AMERICAN ACADEMY
OF OPHTHALMOLOGY®

Longitudinal Study of Visual Function in Dry Age-Related Macular Degeneration at 12 Months

S. Tammy Hsu, BA,[1] Atalie C. Thompson, MD, MPH,[1] Sandra S. Stinnett, DrPH,[1] Ulrich F.O. Luhmann, PhD,[2] Lejla Vajzovic, MD,[1] Anupama Horne, MD,[1] Stefanie G. Schuman, MD,[1] Cynthia A. Toth, MD,[1] Scott W. Cousins, MD,[1] Eleonora M. Lad, MD, PhD[1]

Purpose: To report the 1-year progression of visual impairment on psychophysical tests of visual function in patients with early and intermediate age-related macular degeneration (AMD).

Design: Prospective, observational study.

Participants: Patients with early and intermediate AMD were enrolled from the existing population at the Duke Eye Center, and healthy age-matched control participants were recruited from family members or friends of the AMD patients and from the Duke Optometry and Comprehensive Eye Clinics.

Methods: Patients and control participants recruited during the baseline study were assessed at both 6 and 12 months after the initial study visit. Measurements of visual function included best-corrected visual acuity (BCVA), low-luminance visual acuity (LLVA), low-luminance deficit (LLD), microperimetry percent-reduced threshold (PRT), microperimetry average threshold (AT), and cone contrast tests (CCTs).

Main Outcome Measures: Changes in BCVA, LLVA, LLD, microperimetry PRT, microperimetry AT, and CCT results from baseline to 6 months and to 12 months were assessed.

Results: Eighty-five patients completed the 12-month examination (19 control participants, 27 early AMD patients, and 39 intermediate AMD patients). Longitudinal analysis detected significant changes from baseline within each group in microperimetry PRT and AT and in the intermediate AMD group only for BCVA and CCT results ($P < 0.05$).

Conclusions: Microperimetry and CCT are able to detect functional changes resulting from progression of dry AMD within a period as short as 12 months. These functional markers may be useful end points in future clinical trials that assess the effect of potential treatments for AMD. *Ophthalmology Retina 2019;■:1−12 © 2019 by the American Academy of Ophthalmology*

Age-related macular degeneration (AMD) is the leading cause of blindness in Americans 60 years of age or older[1] and is projected to affect 196 million people worldwide by 2020.[2] Individuals with AMD are classified into early, intermediate, or advanced AMD stages based on the Age-Related Eye Disease Study (AREDS) classification.[3] Age-Related Eye Disease Study vitamins have been shown to slow progression to advanced AMD,[3,4] and the advent of anti−vascular endothelial growth factor medications has led to substantial progress in the treatment of exudative, or wet, AMD characterized by choroidal neovascularization. However, no definitive or curative therapies currently exist for nonexudative or dry early to severe AMD, which affects most patients with AMD.

To develop potential therapeutics to treat dry AMD, end points to monitor progression of disease are necessary, particularly to allow for earlier detection and treatment. Although the rapidly progressive course of exudative AMD can be monitored with changes in best-corrected visual acuity (BCVA), this standard clinical end point used in most ophthalmic clinical trials is not a sensitive measure for visual changes occurring during the slower and earlier course of dry AMD.[5] There is a need for the identification of sensitive functional end points with regulatory acceptance that can detect a clinically meaningful change in early to intermediate dry AMD in a reasonable time frame. End points that meet these needs are necessary for the development of potential treatments for dry AMD.

Previous studies offer insight into potential functional end points that may be used to monitor the natural history of dry AMD as well as potential changes resulting from interventions performed in clinical trials. Many patients with dry AMD report difficulty with vision in dim lighting.[6−8] This is thought to be caused by impaired function in the rod system that may result in impaired scotopic vision and delayed dark adaptation.[9] In all stages of AMD, histopathologic analysis has demonstrated that the rod photoreceptors degenerate earlier than cones.[10,11] Thus, measures that capture rod dysfunction may be useful functional markers of disease progression in dry AMD. In addition to functional visual

https://doi.org/10.1016/j.oret.2019.03.010
ISSN 2468-6530/19

changes in dim lighting and dark adaptation, loss of cones involved in blue color contrast sensitivity also has been reported.[12] Thus, psychophysical tests that monitor such rod-mediated functional deficits in a sensitive manner may prove useful as future clinical trial end points across the different stages of AMD over time.[13–15]

In this prospective, longitudinal, observational natural history study, we aimed to characterize the progression of early and intermediate dry AMD over 1 year using a variety of psychophysical tests that we believed would be able to capture progression of visual function deficits in dry AMD, such as low-luminance visual acuity (LLVA), mesopic microperimetry, and the cone contrast test (CCT). We previously showed that these tests are able to distinguish between early, intermediate AMD and normal control participants in both a pilot study of 30 patients[13] as well as a cross-sectional baseline study of 101 patients.[16] Thus, these measures not only may be able to distinguish early dry AMD stages but also may have the potential to detect their progression. The hypothesis in the current study was that patients with intermediate dry AMD would show a significantly greater decline in performance on specific psychophysical tests of visual function than age-matched control participants after 6 months and 1 year of longitudinal follow-up.

Methods

The participants recruited during the baseline study[16] were followed up and assessed at both 6 and 12 months after the initial study visit. As described previously by Cocce et al,[16] the patients with early and intermediate AMD were enrolled from the existing population at the Duke Eye Center, and healthy age-matched control participants were recruited from family members or friends of the AMD patients and from the Duke Optometry and Comprehensive Eye Clinics. Written informed consent was obtained from all participants. The study (ClinicalTrials.gov identifier, NCT01822873) was approved by the Duke University Health System institutional review board and was conducted in accordance with Good Clinical Practice using the guidance documents and practices offered by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) or applicable international regulatory authority laws, regulations, and guidelines. All research adhered to the tenents of the Declaration of Helsinki. Participants were compensated $20 per visit and were reimbursed for parking costs during the study visit.

A baseline evaluation was performed when the participants were enrolled, and participants were classified as healthy control participants (AREDS category 1), patients with early AMD (AREDS category 2), or patients with intermediate AMD (AREDS category 3).[3] Control participants showed fewer than 5 small drusen measuring less than 63 μm and otherwise no signs of AMD in either eye. Early AMD participants were characterized by showing multiple small drusen measuring 63 to 124 μm in diameter, retinal pigment epithelium (RPE) abnormalities, or both. Intermediate AMD participants showed extensive intermediate drusen and least 1 large drusen of more than 125 μm.[3] Participants meeting the following criteria for advanced AMD were excluded: any geographic atrophy of the RPE and choriocapillaris, neovascular maculopathy, detachment of the sensory retina or RPE, retinal hard exudates, subretinal or sub-RPE fibrovascular proliferation, or a disciform scar in either eye. After the baseline evaluation, follow-up assessments for each participant were performed at approximately 6 and 12 months (±2 months) based on the standard-of-care clinic visits for AMD participants.[17]

At each visit, participants were imaged with stereo color fundus photography (Zeiss FF 450 Plus IR; Carl Zeiss Meditec, Inc, Dublin, CA), fundus autofluorescence (Spectralis 3 mode; Heidelberg Engineering GmbH, Heidelberg, Germany),[15] and spectral-domain OCT (Spectralis 6-mode; Heidelberg Engineering, Franklin, MA, US). Ophthalmologists (EML, SWC, LV, AH) performed clinical examinations, and a medical retinal specialist (EML) evaluated color fundus photographs for the extent of pigmentary changes and drusen size. Study participant demographics, ocular and medical history, and full ophthalmic examination results also were recorded.

