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Retinal Microvascular and Neurodegenerative Changes in Alzheimer's Disease and Mild Cognitive Impairment Compared with Control Participants

Stephen P. Yoon, BS,[1,*] *Dilraj S. Grewal, MD,*[1,*] *Atalie C. Thompson, MD, MPH,*[1] *Bryce W. Polascik,*[1] *Cynthia Dunn, PA,*[2] *James R. Burke, MD, PhD,*[2] *Sharon Fekrat, MD*[1]

Purpose: Evaluate and compare the retinal microvasculature in the superficial capillary plexus (SCP) in Alzheimer's disease (AD), mild cognitive impairment (MCI), and cognitively intact controls using OCT angiography. OCT parameters were also compared.

Design: Cross-sectional study.

Participants: Seventy eyes from 39 AD participants, 72 eyes from 37 MCI participants, and 254 eyes from 133 control participants were enrolled.

Methods: Participants were imaged using Zeiss Cirrus HD-5000 with AngioPlex (Carl Zeiss Meditec, Dublin, CA) and underwent cognitive evaluation with Mini-Mental State Examination.

Main Outcome Measures: Vessel density (VD) and perfusion density (PD) in the SCP within the Early Treatment Diabetic Retinopathy Study 6-mm circle, 3-mm circle, and 3-mm ring were compared between groups. Foveal avascular zone (FAZ) area, central subfield thickness (CST), macular ganglion cell-inner plexiform layer (GC-IPL) thickness, and peripapillary retinal nerve fiber layer (RNFL) thickness were also compared.

Results: Alzheimer's participants showed significantly decreased SCP VD and PD in the 3-mm ring ($P = 0.001$ and $P = 0.002$, respectively) and 3-mm circle ($P = 0.003$ and $P = 0.004$, respectively) and decreased SCP VD in the 6-mm circle ($P = 0.047$) compared with MCI and significantly decreased SCP VD and PD in the 3-mm ring ($P = 0.008$ and $P = 0.004$, respectively) and 3-mm circle ($P = 0.015$ and $P = 0.009$, respectively) and SCP PD in the 6-mm circle ($P = 0.033$) when compared with cognitively intact controls. There was no difference in SCP VD or PD between MCI and controls ($P > 0.05$). FAZ area and CST did not differ significantly between groups ($P > 0.05$). Alzheimer's participants showed significantly decreased GC-IPL thickness over the inferior ($P = 0.032$) and inferonasal ($P = 0.025$) sectors compared with MCI and significantly decreased GC-IPL thickness over the entire ($P = 0.012$), superonasal ($P = 0.041$), inferior ($P = 0.004$), and inferonasal ($P = 0.006$) sectors compared to controls. MCI participants showed significantly decreased temporal RNFL thickness ($P = 0.04$) compared with controls.

Conclusions: Alzheimer's participants showed significantly reduced macular VD, PD, and GC-IPL thickness compared with MCI and controls. Changes in the retinal microvasculature may mirror small vessel cerebrovascular changes in AD. *Ophthalmology Retina 2019;3:489-499 © 2019 by the American Academy of Ophthalmology*

Supplemental material available at www.ophthalmologyretina.org.

Alzheimer's disease (AD) is the most common subtype of dementia (60%–80%), with an estimated 5.5 million individuals affected in the United States alone.[1] The health and societal costs associated with AD are expected to increase, with a projected 13.8 million individuals affected by 2050.[1] Although AD is characterized by memory deficits, aphasia, apraxia, and agnosia,[2] mild cognitive impairment (MCI) is considered a transitional stage between normal aging and dementia with cognitive decline that notably does not interfere with activities of daily life.[3] An estimated 32% of MCI patients will progress to AD within 5 years' follow-up.[4] Because of the increasing prevalence of AD and paucity of effective treatment options, identification of a biomarker for potentially earlier diagnosis and enrollment into interventional clinical trials has become a priority. Current diagnostic methods for AD and MCI are limited by cost (e.g., magnetic resonance imaging, positron emission tomography [PET]), invasiveness (e.g., cerebrospinal fluid examination), suboptimal specificity and sensitivity (e.g., genetic markers, serum amyloid), length of evaluation,

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access to specialists, and neuropsychological evaluation.[5] Faster, more accessible, less invasive diagnostic techniques are a large unmet need for efficient screening of those at risk.

The neuropathologic features of AD consist of the deposition of β-amyloid plaques and neurofibrillary tangles, both of which lead to inflammation and neurodegeneration.[6] Cerebral microvascular changes currently are inaccessible to existing in vivo imaging technologies. In contrast, the retinal microvascular network can be imaged directly and may provide a unique window to study parallel cerebral microvascular pathologic features,[7] because the retinal and cerebral microvasculature share similar embryologic origins as well as anatomic and physiologic properties.[8] Changes in the brain also may be detectable in the retina in individuals genetically predisposed to or demonstrating clinical symptoms of AD.[9] OCT imaging has been used to detect neurodegenerative changes occurring in ganglion cell–inner plexiform layer (GC-IPL) thickness and retinal nerve fiber layer (RNFL) thickness of AD and MCI patients.[10–12] La Morgia et al[13] observed decreased RNFL thickness in AD participants in vivo through OCT and significant loss of melanopsin retinal ganglion cells in postmortem AD retina specimens.

In addition to neurodegenerative changes, the contribution of vascular remodeling to MCI and AD is increasingly recognized.[7,14,15] Postmortem studies of the cerebral microvasculature in AD have shown impairment of the blood–brain barrier and decreased capillary density, length, and mean diameter in comparison with control participants.[16,17] A study using fundus photography showed abnormal retinal vascular parameters in AD participants, which included vascular attenuation, increasing standard deviation of vessel widths, reduced complexity of the branching pattern, reduced optimality of the branching pattern, and less tortuous venules.[18] OCT angiography (OCTA) may permit detection of reduction in capillary vessel and perfusion density before they are visible on retinal photographs.[19] Two previous OCTA studies reported decreased retinal vascular density in AD.[20,21] We sought to identify retinal microvasculature biomarkers using OCTA that may aid in the earlier diagnosis of AD by comparing individuals with AD and MCI with cognitively intact community control participants.

Methods

Participants and Protocol

This cross-sectional study (Clinicaltrials.gov identifier, NCT 03233646) was approved by the Institutional Review Board for Human Research at the Duke University School of Medicine in Durham, North Carolina, and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants or their designated medical power of attorney before enrollment.

