Outer Retinal Thickness Is Associated With Cognitive Function in Normal Aging to Intermediate Age-Related Macular Degeneration

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Purpose. Research on Alzheimer's disease (AD) and precursor states demonstrates a thinner retinal nerve fiber layer (NFL) compared to age-similar controls. Because AD and age-related macular degeneration (AMD) both impact older adults and share risk factors, we asked if retinal layer thicknesses, including NFL, are associated with cognition in AMD.

Methods. Adults ≥ 70 years with normal retinal aging, early AMD, or intermediate AMD per Age-Related Eye Disease Study (AREDS) nine-step grading of color fundus photography were enrolled in a cross-sectional study. Optical coherence tomography (OCT) volumes underwent 11-line segmentation and adjustments by a trained operator. Evaluated thicknesses reflect the vertical organization of retinal neurons and two vascular watersheds: NFL, ganglion cell layer–inner plexiform layer complex (GCL-IPL), inner retina, outer retina (including retinal pigment epithelium–Bruch's membrane), and total retina. Thicknesses were area weighted to achieve mean thickness across the 6-mm-diameter Early Treatment of Diabetic Retinopathy Study (ETDRS) grid. Cognitive status was assessed by the National Institutes of Health Toolbox cognitive battery for fluid and crystallized cognition. Correlations estimated associations between cognition and thicknesses, adjusting for age.

Results. Based on 63 subjects (21 per group), thinning of the outer retina was significantly correlated with lower cognition scores ($P < 0.05$). No other retinal thickness variables were associated with cognition.

Conclusions. Only the outer retina (photoreceptors, supporting glia, retinal pigment epithelium, Bruch's membrane) is associated with cognition in aging to intermediate AMD; NFL was not associated with cognition, contrary to AD-associated condition reports. Early and intermediate AMD constitute a retinal disease whose earliest, primary impact is in the outer retina. Our findings hint at a unique impact on the brain from the outer retina in persons with AMD.

Keywords: age-related macular degeneration, aging, optical coherence tomography, cognition

Clinical retinal imaging in Alzheimer's disease (AD) has repeatedly demonstrated that the nerve fiber layer (NFL) is thinner in AD than in age-similar controls, starting with time-domain optical coherence tomography (OCT)[1–8] and continuing with spectral-domain OCT (SD-OCT).[9,10] Older adults with mild cognitive impairment (MCI),[5] an early stage of cognitive dysfunction in aging, and preclinical AD without cognitive impairment[11,12] also have thinner NFLs. Other retinal layers may thin in AD,[9] and reduced overall macular thickness is associated with worse cognitive performance.[2] Very large community-based cross-sectional studies using scanning laser polarimetry,[13] confocal laser ophthalmoscopy,[14] and SD-OCT[15] have also found that a thinned NFL and reduced cognition are associated. Thus, a consis-

