VISUAL FUNCTION MEASURES IN EARLY AND INTERMEDIATE AGE-RELATED MACULAR DEGENERATION

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Purpose: The objectives of this study were to evaluate 1) the feasibility of performing computerized tests of low luminance visual acuity (LLVA), cone-specific contrast (Cone Contrast Test [CCT]), contrast sensitivity, and microperimetry and 2) the test–retest repeatability of these outcomes in dry age-related macular degeneration (AMD).

Methods: This prospective study enrolled 30 subjects at a single site (8 controls, 8 early AMD, and 12 intermediate AMD). Subjects underwent LLVA, contrast sensitivity, CCT, and microperimetry with eye tracking. Low luminance deficit was defined as best-corrected visual acuity minus LLVA in EDTRS letters. Follow-up testing was administered at approximately 1 month.

Results: There was high test–retest repeatability at one month for all visual function metrics (intraclass correlations >0.7) except log contrast sensitivity (intraclass correlations 0.6). Compared with controls, patients with intermediate AMD showed significant deficits on best-corrected visual acuity, LLVA, low luminance deficit, percent-reduced threshold on microperimetry, and red CCT ($P < 0.05$), but not on contrast sensitivity, green and blue CCT.

Conclusion: This pilot study supports the feasibility and reliability of using LLVA, microperimetry, and CCT in early dry AMD. Our data suggest these measures can be used as alternative future clinical trial endpoints. A larger, prospective natural history study of alternative visual function measures in dry AMD is warranted.

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Age-related macular degeneration (AMD) is the leading cause of severe central vision loss in the United States in people above the age of 50,[1] affecting approximately 30% of individuals above the age of 70 and 60 million people worldwide.[2,3] The main risk factor for developing AMD is increasing age, as previous studies have shown that the prevalence of AMD more than triples for individuals aged 75 to 85 as compared with those aged 43 to 54.[4] With the projected increase in the aging population in the world,[3] the impact of AMD on quality of life and medical cost is substantial.[3,5]

There are two forms of AMD: a "dry" and a "wet" form. The cause of vision loss in each is unique, although patients with the dry form may progress to the wet. In dry AMD, vision loss is associated with the formation of large drusenoid deposits in the macula, which ultimately result in photoreceptor degeneration, retinal pigment epithelium atrophy, and vision loss. The dry form accounts for approximately 85 to 90% of AMD cases.[6] In neovascular or wet AMD, vision loss is induced by onset of neovascularization with resulting subretinal fluid and hemorrhage leading to fibrosis and loss of central vision. Significant advances have been made in the treatment of wet AMD, especially with the introduction of safe and effective anti–vascular endothelial growth factor agents.[5,7]

Despite its prevalence, no treatments exist for most patients affected with its dry form. Because anatomical findings in dry AMD can be uncorrelated to progression or severity, the discovery of therapies for dry AMD is dependent on functional endpoints[8] as standardized biomarkers able to assess the severity, risk of progression, and response to treatment before significant visual changes occur.[9] Current monitoring relies on best-corrected visual acuity (BCVA), however visual acuity is not a sensitive functional measure until the late stages of disease. Alternatively, self-reported

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visual problems under low lighting and at night have been repeatedly documented in this group.[10,11]

Recent studies have shown that low luminance visual acuity (LLVA) is significantly reduced in early AMD.[9,12,13] In early AMD patients with intact BCVA, studies have documented impaired short wavelength cone function,[14] reduced contrast sensitivity for central and peripheral vision,[15,16] and reduced retinal sensitivity as measured by standard microperimetry.[12]

Thus, LLVA, cone-specific contrast, microperimetry, and contrast sensitivity are believed to be more sensitive to early macular changes than BCVA and may be potential endpoints for clinical trials of early dry AMD patients.[12,13,16] However, previous studies have been limited by a lack of comparability between all these testing measures. Herein, we aim to objectively compare these methods within a pilot study of normal controls, early AMD, and intermediate AMD patients. We demonstrate their feasibility and test–retest reliability, as well as suggest the most appropriate functional endpoints for clinical trials of early and intermediate AMD.

Methods

Study Participants

This prospective, controlled exploratory pilot study was approved by the Institutional Review Board of Duke University Medical Center and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants. Study subjects with AMD were identified from patients of the ophthalmology and optometry clinics at Duke Eye Center presenting for consultation. Spouses and friends of AMD subjects as well as Duke Eye Center optometry patients were recruited as control participants.

Inclusion criteria for study participants with AMD were capacity and willingness to provide consent, age >50 years, Snellen visual acuity of 20/40 (logarithm of the minimum angle of resolution, 0.30) or better, diagnosis of early (Age-Related Eye Disease Study, AREDS category 2) or intermediate (AREDS category 3) AMD[17] with the presence of drusen larger than 63 μm and pigmentary anomalies. Drusenoid pigment epithelial detachments and nonfoveal geographic atrophy were allowed. Inclusion criteria for control subjects were identical for age and visual acuity, with no signs of AMD in either eye including reticular pseudodrusen, although fewer than 10 drusen $\leq$63 μm were allowed.