Eligible AD and MCI participants 50 years of age or older were enrolled from the Duke Memory Disorders Clinic. Alzheimer's disease and MCI participants initially were evaluated and diagnosed clinically by an experienced neurologist (J.R.B.) based on the diagnostic guidelines and recommendations of the National Institute on Aging-Alzheimer's Association.[22,23] Clinical history, cognitive testing, and neuroimaging were reviewed for diagnostic accuracy by an experienced neurologist (J.R.B.) with a specialization in memory disorders. Participants did not undergo PET imaging or lumbar puncture for assessment of biomarker status. Community control participants were healthy volunteers 50 years of age or older without subjective memory symptoms who either were spouses of or attendants to patients at the Duke Memory Disorders Clinic or who were from the Bryan Alzheimer's Disease Research Center registry of control participants. The Bryan Alzheimer's Disease Research Center maintains a registry of cognitively normal community control participants based on extensive cognitive tests, which include Montreal Cognitive Assessment, Trail Making Test, and Delayed Recall from the Consortium to Establish a Registry for Alzheimer's Disease Word-List.[10] Exclusion criteria for all study participants included a history of non–AD-associated dementia, diabetes mellitus, uncontrolled hypertension, demyelinating disorders, glaucoma, age-related macular degeneration, other vitreoretinal pathologic features that could interfere with OCT and OCTA analysis, and corrected Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity worse than 20/40 on the day of image acquisition. All participants underwent a Mini-Mental State Examination (MMSE) to evaluate cognitive function on the same day as image acquisition. Years of education were collected from each patient and were calculated from the first grade onward.

OCT Angiography Image Acquisition

All participants were imaged with the Zeiss Cirrus HD-5000 Spectral-Domain OCT with AngioPlex OCT Angiography (Carl Zeiss Meditec, Dublin, CA) that has a scan rate of 68 000 A-scans per second, central wavelength of 840 nm, motion tracking to reduce motion artifact, and an optical microangiography algorithm for analysis.[19] Both 3 × 3-mm and 6 × 6-mm images centered on the fovea were acquired. OCT angiography images that were of poor scan quality (less than 7/10 signal strength) because of low resolution or poor saturation and those that exhibited motion artifacts because of poor cooperation were excluded. The inner boundary of the superficial capillary plexus (SCP) slab was defined as the internal limiting membrane and the outer boundary was defined as the inner plexiform layer, which was calculated as 70% of the distance from the internal limiting membrane to the estimated boundary of the outer plexiform layer, which in turn was determined as being 110 μm higher than the retinal pigment epithelium boundary as automatically detected by the software (Carl Zeiss Meditec, version 10.0.0.14618). The software quantified the average vessel density (VD) and perfusion density (PD) using a grid overlay according to the standard ETDRS subfields. Vessel density was defined as the total length of perfused retinal microvasculature per unit area in the region of measurement, whereas PD was defined as the total area of perfused retinal microvasculature per unit area in a region of measurement. Vessel density and PD were calculated for the 3-mm circle and 3-mm ring for 3 × 3-mm images and over the entire ETDRS 6-mm circle for 6 × 6-mm scans (Fig 1). The foveal avascular zone (FAZ) boundaries were calculated automatically by the software; values with inaccurate boundaries identified on manual review were excluded.

OCT Image Acquisition

For all participants, the same Cirrus HD-OCT 5000 device was used to acquire a 512 × 128 macular cube and 200 × 200 optic disc cube. OCT images with poor quality (less than 7/10) or motion artifacts were excluded. OCT software automatically quantified central subfield thickness (CST) as the thickness between the inner limiting membrane and retinal pigment epithelium at the fovea from the macular cube. Average GC-IPL thickness was quantified automatically over the 14.13-mm^2 elliptical annulus area centered

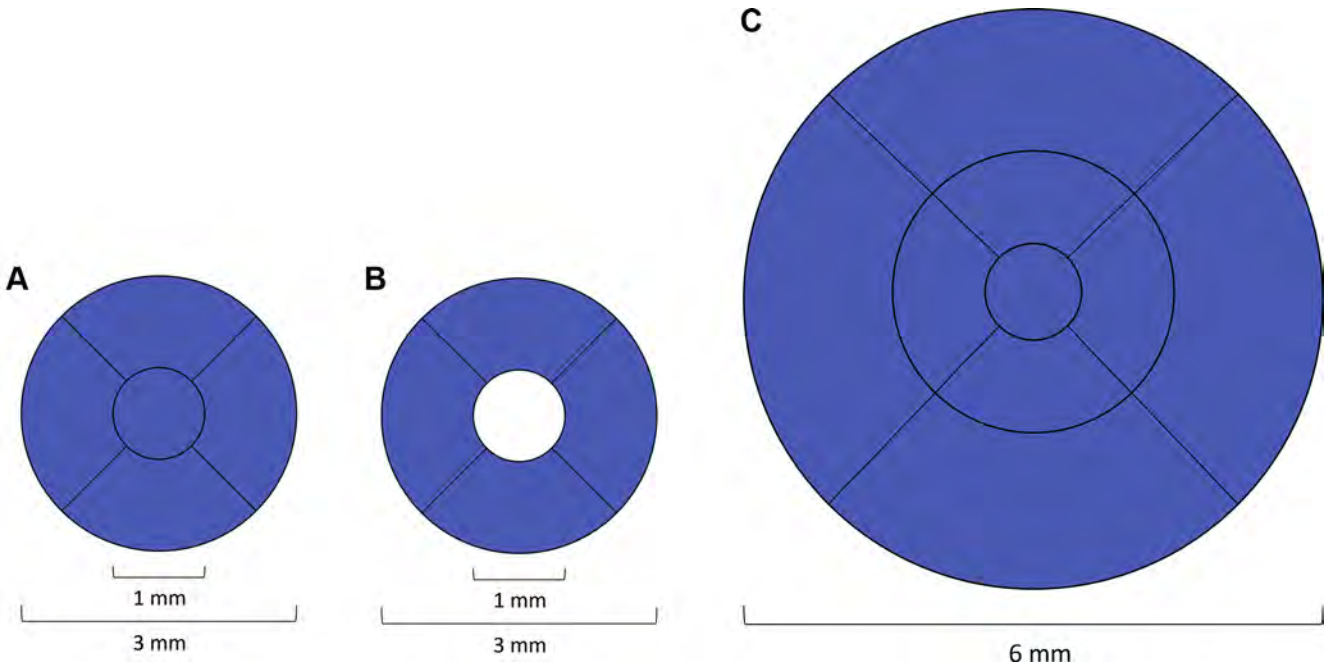

Figure 1. Early Treatment Diabetic Retinopathy Study (ETDRS) grid regions used for (**A**) 3-mm circle, (**B**) 3-mm ring, and (**C**) 6-mm circle regions. Vessel density and perfusion density were averaged over the highlighted blue area for each respective region.

on the fovea and over 6 sectors of the annulus, including the superotemporal, superior, superonasal, inferotemporal, inferior, and inferonasal sectors. Average RNFL thickness was quantified automatically over a 3.46-mm diameter circle centered on the optic disc and over 4 sectors of the circle, including the superior, temporal, nasal, and inferior sectors.