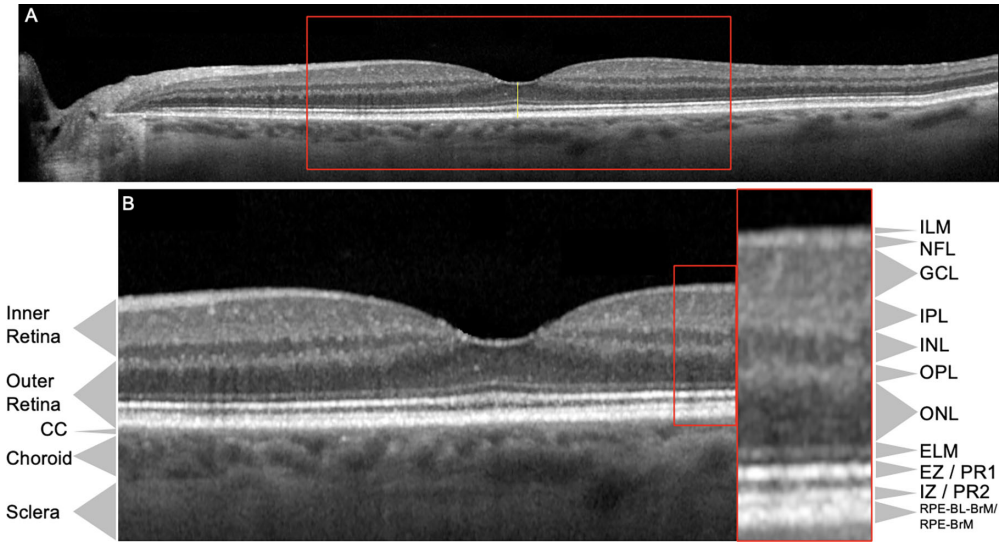

Figure 1. Retinal layer segmentation in a healthy aged macula. Left eye of a 77-year-old male participant, assessed at AREDS1 by color fundus photography. (**A**) In a foveal B-scan, the foveal center is defined as the rise of the ELM as demarcated with a *vertical yellow line*. (**B**) Enlarged view of the *red rectangle* in **A**. Individual layer segmentation reflects the vertical organization of retinal neurons and the two vascular watersheds: inner retina (NFL, GCL, IPL, and INL) and outer retina (OPL, ONL, ELM, EZ, IZ, RPE and its BL, BrM). We use the IN•OCT nomenclature.[29] In the Heidelberg Eye Explorer software, EZ is referred to as PR1, and IZ is referred to as PR2. CC, choriocapillaris; ILM, internal limiting membrane; NFL, nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; ELM, external limiting membrane; EZ, ellipsoid zone; IZ, interdigitation zone; RPE, retinal pigment epithelium; BL, basal lamina; BrM, Bruch's membrane; PR1, photoreceptor 1; PR2, photoreceptor 2.

tent finding across technologies and populations is thinning of the NFL, particularly in the peripapillary and/or macular region.[16]

Retinal imaging studies such as these have excluded participants with age-related macular degeneration (AMD), the major cause of irreversible vision loss in older adults in the United States.[16–18] Attempts to verify characteristic AD pathology such as amyloid within the retina have been controversial[19]; however, associations between AMD and AD have been reported in several epidemiologic studies.[20–22] AMD and AD both include neurodegeneration and share some risk factors such as advanced age, smoking, hypertension, vascular changes, oxidative stress, and inflammation.[16,23,24] The common risk factors are intriguing, given a conflicting association with the *APOE* genotype between the two conditions, a major paradox in human genetics. The *APOE* $\varepsilon 4$ allele is associated with an increased risk of AD and atherosclerotic cardiovascular disease and decreased risk of AMD, whereas the opposite is true for *APOE* $\varepsilon 2$.[25,26] One proposed explanation is opposing effects of cholesterol efflux into systemic artery walls versus Bruch's membrane of the eye.[27] Neuropathologists have shown that cerebrovascular disease is common in AD and not in other neurodegenerations,[28] suggesting that this idea can be extrapolated to brain vessels.

Previous studies focused on the NFL, which contains ganglion cell axons passing to the brain, where many primary neurodegenerations begin. As OCT is non-invasive, repeatable, user friendly, and available in office settings, quantification of retinal layer thickness with OCT might be an easy and affordable tool to detect retinal abnormalities, including neurodegeneration in patients with brain disorders. A standardized nomenclature for SD-OCT is widely used (Fig. 1).[29] OCT is most useful for detection if cellular components of the hyper- and hyporeflective bands are incorporated into study designs. For example, the NFL also contains astrocytes, Müller glia, and microglia that may participate in overall thinning in addition to axon loss.

For our thickness studies, we have chosen to divide the retina into its two vascular watersheds. The inner retina is supplied by an intrinsic circulation functionally analogous to the cerebral vasculature, and the outer retina is supplied by the choroidal circulation, which features the highest blood flow per unit weight in the body. The choroid serves the prodigious energy needs of numerous photoreceptors, which share a metabolic ecosystem dependent on aerobic glycolysis by the retinal pigment epithelium (RPE) and Müller glia. Of note, some molecules, such as cholesterol, that are almost entirely synthesized endogenously by the brain are instead readily taken up on plasma lipoproteins by the RPE for transfer to retina.[30,31] Thus, the two vascular watersheds of retina reflect different metabolic requirements of the nearby cells, offering both a comparison and contrast to the brain.