Individuals were excluded if they demonstrated any ocular abnormality other than AMD or cataracts, in addition to not being able to perform any of the designated tests or complete the consent form for other health reasons. When both eyes met the inclusion criteria, the eye with better visual acuity was chosen as the study eye or the following algorithm was used if both had the same visual acuity: odd birth month—right eye and even birth month—left eye.

Functional Testing

Visual acuity evaluation and all functional tests were performed before fundus imaging to prevent bleaching of the retina. Subjects wore their best correction for all tests. Best-corrected visual acuity was assessed by the Electronic Visual Acuity tester (EVA; JAEB Center, Tampa, FL)[18] under photopic conditions (100 cd/m^2) and expressed in number of letters read. The tester runs the visual acuity testing program E-ETDRS (Electronic Early Treatment of Diabetic Retinopathy) that provides a visual acuity letter score comparable with the standard ETDRS chart testing score with additional benefits of electronic data capture and single distance testing from Snellen 20/12 to 20/800 range, as well as reduced testing time and technician-related bias.[18,19] Testing began with a screening phase to determine the approximate visual acuity threshold. This phase was followed by threshold testing to determine an upper acuity level at which 5 of 5 letters were correctly identified and a lower level at which 0 of 5 letters were correctly identified. A letter score to approximate the standard ETDRS score was computed as the number of letters correctly identified during threshold testing at the most difficult level, plus 5 letters for each acuity line above the upper boundary through 20/800. Testing was conducted in the same room with dim incandescent lighting for all patients across visits and monitor luminance for BCVA testing was 85 to 105 cd/m^2 with a contrast of 98%.

Low luminance visual acuity, Cone Contrast Test (CCT), and contrast sensitivity testing were performed monocularly at near distance (1 m) using computerized tests developed by Innova Systems (Burr Ridge, IL). During the LLVA test, subjects were presented with a succession of lines composed of 5 Snellen letters of decreasing size on a PC (Dell Optiplex 9010, Dell, Plano, TX) screen with the initial background luminance of 16 cd/m^2 followed by a different set of Snellen lines on a background with luminance of 5 cd/m^2. The resulting BCVA and LLVA in Snellen letters were recorded and converted to ETDRS letters.[20] Low luminance deficit (LLD) was calculated by subtracting LLVA from BCVA.

Next, subjects underwent CCT assessment, a computerized method of quantifying color vision and deficits in cone color discrimination at the

photoreceptor level.[21] The test was conducted in a dark room while wearing best correction. After instruction and demonstration with L, M, and S letter appearance, the CCT presents a randomized series of colored letters visible to a single cone type (long L, medium M, or short S wavelength photoreceptors) in decreasing steps of cone contrast to determine the threshold for letter recognition. These cone scores are logarithmically normalized to a 100-point scale, 100 being the maximum score achievable.[21]

After CCT, a computerized contrast sensitivity test was performed. This is a rapid self-test measuring low-contrast threshold using low-contrast letters on a white background. A staircase method is used to reduce contrast level to at or near threshold. Results are displayed in log contrast scores ranging from 2.0 log contrast (normal) to 1.0 log contrast (visual disability requiring 10 times as much contrast as compared with a person with normal contrast sensitivity).

Last, microperimetry testing was administered after pupillary dilation with 1 drop of tropicamide 1% and phenylephrine 2.5% each. Retinal sensitivity assessment was performed using a microperimeter with eye tracking (Macular Integrity Assessment [MAIA]; CenterVue, San Jose, CA).[9] Subjects underwent a 2-minute microperimetry training session to demonstrate the principles of the MAIA examination before the full test. This brief training examination is generally used before all MAIA testing sessions to familiarize the patient with the MAIA testing environment. The grid used during the full examination was designed to evaluate the macular region and consists of 37 points arranged in concentric circles and located at the fovea and 1, 3, and 5° from fixation (Figure 2). Three microperimetry measures were derived: average threshold, percent-reduced threshold (PRT), and central retinal sensitivity (CRS). Average threshold is the average of all retinal sensitivities from all loci tested, whereas CRS is the average of only the foveal locus and 12 points located at 1° from fixation. Percent-reduced threshold is a derived functional index representing the percentage of measured thresholds below 25 dB.

In all study participants, functional measurements were repeated on a separate visit at approximately 1 month (±10 days) from the initial visit to determine test–retest repeatability. This interval was chosen because no significant changes in disease progression or ocular health are expected, and most subjects were not able to return sooner for a follow-up study appointment which was not a standard of care examination. No patients were lost to follow-up during the duration of the study.