The following psychophysical tests of visual function that had been performed at baseline, as previously described by Cocce et al,[16] were repeated at the 6- and 12-month visits in the following order: BCVA; LLVA; CCT for red, green, and blue cones; and mesopic microperimetry. Best-corrected visual acuity, assessed using an Early Treatment in Diabetic Retinopathy Study chart (85 cd/m^2; Good-Lite, Elgin, IL), was reported as the number of letters read.[18,19] Low-luminance visual acuity was assessed by having participants read the Early Treatment in Diabetic Retinopathy Study chart through a 2.0-log neutral density filter that reduces luminance by 100-fold.[20] Low-luminance deficit (LLD) was defined as the difference between BCVA and LLVA in Early Treatment in Diabetic Retinopathy Study letters. The CCT (Innova Systems, Burr Ridge, IL) assessed for deficits in cone color discrimination[13] by requiring participants to distinguish colored letters that were detectable by a single cone type (long-, medium-, or short-wavelength photoreceptors for red, green, and blue colors, respectively) on a gray background. Letters were shown in decreasing steps of color contrast to determine the threshold for distinguishing color. The CCT results were scored on a normalized 100-point scale, with 90% to 100% representing normal cone function and scores of less than 75% representing color deficiency. This allowed the assessment of the function of each of the different cone types. Participants then underwent dilation with tropicamide 1% and phenylephrine 2.5% for mesopic microperimetry testing (Macular Integrity Assessment; CenterVue, San Jose, CA), during which retinal sensitivity was assessed using the standard 37 loci, 10° (37-10) Macular Integrity Assessment grid. Microperimetry results were reported as percent reduced threshold (PRT), defined as the percentage of abnormal retinal sensitivity thresholds less than 25 dB, and as average threshold (AT), defined as the average of retinal sensitivity values from all loci tested.

All statistical analyses were performed using SAS software version 9.4 (SAS Institute, Inc, Cary, NC). Before the start of the study, we determined the necessary sample size using standard LLVA values in letters from all the 3 groups that were obtained in our pilot study[13] for a 2-sample t test with an α level of 0.05 and 80% power. The resulting sample size was inflated by 5% to account for the use of the nonparametric Wilcoxon rank-sum test in the analysis and potential participant attrition at 12 months of up to 20%. The resulting necessary sample size was approximately 13 to 25 in the control group, 25 to 57 in the early group, and 13 to 57 in the intermediate group. The number of participants subsequently enrolled in each group matched the planned sample size determined by the power calculation. Data collected from case report forms were entered into the Research Electronic Data Capture database by certified data entry analysts from the Duke Office of Clinical Research.

Cross-sectional analyses of the psychophysical test results from the 6- and 12-month study visits were performed for the normal control, early AMD, and intermediate AMD groups. An overall P

value comparing the 3 groups was calculated, and correction for multiple comparisons for each variable was applied with a Bonferroni test (adjusted α level of $P = 0.025$ for the P value overall, and adjusted α level of $P = 0.0167$ for the pairwise comparisons). Furthermore, because of the exploratory, observational nature of this study, we also compared the groups in a pairwise fashion using the nonparametric Wilcoxon rank-sum test to detect any potential associations of interest, especially between the intermediate AMD and normal control groups and between intermediate and early AMD groups, with the expectation that these exploratory analyses will be reassessed and confirmed in a future larger study.[21–23]

Longitudinal analyses of the change from baseline to 12 months within each group were performed using Wilcoxon signed-rank tests. Comparisons between groups of the change from baseline to 12 months for results from each psychophysical test were assessed using the Wilcoxon rank-sum test. To assess for possible progression of dry AMD more than would be expected because of normal aging on each psychophysical test, the mean plus twice the standard deviation of the normal control group at baseline was computed as a cut point. The Fisher exact test was used to assess the significance of the difference among groups with respect to proportions exceeding the cut points.

Results

A total of 85 participants (19 normal control participants, 27 early AMD patients, and 39 intermediate AMD patients) enrolled at baseline and completed the 12-month study visit (Table 1). Four of the 19 study eyes in the control group showed fewer than 5 drusen less than 65 μm at baseline. All 85 participants underwent a dilated fundus examination along with assessment of BCVA, LLVA, and LLD during the baseline and 12-month visit. Of the 85 participants, 78 participants completed the microperimetry testing, and 83 participants completed cone contrast testing at the 12-month visit.

Cross-sectional disease classification of participants was confirmed using structural assessments (dilated fundus examination, color fundus photography, and spectral-domain OCT), none of which showed obvious progression at the 12-month visit. Overall, the results of cross-sectional analysis of the psychophysical test results were similar from baseline to the 12-month visit, with a few exceptions: microperimetry PRT and AT showed significant differences at 6 and 12 months between the intermediate AMD group as compared with the control and early AMD groups; LLVA and LLD showed significant difference at 12 months between the intermediate AMD and control groups, and CCT red and blue were significantly different at 12 months for the early versus the intermediate AMD groups. Best-corrected visual acuity did not differ significantly between the groups at the baseline, 6-month, and 12-month follow-up visits ($P > 0.0167$; Table 2), and LLVA and LLD were unrevealing as well, with the exception of LLVA and LLD at 12 months for control versus intermediate AMD. None of the tests displayed significant differences between the early AMD versus control group at any of the visits. Significant differences between the intermediate and early AMD groups were detected in microperimetry PRT and AT results at 6 and 12 months and for the red and blue CCT results at 12 months ($P < 0.0167$).

Longitudinal analyses of the visual function tests were performed to evaluate for significant changes from baseline to 12 months within each of the control, early AMD, and intermediate AMD groups. The intermediate AMD group demonstrated a statistically significant decline in some of the visual function measures over the first year as assessed by several psychophysical tests (Tables 2, 3, and 4): BCVA (Fig 1); microperimetry PRT and AT (Fig 2); and CCT red, green, and blue (Fig 3; $P < 0.05$ for all metrics) showed a significant worsening compared with baseline, whereas LLVA and LLD did not decline significantly from baseline to 12 months. In the univariate analyses, changes from baseline to 12 months for BCVA ($P = 0.021$), microperimetry AT ($P = 0.047$), CCT red ($P = 0.012$), and CCT blue ($P = 0.019$) in the intermediate AMD group were significantly larger compared with those in the early AMD group. Compared with the change observed over 12 months in the normal control group, the intermediate AMD group showed a significantly

Table 1. Participant Demographics at the 1-Year Follow-up Visit

Variable	Normal Control Group	Early Age-Related Macular Degeneration Group	Intermediate Age-Related Macular Degeneration Group
Age (yrs)			
No.	19	27	39
Mean (SD)	71.8 (7.2)	72.7 (8.4)	70.8 (6.7)
Minimum, median, maximum	61. 74, 81	58, 72, 90	51, 70, 82
Gender, no. (%)			
Male	13 (68)	16 (59)	26 (67)
Female	6 (32)	11 (41)	13 (33)
Race, no. (%)			
White	19 (100)	27 (100)	37 (95)
Black	0	0	1 (3)
Native Hawaiian or Pacific Islander	0	0	1 (3)
Ethnicity, no. (%)			
Not Hispanic or Latino	18 (95)	22 (85)	37 (95)
Hispanic or Latino	0	1 (4)	0
Unknown/not reported	1 (5)	3 (12)	2 (5)

SD = standard deviation.