Herein, we describe a cross-sectional study on the association between the thickness of retinal layers using OCT and cognitive function in persons on the AMD trajectory from normal aging to early and intermediate AMD. Given the focus of prior literature on NFL thickness, we assess the NFL, as well as the outer retina, inner retina, ganglion cell layer–inner plexiform layer complex (GCL-IPL), and the overall neurosensory retina, combining OCT reflectivity bands as shown in Figure 1. To our knowledge, this is the first examination of the question of which retinal layers are associated with cognitive function in eyes of known AMD status.

Methods

The protocol was approved by the Institutional Review Board of the University of Alabama at Birmingham (UAB)

and followed the tenets of the Declaration of Helsinki. Participants provided written informed consent after the nature and purpose of the study were described.

Participants ages 70 years old or older were recruited from the Callahan Eye Hospital Clinics at UAB, the faculty practice of the Department of Ophthalmology and Visual Sciences. All participants lived independently in the community. Three groups of participants were recruited: those with normal retinal aging and those with early or intermediate AMD. The electronic medical record (EMR) of the clinic was used to search patients with early or intermediate AMD using *International Classification of Diseases*, Tenth Revision (ICD-10) codes for these conditions (H35.30*, H35.31*, H35.36*). An investigator (C.O.) screened each EMR for eligibility. Exclusion criteria were (1) diagnoses of any eye condition or disease in either eye in the EMR that can impair vision, including diabetic retinopathy, glaucoma, ocular hypertension, and a history of retina diseases other than AMD; (2) diagnoses of neurological conditions such as AD, frontotemporal dementia, preclinical AD, MCI, or cerebrovascular accident; (3) psychiatric disorders that could impair the ability to follow instructions; (4) diabetes; or (5) any medical condition that causes significant frailty or is thought to be terminal. Persons in normal macular health were recruited with the same criteria but did not have ICD-10 codes for AMD. All participants had intraocular lenses in both eyes.

Classification into the three groups was based on fundus photography on each eye with a digital camera (FF 450 Plus; Carl Zeiss Meditec, Jena, Germany) under dilation with 1% tropicamide and 2.5% phenylephrine hydrochloride. Group classification per eye was determined using the Age-Related Eye Disease (AREDS) nine-step classification system[32] by a trained and experienced grader. As previously described,[33] intragrader agreement was $K = 0.88$, and intergrader agreement with a second grader was $K = 0.75$. Normal aging was defined as grade 1, early AMD as grades 2 to 4, and intermediate AMD as grades 5 to 8.

The study visit consisted of a demographic review, cognitive testing, and multimodal imaging including OCT. Because fundus photography and OCT imaging were captured under dilated conditions, they were conducted at the end of the visit so dilation would not impact vision during the visual acuity and cognitive testing. A self-administered demographic questionnaire collected information on birthdate, gender, race, and ethnicity. Best-corrected visual acuity for each eye was measured with the Electronic Visual Acuity tester (M&S Technologies, Niles, IL, USA)[34] and was scored as the logarithm of the minimum angle of resolution (logMAR). Acuity was measured under photopic conditions at the same luminance level under which the cognitive tests were performed. Photopic contrast sensitivity was assessed in each eye using the Mars Chart,[35] scored letter by letter, and expressed as log sensitivity. The eye with better acuity and better contrast sensitivity defined these measures, as visual task performance in the cognitive tasks is driven by the eye with better function.

Cognition was assessed binocularly using the cognitive domain of the National Institutes of Health (NIH) Toolbox for Assessment of Neurological and Behavioral Function.[36,37] These tests included picture vocabulary, flanker inhibitory control and attention, list sorting working memory, dimensional change card sort, pattern comparison processing speed, picture sequence memory, oral reading recognition, oral symbol digit, and auditory verbal learning. The NIH Cognitive Toolbox was originally designed for the general population, including adults living independently in the community, such as in our sample. Cognitive tests were administered using an app on an iPad (Apple, Cupertino, CA, USA) following the good testing practices provided in the Toolbox Administrator's Manual.[38] The app computes separate composite scores for both fluid cognitive abilities and crystallized cognitive abilities. Both scores are provided with and without age correction. Fluid cognitive ability refers to a person's capacity to process new information, learn, and solve problems. Crystallized ability refers to a person's knowledge, vocabulary, and reasoning based on acquired information. The age-corrected score compares the person's score to those in the NIH nationally representative normative sample at the same age, where a score of 100 indicates performance at the national average for persons in that age group. For example, a score of 115 or 85 would indicate that the score is 1 standard deviation above or below the national average of age-similar participants. Scores that are not age corrected compare the participant's score to those in the entire NIH Toolbox nationally representative normal score, regardless of age or any other variable. Higher scores mean better cognitive performance.[39]