Statistical Analysis

Multivariate statistical analysis was completed in STATA software version 15.1 (StataCorp, College Station, TX). Baseline demographic variables of study participants were compared overall across groups using the chi-square test for categorical variables and the analysis of variance or K-Wallis test for continuous variables. Scores on the MMSE also were compared between groups using a multivariate tobit regression analysis that controlled for years of education. Multivariate generalized estimating equations for each of the OCT and OCTA imaging parameters with adjustment for age and gender were used to compare AD, MCI, and control participants. The logarithm of the minimum angle of resolution (logMAR) visual acuity also was compared between groups using generalized estimating equation models. A P value less than 0.05 was considered statistically significant. Ordinary least squares regression analysis and Spearman's correlation were performed to explore the relationship between each of the averaged VD, PD, or FAZ area SCP parameters in the 6 × 6-mm or 3 × 3-mm circles with MMSE scores among all participants. The relationships between average RNFL thickness, average GC-IPL thickness, and CST with MMSE score also were explored with Spearman's correlation among all participants.

Results

A total of 90 eyes from 52 AD participants, 79 eyes from 41 MCI participants, and 269 eyes from 142 healthy control participants were enrolled and imaged. A total of 20 eyes from 13 AD participants, 7 eyes from 4 MCI participants, and 15 eyes from 11 control participants were excluded from the analysis because of poor OCTA image quality or motion artifact. Of these 42 excluded eyes, 22 were excluded because of poor scan quality and 20 because of motion artifact. Some AD patients were fatigued easily and were more prone to fixation errors; as a result, 22.2% of imaged AD eyes were excluded from analysis because of poor scan quality (less than 7/10) or motion artifacts. A total of 70 eyes from 39 AD participants, 72 eyes from 37 MCI participants, and 254 eyes from 133 healthy control participants were analyzed. The average corrected ETDRS visual acuity in AD participants (0.20±0.10 logMAR) was not significantly different than that of MCI participants (0.16±0.10 logMAR; $P = 0.11$), but was lower that of control eyes (0.11±0.10 logMAR; $P < 0.001$), and the difference between MCI and control participants also was significant ($P = 0.001$).

There was a significant difference in age, but no significant difference in gender, among the groups (Table 1). The average age of the AD group (72.8±7.7 years) was older than that of the MCI group (71.1±7.6 years) and control group (69.2±7.8 years). The MMSE score was lower in both the AD ($P < 0.001$) and MCI ($P < 0.001$) groups compared with the control group, even after controlling for years of education (Table 1). In addition, the AD group showed a lower MMSE score compared with the MCI group ($P = 0.033$), and the MCI group showed a lower MMSE score than the control participants ($P < 0.001$), after adjusting for years of education.

Comparing AD with MCI participants, the AD group showed significantly decreased 3-mm circle VD ($P = 0.003$) and 3-mm ring VD ($P = 0.001$) as well as decreased 3-mm circle PD ($P = 0.004$) and 3-mm ring PD ($P = 0.002$) compared with the MCI group (Fig 2). The AD group also showed significantly reduced 6-mm circle VD ($P = 0.047$) compared with the MCI

Table 1. Patient Demographic and Clinical Characteristics

Parameter	Alzheimer's Disease (n = 39)	Mild Cognitive Impairment (n = 37)	Control (n = 133)	P Value
Age (yrs), mean ± standard deviation	72.8±7.7	71.1±7.6	69.2±7.8	0.03*
Female gender, % (no./total no.)	66.6 (26/39)	54.1 (20/37)	72.9 (97/133)	0.09†
Years of education, mean ± standard deviation	15.6±2.4	15.1±1.9	17.2±2.3	<0.001§
MMSE score, mean ± standard deviation	20.1±5.9	22.6±4.7	29.2±1.1	<0.001‡,§

MMSE = Mini-Mental State Examination.
*Analysis of variance for age.
†Chi-square test for gender.
‡K-Wallis test for years of education and MMSE.
§*P* value statistically significant after controlling for years of education in a tobit multivariate regression model.

group (Table 2). The 6-mm circle PD ($P = 0.053$) was not significantly different between the AD and MCI groups. The AD group showed significantly decreased inferior GC-IPL ($P = 0.032$) and inferonasal GC-IPL ($P = 0.025$) thicknesses compared with the MCI group (Supplemental Table 1, available at www.ophthalmologyretina.org). There were no significant differences between AD and MCI participants in RNFL thickness ($P > 0.05$).

Comparing AD with control participants, the AD group showed significantly decreased 3-mm circle VD ($P = 0.015$), 3-mm circle PD ($P = 0.009$), 3-mm ring VD ($P = 0.008$), and 3-mm ring PD ($P = 0.004$) compared with the control group (Fig 2). The AD group also showed significantly reduced 6-mm circle PD ($P = 0.033$) compared with the control group (Table 2). The 6-mm circle VD ($P = 0.053$) was not significantly different between the AD and control groups ($P = 0.053$) (Fig 3). The AD group showed significantly reduced GC-IPL thickness over the entire ($P = 0.012$), superonasal ($P = 0.041$), inferior ($P = 0.004$), and inferonasal ($P = 0.006$) sectors compared with the control group (Fig 4). There were no significant differences between AD and control participants in RNFL thickness ($P > 0.05$).

Comparing MCI with control participants, there were no significant differences between the MCI and control groups for VD and PD in the 3-mm circle, 3-mm ring, and 6-mm circle (all $P > 0.05$). Temporal RNFL thickness was decreased significantly in the MCI participants compared with the control participants ($P = 0.04$). There were no significant differences between MCI and control participants in GC-IPL thickness ($P > 0.05$). Foveal avascular zone area and CST were not significantly different between any of the groups (all $P > 0.05$; Table 2; Supplemental Table 1).