Imaging

Fundus imaging included color fundus photography (Zeiss FF 450 Plus IR; Carl Zeiss Meditec Inc., Dublin, CA) and fundus autofluorescence (Spectralis 3-mode; Heidelberg Engineering US, Carlsbad, CA). Spectral domain optical coherence tomography was performed on a Spectralis optical coherence tomography (Spectralis 6-mode; Heidelberg Engineering US). Two retinal specialists (E.M.L. and C.A.T.) performed all clinical examinations. Color fundus photographs were graded by a medical retinal specialist (EML) by evaluating the extent of pigmentary changes and drusen size.[17] Early AMD (AREDS category 2) was defined by the presence of many small drusen, few intermediate drusen, and/or retinal pigment epithelium abnormalities, whereas intermediate AMD (AREDS category 3) was defined by extensive intermediate drusen, at least 1 large drusen, and/or the presence of nonfoveal geographic atrophy.[17] Fundus autofluorescence and spectral domain optical coherence tomography images were used to confirm the color fundus grading.

Statistical Analysis

Descriptive statistics for functional measures were computed for all groups. Comparisons between groups were assessed using the Wilcoxon rank-sum test of difference between medians; a correction was performed to account for multiple comparisons. Linear regression analysis was conducted to examine the relationship between different functional measures. The correlation coefficient R was computed to measure the strength and direction of the linear relationship between two variables.

The intersession test–retest repeatability of visual acuity and other functional measures was determined by calculating intraclass correlations (ICCs) with 95% confidence intervals and with the coefficients of repeatability (CoRs). The ICC represents the ratio of variance between subjects and the total variance, and best demonstrates quantifiable reproducibility. We used the Bland–Altman analysis and plots (not shown) and calculated the CoR related to the plots. The CoR, also called as the smallest real difference,[22] is an index that quantifies absolute reliability measurement error. If the differences between two measurements made on a subject are approximately normally distributed, we expect the absolute difference between two measurements on a subject to differ by no more than the CoR on 95% of occasions. The value of the CoR depends on the values of each measurement. The relationship between ICC and CoR is demonstrated below:

$$\mathrm{ICC} = \frac{\text{Between} - \text{subject variance}}{\text{Between} - \text{subject variance} + \text{within} - \text{subject variance}}$$

$$\mathrm{CoR} = 1.96 \times \sqrt{2} \times \text{Standard Error of the Mean (SEM)}$$

$$\mathrm{SEM} = \sqrt{\text{within} - \text{subject variance}} - \sqrt{\text{total variance}}$$

The CoR of a tool is directly related to the 95% limits of agreement proposed by Bland and Altman[23,24] and demonstrates the precision of measured values between initial and follow-up visits.[25] All statistical analyses for this article were performed in SAS version 9.3 (SAS Institute, Cary, NC).

Results

A total of 30 participants were enrolled in the study (20 AMD subjects and 10 healthy controls) and underwent visual function testing on the same study eye at approximately 1 month (±10 days) after the baseline examination. Of the subjects with AMD, 8 had the diagnosis of AREDS Stage 2 (early AMD) and 12 were characterized with AREDS Stage 3 (intermediate AMD). Demographic characteristics and lens status are presented in Table 1; there was no overall significant intergroup variation about age, sex, visual acuity, or cataract status. Average age for control subjects was 69.2 ± 8.6 years, 67.5 ± 7.58 years for the early AMD group, and 71.8 ± 6.8 years for the intermediate AMD group. Sex distributions (percent male) ranged from 58.3% in the intermediate AMD group to 62.5% in the early AMD group, ($P = 1.00$). The range of visual acuities was Snellen 20/13 to 20/25 for the control group, 20/13 to 20/40 for the early AMD group, and 20/16 to 20/40 for the intermediate AMD group (Table 1 and Figure 1; P-value overall 0.112, however P-value between control and intermediate group was 0.029). Proportion of phakic subjects present among groups did not differ (Fisher's exact test $P = 0.448$). Pseudophakia was documented in 1 control patient, 1 early AMD patient, and 4 intermediate AMD patients.

Test–Retest Variability

To examine test–retest variability between the two testing sessions, ICC and CoR were calculated both among all patients as well within each study group (Table 2). Among all subjects, LLVA and LLD demonstrated ICCs of 0.86 and 0.87, respectively. Microperimetry measure ICCs ranged from 0.73 to 0.90 (Table 2), with average threshold exhibiting the highest of all overall ICCs at 0.90. All CCT measures (red, blue, and green) had ICCs of ≥0.75. Red color testing on CCT demonstrated the best ICC at 0.83, followed by blue (0.79) and green (0.75) color testing. Log Contrast test exhibited the least substantial agreement (ICC 0.60).

Within individual comparison and control groups, each test's ICC measures displayed greater variability than overall ICC. The control group demonstrated a weaker array of ICCs (Table 2). Among patient groups, the intermediate AMD group paralleled the aggregate measures most closely, where log contrast sensitivity had the lowest ICC (0.71). Similar ICC trends were maintained within the early AMD group,

Table 1. Subject Demographics and Group Designation

Group	Control	Early AMD	Intermediate AMD	*P*
Number of subjects	10	8	12	
Mean age (SD)	69.2 (8.59)	67.5 (7.58)	71.83 (6.77)	0.45
Sex (% male)	60	62.5	58.3	1.00
BCVA range Snellen (ETDRS letters)	20/13 (97)-20/25 (83)	20/13 (94)-20/40 (72)	20/16 (90)-20/40 (72)	0.112
Cataract status				
Phakic N (%)	9 (90%)	7 (87.5%)	8 (66.7%)	0.45
Clear	1	1	0	
Trace NS	1	1	0	
1+ NS	2	4	5	
s≥2+ NS	5	1	3	
Pseudophakic	1	1	4	

NS, nuclear sclerosis.