Table 2. Cross-sectional Analysis at the 6- and 12-Month Visits for the Normal Control, Early Age-Related Macular Degeneration, and Intermediate Age-Related Macular Degeneration Groups for Best-Corrected Visual Acuity, Low-Luminance Visual Acuity, and Low-Luminance Deficit

Test	Normal Control Group	Early Age-Related Macular Degeneration Group	Intermediate Age-Related Macular Degeneration Group	Overall *P* Value	Normal Control vs. Early Age-Related Macular Degeneration Groups	Normal Control vs. Intermediate Age-Related Macular Degeneration Groups	Early vs. Intermediate Age-Related Macular Degeneration Groups
BCVA							
Baseline							
No.	19	27	39				
Mean (SD)	83.5 (4.5)	81.4 (5.4)	81.4 (5.8)	0.400	0.223	0.230	0.927
Minimum, median, maximum	73, 83, 90	69, 82, 90	64, 83, 92				
6 mos							
No.	17	27	37				
Mean (SD)	84.0 (5.7)	80.4 (5.1)	81.1 (6.4)	0.072	0.020	0.078	0.668
Minimum, median, maximum	73, 85, 95	72, 81, 91	67, 81, 95				
12 mos							
No.	19	27	39				
Mean (SD)	82.2 (5.5)	82.4 (4.9)	79.8 (7.7)	0.299	0.671	0.242	0.180
Minimum, median, maximum	68, 84, 89	71, 82, 90	54, 80, 91				
LLVA							
Baseline							
No.	19	27	39				
Mean (SD)	72.1 (4.8)	70.8 (6.6)	68.0 (7.5)	0.134	0.531	0.045	0.249
Minimum, median, maximum	63, 73, 80	60, 70, 83	41, 70, 80				
6 mos							
No.	17	27	37				
Mean (SD)	71.5 (8.9)	70.8 (6.0)	67.4 (11.2)	0.173	0.426	0.095	0.208
Minimum, median, maximum	47, 73, 82	56, 71, 82	16, 69, 81				
12 mos							
No.	19	27	39				
Mean (SD)	73.0 (5.6)	70.4 (6.4)	65.8 (10.2)	**0.018**	0.282	**0.008**	0.069
Minimum, median, maximum	63, 72, 84	55, 71, 82	28, 67, 80				
LLD							
Baseline							
No.	19	27	39				
Mean (SD)	11.4 (3.2)	10.6 (4.2)	13.4 (6.1)	0.147	0.453	0.262	0.065
Minimum, median, maximum	7, 11, 17	4, 11, 20	4, 12, 34				
6 mos							
No.	17	27	37				
Mean (SD)	12.5 (8.5)	9.6 (5.1)	13.7 (9.5)	0.110	0.460	0.277	0.042
Minimum, median, maximum	5, 10, 42	−3, 10, 19	3, 12, 64				
12 mos							
No.	19	27	39				
Mean (SD)	9.2 (4.5)	12.0 (2.8)	14.0 (5.3)	**0.003**	0.027	**0.002**	0.062
Minimum, median, maximum	−2, 9, 16	7, 11, 20	4, 14, 26				

BCVA = best-corrected visual acuity; LLD = low-luminance deficit; LLVA = low-luminance visual acuity; SD = standard deviation.
Data are units of BCVA, LLVA, and LLD are Early Treatment in Diabetic Retinopathy Study letters. *P* values for pairwise comparisons between groups were performed using the Wilcoxon rank-sum test. Bold values denote statistical significance.

Table 3. Cross-sectional Analysis at the 6- and 12-Month Visits for Normal Control, Early Age-Related Macular Degeneration, and Intermediate Age-Related Macular Degeneration Groups for Microperimetry Percent Reduced Threshold and Average Threshold

Test	Normal Control	Early Age-Related Macular Degeneration Group	Intermediate Age-Related Macular Degeneration Group	Overall *P* Value	Normal Control vs. Early Age-Related Macular Degeneration Groups†	Normal Control vs. Intermediate Age-Related Macular Degeneration Groups†	Early vs. Intermediate Age-Related Macular Degeneration Groups†
MP PRT (%)							
Baseline							
No.	18	25	35				
Mean (SD)	16.96 (26.59)	23.56 (27.00)	41.78 (34.44)	**0.005**	0.242	**0.003**	0.028
Minimum, median, maximum	0.0, 2.7, 94.6	0.0, 21.6, 97.3	0.0, 37.8, 100.0				
6 mos							
No.	17	23	32				
Mean (SD)	24.8 (29.3)	32.3 (23.0)	57.2 (34.0)	**0.001**	0.138	**0.001**	**0.009**
Minimum, median, maximum	0.0, 10.8, 100.0	2.7, 29.7, 91.9	2.7, 70.3, 100.0				
12 mos							
No.	18	25	35				
Mean (SD)	30.6 (30.1)	33.5 (27.8)	67.0 (31.4)	**<0.001**	0.639	**<0.001**	**<0.001**
Minimum, median, maximum	0.0, 23.0, 100.0	0.0, 29.7, 94.6	5.4, 81.1, 100.0				
MP AT (dB)							
Baseline							
No.	18	25	35				
Mean (SD)	26.90 (3.80)	26.85 (2.49)	25.30 (3.07)	0.028	0.382	0.019	0.049
Minimum, median, maximum	14.4, 27.7, 31.3	21.3, 26.4, 31.0	17.9, 25.2, 30.1				
6 mos							
No.	17	23	32				
Mean (SD)	25.4 (3.8)	25.8 (1.6)	23.5 (2.9)	**0.001**	0.245	**0.003**	**0.002**
Minimum, median, maximum	14.4, 26.9, 28.1	22.5, 25.8, 28.9	14.1, 23.1, 28.1				
12 mos							
No.	18	25	35				
Mean (SD)	25.4 (3.5)	25.5 (2.1)	22.3 (4.1)	**<0.001**	0.232	**<0.001**	**<0.001**
Minimum, median, maximum	13.5, 26.7, 28.5	19.8, 25.9, 28.7	9.4, 22.5, 27.9				

AT = average threshold; MP = microperimetry; PRT = percent reduced threshold; SD = standard deviation.
Bold numbers denote statistical significance.
†*P* values for pairwise comparisons between groups were performed using the Wilcoxon rank-sum test.