We acquired SD-OCT volumes (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany; $\lambda = 870$ 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 > 12 and quality (signal-to-noise ratio) of 27 to 46 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. OCT was accomplished on all study eyes. The software allowed viewing of the 11-line segmentation available in the Heidelberg HEYEX software as an overlay on individual B-scans for adjustment by a trained operator (author T.T.). The foveal center was defined as the rise of the external limiting membrane (ELM), where cone photoreceptors are at their longest.[40,41] The adjusted 11-line segmentation was exported in .xml format and processed using custom software (LayerThickness_ETDRS, available at https://sites.imagej.net/CreativeComputation) written in FIJI (Fiji Is Just ImageJ 2.0.0-rc-69/1.52p; National Institutes of Health, Bethesda, MD, USA).[42] The output was thicknesses in millimeters in the nine subfields of the Early Treatment of Diabetic Retinopathy Study (ETDRS)[43] grading grid. The thicknesses combined the outputs of the HEYEX segmentation (in parentheses) to reflect the vertical organization of retinal neurons and vascular watersheds (Fig. 1), as follows: NFL, GCL-IPL, inner retina (NFL + GCL + IPL + INL), outer retina (OPLtoELM + ELMtoPR1 + PR1toRPE + RPEtoBrM), and retina (inner retina + outer retina). Figure 1 illustrates the implementation of band segmentation in the context of the consensus nomenclature. Thicknesses were combined in an area-weighted manner in the statistical analysis (see next paragraph) to achieve mean thickness across the ETDRS grid. The area ratio of the central subfield, inner ring, and outer ring was 1:8:27.

Continuous and categorical data were summarized using mean (SD) and number (percent). The eye with the worse AREDS grade was chosen to define the retinal thickness variables; if the grades were the same, then one eye was randomly chosen. Analysis of covariance was used to compare cognition, visual function, and retinal thickness measures by AREDS category adjusting for age. Associations

between cognition scores and retinal layer thicknesses were evaluated using Spearman correlations, adjusting for age. The level of significance was $P \leq 0.05$ (two-sided). All analyses were completed using SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

There were 63 participants enrolled in the study: 21 with normal aging, 21 with early AMD, and 21 with intermediate AMD (Table 1). Those with early or intermediate AMD were significantly older than those with normal aging. The distributions of gender and race were similar in the three groups; approximately 2/3 of the sample were women, and approximately 90% were white of non-Hispanic origin. Visual acuity and contrast sensitivity for the better eye are shown in Table 2. There were no significant differences for visual acuity or contrast sensitivity when compared among groups. Visual acuity was approximately 20/20 in all groups. Contrast sensitivity was also highly similar in all groups, averaging 1.57 log sensitivity. Of our 63 participants, 62 were never-smokers (98.4%).

Fluid cognition and crystallized cognition scores did not differ among the groups, regardless of whether or not these scores were age corrected (Fig. 2). The outer retina was thinner in eyes with intermediate AMD compared to normal aging and early AMD but did not reach statistical significance ($P = 0.053$); no other retinal thickness variables differed among the three groups (Fig. 3). Figure 4 shows representative fundus photographs and OCT scans for normal aging, early AMD, and intermediate AMD.

Table 3 lists the age-adjusted Spearman correlations for each retinal thickness variable with each of the cognition scores. The only significant correlation between retinal thickness and the cognition scores was for the outer retina ($P < 0.05$); these correlations were of similar magnitudes for all cognition scores. No other retinal thickness variables were associated with cognition scores.