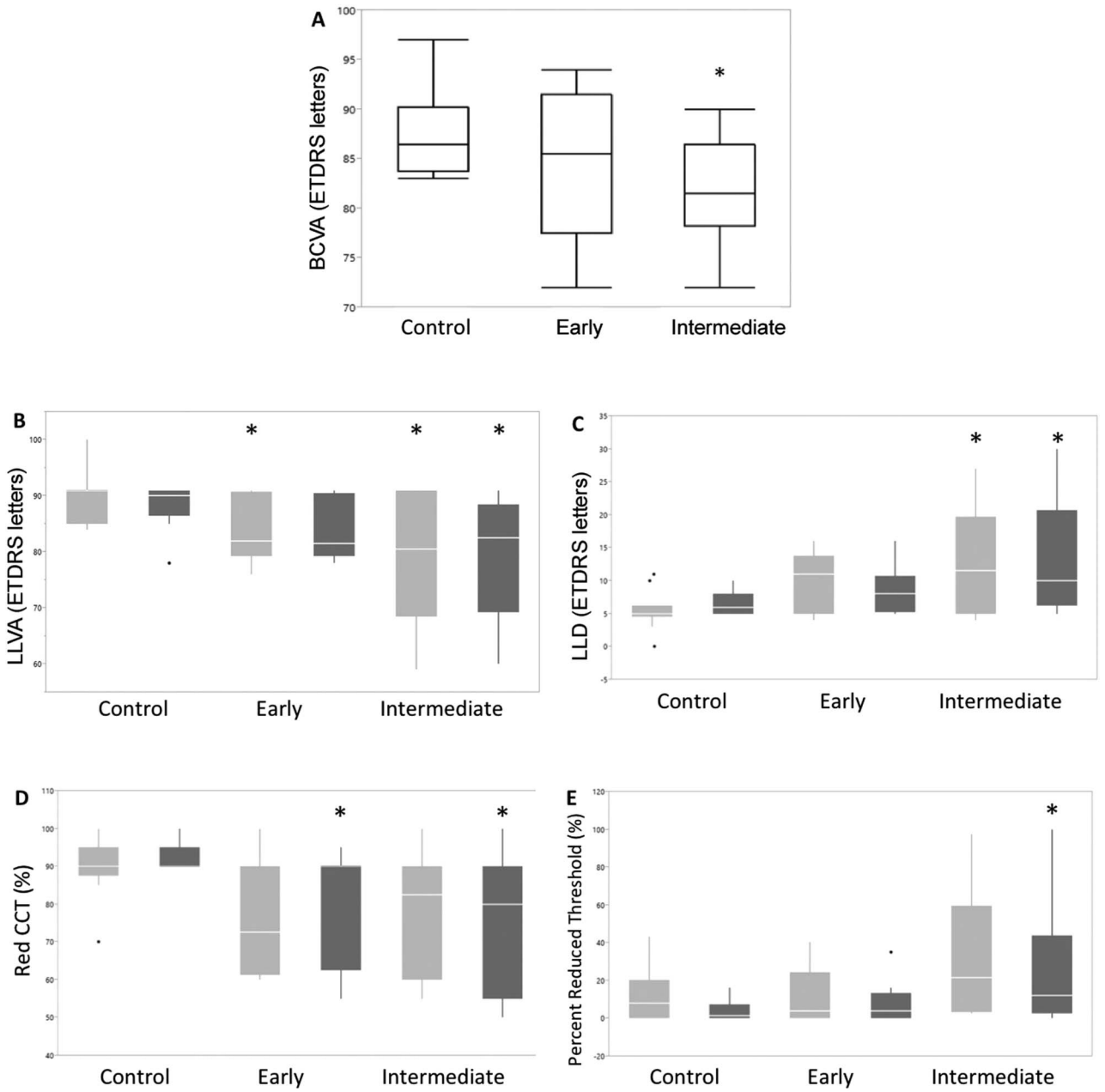

Fig. 1. Boxplots showing (**A**) BCVA, (**B**) LLVA, (**C**) LLD, (**D**) red CCT, and (**E**) PRT on microperimetry testing for control and each AMD AREDS clinical group. Each boxplot demonstrates outliers along with the maximum, upper quartile, median, lower quartile, and minimum values. (**A**) displays values collected during visit 1 only, whereas (**B**) to (**E**) show values from Visits 1 and 2 in light and dark gray, respectively. Black dots represent outliers while 1 asterisk denotes significance at the $P < 0.05$ level when compared with controls at the same visit. Groups did not vary with respect to baseline BCVA (A), however LLVA (B) and LLD (C) showed significant differences between control and intermediate populations. **D.** Both early and intermediate groups had differing performance compared with controls on the red CCT test during the second visit. **E.** The intermediate AMD group was characterized by a significantly higher PRT as compared with the control group. Best-corrected visual acuity and LLD are reported as aggregate letters; PRT and red CCT are both measured as percent.

with the exception of blue and green CCT, which displayed a low ICC in this population (<0.4). Red CCT, low luminance, and microperimetry measures were characterized by ICC > 0.7, with microperimetry's average threshold being the highest (0.96) (Table 2).