Table 4. Cross-sectional Analysis at the 6- and 12-Month Visits for Normal Control, Early Age-Related Macular Degeneration, and Intermediate Age-Related Macular Degeneration Groups for Cone Contrast Tests of Red, Green, and Blue Cones

Test	Normal Control Group	Early Age-Related Macular Degeneration Group	Intermediate Age-Related Macular Degeneration Group	Overall *P* Value	Normal Control vs. Early Age-Related Macular Degeneration Group[†]	Normal Control vs. Intermediate Age-Related Macular Degeneration Group[†]	Early vs. Intermediate Age-Related Macular Degeneration Group[†]
CCT red (%)							
Baseline							
No.	18	27	38				
Mean (SD)	70.28 (20.40)	65.56 (11.29)	56.71 (18.43)	**0.011**	0.100	**0.005**	0.095
Minimum, median, maximum	5, 70, 95	50, 70, 90	10, 60, 85				
6 mos							
No.	16	25	35				
Mean (SD)	71.9 (23.4)	64.6 (17.3)	55.7 (21.3)	**0.002**	0.028	**<0.001**	0.084
Minimum, median, maximum	0, 80, 90	10, 65, 90	0, 55, 90				
12 mos							
No.	18	27	38				
Mean (SD)	62.8 (18.3)	64.3 (8.7)	48.4 (22.5)	**<0.001**	0.704	**0.004**	**0.001**
Minimum, median, maximum	0, 67.5, 90	50, 60, 85	0, 55, 75				
CCT green (%)							
Baseline							
No.	18	27	38				
Mean (SD)	80.00 (9.07)	71.85 (12.57)	59.34 (23.71)	**<0.001**	0.021	**<0.001**	0.056
Minimum, median, maximum	60, 80, 95	30, 75, 90	0, 70, 90				
6 mos							
No.	16	25	35				
Mean (SD)	77.5 (13.4)	71.0 (16.8)	58.9 (29.0)	0.085	0.280	0.037	0.200
Minimum, median, maximum	50, 80, 100	35, 75, 95	0, 65, 95				
12 mos							
No.	18	27	38				
Mean (SD)	73.3 (13.9)	68.2 (13.0)	52.6 (26.7)	**0.009**	0.188	**0.008**	0.033
Minimum, median, maximum	50, 75, 100	30, 70, 90	0, 57.5, 90				
CCT blue (%)							
Baseline							
No.	18	27	38				
Mean (SD)	84.44 (15.14)	76.11 (16.54)	66.97 (23.32)	**0.008**	0.072	**0.003**	0.137
Minimum, median, maximum	50, 90, 100	45, 75, 100	0, 70, 100				
6 mos							
No.	16	25	35				
Mean (SD)	80.0 (16.8)	74.0 (18.5)	68.0 (23.0)	0.154	0.280	0.062	0.346
Minimum, median, maximum	45, 90, 95	30, 75, 95	15, 70, 100				
12 mos							
No.	18	27	38				
Mean (SD)	79.4 (18.1)	75.4 (19.9)	55.5 (26.8)	**0.001**	0.514	**0.002**	**0.004**
Minimum, median, maximum	30, 82.5, 100	35, 80, 100	5, 50, 100				

CCT = cone contrast test; SD = standard deviation.
Bold values denote statistical significance.
[†]*P* values for pairwise comparisons between groups were performed using the Wilcoxon rank-sum test.