Discussion

In examining the association between cognition and retinal thickness in participants ranging from normal aging to intermediate AMD, we report that, among the current measures, only outer retinal thickness was associated with cognition. This contrasts with frequently reported findings that NFL thickness is associated with AD and cognitive function,[1–10] which we did not observe. In a community-based sample based in the Rhineland area of Germany,[44] outer retinal thickness was associated with magnetic resonance imaging variables including hippocampus and grey matter volumes. However, that study did not assess the aging to intermediate AMD trajectory, as we did, nor did it assess the association between retinal thickness and cognitive status. Others have studied brain images of AMD patients but without detailed retinal analysis.[45]

Early and intermediate AMD are stages of a disease whose first-order and primary impact is in the outer retina and its supporting vasculature, with functional changes in inner retinal layers occurring as circuitry eventually degrades.[46] How could outer retinal thickness reductions impact cognitive function? Our findings may hint at a uniquely shared factor impacting brain and outer retina in persons with AMD whose outer retina is degenerating. For example, Kim and colleagues[47] identified a thinning of the outer nuclear layer (ONL) and ellipsoid zone (EZ) in patients affected by frontotemporal dementia compared to healthy controls ($n = 44$ and 37, respectively), whereas no differences in inner retinal layer thickness were apparent. Further, thinning correlated significantly with Mini-Mental State Examination results (Spearman $r = 0.44$).[47] The same authors hypothesized a role for microtubule-associated defects in these findings, and they identified ONL thinning and EZ abnormalities in mice with a confirmed *Rp1* mutation.[48] As RP1 and tau are both microtubule-associated proteins in photoreceptor cilia, this may be a possible explanation.[49]

Table 1. Demographic Characteristics of Sample Stratified by AMD Presence and Severity for the Worse Eye

	Total Sample ($n = 63$)	Normal Aging ($n = 21$)	Early AMD ($n = 21$)	Intermediate AMD ($n = 21$)	P^*
Age, mean (SD)	75.9 (3.7)	74.2 (2.9)	77.2 (4.17)	76.7 (4.5)	0.025
Gender, *n* (%)					1.0
Men	17 (27.0)	6 (28.6)	5 (23.8)	6 (28.6)	
Women	46 (73.0)	15 (71.4)	16 (76.2)	1 (71.4)	
Race, *n* (%)					1.0
Black	7 (11.1)	3 (14.3)	2 (9.1)	2 (9.1)	
White	56 (88.9)	18 (85.7)	30 (90.9)	20 (90.9)	

* Comparison among the three groups.

Table 2. Visual Acuity, Contrast Sensitivity, and Fluid and Crystallized Cognitive Scores Stratified by AMD Presence

	Mean (SD)				
	Total Sample ($n = 63$)	Normal Aging ($n = 21$)	Early AMD ($n = 21$)	Intermediate AMD ($n = 21$)	Age-Adjusted P^*
Visual acuity (logMAR)	0.01 (0.10)	0.01 (0.1)	−0.01 (0.11)	0.03 (0.08)	0.368
Contrast sensitivity (log sensitivity)	1.57 (0.11)	1.56 (0.11)	1.57 (0.12)	1.58 (0.11)	0.675
Fluid cognition	85.1 (9.4)	87.8 (10.9)	81.3 (5.4)	86.2 (10.1)	0.197
Fluid cognition age-corrected	96.8 (13.5)	99.9 (15.5)	91.2 (7.4)	99.3 (14.9)	0.086
Crystalized cognition	109.0 (8.3)	110.6 (8.5)	107.8 (8.0)	108.8 (8.6)	0.430
Crystalized cognition age-corrected	105.7 (13.3)	108.1 (13.9)	103.7 (12.6)	105.4 (13.7)	0.444

* Comparison among the three groups.