Functional Measures Between Groups

The functional measures that showed significant differences among all groups were BCVA, LLVA, LLD, red CCT, and PRT on microperimetry testing (Figure 1). Best-corrected visual acuity performance at baseline was similar between control and early groups

Table 2. Intrasession Test–retest Variability as Described by ICC Analysis and CoR for All Subjects (A), Control group (B), Early AMD Group (C), and Intermediate AMD Group (D)

Variable	N	ICC	Lower Confidence Limit	Upper Confidence Limit	CoR
All subjects					
LLVA	30	0.86	0.73	0.93	9.34
LLD	30	0.87	0.76	0.94	6.27
Log contrast sensitivity	30	0.60	0.27	0.76	0.28
CCT red	28	0.83	0.67	0.92	17.36
CCT blue	28	0.79	0.60	0.90	15.37
CCT green	28	0.75	0.53	0.87	20.94
PRT	30	0.83	0.68	0.92	27.77
Average threshold	30	0.90	0.79	0.95	2.78
Central foveal sensitivity	30	0.73	0.51	0.86	4.27
Variable	N	ICC	Lower Confidence Limit	Upper Confidence Limit	CoR
Control group					
LLVA	10	0.51	0.00	0.85	8.69
LLD	10	0.52	0.00	0.85	4.88
Log contrast sensitivity	10	0.00	0.00	0.56	0.28
CCT red	9	0.18	0.00	0.70	15.99
CCT blue	9	0.87	0.56	0.97	7.30
CCT green	9	0.67	0.12	0.91	13.46
PRT	10	0.21	0.00	0.72	29.25
Average threshold	10	0.68	0.17	0.91	3.21
Central foveal sensitivity	10	0.32	0.00	0.77	4.44
Variable	N	ICC	Lower Confidence Limit	Upper Confidence Limit	CoR
Early AMD group					
LLVA	8	0.96	0.84	0.99	3.10
LLD	8	0.71	0.12	0.93	6.35
Log contrast sensitivity	8	0.67	0.02	0.92	0.28
CCT red	8	0.79	0.32	0.96	19.59
CCT blue	8	0.09	0.00	0.71	22.71
CCT green	8	0.31	0.00	0.81	30.97
PRT	8	0.93	0.70	0.98	10.58
Average threshold	8	0.96	0.84	0.99	1.07
Central foveal sensitivity	8	0.86	0.50	0.97	1.75
Variable	N	ICC	Lower Confidence Limit	Upper Confidence Limit	CoR
Intermediate AMD group					
LLVA	12	0.84	0.56	0.95	12.21
LLD	12	0.90	0.70	0.97	7.20
Log contrast sensitivity	12	0.71	0.10	0.87	0.28
CCT red	11	0.88	0.64	0.96	16.70
CCT blue	11	0.91	0.71	0.97	13.53
CCT green	11	0.90	0.69	0.97	16.44
PRT	12	0.86	0.60	0.96	33.77
Average threshold	12	0.92	0.75	0.98	3.17
Central foveal sensitivity	12	0.76	0.37	0.92	5.20

but significantly lower in the intermediate AMD group as compared with controls. Significant differences were noted between early AMD and controls as well as between intermediate AMD and control groups for LLVA and LLD during at least 1 of the two visits ($P < 0.05$; Figure 1). Red CCT also demonstrated a statistically significant difference between these groups within Visit 2 ($P = 0.020$), although not at visit 1 ($P = 0.115$).

Microperimetry deficits varied within each clinical severity group. Specifically, PRT showed differences between control and intermediate AMD groups at Visit 2 only ($P = 0.055$). Figure 2 demonstrates a typical microperimetry examination in the three groups (control, early AMD, and intermediate AMD) as exemplified by three representative subjects, as well as the corresponding functional measures.

