3884–3892.
25. Cideciyan AV, Hufnagel RB, Carroll J, et al. Human cone visual pigment deletions spare sufficient photoreceptors to warrant gene therapy. *Hum Gene Ther*. 2013;24:993–1006.
26. Gardner JC, Liew G, Quan YH, et al. Three different cone opsin gene array mutational mechanisms with genotype-phenotype correlation and functional investigation of cone opsin variants. *Hum Mutat*. 2014;35:1354–1362.
27. Luo X, Cideciyan AV, Iannaccone A, et al. Blue cone monochromacy: visual function and efficacy outcome measures for clinical trials. *PLoS One*. 2015;10:1–18.
28. Sumaroka A, Garafalo AV, Cideciyan AV, et al. Blue cone monochromacy caused by the C203R missense mutation or large deletion mutations. *Invest Ophthalmol Vis Sci*. 2018;59:5762–5772.
29. Calabrèse A, To L, He Y, Berkholtz E, Rafianm P, Legge GE. Comparing performance on the MNREAD iPad application with the MNREAD acuity chart. *J Vis*. 2018;18:8.
30. Legge GE, Ross JA, Luebker A, Lamay JM. Psychophysics of reading. VIII. The Minnesota low-vision reading test. *Optom Vis Sci*. 1989;66:843–853.
31. Mansfield J, Legge G, Luebker A, Cunningham K. *MNRead Acuity Charts: Continuous-Text Reading-Acuity Charts for Normal and Low Vision*. Duluth, MN: Lighthouse Low Vision Products; 1994.
32. Cideciyan AV, Aleman TS, Boye SL, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci USA*. 2008;105:15112–15117.
33. Crossland Dunbar HM, Rubin GS. Fixation stability measurement using the MP1 microperimeter. *Retina*. 2009;29(5):651–656.
34. Calabrèse A, Mansfield J, Legge GE. mnreadR, an R package to analyze MNREAD data, version 2.1.4. Available at: https://CRAN.R-project.org/package=mnreadR.
35. Calabrèse A, Cheong AMY, Cheung SH, et al. Baseline MNREAD measures for normally sighted subjects from childhood to old age. *Invest Ophthalmol Vis Sci*. 2016;57:3836–3843.
36. Calabrèse A, Owsley C, McGwin G, Legge GE. Development of a reading accessibility index using the MNREAD acuity chart. *JAMA Ophthalmol*. 2016;134:398–405.
37. Bonett DG, Wright TA. Sample size requirements for estimating Pearson, Kendall and Spearman correlations. *Psychometrika*. 2000;65:23–28.
38. Rubin GS. Measuring reading performance. *Vision Res*. 2013;90:43–51.
39. Varadaraj V, Lesche S, Ramulu PY, Swenor BK. Reading speed and reading comprehension in age-related macular degeneration. *Am J Ophthalmol*. 2018;186:138–143.

40. Patel PJ, Chen FK, da Cruz L, Rubin GS, Tufail A. Test-Retest variability of reading performance metrics using MNREAD in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:3854–3859.
41. Subramanian A, Pardhan S. Repeatability of reading ability indices in subjects with impaired vision. *Invest Ophthalmol Vis Sci*. 2009;50:3643–3647.
42. Legge GE. Reading digital with low vision. *Visible Lang*. 2016;50:102–125.
43. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet*. 2018;392:1147–1159.
44. Sunness JS, Rubin GS, Zuckerbrod A, Applegate CA. Foveal-sparing scotomas in advanced dry age-related macular degeneration. *J Vis Impair Blind*. 2008;102:600–610.
45. Kimel M, Leidy NK, Tschosik E, et al. Functional Reading Independence (Fri) index: a new patient-reported outcome measure for patients with geographic atrophy. *Invest Ophthalmol Vis Sci*. 2016;57:6298–6304.
46. Varma R, Souied EH, Tufail A, et al. Maximum reading speed in patients with geographic atrophy secondary to age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2018;59:AMD195–AMD201.
47. Stelmack JA, Tang XC, Wei Y, et al. Outcomes of the Veterans Affairs Low Vision Intervention Trial II (LOVIT II). *JAMA Ophthalmol*. 2017;135:96.
48. Tarita-Nistor L, González EG, Mandelcorn MS, Brent MH, Markowitz SN, Steinbach MJ. The reading accessibility index and quality of reading grid of patients with central vision loss. *Ophthalmic Physiol Opt*. 2018;38:88–97.
49. Chung STL. Reading in the presence of macular disease: a mini-review. *Ophthalmic Physiol Opt*. 2020;40:171–186.
50. Cheong AMY, Legge GE, Lawrence MG, Cheung SH, Ruff MA. Relationship between visual span and reading performance in age-related macular degeneration. *Vision Res*. 2008;48:577–588.
51. Murro V, Sodi A, Giacomelli G, et al. Reading ability and quality of life in Stargardt disease. *Eur J Ophthalmol*. 2017;27:740–745.
52. Subramanian A, Pardhan S. The repeatability of MNREAD acuity charts and variability at different test distances. *Optom Vis Sci*. 2006;83:572–576.
53. Smithson HE. S-cone psychophysics. *Vis Neurosci*. 2014;31:211–225.
54. Stromeyer CF, Kranda K, Sternheim CE. Selective chromatic adaptation at different spatial frequencies. *Vision Res*. 1978;18:427–437.
55. Daw NW, Enoch JM . Contrast sensitivity, Westheimer function and Stiles-Crawford effect in a blue cone monochromat. *Vision Res*. 1973;13:1669–1680.
56. Hess RF, Mullen KT, Sharpe LT, Zrenner E. The photoreceptors in atypical achromatopsia. *J Physiol*. 1989;417:123–149.
57. Hess RF, Mullen KT, Zrenner E. Human photopic vision with only short wavelength cones: postreceptoral properties. *J Physiol*. 1989;417:151–172.
58. Curcio CA, Allen KA, Sloan KR, et al. Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. *J Comp Neurol*. 1991;312:610–624.
59. Ahnelt PK, Kolb H. The mammalian photoreceptor mosaic-adaptive design. *Prog Retin Eye Res*. 2000;19:711–777.
60. Hunt DM, Peichl L. S cones: evolution, retinal distribution, development, and spectral sensitivity. *Vis Neurosci*. 2014;31:115–138.
61. Huchzermeyer C, Kremers J. Perifoveal S-cone and rod-driven temporal contrast sensitivities at different retinal illuminances. *J Opt Soc Am A*. 2017;34:171–183.
62. Hirji N, Aboshiha J, Georgiou M, Bainbridge J, Michaelides M. Achromatopsia: clinical features, molecular genetics, animal models and therapeutic options. *Ophthalmic Genet*. 2018;39:149–157.