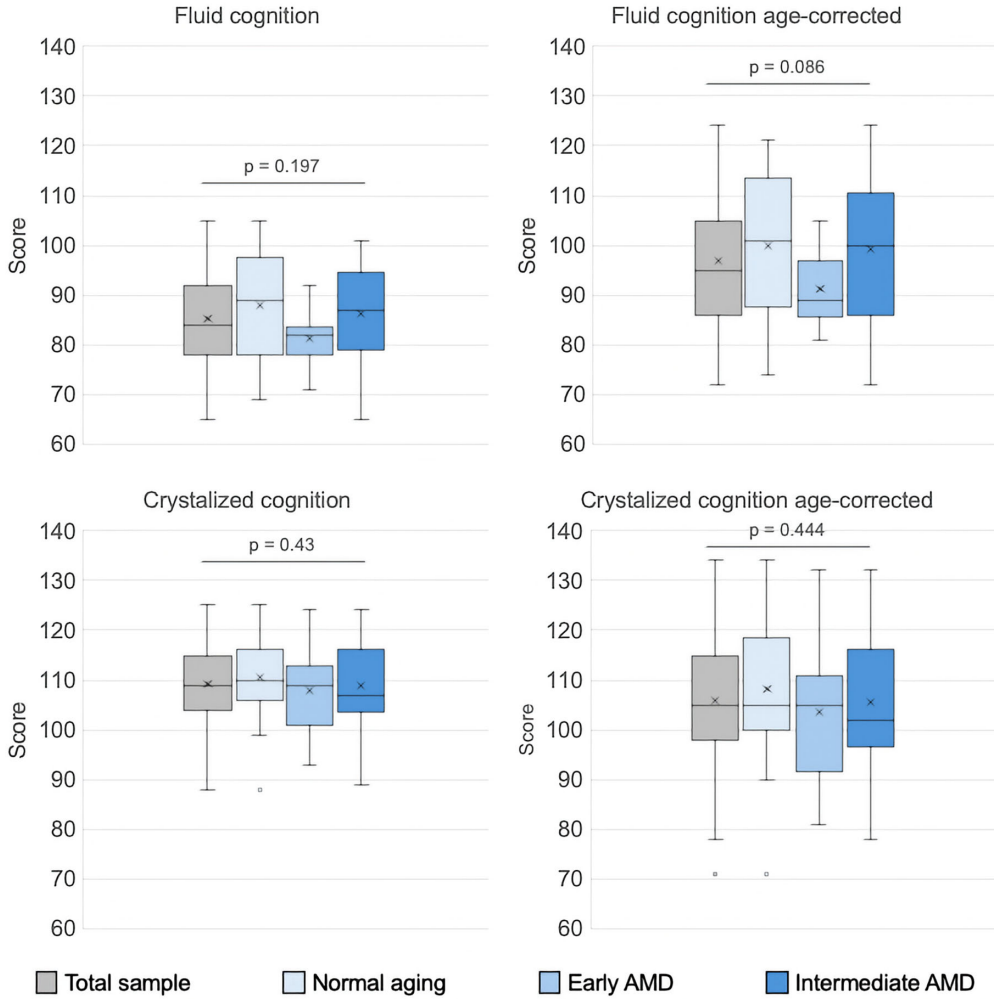

Figure 2. Boxplots for NIH Toolbox scores for fluid cognition, fluid cognition-adjusted, crystalized cognition, and crystallize cognition-adjusted, stratified by the three groups (normal aging, early AMD, intermediate AMD). Each group had 21 participants for a total of 63 participants. None of the cognition scores differed by group.

Although we did not find reduced thicknesses of the inner retina or its components, we note that retinal vascular changes in relation to cognition and AD are well documented. In a population-based study of 12,317 persons, rates of 20-year cognitive change adjusted for attrition were associated with moderate-to-severe retinopathy and aspects of arteriolar disintegrity (e.g., focal narrowing) observable in color fundus photographs.[50] In this same large population, retinopathy and generalized retinal arteriolar narrowing at midlife were associated with greater risk of all-cause dementia at 20 years.[51] Newer technology affords potentially greater vascular detail in necessarily smaller samples. Employing OCT angiography (OCTA), Belmut et al.[52] identified reduced retinal capillary vascular density and an enlarged foveal avascular zone in patients with AD compared to healthy controls ($n = 26$ each). Enlargement was also seen in one study of preclinical biomarker-positive AD cases ($n = 50$) [53] and not in another ($n = 13$).[54]

There is reason to suspect microvascular involvement in the outer retinal thinning seen in our study. Degraded microvascular activities such as provision of oxygen, nutrients, and essential angiocrine factors, as well as waste removal, can be expected to impact retinal function. Dropout and dysfunction of the choriocapillaris endothelium is a prominent histologic feature of aging and early-to-intermediate AMD[55] that contributes to both drusen formation[56] and vision impairment.[57] Choriocapillaris flow signal by OCTA is diminished in patients with early-onset AD relative to controls.[58] In laboratory animals, healthy vessels can delay "inflammaging" and capillary rarefaction that otherwise result in inadequate multiorgan perfusion in advanced age.[59] Regarding macro vessels in the eye, choroidal vascularity (proportion of choroid that is vascular cross-sections) but not thickness differed significantly between aged normal and dry AMD eyes ($n = 121$ and 175, respectively).[60]