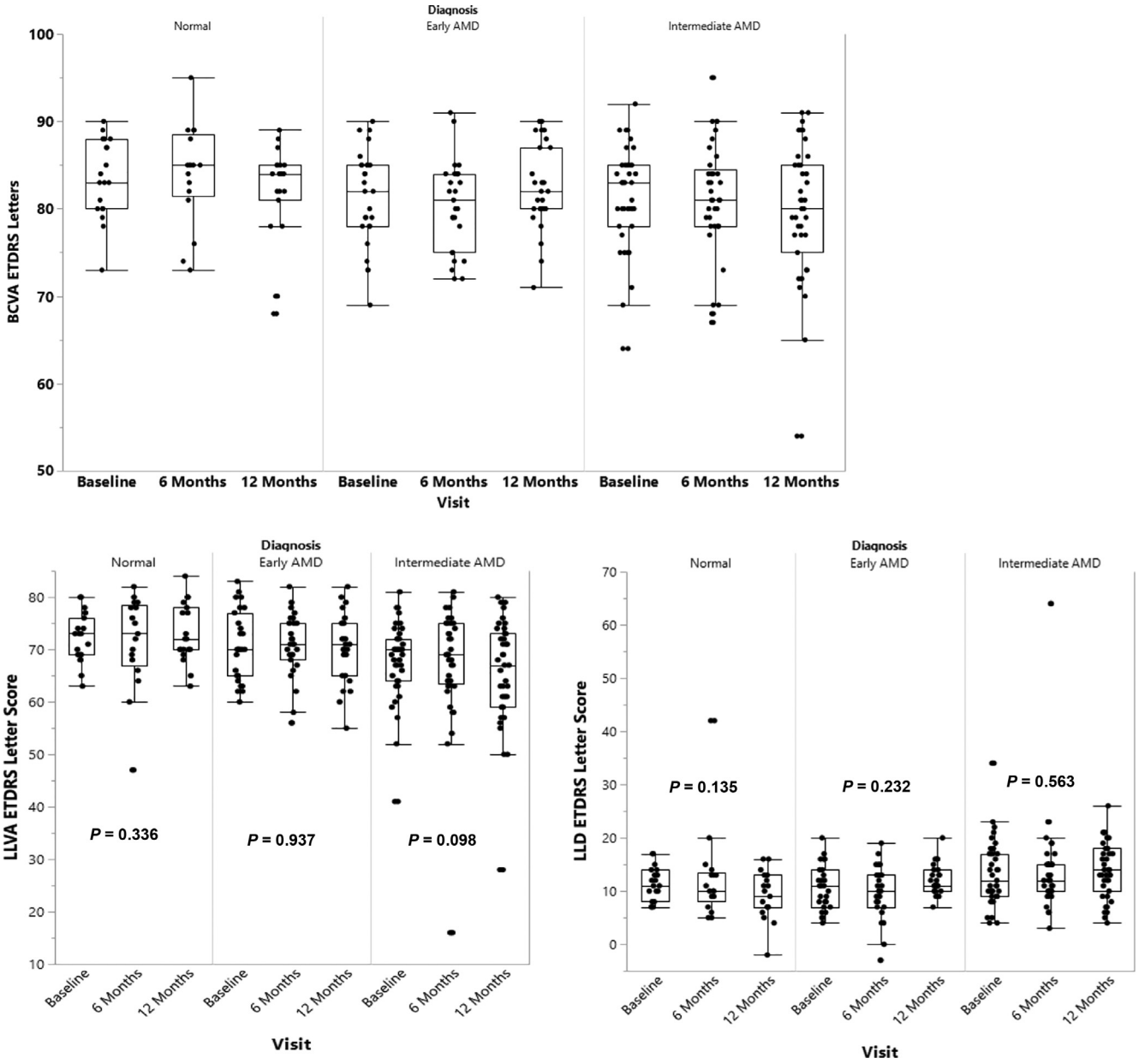

Figure 1. Box-and-whisker plots showing longitudinal progression of best-corrected visual acuity (BCVA), low-luminance visual acuity (LLVA), and low-luminance deficit (LLD) at baseline, 6 months, and 12 months for the normal control, early age-related macular degeneration (AMD), and intermediate AMD participants. Low-luminance visual acuity was reported as the number of Early Treatment Diabetic Retinopathy (ETDRS) letters the participant could read through a 2.0-log neutral density filter. Low-luminance deficit was calculated as the difference in the number of ETDRS letters the participant was able to read under standard conditions (BCVA) and through the neutral density filter (LLVA). *P* values are calculated to assess for significant changes between baseline and 12 months within each group using the Wilcoxon signed-rank test.

greater change in microperimetry AT from baseline to 12 months ($P = 0.029$), thus emphasizing the robustness of the change observed in microperimetry.

For the normal control and early AMD groups, no significant changes were observed between baseline and 12 months for BCVA, LLVA, LLD, and CCT red and blue ($P > 0.0167$; Tables 2 and 3). For all the groups, microperimetry PRT and AT were significantly different between baseline and 12 months ($P < 0.0167$; Table 4), with the exception that microperimetry PRT did not change for the early AMD participants ($P = 0.125$). Additionally, the change in LLD from baseline to 12 months was significantly greater for the early AMD group than the normal control group ($P = 0.037$).

To characterize further the progression in each of the 3 groups (control, early AMD, and intermediate AMD) from baseline to 12 months, we calculated the proportion of participants from each group that showed results outside the range of 95% of the normal values, defined as more than 2 standard deviations plus the mean of baseline normal control values (Table 5). The proportion of intermediate AMD participants who showed results out of this

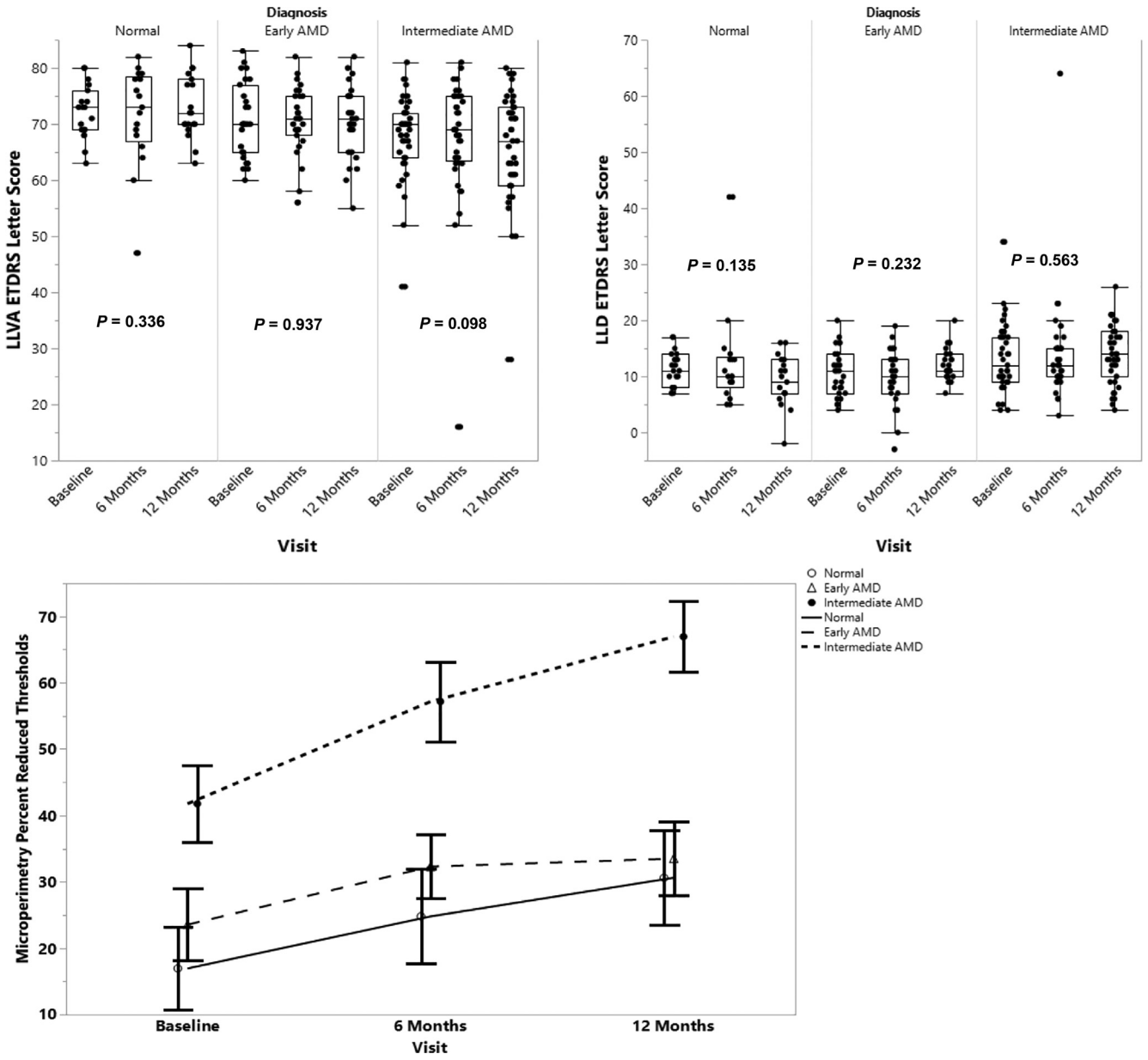

Figure 2. Box-and-whisker plot (top) and line graph (bottom) showing longitudinal progression of microperimetry percent reduced threshold (PRT) and average threshold (AT) at baseline, 6 months, and 12 months for the normal control, early age-related macular degeneration (AMD), and intermediate AMD participants. Percent reduced threshold is defined as the percentage of abnormal retinal sensitivity thresholds less than 25 dB, and AT is the average of retinal sensitivity values from all loci tested. *P* values are calculated to assess for significant changes between baseline and 12 months within each group using the Wilcoxon signed-rank test. LLD = low-luminance deficit; LLVA = low-luminance visual acuity.

normal range was significantly greater than the proportion of normal control participants for both LLD and microperimetry PRT ($P < 0.0167$; Table 6).

