Supplementary Materials

Potential Structural Biomarkers in 3D Images Validated by the First Functional Biomarker for Early Age-Related Macular Degeneration - ALSTAR2 Baseline

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Supplementary Methods:

The Alabama Study on Early Age-Related Macular Degeneration 2 (ALSTAR2) is a prospective cohort study on normal aging and early and intermediate AMD whose purpose is to validate retinal imaging biomarkers in these conditions with visual function measures (Clinicaltrials.gov identifier NCT04112667, October 7, 2019)29. The study was approved by the Institutional Review Board of the University of Alabama at Birmingham. All participants provided written informed consent after the nature and purpose of the study were explained. Conduct of the study followed the Declaration of Helsinki. The baseline data from ALSTAR2 were collected between October 2019 and September 2021, which included a 4-month pause in enrollment due to the coronavirus pandemic (March-June 2020).

Participants ≥ 60 years old were recruited from the Callahan Eye Hospital Clinics, the clinical service of the University of Alabama at Birmingham Department of Ophthalmology and Visual Sciences. Three groups were recruited: those with early AMD and intermediate AMD, and those in normal macular health. The clinic's electronic health record was used to search for patients with early or intermediate AMD using International Classification of Diseases 10 codes

(H35.30*; H35.31*; H35.36*). One of the investigators (C.O.) screened charts to confirm that participants met the eligibility criteria. Exclusion criteria were (1) any eye condition or disease in either eye (other than early cataract) in the medical record that can impair vision including diabetic retinopathy, glaucoma, ocular hypertension, history of retinal diseases (e.g., retinal vein occlusion, retinal degeneration), optic neuritis, corneal disease, previous ocular trauma or surgery, and refractive error ≥ 6 diopters; (2) neurological conditions that can impair vision or judgment including multiple sclerosis, Parkinson's disease, stroke, Alzheimer's disease, seizure disorders, brain tumor, traumatic brain injury; (3) psychiatric disorders that could impair the ability to follow directions, answer questions about health and functioning, or to provide informed consent; (4) diabetes; (5) any medical condition that causes significant frailty or was thought to be terminal. Persons in normal macular health met the same eligibility criteria except they were not classified with the International Classification of Diseases – 10 (ICD-10) codes indicative of AMD. Letters were sent to potential participants, with the study coordinator following up by phone to determine interest.

One eye was tested for each participant, with the eye selected for testing being the eye with better acuity. If the eyes had the same acuity, then an eye was randomly selected. Classification into the 3 groups was based on a trained grader's (M.E.C.) evaluation of 3-field color fundus photographs taken with a digital camera (FF-450, Carl Zeiss Meditec) following dilation with 1% tropicamide and 2.5% phenylephrine hydrochloride. The Age-Related Eye Disease Study (AREDS) 9-step classification system30 was used by the grader to identify AMD presence and severity. Group membership was determined, as follows: eyes in normal macular health had AREDS grade 1, early AMD had grades 2 to 4, and intermediate AMD had grades 5 to 8. We also used the Beckman classification system31 with normal aging defined to include grades 1 to 2, early AMD as grade 3, and intermediate AMD as grade 4. The grader was masked to all other participant characteristics. As previously described,32 intra-grader agreement was K = 0.88; intergrader agreement with a second grader was K = 0.75. Demographic information for birthdate, gender, and race/ethnicity were obtained through a self-administered questionnaire.

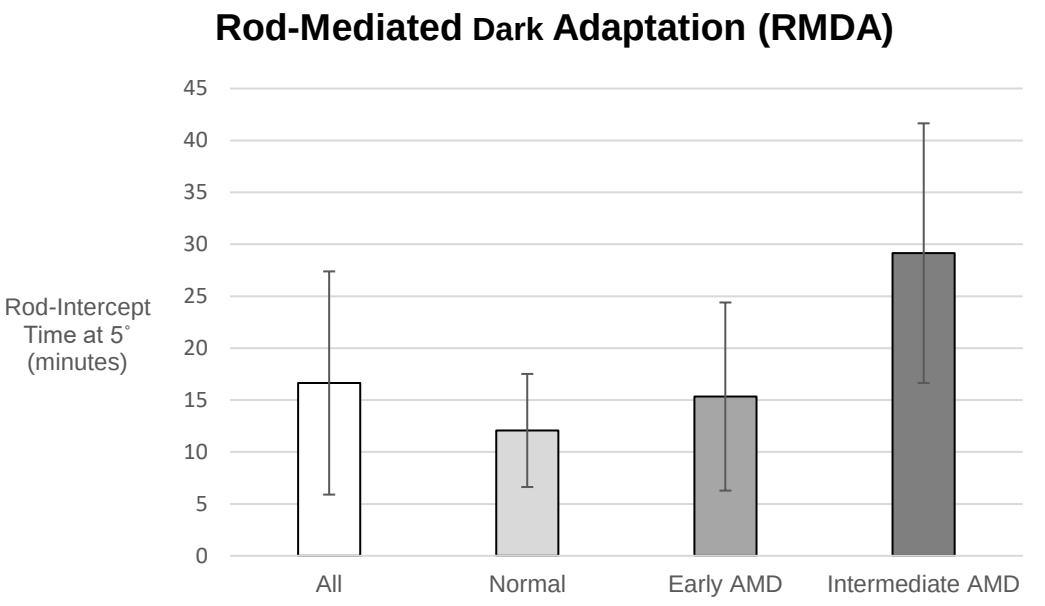
Rod-mediated dark adaptation was assessed with the AdaptDx device (Lumithera, Poulsbo WA). Testing occurred in a dark, light-tight room after dilation. RMDA was measured on the superior vertical meridian at 5˚ eccentricity to probe the area of proportionately greatest rod loss in aging and AMD.33, 34 The procedure began with a photo-bleach exposure to a 6˚ diameter flash centered at each test target location (equivalent ~83% bleach; 50 ms duration, 58,000 scotopic cd/m2 s intensity35) while the participant focused on the fixation light. Threshold