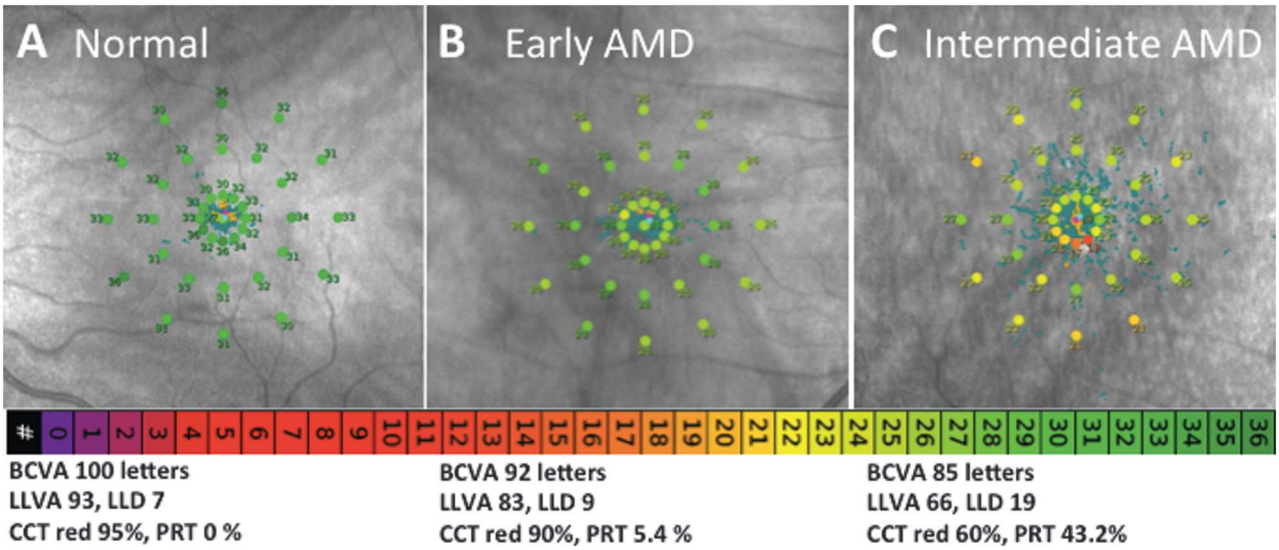

Fig. 2. A–C. retinal sensitivity on microperimetry testing of participants from each of the three groups (control, early AMD, and intermediate AMD). Color scale bar on the bottom of the image represents retinal sensitivity value range for microperimetry. Note the difference in retinal sensitivity between the three subjects with increasing AMD pathology. The corresponding values for BCVA, LLVA, LLD (all expressed in ETDRS letters), CCT red (%), and PRT are shown for each subject.

Correlations Between Functional Measures

Bivariate fit analysis evidenced significant correlations between LLD and BCVA (R = −0.71, slope −0.75, $P < 0.001$) among all groups, as well as between LLVA and BCVA, LLD, PRT, and central foveal sensitivity, respectively, (Figure 3A–E). The strength of correlations between these variables differed within early and intermediate AMD groups. Analysis of the early AMD group showed a significant correlation only between LLD and BCVA, LLVA and BCVA, and LLVA and LLD (R = −0.88, 0.75, and −0.84, respectively). All significant correlations demonstrated using aggregate data (Figure 3) were present within the intermediate group analysis alone ($R > 0.8$), except for the correlation between LLD and BCVA, which was demonstrated between early and control groups (R = −0.88).

Discussion

As researchers work to develop new treatments for dry AMD, they will require reliable and effective functional outcome measures. Recent studies have suggested a variety of alternative early endpoints,[8,26,27] although all measures warranted further testing to establish accuracy, precision, and applicability to drug discovery and early functional disparities. Few studies have addressed the feasibility of implementing these tests in the clinical setting and their intrasession variability,[28,29] thus, our goal was to address this gap and objectively examine feasibility and test–retest reliability.

The psychophysical tests used in our study, LLVA, MAIA microperimetry, CCT, and cone contrast sensitivity, captured a broad expression of AMD-related deficiencies. Low luminance visual acuity is a quick reproducible measure of central cone-mediated function under conditions of reduced illumination and can serve as a predictor of risk of vision loss even in eyes with normal BCVA.[13] Using a computerized LLVA method allowed standardization of background luminance and testing conditions across various clinics, minimizing operator and patient-related factors (such as variability in angle and distance between the filter and the subject's eye, sight around the filter, difficulties holding the filter, etc.) and enabled multiple testing, such as BCVA, CCT, and LLVA through the same interface. Retinal sensitivity as determined by MAIA microperimetry has recently been shown to detect a greater extent of foveal dysfunction than BCVA and standard LLVA, while also providing topographic information about functional defects.[9] The CCT is unique in its ability to accurately indicate the type and severity of color deficiencies as well as disease progression even in individuals with normal visual acuity.[21,30]

In our study, we found that computerized LLVA and LLD, CCT and MAIA microperimetry all demonstrated significant test–retest reliability (ICC > 0.7). Wu et al[28] recently demonstrated similarly good intrasession test–retest reliability for MAIA microperimetry, however they also reported a "learning effect" influencing mean