Discussion

Individuals affected by dry AMD currently lack effective treatment options to halt or reverse the course of this prevalent degenerative disease definitively, which becomes disabling in the advanced stages. This partly stems from a paucity of sensitive functional end points that can be used in clinical trials to assess disease progression in a short period and to intervene in the early stages. In this natural history study, we compared the results of several psychophysical measures of visual function at 6 months and 1 year between early and intermediate AMD participants and normal age-matched control participants, and we documented the longitudinal changes in these measures over the study period. Overall, the data show that, although AMD progression is slow and the AREDS staging for the individual patients did not change significantly, mesopic microperimetry and CCT

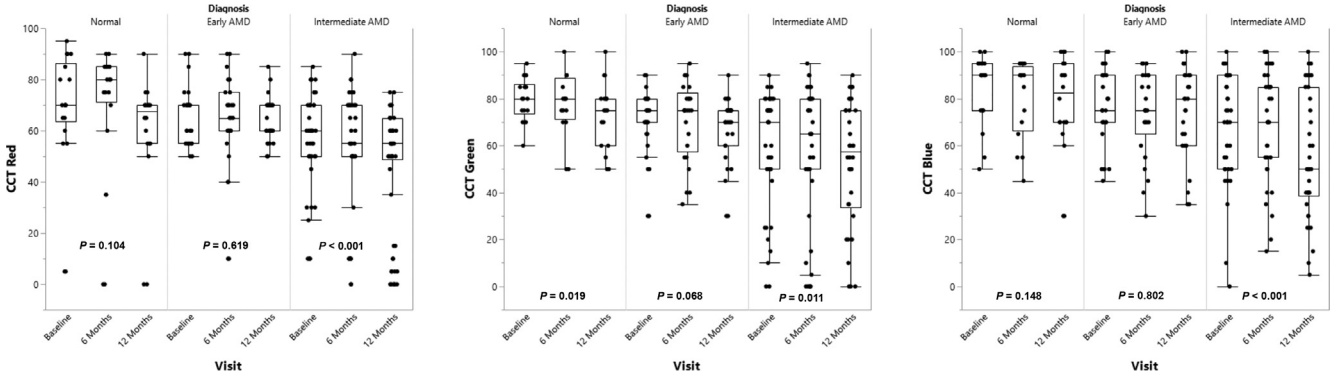

Figure 3. Box-and-whisker plots showing longitudinal progression of cone contrast test (CCT) results for red, green, and blue cones at baseline, 6 months, and 12 months for the normal control, early age-related macular degeneration (AMD), and intermediate AMD participants. The CCT results were scored on a 100-point scale, with 90% to 100% representing normal cone function and scores of less than 75% representing color deficiency. *P* values are calculated to assess for significant changes between baseline and 12 months within each group using the Wilcoxon signed-rank test.

are able to detect functional changes because of progression of dry AMD within a period as short as 12 months. Thus, this is a strong argument for inclusion of functional measures in addition to structural assessments of disease progression in intermediate AMD.

In this work, we demonstrated at 6 and 12 months that functional measures in cross-sectional analyses can distinguish between intermediate AMD and control participants as well as between intermediate AMD and early AMD disease stages. Microperimetry PRT, a metric that illustrates the percentage of loci with abnormal retinal sensitivity over the entire area tested, may be the most robust indication of progression of AMD, because it showed a significant decline in function across all 3 disease groups and time points (Fig 2). As expected, the progression of the intermediate AMD group was most pronounced and statistically robust in microperimetry PRT, because significant changes were seen in both methods of statistical analysis. In a cross-sectional analysis, Wu et al[15] demonstrated significant differences for BCVA, LLVA, and microperimetry for all AMD groups except AREDS stage 2 or early AMD relative to control participants. They subsequently reported that eyes with intermediate AMD demonstrate a statistically significant decline in mesopic microperimetry mean pointwise sensitivity over a 12-month follow-up period (mean, −0.42 dB; standard error, 0.12 dB; $P < 0.001$) but that LLVA and BCVA do not change significantly.[14] Similarly, Vujosevic et al[24] found that mean retinal sensitivity decreased over 6 years in eyes with AREDS 2 and 3 disease (mean, −10.8 dB; standard deviation 9.2; $P = 0.0028$). In our study, we observed a decline in microperimetry mean pointwise sensitivity, also termed *average threshold*, of −3.0 dB (standard deviation, 3.3 dB; $P < 0.001$). Although the direction of the change in microperimetry is similar across these 3 studies, the magnitude differs slightly between the studies. Disparities in the magnitude could be related to variation in the level of severity and visual impairment in intermediate AMD patients at baseline or to differences in the testing technique, grid used, and length of follow-up. For example, Wu et al used a customized grid pattern for Macular Integrity Assessment that tested points in locations different than the 10° standard grid pattern used in our study, and Vujosevic et al followed up patients for a much longer period (i.e., 6 years).

Although none of the participants in our study showed evidence of progression on clinical dilated fundus examination, the longitudinal changes observed in microperimetry and CCT among participants with intermediate or early AMD, or both, suggest that these psychophysical tests may detect subclinical progression in individuals with dry AMD. The fact that we noted significant declines in BCVA for the intermediate AMD group within a 12-month window underscores the importance of timely therapeutic intervention in intermediate AMD. The psychophysical metrics of BCVA and CCT also can be used to monitor the stability of the disease in patients with early AMD, because no significant changes and moreover no improvements were appreciated in this subgroup over the 12-month study period. Given the sensitive nature of microperimetry,[15] it is possible that the decline in visual function observed in the normal control participants for microperimetry AT from baseline to 12 months may be evidence of subclinical progression to dry AMD. Longer follow-up in this study and other longitudinal natural history studies are needed to assess whether these changes in visual function on microperimetry in fact do precede structural changes on examination. The CCT results of all cone types (red, green, and blue) also consistently differentiated between the normal control and intermediate AMD groups at baseline and 12 months and showed significant longitudinal changes at 12 months within the intermediate AMD group. To our knowledge, our study is the most comprehensive longitudinal study to date to evaluate a range of psychophysical tests in early and intermediate AMD participants, using CCT in addition to LLVA and microperimetry tests. To our knowledge, to date, this study is the only one to assess longitudinal changes in CCT in early intermediate AMD patients.

This study has limitations that may impact the interpretation of our results. First, the necessary sample size was based on a pilot study[13] that focused on detecting the difference in LLVA between groups and used a smaller

Table 5. Longitudinal Changes from Baseline to 1 Year within Group for Normal Control, Early Age-Related Macular Degeneration, and Intermediate Age-Related Macular Degeneration Patients