Correlations between OCTA and OCT parameters with MMSE score were analyzed through Spearman's correlation coefficients. For 3 × 3-mm OCTA parameters, 3-mm circle VD ($\rho = 0.153$; $P = 0.003$), 3-mm ring VD ($\rho = 0.168$; $P = 0.001$), 3-mm circle PD ($\rho = 0.146$; $P = 0.004$), and 3-mm ring PD ($\rho = 0.160$; $P = 0.002$) were correlated significantly with MMSE scores among all participants (all $P < 0.005$; Supplemental Table 2, available at www.ophthalmologyretina.org). No significant correlations were found between 3 × 3-mm FAZ area or 6 × 6-mm VD or PD parameters with MMSE score (all $P > 0.05$). For OCT parameters, average RNFL thickness ($\rho = 0.132$; $P = 0.009$) and average GC-IPL thickness ($\rho = 0.234$; $P < 0.001$) were correlated significantly with MMSE score (Supplemental Table 2). Central subfield thickness was not significantly correlated with MMSE score ($P > 0.05$).

Discussion

In this large cross-sectional study of OCTA findings in AD and MCI, we observed significantly decreased VD and PD in both the 3 × 3-mm and 6 × 6-mm scans using ETDRS subfields in AD participants when compared with both MCI and control participants. These differences suggest that VD and PD may be imaging biomarkers useful in screening for AD in symptomatic individuals and may be able to distinguish between MCI and AD. We evaluate both VD and PD of the SCP in AD, MCI, and control participants.

The mechanism underlying reduced retinal vessel density in AD is unknown, but decreased angiogenesis from sequestration of vascular endothelial growth factor in β-amyloid plaques and competitive binding of β-amyloid to vascular endothelial growth factor receptor 2 has been proposed.[17] Koronyo-Hamaoui et al[6] identified β-amyloid plaques in the retina of 8 postmortem AD patients and 5 suspected early-stage patients. Furthermore, Koronyo-Hamaoui et al[6] detected β-amyloid plaques in the retina of $APP_{SWE}/PS1_{\Delta E9}$ transgenic mice earlier than plaques in the brain that accumulated with disease progression. β-Amyloid deposition around vascular walls disrupts the basement membrane of small vessels, causing endothelial damage and reducing the vascular lumen.[17] In a follow-up histologic study by Koronyo et al[24] of 37 definite AD and control participants, β-amyloid plaques in the retina were associated with blood vessels and were observed inside blood vessels, in the perivascular area, and along blood vessel walls. The study also used systemic administration of curcumin in 16 living human participants to show marked increase in fluorescence of retinal β-amyloid deposits at 10 days through a scanning laser ophthalmoscope.[24] Although curcumin allows for direct visualization of β-amyloid plaques, the systemic administration of curcumin is invasive and requires significant time for optimal fluorescence of plaques, which make it difficult to adapt to clinical practice. The association of β-amyloid plaques to retinal blood vessels in histologic studies strengthens the case for the use of OCTA to detect microvascular abnormalities in AD pathologic features.

Our findings support a previous, smaller study that reported decreased vascular density of the SCP in 26 eyes of AD participants when compared with 26 eyes of control