measurement (3-down/1 up threshold strategy) for a 2˚ diameter, 500 nm circular target began 15 seconds after bleach offset. The participant was instructed to maintain fixation and press a button when the flashing target first became visible. Log thresholds were expressed as sensitivity in decibel units as a function of time since bleach offset. Threshold measurement continued at 30-second intervals until the rod intercept time (RIT) was reached. Rod intercept time is the duration in minutes required for sensitivity to recover to a criterion value of 5.0 x 10-3 scotopic cd/m2,23, 36 located in the latter half of the second component of rod-mediated recovery.27, 37 If RIT was not reached, the threshold measurement procedure stopped at 45 minutes. For some participants where the threshold measurement procedure was stopped, the AdaptDx's algorithm generated a RIT if it could be computed based on previous thresholds. Participants with fixation errors > 30% were excluded from analysis.

We acquired spectral-domain OCT volumes (Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany; λ = 870 nm; scan depth, 1.9 mm; axial resolution, 3.5 μm per pixel in tissue; lateral resolution, 14 μm per pixel in tissue), with Automatic Real-Time averaging > 9, and quality (signal-to-noise) 20–47 dB. B-scans (n = 121 scans, spacing =60 μm) were horizontally oriented and centered over the fovea in a 30°× 25° (8.6 × 7.2 mm) area.

Supplementary Results:

Supplemental Figure 1:



Supplemental Figure 1: Mean and standard deviation of rod-intercept time at 5˚ for all eyes, normal eyes, early AMD eyes, and intermediate AMD eyes

Supplemental Table 1:

Supplemental Table 1	Rod-Mediated Dark Adaptation (RMDA)			
		Mean	Std Dev	p-value
RIT at 5˚	All	16.642	10.746	
	Normal	12.070	5.445	<.0001
	Early AMD	15.338	9.057	
	Intermediate AMD	29.149	12.500	

Supplemental Table 1: Mean and standard deviation of rod-intercept time at 5˚ for all eyes, normal eyes, early AMD eyes, and intermediate AMD eyes.

Supplemental Table 2:

Supplemental Table 2	Normal	Early AMD	Intermediate AMD
Normal		0.0006	<.0001
Early AMD	0.0006		<.0001
Intermediate AMD	<.0001	<.0001	

Supplemental Table 2: Pairwise p-values for mean rod-intercept time at 5˚.

Supplemental Table 3:

Supplemental Table 3					
Layer	ETDRS Subfield	AREDS Category	Normal	Early	Intermediate
Ellipsoid Zone (EZ) Area	Entire Grid	Normal		0.842	<.0001
		Early	0.842		<.0001
		Intermediate	<.0001	<.0001	
	Central Subfield	Normal		0.552	<.0001
		Early	0.552		<.0001
		Intermediate	<.0001	<.0001	
	Inner Ring	Normal		0.796	<.0001
		Early	0.796		<.0001
		Intermediate	<.0001	<.0001	
	Outer Ring	Normal		0.889	<.0001
		Early	0.889		<.0001
		Intermediate	<.0001	<.0001	
Interdigitation Zone (IZ) Area	Entire Grid	Normal		0.251	<.0001
		Early	0.251		<.0001
		Intermediate	<.0001	<.0001	
	Central Subfield	Normal		0.129	<.0001
		Early	0.129		<.0001
		Intermediate	<.0001	<.0001	
	Inner Ring	Normal		0.243	<.0001
		Early	0.243		<.0001
		Intermediate	<.0001	<.0001	
	Outer Ring	Normal		0.326	<.0001
		Early	0.326		<.0001
		Intermediate	<.0001	<.0001	
Interdigitation Zone (IZ) Thickness	Entire Grid	Normal		0.820	<.0001
		Early	0.820		<.0001
		Intermediate	<.0001	<.0001	
	Central Subfield	Normal		0.969	<.0001
		Early	0.969		<.0001
		Intermediate	<.0001	<.0001	
	Inner Ring	Normal		0.841	<.0001
		Early	0.841		<.0001
		Intermediate	<.0001	<.0001	
	Outer Ring	Normal		0.843	<.0001
		Early	0.843		<.0001
		Intermediate	<.0001	<.0001	

Supplemental Table 3: Pairwise p-values for mean area of EZ, mean area of IZ, and mean thickness of IZ in the entire grid, central subfield, inner ring, and outer ring.