Our current data, due to sample size and cross-sectional study design, cannot establish or exclude any one mechanism underlying retinal involvement in brain disease. Research on the nerve fiber layer was launched by early reports of loss of optic nerve axons and retinal ganglion cells in eyes that lacked direct evidence of typical AD pathology.[61–63] Subsequent authors using OCT sought evidence of retrograde degeneration; for example, as the brain atrophies due to aging and neurodegeneration, ganglion cell axons in the NFL may die back in concert.[9] In our sample, any differences in NFL thickness between stages of AMD were small

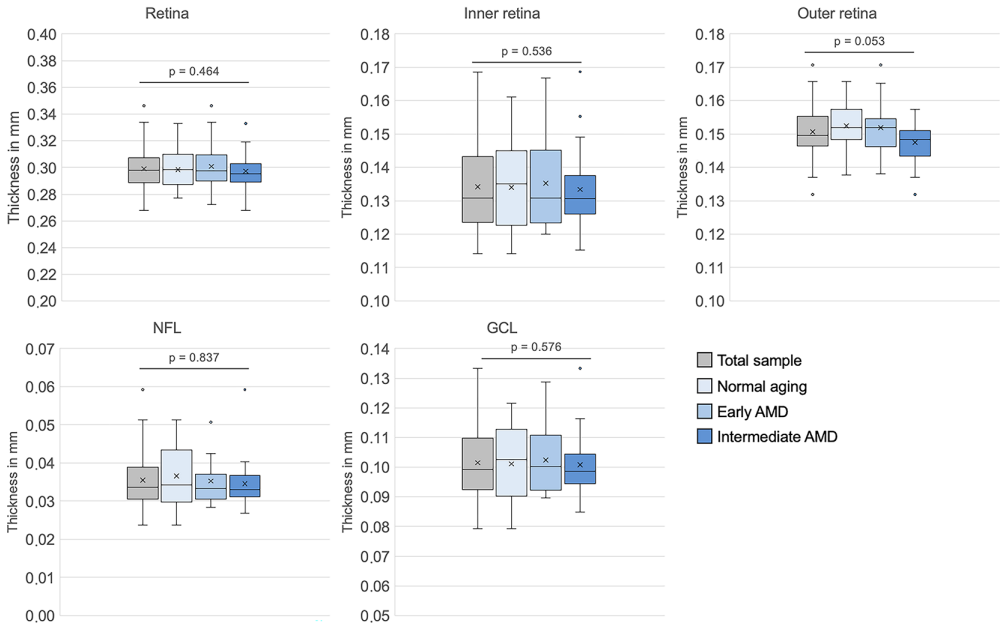

Figure 3. Boxplots for retinal thickness (in mm) for the overall retina, inner retina, outer retina, NFL, and GCL stratified by the three groups (normal aging, early AMD, intermediate AMD). Each group had 21 participants for a total of 63 participants. None of the retinal thickness variables differed by group.