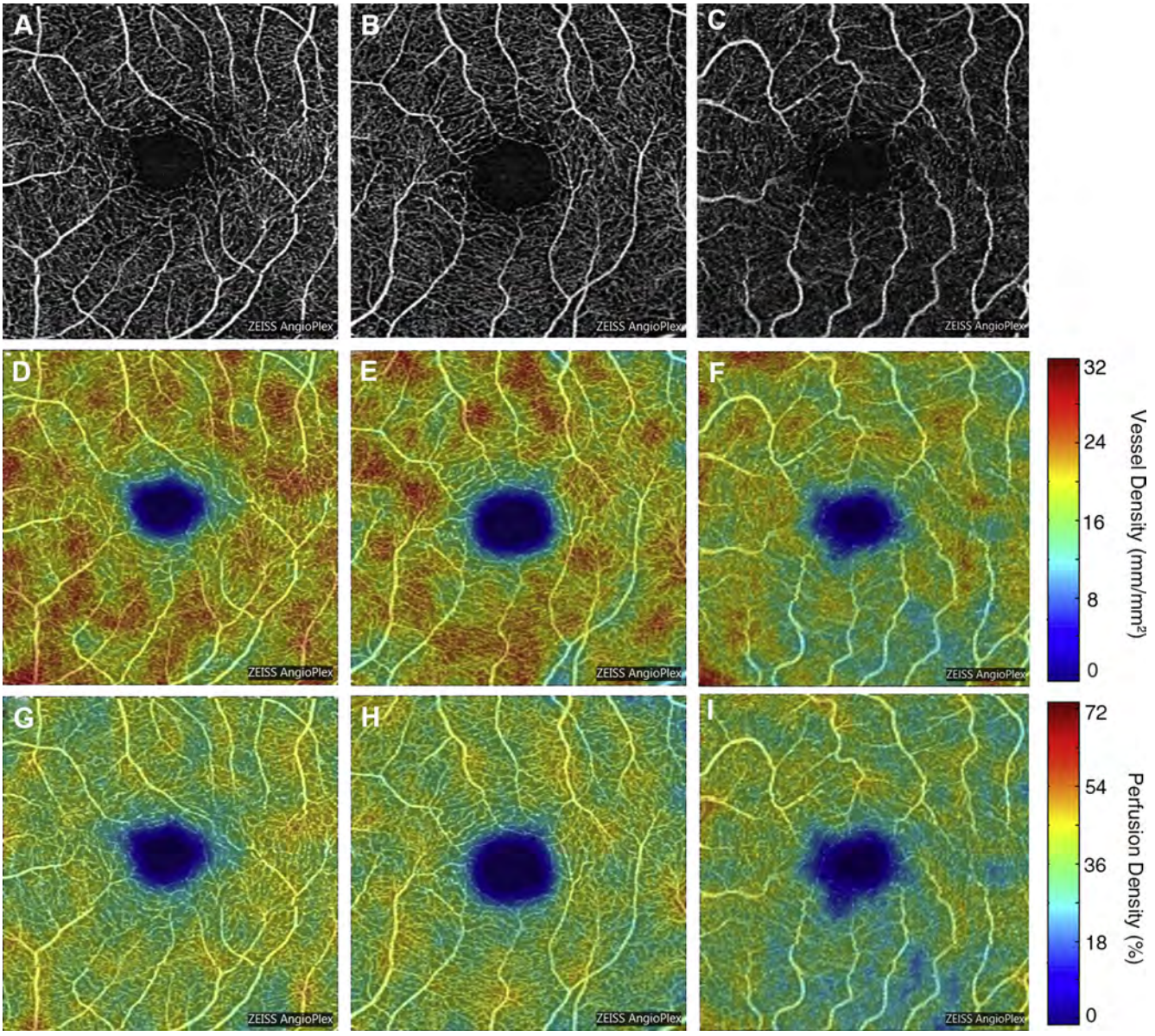

Figure 2. Representative OCT angiography 3 × 3-mm images of the superficial capillary plexus (SCP) of the left eye from (**A**) a community control participant, (**B**) a mild cognitive impairment (MCI) participant, and (**C**) an Alzheimer's disease (AD) participant. Corresponding quantitative color maps (Carl Zeiss Meditec, Dublin, CA) of (**D**–**F**) vessel density and (**G**–**I**) perfusion density of the SCP, with the scale on the right, showing decreased vessel density and perfusion density in (**F**, **I**) the participant with AD compared with (**D**, **G**) the control participant and (**E**, **H**) MCI participant.

participants using OCTA, with exclusion of participants with glaucoma, diabetes, and macular degeneration.[20] They used a 6 × 6-mm OCTA scan (RTVue XR100-2; Optovue, Fremont, CA) and reported differences in the vascular density of foveal, parafoveal, and whole regions. In addition to decreased PD in the 3-mm ring, 3-mm circle, and 6-mm circle between the AD group and control group, our study observed significantly decreased VD in eyes of participants with AD in the 3-mm ring and 3-mm circle compared with control participants. Our study also showed decreased 3-mm ring, 3-mm circle, and 6-mm circle VD in AD patients compared with MCI participants. The VD measurement quantifies density by considering the total length of the vessels and does not account for vessel diameter. Both large vessels and small capillaries contribute equally to VD. Therefore, VD may be a more sensitive measure for perfusion changes at the capillary level when assessing the retinal microvasculature in AD.[25] Another smaller OCTA study by Jiang et al[21] found reduced fractal dimension in the SCP and deep capillary plexus of 12 AD participants and in 1 quadrant of the deep capillary plexus in 19 MCI participants compared with 21 control participants using the Zeiss Angioplex OCTA. However, their study did not exclude glaucoma or controlled type 2 diabetics, which may affect OCTA parameters.[25,26] In addition, fractal dimension is a measure of global branching complexity of

Table 2. Comparison of OCT Angiography Parameters for 3 × 3-mm Circle and Ring Regions and 6 × 6-mm Circle Region among Participants with Alzheimer's Disease, Participants with Mild Cognitive Impairment, and Cognitively Intact Community Controls by Generalized Estimating Equation Multivariate Analysis with Adjustment for Age and Gender

OCT Angiography Parameter	Alzheimer's Disease	Mild Cognitive Impairment	Control	β Coefficient (95% Confidence Interval)		
				Alzheimer's Disease vs. Controls	*Alzheimer's Disease vs. Mild Cognitive Impairment*	*Mild Cognitive Impairment vs. Controls*
3-mm Circle VD (/mm)	19.0±2.3	20.3±1.5	20.2±1.6	−0.87 (−1.57 to −0.17), $P = 0.015$	−1.18 (−1.96 to −0.40), $P = 0.003$	0.27 (−0.15 to 0.70), $P = 0.20$
3-mm Circle PD	0.347±0.037	0.366±0.024	0.365±0.026	−0.015 (−0.026 to −0.004), $P = 0.009$	−0.018 (−0.03 to −0.006), $P = 0.004$	0.003 (−0.004 to 0.009), $P = 0.44$
3-mm Ring VD (/mm)	20.1±2.3	21.4±1.5	21.3±1.5	−0.93 (−1.62 to −0.24), $P = 0.008$	−1.26 (−2.03 to −0.48), $P = 0.001$	0.30 (−0.12 to 0.272), $P = 0.17$
3-mm Ring PD	0.366±0.036	0.386±0.024	0.386±0.025	−0.016 (−0.026 to −0.005), $P = 0.004$	−0.019 (−0.031 to −0.007), $P = 0.002$	0.003 (−0.004 to 0.009), $P = 0.38$
6-mm Circle VD (/mm)	17.4±1.5	17.9±1.0	17.9±1.1	−0.42 (−0.84 to −0.006), $P = 0.053$	−0.47 (−0.94 to −0.006), $P = 0.047$	0.11 (−0.18 to 0.39), $P = 0.46$
6-mm Circle PD	0.427±0.041	0.441±0.025	0.440±0.026	−0.012 (−0.023 to −0.001), $P = 0.033$	−0.011 (−0.023 to 0.0001), $P = 0.053$	−0.0008 (−0.006 to 0.007), $P = 0.81$
FAZ area (mm^2)	0.25±0.13	0.24±0.10	0.25±0.11	−0.002 (−0.045 to −0.04), $P = 0.91$	0.003 (−0.045 to 0.05), $P = 0.92$	−0.001 (−0.037 to 0.034), $P = 0.94$

FAZ = foveal avascular zone; OCTA = OCT angiography; PD = perfusion density; VD = vessel density.

the OCTA image that may not reflect early microvascular changes.[27] We did not evaluate the middle or deep capillary plexus in this study, and our analysis is limited to the SCP because it is easier to segment and provides fewer artifacts such as projection artifact and issues with low resolution. We are currently evaluating the middle and deep plexuses.