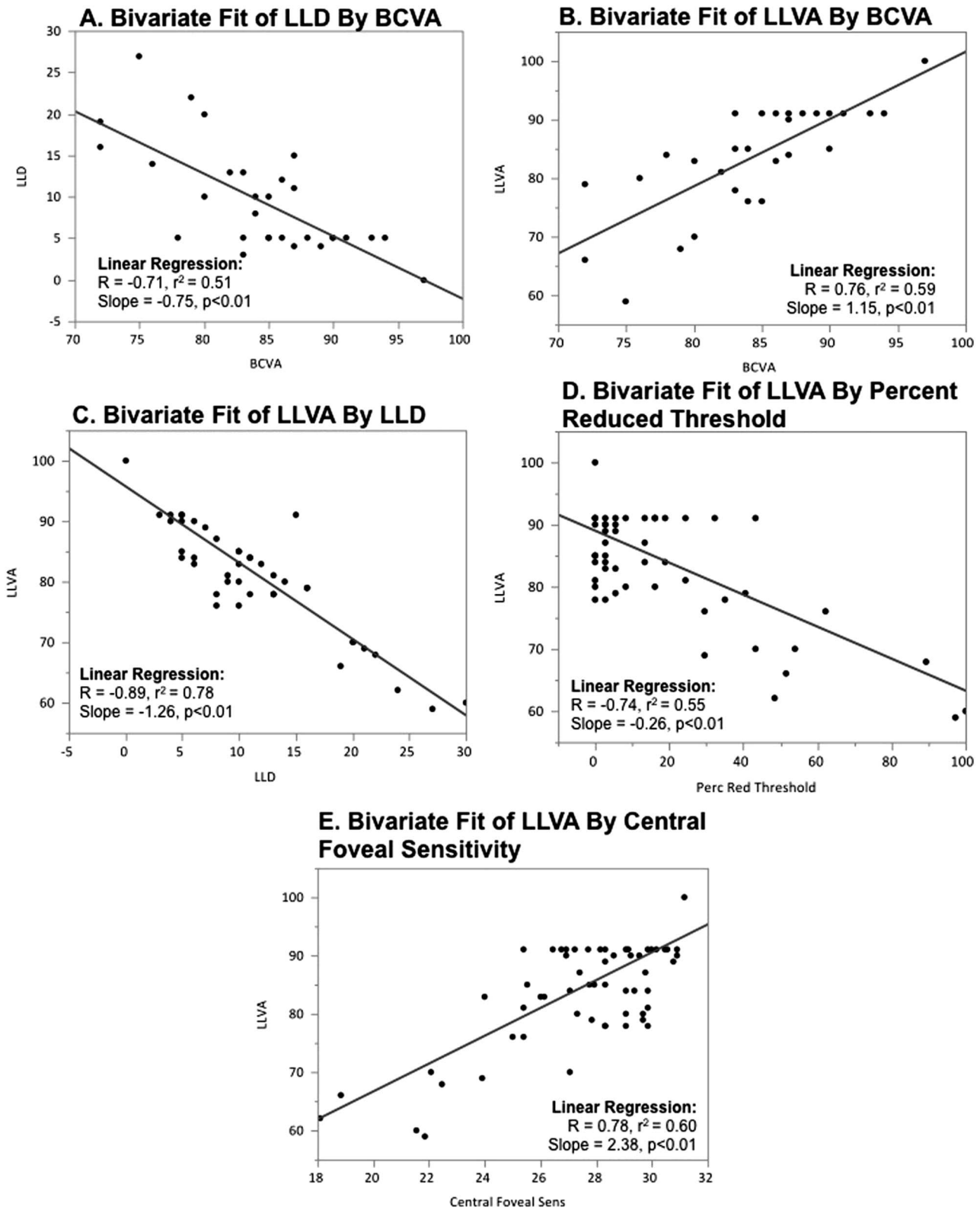

Fig. 3. Scatterplots examining the relationship between functional measures among all subjects. **A** and **B** show correlations indicating worse BCVA is associated with worse low light visual function. **C–E.** demonstrate strong relationships between LLVA and LLD (reported in letters) and MAIA measures PRT (%) and central foveal sensitivity (dB).

sensitivity between the first two MAIA administrations. Although there seemed to be a trend across groups toward improved PRT during the second visit, this effect was not reflected in ICC measures in our study, nor was it replicated in other previous studies.[23,31,32] In addition to using different testing parameters, the patients enrolled in our study underwent a brief training session before the first examination, which likely reduced any learning effect during the first visit.[28]

The CoRs obtained in our study for LLVA (9.34) and LLD (6.27) were similar to previous studies (0.13 logarithm of the minimum angle of resolution or 6.5 ETDRS letters in the study of Wu and colleagues[28]). The CoR for the microperimetry measure CRS (±4.27 dB) was comparable with findings from previous studies of ± 4.76 dB reported by Wu and collaborators for pointwise sensitivities with MAIA microperimetry[28] and ± 5.56 dB documented by Chen and colleagues[32] using MP-1. The higher CoR obtained by Chen et al[32] with the Nidek MP-1 may be attributed to a greater number of stimuli tested (68 vs. 37 used by Wu et al and our study), although this also significantly lengthens test duration. The CoR for average threshold in our study was more favorable than in all previous studies at 2.78 dB.