Test	Normal Control Group	Early Age-Related Macular Degeneration Group	Intermediate Age-Related Macular Degeneration Group
BCVA			
No.	19	27	39
Mean (SD)	−1.3 (5.2)	1.0 (4.7)	−1.6 (6.8)
Minimum, median, maximum	−15.0, −2.0, 6.0	−9.0, 1.0, 15.0	−15.0, −3.0, 25.0
P value	0.497	0.420	**0.033**
LLVA			
No.	19	27	39
Mean (SD)	0.9 (5.1)	−0.4 (5.6)	−2.2 (7.5)
Minimum, median, maximum	−12.0, 2.0, 8.0	−16.0, 0.0, 9.0	−24.0, −1.0, 12.0
P value	0.336	0.937	0.098
LLD			
No.	19	27	39
Mean (SD)	−2.2 (5.8)	1.4 (5.4)	0.6 (7.1)
Minimum, median, maximum	−16.0, −2.0, 9.0	−8.0, 1.0, 13.0	−14.0, 0.0, 15.0
P value	0.135	0.232	0.563
Microperimetry PRT (%)			
No.	18	25	35
Mean (SD)	13.7 (20.5)	10.0 (35.0)	25.2 (27.4)
Minimum, median, maximum	−18.9, 8.1, 62.2	−75.7, 8.1, 73.0	−18.9, 18.9, 94.6
P value	**0.003**	0.125	**<0.001**
Microperimetry AT (dB)			
No.	18	25	35
Mean (SD)	−1.5 (1.7)	−1.4 (2.7)	−3.0 (3.3)
Minimum, median, maximum	−4.5, −0.9, 1.2	−6.1, −2.1, 4.6	−18.5, −2.6, 1.2
P value	**0.002**	**0.017**	**<0.001**
CCT (%)			
Red			
No.	18	27	38
Mean (SD)	−7.5 (17.8)	−1.3 (11.0)	−8.3 (12.7)
Minimum, median, maximum	−40.0, −5.0, 20.0	−30.0, 0.0, 25.0	−45.0, −10.0, 20.0
P value	0.104	0.619	**<0.001**
Green			
No.	18	27	38
Mean (SD)	−6.7 (11.4)	−3.7 (10.1)	−6.7 (15.1)
Minimum, median, maximum	−35.0, −5.0, 15.0	−30.0, −5.0, 20.0	−50.0, −5.0, 20.0
P value	**0.019**	0.068	**0.011**
Blue			
No.	18	27	38
Mean (SD)	−5.0 (13.6)	−0.7 (17.4)	−11.5 (15.4)
Minimum, median, maximum	−30.0, −5.0, 25.0	−35.0, 0.0, 40.0	−45.0, −10.0, 30.0
P value	0.148	0.802	**<0.001**

AT = average threshold; BCVA = best-corrected visual acuity; CCT = cone contrast test; LLD = low-luminance deficit; LLVA = low-luminance visual acuity; PRT = percent reduced threshold; SD = standard deviation.
P values are calculated to assess for significant change within each group over 1 year using the Wilcoxon signed-rank test.
Bold values denote statistical significance.

number of control participants and AMD patients, even though the study population was very similar to the current larger study. Second, some participants were unable or unwilling to complete parts of the study, such as the microperimetry tests, because of the need for pupil dilation or because of time constraints. For the 6-month visit, several participants elected not to return or to complete all of the tests; this resulted in a smaller sample size that likely decreased the ability to detect potential differences between groups at this visit. Furthermore, to understand the reliability of predicting the changes assessed over time, test–retest reliability of the functional measurements will require rigorous assessment before serving as clinical trial end points. Continued longitudinal analysis of the groups at future time points will be necessary to confirm persistent changes between and within groups over time, as well as to provide additional important insights into the natural course of visual impairment in dry AMD. Furthermore, it is important to recognize the distinction between statistical and clinical significance. Although the observed changes in microperimetry over the 12-month period were small and subclinical, they represent a statistically significant decline in retinal sensitivity that may become more clinically significant as patients are followed up over a longer period. If microperimetry is able to detect early alterations in visual function before the patient appreciates a

Table 6. Percentage of Participants at 1 Year Whose Results Were Greater Than the Mean Plus 2 Standard Deviations of the Normal Control Values at Baseline for Each of the Psychophysical Tests: Best-Corrected Visual Acuity, Low-Luminance Visual Acuity, Low-Luminance Deficit, Microperimetry Percent-Reduced Threshold and Average Threshold, and Cone Contrast Test for Red, Blue, and Green Cones

Test	Normal Control Group	Early Age-Related Macular Degeneration Group	Intermediate Age-Related Macular Degeneration Group	*P* Value*
BCVA ≥ 92.48	0	0	0	—
LLVA ≥ 81.65	1 (5%)	1 (4%)	0	0.328
LLD ≥ 17.74	0	1 (4%)	11 (28%)	**0.011**
Microperimetry PRT ≥ 70.15	2 (11%)	3 (12%)	21 (60%)	**0.001**
Microperimetry AT ≥ 34.51	0	0	0	—
CCT				
Red ≥ 111.08	0	0	0	—
Green ≥ 98.15	1 (6%)	0	0	0.321
Blue ≥ 114.72	0	0	0	—

AT = average threshold; BCVA = best-corrected visual acuity; CCT = cone contrast test; LLD = low-luminance deficit; LLVA = low-luminance visual acuity; PRT = percent reduced threshold; — = *P* values not calculated.
Bold values denote statistical significance.
*Calculated to assess for significant differences between the normal and intermediate AMD groups using the Fisher exact test of difference in proportions.

subjective change, interventions may be targeted better toward the prevention of disease progression and resulting subjective visual dysfunction.

Future analyses over an additional 12 months of longitudinal follow-up in this study will explore the relationship between structure and function in AMD over time by correlating psychophysical test results with findings on OCT such as geographic atrophy, RPE drusen complex,[25] photoreceptor layer thickness, and hyperreflective foci overlying drusen.[26] This either may confirm that functional impairment proceeds the current structural grading or may aid in the search for morphologic changes of finer granularity that predict functional impairment and progression in AMD. Suggestions of possible changes in function preceding changes in structure arise in our results that showed significant decline in visual function in the control and intermediate AMD groups as measured on microperimetry PRT and AT but without any observed changes on dilated fundus examinations or examination of retinal images. Additionally, longitudinal changes on the macular pigment optical density test, a measure of the macular carotenoid pigments lutein and zeaxanthin that are thought to be protective factors in AMD,[27,28] should be correlated to the progression of visual impairment on tests of visual function explored in our study. In the future, these psychophysical tests should be evaluated in participants with genetically subtyped AMD to allow possibly early identification of patients at risk for disease progression.

In summary, the natural progression of disease in patients with intermediate AMD differs from those of normal aging control participants and early AMD. Our study investigated functional markers that could be sensitive to diagnosing disease stage as well as monitoring disease progression in the shortest length of time possible. Of the psychophysical tests of visual function, microperimetry and sensitive tests of cone function such as the CCT may be the most likely end points to deliver on this goal. Further follow-up of our cohorts is needed to verify these findings over longer periods. The 1-year results of this study provide foundational knowledge that should be built on by ongoing long-term studies of AMD. Such sensitive psychophysical tests may represent useful end points for future clinical trials of potential therapies for dry AMD, particularly as we seek to intervene as soon as possible in the early stages of disease.