Our OCTA findings also support previous studies that evaluated the retinal vasculature using fundus photographs wherein AD patients had narrower venular caliber, decreased arteriolar and venular fractal dimensions, and increased arteriolar and venular tortuosity.[7,14] Williams et al[28] observed lower venular fractal dimension and decreased arteriolar tortuosity in the largest case-control study of AD using fundus photography. Frost et al[18] reported differences in 13 retinal vascular parameters in 25 AD participants compared with 123 healthy control participants through analysis of fundus photographs. Key vascular parameters found to be different included central retinal artery and vein caliber, standard deviation of vessel width, arteriole-to-venule ratio, fractal dimension of venular network, and asymmetry factor of the venule network. Retinopathy on fundus photography was associated with cognitive decline.[29] However, visible retinopathy is a relatively late indicator of target organ damage and probably reflects advanced stages of structural microvascular damage.[30] Quantitative metrics of VD and PD of the SCP using OCTA may detect areas of vascular loss that are not yet visible on fundus photographs.[31]

There were no significant differences observed for VD or PD in the 3-mm ring, 3-mm circle, and 6-mm circle between the MCI and control groups in our study. However, Feke et al[14] observed retinal blood flow differences between AD and MCI participants, AD and control participants, and MCI and control participants using laser Doppler on the largest temporal vein 1 disc diameter away from the optic disc margin. The MCI participants were found to have a 19.4% decrease in retinal blood flow compared with control participants, whereas AD participants showed a 39% decrease relative to control participants. Venous blood column diameter was not different in MCI versus control groups, but it was decreased in AD participants.[14] The lack of difference between MCI and control participants in our study in part may be the result of the location of the blood vessels measured and the different metrics being analyzed. The laser Doppler measurements were obtained near the optic disc, whereas our measurements of the SCP were centered at the macula. The radial peripapillary capillary plexus is distinguished from the SCP by its concentration in the inferotemporal and superotemporal peripapillary areas and absence in the fovea.[32] Radial peripapillary capillaries have fewer anastomoses when compared with the SCP, which may make them more susceptible to vascular dysfunction.[32] We currently are evaluating whether peripapillary OCTA measurements may be more sensitive in detecting changes in AD and MCI participants. Furthermore, VD and PD assess the retinal microvascular structure, which may not be mediated by the same mechanism of decreased blood flow. Structural biomarkers such as VD, PD, and venous blood column diameter may change later in the pathologic processes of AD.

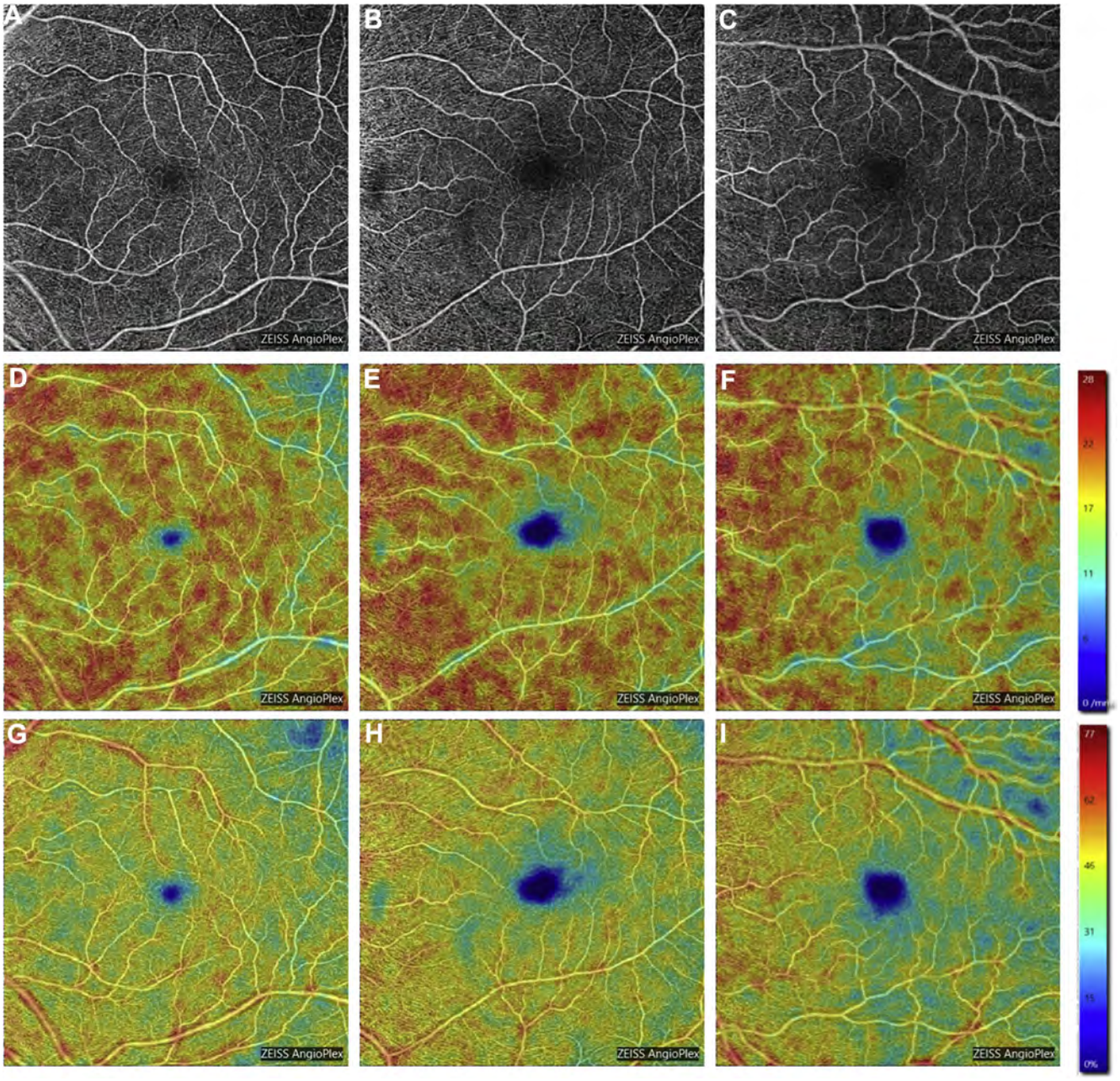

Figure 3. Representative OCT angiography 6 × 6-mm images of the superficial capillary plexus (SCP) of the left eye from (**A**) a community control participant, (**B**) a mild cognitive impairment (MCI) participant, and (**C**) an Alzheimer's disease (AD) participant. Corresponding quantitative color maps (Carl Zeiss Meditec, Dublin, CA) of (**D**–**F**) vessel density and (**G**–**I**) perfusion density of the SCP, with the scale on the right, showing decreased vessel density and perfusion density in (**F**, **I**) the participant with AD compared with (**D**, **G**) the control participant and (**E**, **H**) MCI participant.