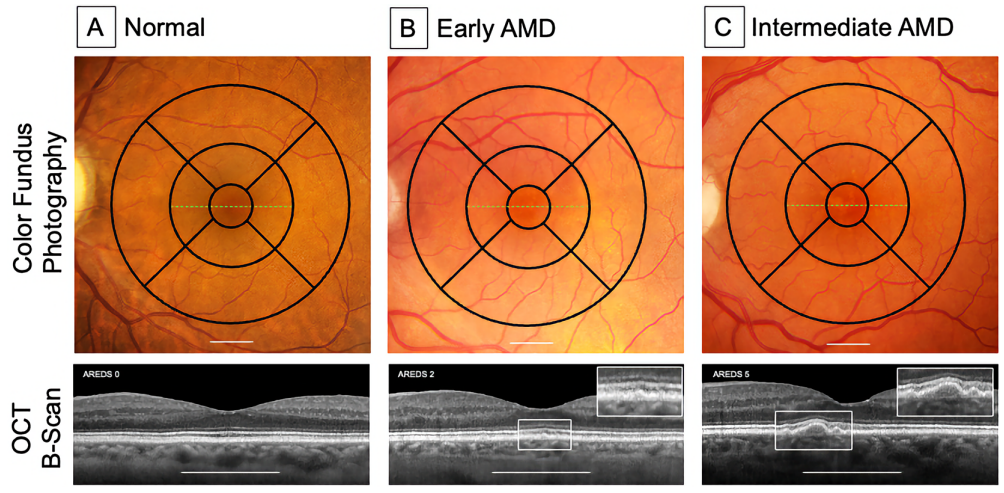

Figure 4. AMD disease stages by AREDS nine-step classification system for three left eyes representing normal aging, early AMD, and intermediate AMD. *Green dotted line* shows OCT B-scan position and extent. *White insets* highlight drusen on OCT B-scans (magnification, 250%). *Scale bar*: 1 mm (*white line*). (**A**) Male, 77 years old; (**B**) female, 82 years old; (**C**) female, 74 years old.

Table 3. Age-Adjusted Spearman Correlations Between Cognition Variables and Retinal Thickness Variables

	Retinal Thickness (mm) Variables									
	Outer Retina		Inner Retina		NFL		GCL		Retina	
Cognitive Variables	**Correlation**	***P***	**Correlation**	***P***	**Correlation**	***P***	**Correlation**	***P***	**Correlation**	***P***
Fluid cognitive composite	0.260	0.041*	0.156	0.226	0.199	0.120	0.202	0.115	0.233	0.068
Age-corrected fluid cognitive composite	0.263	0.039*	0.166	0.197	0.199	0.120	0.209	0.103	0.243	0.057
Crystallized cognitive composite	0.263	0.039*	−0.051	0.692	−0.071	0.584	−0.015	0.912	0.041	0.752
Age-corrected crystallized cognitive composite	0.272	0.032*	−0.039	0.766	−0.048	0.711	0.002	0.991	0.050	0.698

* Statistically significant $P < 0.05$.

relative to those seen in outer retina. Although our project was conceived with vascular attenuation or pathology as possible mechanisms affecting both retina and brain,[64] direct tests of these ideas in this cohort are intended for the future. Other mechanisms that may impact both retina and brain are populations of microglia that reflect specific niches and phases of neurodegenerative disease, infiltration by circulating immune cells, long-term effects of prior infection, and the impact of microbiota on inflammation and nutrient absorption.[65–67] Some of these processes may be visible in the retina with new imaging technologies.

A strength of our study is that we documented that visual acuity and contrast sensitivity are at a sufficient level for valid cognitive testing and are similar across groups. Thus, detection of the test stimuli was not impacted by visual impairment in these participants. Another strength is the use of a standardized color fundus photography grading system for AMD pathology, which, despite the limitations, is repeatable and widely used until such a time when OCT-based grading systems became available. We expanded the examination of retinal layer thickness beyond the NFL to include layers affected by early and intermediate AMD. OCT layer segmentation was manually checked by a trained reviewer on images centered on the longest and finest foveal cones, rather than the point of fixation or the thinnest point in the central subfield; we view these cones as the most biologically defensible origin of a retinal coordinate system. A limitation is the relatively small sample of participants across groups, yet we were able to identify significant associations in four key cognitive variables from the NIH Toolbox assessment with the outer retina. A larger sample size with greater statistical power may have revealed associations with other retinal layers, yet we saw cognition–outer retinal layer associations in the current sample. Another limitation is that our participants may have had undiagnosed AD, preclinical AD, frontotemporal dementia, or MCI which was not listed in the EMR.

In a forthcoming analysis of this sample of older adults on the AMD trajectory but not having AD, we explore a relationship between outer retina thinning and both hippocampal volume loss and cortical thinning in brain regions typically associated with AD. We are also currently examining OCTA in the retinas of these participants to examine potential associations with brain angiographic features. Together these studies can serve as preliminary information to motivate hypotheses for a more comprehensive examination of the potential associations between AMD and AD, two of the most common and devastating neurodegenerations of aging.

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