In this study, only contrast sensitivity testing demonstrated lower intersession reliability (ICC 0.6). This may be related to the variability of nuclear sclerotic cataract in our study population. The potential difficulty of this computerized test is its reliance on subject attention and familiarity with the test. Unlike microperimetry and the CCT test, this psychophysical test did not begin with a training session. Future studies should be performed using either the current methodology with an added training test or the Spaeth/Richman Contrast Sensitivity test—an internet-based method of testing contrast sensitivity that was recently demonstrated to be valuable for evaluating contrast sensitivity in AMD.[15] An additional limitation of our study was that all patients received the same order of testing across visits. This nonrandomized administration may have influenced performance and intertest variability due to order effects or fatigue. A future larger study in which testing order is randomized and perhaps repeated in a reverse order at follow-up is warranted. However, the consistent order of tests performed between visits in this pilot study created parallel testing conditions where such effects would be present in both visits and minimally affect test–retest reliability of a single test.

Another limitation of our pilot study was that the control group may have included some patients with AREDS Category 1 because fewer than 10 drusen <63 microns were allowed. The decision to tolerate <10 small drusen in our control group is consistent with criteria used by previous natural history studies[9,12] and based on the current recommendations on clinical classification of AMD versus controls.[17] A future larger, longitudinal natural history study specifying strict inclusion criteria for the study groups is needed. This study should recruit subjects with good, intermediate, and poor visual acuities to assess alternative visual function outcomes in the patients with a range of disease severity and visual acuities.

Although the main goal of our pilot study was to evaluate test–retest repeatability for the functional endpoints across all subjects, we noted significant differences between the intermediate AMD group and age-matched controls in BCVA, LLVA, LLD, CCT red, and microperimetry PRT. Our findings were consistent with previous studies[9,33] in which intermediate stage patients demonstrated significantly worse LLVA and BCVA. A cone-mediated mechanism of LLD in early AMD suggests that LLVA testing may better capture nuanced functional deficits.[13,34,35] Low luminance deficit has also been shown to strongly predict subsequent visual acuity loss at all levels of baseline visual acuity.[13]

We also found a significant increase in LLD in both early and intermediate AMD groups as compared with controls during at least 1 of the two visits. Although a previous study found a difference in LLD between early stage AMD (defined by the presence of intermediate-large drusen ± retinal pigment epithelium abnormalities) and controls,[35] others found no significant difference between nonneovascular AMD groups and controls except for those with nonfoveal geographic atrophy.[9] However, in this previous work, 20% of participants with their definition of early AMD had an LLD worse by 2 SD as compared with normal, suggesting that LLD may indeed identify individual AMD eyes at risk for vision loss.

Our findings of a red cone contrast deficit in both AMD groups compared with healthy controls differed from an earlier study demonstrating blue color impairments in early AMD,[14] and another documenting function deficiencies in all three types of photoreceptors.[26] This discrepancy may be explained by the small sample size of our study, or by the presence of lens opacification in some of the subjects as pseudophakic cases were concentrated in our study's intermediate AMD group, while controls had most patients with $\geq$2+ nuclear sclerosis. Indeed, earlier work has noted that the blue stimulus has good reproducibility but only moderate specificity, and testing performance along the blue–yellow axis is significantly impacted by the presence of cataract.[36] Our AMD groups had a lower cataract burden than controls, which may have decreased their blue contrast test performance to the degree of AMD patients. Further studies enrolling

subjects with pseudophakia or nonvisually significant cataract are required to understand the functional differences in all photoreceptor types between normal, early, and intermediate AMD cohorts over time.

In the intermediate AMD group as compared with normal subjects, we also noted a significant difference only in MAIA microperimetry measures of PRT. In contrast, earlier work demonstrated significant reduction in CRS in a subset of intermediate AMD patients with large drusen and nonfoveal geographic atrophy, but not in subjects with early or intermediate AMD characterized by intermediate drusen.[9] Our small sample size precluded this type of subset analysis, however, our results suggest that PRT may be a superior microperimetry indicator of early dysfunction by distinguishing local sensitivity changes associated with focal pathology in early AMD.

Another important goal of our study was to examine correlations between visual function endpoints to assess possible surrogate markers of functional decline. All microperimetry indices appropriately correlated with one another, further emphasizing consistent measurements across this testing modality. The strong correlations between CRS and PRT with LLVA suggest that LLVA may be able to translate subtle changes in foveal pathology to a functional deficit. Nevertheless, others warned that LLVA might be limited in its ability to assess retinal function outside the fovea, where the loss of function and atrophic changes occur first.[37–39] Yet in this pilot study, both early and intermediate groups demonstrated lower LLVA than control populations, supporting LLVA as a sensitive functional marker of early AMD.

In summary, our study supports the feasibility of using computerized low luminance testing (LLVA and LLD), CCT, and MAIA microperimetry in subjects with early and intermediate AMD. These tests were well tolerated and had high test–retest reliability in this population. Our data suggest that this battery of psychophysical measures, in addition to other promising tests such as dark adaptation,[40] may be used as alternative early endpoints for future interventions in dry AMD. A larger prospective study fully investigating the natural history of these functional endpoints in early and intermediate AMD is warranted.

Key words: age-related macular degeneration, visual function testing, low vision, microperimetry, low light visual acuity, low light deficit, contrast sensitivity.

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