The FAZ area was not significantly different among the groups in our study. We manually reviewed all FAZ boundaries constructed by the software to ensure anatomic accuracy. Two studies previously evaluated the relationship between AD pathologic features and FAZ area. A small study by Bulut et al[20] with 26 AD and 26 control participants showed a larger FAZ area in the AD group compared with the control group. Another small study by O'Bryhim et al[33] with 58 eyes from 30 cognitively healthy participants reported an enlarged FAZ area in the group of 14 subjects with positive results for amyloid biomarkers (cerebrospinal fluid analysis and PET imaging) compared with 16 subjects in the control group. Furthermore, significant variation in FAZ area in normal eyes has been reported in prior studies, which may be associated with gender, central retinal thickness, and retinal VD.[34,35] In addition, enlargement in FAZ area may be the result of confounding factors that were not controlled for, and additional studies with larger sample sizes may be needed to confirm if the finding is related to AD pathologic features.

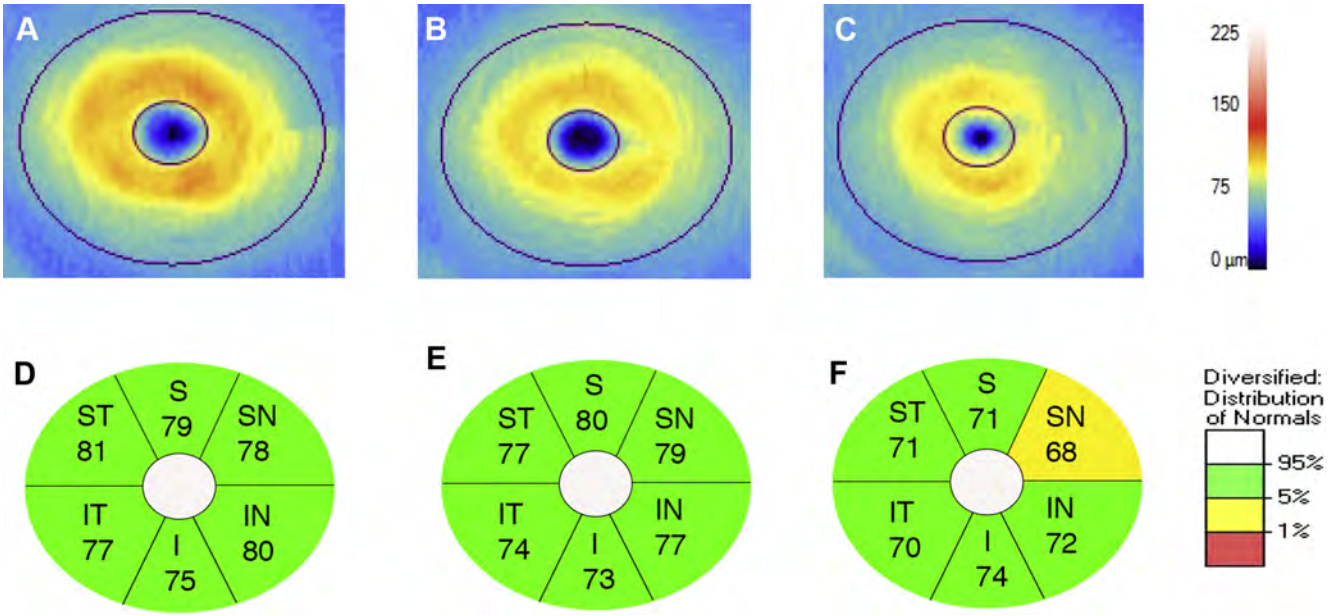

Figure 4. Ganglion cell analysis (Carl Zeiss Meditec, Dublin, CA) of representative OCT images of the macula of the left eye in (**A**) a control participant, (**B**) a mild cognitive impairment (MCI) participant, and (**C**) an Alzheimer's disease (AD) participant. Corresponding ganglion cell–inner plexiform layer thickness for each elliptical annular region showing (**F**) diffuse thinning in the AD participant compared with (**D**) the control participant and (**E**) the MCI participant. I = inferior; IN = inferonasal; IT = inferotemporal; S = superior; SN = superonasal; ST = superotemporal.

Although CST was decreased slightly in both AD and MCI groups relative to control participants, this relationship did not reach statistical significance. Our results differ from those of previous studies that found significantly decreased CST in AD.[36–38] A systematic review and meta-analysis of 302 AD patients and 241 control participants from 7 OCT studies showed decreased macular thickness in AD, which was most prominent in the outer ring of the ETDRS grid.[37] The decrease in macular thickness in the outer ring may reflect RNFL loss more peripherally, whereas the central macula was the region with the least relative thinning compared with control participants. Open-angle glaucoma is a risk factor for AD,[39] and the outer ring is the first region of thinning in early glaucoma patients.[40,41] The rigor of adjustment for confounders such as glaucoma may be a factor in the macular thinning observed in previous studies.

We found significantly decreased GC-IPL thickness in AD compared with MCI and control participants. Our finding is supported by a systematic review and meta-analysis reporting decreased GC-IPL thickness in 201 AD participants when compared with 311 control participants.[12] Morphometric analysis in postmortem AD eyes also revealed a 25% decrease in the total number of ganglion cell layer neurons compared with control participants in the central retina.[42] Williams et al[43] reported that retinal ganglion cell dendritic atrophy preceded cell loss in a mouse model of AD, which suggests that GC-IPL thickness may be a useful biomarker for early detection of AD. Thinning of the GC-IPL has been associated with lower gray matter volumes in the visual cortex and cerebellum, as well as lower white matter microstructure integrity on magnetic resonance imaging.[44] Thinning of GC-IPL is a retinal biomarker that may reflect neurodegenerative changes in the brain and may improve screening of AD when combined with evaluation of the retinal microvasculature with OCTA.