References

1. Garrett D. *Report released on one of the most-feared disabilities*. Prevent Blindness America: National Eye Institute; 2002. https://nei.nih.gov/news/pressreleases/032002. Accessed October 1, 2018.
2. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Global Health*. 2014;2:e106–e116.
3. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch Ophthalmol*. 2001;119:1417–1436.
4. Chew EY, Clemons TE, Agrón E, et al. Long-term effects of vitamins C and E, β-carotene, and zinc on age-related macular degeneration: AREDS report no. 35. *Ophthalmology*. 2013;120:1604–1611.e1604.
5. Lesmes LA, Jackson ML, Bex P. Visual function endpoints to enable dry AMD clinical trials. *Drug Discov Today Ther Strateg*. 2013;10:e43–e50.
6. Scilley K, Jackson GR, Cideciyan AV, et al. Early age-related maculopathy and self-reported visual difficulty in daily life. *Ophthalmology*. 2002;109:1235–1242.
7. Owsley C, McGwin JG, Scilley K, Kallies K. Development of a questionnaire to assess vision problems under low luminance in age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2006;47:528–535.
8. Steinmetz RL, Haimovici R, Jubb C, et al. Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change. *Br J Ophthalmol*. 1993;77:549–554.
9. Owsley C, Jackson GR, Cideciyan AV, et al. Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41:267–273.

10. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996;37:1236−1249.
11. Medeiros NE, Curcio CA. Preservation of ganglion cell layer neurons in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2001;42:795−803.
12. Holz FG, Gross-Jendroska M, Eckstein A, et al. Colour contrast sensitivity in patients with age-related Bruch's membrane changes. *Ger J Ophthalmol*. 1995;4:336−341.
13. Chandramohan A, Stinnett SS, Petrowski JT, et al. Visual function measures in early and intermediate age-related macular degeneration. *Retina*. 2016;36:1021−1031.
14. Wu Z, Ayton LN, Luu CD, Guymer RH. Longitudinal changes in microperimetry and low luminance visual acuity in age-related macular degeneration. *JAMA Ophthalmol*. 2015;133:442−448.
15. Wu Z, Ayton LN, Guymer RH, Luu CD. Low-luminance visual acuity and microperimetry in age-related macular degeneration. *Ophthalmology*. 2014;121:1612−1619.
16. Cocce KJ, Stinnett SS, Luhmann UFO, et a l. Visual function metrics in early and intermediate dry age-related macular degeneration for use as clinical trial endpoints. *Am J Ophthalmol*. 2018;189:127−138.
17. Panel American Academy of Ophthalmology Retina/Vitreous Panel. *Preferred Practice Pattern Guidelines: Age-Related Macular Degeneration*. San Francisco, CA: American Academy of Ophthalmology; 2015.
18. Early Treatment Diabetic Retinopathy Study Research Group. Early Treatment Diabetic Retinopathy Study design and baseline patient characteristics. ETDRS report number 7. *Ophthalmology*. 1991;98(5 Suppl):741−756.
19. Ferris 3rd FL, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. *Am J Ophthalmol*. 1982;94:91−96.
20. Sunness JS, Rubin GS, Broman A, et al. Low luminance visual dysfunction as a predictor of subsequent visual acuity loss from geographic atrophy in age-related macular degeneration. *Ophthalmology*. 2008;115:1480−1488, 1488.e1481−1482.
21. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology*. 1990;1:43−46.
22. Rothman KJ. Six persistent research misconceptions. *J Gen Intern Med*. 2014;29:1060−1064.
23. Althouse AD. Adjust for multiple comparisons? It's not that simple. *Ann Thorac Surg*. 2016;101:1644−1645.
24. Vujosevic S, Pucci P, Casciano M, et al. Long-term longitudinal modifications in mesopic microperimetry in early and intermediate age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 2017;255:301−309.
25. Farsiu S, Chiu SJ, O'Connell RV, et al. Quantitative classification of eyes with and without intermediate age-related macular degeneration using optical coherence tomography. *Ophthalmology*. 2014;121:162−172.
26. Schuman SG, Koreishi AF, Farsiu S, et al. Photoreceptor layer thinning over drusen in eyes with age-related macular degeneration imaged in vivo with spectral-domain optical coherence tomography. *Ophthalmology*. 2009;116:488−496.e482.
27. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 1994;272:1413−1420.
28. Moeller SM, Parekh N, Tinker L, et al. Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-Related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. *Arch Ophthalmol*. 2006;124:1151−1162.

Footnotes and Financial Disclosures

Originally received: October 21, 2018.
Final revision: March 8, 2019.
Accepted: March 12, 2019.
Available online: ■■■. Manuscript no. ORET_2018_418.

[1] Department of Ophthalmology, Duke University Medical Center, Durham, North Carolina.

[2] Roche Pharmaceutical Research and Early Development, Translational Medicine Neuroscience & Ophthalmology & Rare Diseases Biomarkers, Roche Innovation Center Basel, Basel, Switzerland.

Financial Disclosure(s):
The author(s) have made the following disclosure(s): U.F.O.L.: Employee − F. Hoffmann − La Roche Ltd. (Basel, Switzerland).

Lejla Vajzovic: Financial support − Janssen Pharmaceutical, Roche, DORC, Alcon, Genentech.

C.A.T.: Consultant and Financial support − Emmes; Royalties − Alcon.

S.W.C.: Consultant − Bausch + Lomb, Stealth BioTherapeutics, Alimera Sciences, PanOptica, TheraKine, Eyedesis; Financial support − B&L, Stealth BioTherapeutics; Equity owner − PanOptica, Eyedesis; Board member − TheraKine; Founder − Eyedesis.

E.M.L.: Consultant − Stealth BioTherapeutics; Financial support − F. Hoffman − La Roche (Basel, Switzerland), Apellis, Novartis.

Supported by the National Eye Institute, National Institutes of Health, Bethesda, Maryland (grant no.: K23EY026988); Research to Prevent Blindness, Inc, New York, New York; the Knights Templar Eye Foundation (L.V.); the Second Sight Foundation (L.V.); Duke University, Durham, North Carolina (Private Diagnostic Clinic Enable Award to L.V.); F. Hoffmann − La Roche Ltd., Basel, Switzerland (through Duke University).

HUMAN SUBJECTS: Human subjects were included in this study. The human ethics committees at the Duke University Health System approved the study. All participants provided informed consent. All research adhered to the tenets of the Declaration of Helsinki.

No animal subjects were included in this study.

Author Contributions:

Conception and design: Stinnett, Luhmann, Cousins, Lad

Analysis and interpretation: Hsu, Thompson, Stinnett, Luhmann, Lad

Data collection: Hsu, Stinnett, Vajzovic, Horne, Schuman, Toth, Lad

Obtained funding: Lad

Overall responsibility: Hsu, Thompson, Stinnett, Luhmann, Vajzovic, Horne, Schuman, Toth, Cousins, Lad

Abbreviations and Acronyms:
AMD = age-related macular degeneration; **AREDS** = Age-Related Eye Disease Study; **AT** = average threshold; **BCVA** = best-corrected visual acuity; **CCT** = cone contrast test; **LLD** = low-luminance deficit; **LLVA** = low-luminance visual acuity; **PRT** = percent reduced threshold; **RPE** = retinal pigment epithelium.

Correspondence:
Eleonora M. Lad, MD, PhD, Department of Ophthalmology, Duke University Medical Center, DUMC 3802, Durham, NC 27710. E-mail: nora.lad@duke.edu.