Our study found a significantly decreased temporal peripapillary RNFL thickness in MCI participants compared with control participants, but no significant differences in peripapillary RNFL thickness between the AD group and the MCI or control groups. In a systematic review and meta-analysis, Chan et al[12] reported a significantly decreased peripapillary RNFL thickness of 1061 AD participants compared with 1130 control participants, as well as no significant difference in RNFL thickness between 198 MCI participants and 1130 control participants. The different results in our study may be attributed to the variability of case identification, exclusion criteria of confounding factors (glaucoma and retinal disease), OCT models, and segmentation algorithms in other studies.[10,45] Because the macula contains approximately 50% of the total retinal ganglion cell population whose cell bodies are 10 to 20 times the diameter of their axons, macular GC-IPL thickness may be a more sensitive marker of AD-related neurodegeneration than RNFL thickness.[11,46]

There were no significant differences in VD, PD, FAZ area, CST, or GC-IPL thickness between MCI participants and control participants in our study. Mild cognitive impairment is a complex, heterogeneous group that includes patients who may not progress to AD, may revert back to being cognitively normal, or may demonstrate non-AD dementia.[47] The MCI group in our study showed a significantly lower MMSE score than the control group, which supports the clinical diagnosis of MCI and associated decreased cognitive function. We carefully excluded other major causes of MCI, such as frontotemporal dementia, dementia

with Lewy bodies, and vascular dementia, before enrollment. The role of β-amyloid plaques in MCI is still unanswered, with aged control participants exhibiting a similar extent of β-amyloid deposition as MCI patients in postmortem brain tissue and brain PET imaging.[48–50] Although our study was the largest OCTA study of AD and MCI to date, additional studies with larger sample sizes may be needed to detect differences in the MCI group, possibly with segregation into amnestic and nonamnestic groups, relative to control participants with regard to OCTA and OCT parameters.

The strengths of this study include that it is the largest prospectively imaged cohort of AD and MCI individuals using OCTA to date with stringent adherence to scan quality. In addition, we had a robust control group with approximately a 4:1 control-to-case ratio. We also adjusted for age and gender during multivariate analysis and excluded possible confounding factors, such as diabetes, uncontrolled hypertension, glaucoma, age-related macular degeneration, and other vitreoretinal diseases, through our enrollment criteria. Although age was statistically different among the groups, the groups were still relatively close in age, with the mean difference between AD and control participants being 3.6 years and that between MCI and control participants being 2.6 years.

Advanced AD patients are easily fatigued by imaging and are more prone to fixation errors; this led to 22.2% of the AD patients imaged in our study being excluded from analysis because of poor scan quality as compared with 8.9% of MCI and 5.6% of control participants. OCT angiography may not be feasible, or perhaps even necessary, in participants with advanced dementia and may be most useful for targeting patients with milder disease. Because of the cross-sectional design of the study, it was limited by not being able to examine causal or temporal relationships between the retinal microvasculature in AD or MCI, nor were we able to assess progression. In addition, the use of brain amyloid PET scanning could produce a more homogenous group of MCI participants in future work that may demonstrate a difference between MCI and control participants.[51]

In conclusion, AD participants demonstrated a significantly reduced macular VD and PD compared with MCI and control participants, but there were no significant differences in either the FAZ or CST. Changes in the retinal microvasculature may mirror small vessel cerebrovascular changes in AD. These parameters may serve as surrogate noninvasive biomarkers for the diagnosis of AD. Future studies are needed to determine whether such tests will be able to detect progression of MCI to AD.

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

[2] Department of Neurology, Duke University, Durham, North Carolina.

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*Both authors contributed equally as first authors.

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Author Contributions:

Conception and design: Grewal, Polascik, Burke, Fekrat

Analysis and interpretation: Yoon, Grewal, Thompson, Burke, Fekrat

Data collection: Yoon, Grewal, Thompson, Polascik, Dunn, Burke, Fekrat

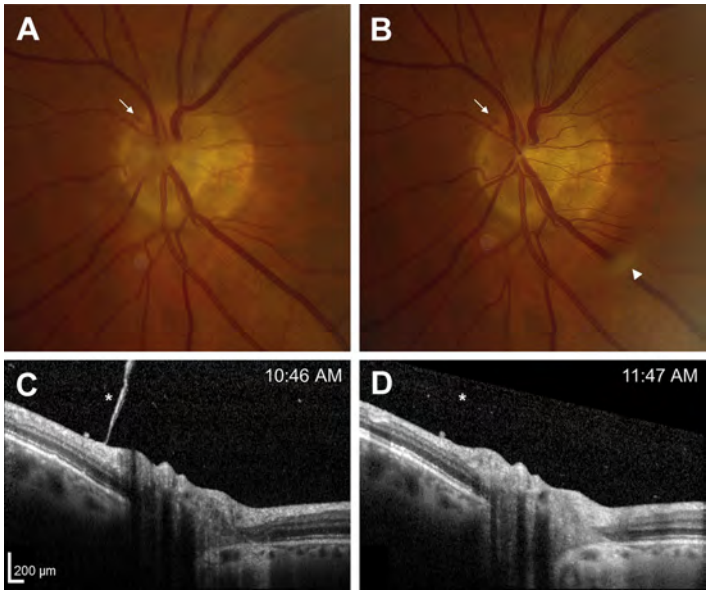
Obtained funding: Yoon, Grewal, Fekrat

Overall responsibility: Yoon, Grewal, Thompson, Fekrat

Abbreviations and Acronyms:
AD = Alzheimer's disease; **CST** = central subfield thickness; **ETDRS** = Early Treatment Diabetic Retinopathy Study; **FAZ** = foveal avascular zone; **GC-IPL** = ganglion cell–inner plexiform layer; **logMAR** = logarithm of the minimum angle of resolution; **MCI** = mild cognitive impairment; **MMSE** = Mini-Mental State Examination; **OCTA** = OCT angiography; **PD** = perfusion density; **PET** = positron emission tomography; **RNFL** = retinal nerve fiber layer; **SCP** = superficial capillary plexus; **VD** = vessel density.

Correspondence:
Sharon Fekrat, MD, Box 3802, Duke University Medical Center, Durham, NC 27710-3802. E-mail: sharon.fekrat@duke.edu.

Pictures & Perspectives



Separation Anxiety

A 62-year-old male physician presented with a 1-day history of floaters and flashes in his left eye. Examination, photography, and OCT revealed a partial posterior vitreous detachment with nasal adhesion (**A**; **C**, asterisk). There was a small flame hemorrhage on the margin of the nerve at 11 o'clock (**A**, **B**, arrow). The peripheral depressed examination was unremarkable. Upon return from initial photography, the vitreous attachment had separated (**D**, asterisk) and a Weiss ring was visible (**B**, arrowhead). The patient endorsed a prominent new floater. A repeat examination was without any new tears. One month later, his examination was stable.

Drew Scoles, MD, PhD
Paul J. Tapino, MD

Department of Ophthalmology, Scheie Eye Institute, Raymond and Ruth Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania