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Retinal Imaging

Change in retinal structural anatomy during the preclinical stage of Alzheimer's disease

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Abstract

Introduction: We conducted a 27-month longitudinal study of mid-life adults with preclinical Alzheimer's disease (AD), using spectral domain optical coherence tomography to compare changes in volume and thickness in all retinal neuronal layers to those of age-matched healthy control subjects.
Methods: Fifty-six older adults (mean age = 65.36 years) with multiple risk factors for AD completed spectral domain optical coherence tomography retinal imaging and cognitive testing at baseline. Twenty-seven months later, they completed the same examinations and an ^{18}F-florbetapir positron emission tomography imaging study.
Results: Compared to healthy control subjects, those in the preclinical stage of AD showed a significant decrease in macular retinal nerve fiber layer (mRNFL) volume, over a 27-month follow-up interval period, as well as a decrease in outer nuclear layer and inner plexiform layer volumes and thickness in the inferior quadrant. However, only the mRNFL volume was linearly related to neocortical positron emission tomography amyloid standardized uptake value ratio after controlling for any main effects of age ($R^2 = 0.103$; $\rho = 0.017$). Furthermore, the magnitude of mRNFL volume reduction was significantly correlated with performance on a task of participants' abilities to efficiently integrate visual and auditory speech information (McGurk effect).
Discussion: We observed a decrease in mRNFL, outer nuclear layer, and inner plexiform layer volumes, in preclinical AD relative to controls. Moreover, the largely myelinated axonal loss in the RNFL is related to increased neocortical amyloid-β accumulation after controlling for age. Volume loss in the RNFL, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving. However, this retinal change does appear to be modestly related to relative decrements in performance on a measure of audiovisual integration efficiency that has been recently advanced as a possible early cognitive marker of mild cognitive impairment.

Keywords: Preclinical; Alzheimer's disease; Retinal; Cognition; RNFL; OCT; Optical coherence tomography; McGurk effect; Amyloid

1. Background

The formation of the eye begins in the third week of human embryologic development, with the retina being a crucial component of the ocular globe and the central nervous system. The retina is derived from pluripotent neuroectodermal

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cells that migrate from the diencephalic invagination of the neural tube [1], and so it is structurally, physiologically, and functionally brain tissue—with the retina sometimes described as a "protrusion" from the brain. As is the case throughout the neocortex, the retina consists of discrete neuronal cell layers, with multiple types of neurons and neurotransmitter systems, glial cells, and microvasculature. Unlike the rest of the central nervous system, however, the retinal neuronal cell layers can be noninvasively visualized through high-resolution optical methods such as spectral domain optical coherence tomography (SD-OCT) [2].

A recent review [3] cites the wide variety of retinal biomarkers that have been explored in patients with Alzheimer's disease (AD), ranging from retinal anatomical and vascular markers to curcumin binding studies [4] and retinal oximetry and electroretinogram studies. Clinically, it is reasonable to suspect the presence of early ocular involvement in AD because visual system changes such as decreased vision, abnormal pupillary reaction, visual field changes, motion detection abnormalities, impaired color vision, and decreased contrast sensitivity have been identified in mild-to-moderate AD [3,5].

SD-OCT allows for the precise segmentation and measurement of the retinal cell layers, thereby promoting exploration of neuronal changes that might be directly related to specific neurodegenerative diseases. Over the past 2 decades, there have been over 70 peer-reviewed publications exploring retinal optical coherence tomographic (OCT) correlates of AD, in humans, nonhuman primates, and other animal models of AD disease. With respect to humans, most prior reports have consisted of cross-sectional studies comparing groups of AD patients to groups of seemingly "healthy controls". When compared with healthy age-matched controls, patients with AD have reduced numbers of ganglion cell axons and are three times more likely to have an increased optic nerve cup-to-disc ratio, a potential consequence of ganglion cell and nerve fiber loss [6]. Such reports have confirmed an earlier report of substantial loss of ganglion cells in AD patients, based on histopathology of autopsy materials [7]. Furthermore, peripapillary retinal nerve fiber layer (pRNFL) thickness has been found to be significantly thinner, suggesting the presence of optic atrophy in patients with mild cognitive impairment (MCI) and mild-to-moderate AD when compared with age-matched controls [8]. Reduction of macular RNFL (mRNFL) volume also has been identified in AD [9]. The loss of RNFL tissue in the retina may constitute an early biomarker of AD. If so, a reduction in RNFL thickness or volume may be observed before widespread damage to the mesiotemporal central nervous memory system that is characteristic of AD [10–12].

With respect to the RNFL, we have surveyed all available literature, including those studies relying on other imaging approaches, such as 2D fundus photography and histological analyses, and we have found variable reports of decreased RNFL and/or ganglion cell layer (GCL) thicknesses in AD and MCI. A search on PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) was performed (25 August, 2017) to find all published articles, using the search terms "Alzheimer's" and "retinal layer". This search led to 134 articles identified and, of these, only 34 articles consisted of human studies that compare retinal layer morphology changes in Alzheimer's patients to healthy individuals (see Table 1).

Nearly all of these 34 publications resulted from cross-sectional studies based on comparisons of cases to putatively "healthy controls". Most often the healthy controls had no biomarker confirmation to indicate that they did not fall within the preclinical stage of AD. One study did search for thickness differences in all 10 retinal layers [20], with the remaining 33 studies concentrating on measurement differences for the mRNFL, pRNFL, and the GCL. Of note, the GCL was only once reported as a single layer [20], being most often considered in conjunction with an adjacent cell layer, either as the ganglion cell–inner plexiform layer complex (GC-IPL) or as the retinal nerve fiber layer–GCL complex (RGCL or GCC).

From the 33 articles seeking to compare group differences for the RNFL, 29 found RNFL thinning in AD compared with age-matched controls (see Table 1). Only seven of these 29 published reports appear to have accounted for participants' age as a statistical covariate in their analyses. This is important because there is normal age-related thinning of the RNFL and other retinal layers [44]. Of these seven publications that did account for effects of aging, one determined that the observed thinning was due primarily to the main effect of aging rather than disease burden [26]; one study found RNFL thinning in MCI but not in AD [30]; two studies reported RNFL thinning solely in one or two specific quadrants [13,32]; and three studies reported robust disease burden after accounting for age [15,17,18]. Only two of the 33 reviewed studies reported within-subjects longitudinal results (both in symptomatic AD patients vs. controls), and they both reported increased RNFL thinning compared with controls [18,27].

AD-related amyloid-β (Aβ) plaques can start to accumulate abnormally in the brain up to 20 years before symptom onset, and this stage is classified as preclinical AD [45,46]. Only two of the studies reviewed previously, both relying solely on cross-sectional data, recruited individuals in the preclinical stage of the disease. One from the same larger study that we draw from in the current report [16] found a thicker inner plexiform layer (IPL) in preclinical AD compared with healthy controls. Another study [14] found no difference in the RNFL thickness between AD, preclinical AD, and healthy controls. Golzan et al. reported a significant difference in the RGCL thickness across the three groups, and yet they found no association between retinal structural measurements and positron emission tomography (PET) Aβ binding in the neocortex. Aside from these two cross-sectional studies of preclinical AD [14,16], none of the other published reports compared PET imaging evidence of neocortical amyloidosis with the retinal OCT measurements for this earliest stage of the disease. In this

Table 1
Review of articles published until 25 July 2017, using the search terms "Alzheimer's" and "retinal layer"

Publication	Layers	Results	Cross-sectional	Subjects	Age-matched controls
Cunha et al., 2017 [13]	pRNFL and retinal	Thinner pRNFL, and superior pericentral and peripheral retinal sectors	Yes	AD	Yes, age as covariate
Golzan et al., 2017 [14]	RNFL and RGCL	RGCL thinner, no difference in RNFL	Yes	preclinical AD	Yes, age as covariate
Ferrari et al., 2017 [15]	pRNFL and GC-IPL	Thinning	Yes	MCI and AD	Yes, age as covariate
Snyder et al., 2016 [16]	IPL	Thicker in preclinical	Yes	preclinical AD	Yes
Choi et al., 2016 [17]	pRNFL and GC-IPL	Thinner in the temporal quadrant	Yes	MCI and AD	Yes, age as covariate
Trebbastoni et al., 2016 [18]	pRNFL	Thinner	No, 1 year	AD	Yes, age as covariate
Feke et al., 2015 [19]	pRNFL	No difference	Yes	MCI and AD	Yes
Cunha et al., 2016 [5]	Macular and GCL+ (GC-IPL)	Thinner	Yes	AD	Yes
Garcia-Martin et al., 2016 [20]	pRNFL, GCL, INL, IPL, ONL, OPL	Thinner RNFL, GCL, and IPL	Yes	AD	Yes
Pillai et al., 2016 [21]	pRNFL, macular, and GCLIPL	No difference	Yes	MCI and AD	Yes
Eraslan et al., 2015 [22]	pRNFL and GCC (RGCL)	Thinner	Yes	AD	Yes
Güneş et al., 2015 [23]	pRNFL	Thinner	Yes	AD	Yes
Cesareo et al., 2015 [24]	pRNFL	Thinner	Yes	AD	Yes
Salobrar-Garcia et al., 2015 [25]	pRNFL and macular	Thinner	Yes	AD	Yes
La Morgia et al., 2016 [26]	pRNFL	Age-related thinner	Yes	AD	Yes, age as covariate
Shi et al., 2016 [27]	pRNFL	Thinner in the inferior quadrant	No, 27 months	AD	Yes
Liu et al., 2015 [28]	pRNFL	Thinner in the superior and superior quadrant	Yes	MCI and AD	Yes
Oktem et al., 2015 [29]	pRNFL	Thinner, but no differences between MCI and AD	Yes	MCI and AD	Yes
Gao et al., 2015 [30]	pRNFL and macula lutea	Thinner specially in MCI	Yes	MCI and AD	Yes, age as covariate
Cheung et al., 2015 [31]	pRNFL and GC-IPL	Thinner pRNFL superior quadrant, MCI had thinner GC-IPL	Yes	MCI and AD	Yes
Kromer et al., 2014 [32]	pRNFL	Thinner in nasal superior	Yes	AD	Yes, age as covariate
Bambo et al., 2014 [33]	pRNFL	Thinner in the inferior and inferiotemporal	Yes	AD	Yes
Marziani et al., 2013 [34]	pRNFL + GCL	Thinner	Yes	AD	Yes
Kirbas et al., 2013 [35]	pRNFL	Thinner	Yes	AD	Yes
Moschos et al., 2012 [36]	pRNFL and macular	Thinner	Yes	AD	Yes
Kesler et al., 2011 [37]	pRNFL	Thinner	Yes	MCI and AD	Yes
Lu et al., 2010 [10]	pRNFL	Thinner	Yes	AD	Yes
Chi et al., 2010 [38]	pRNFL	Thinner	Yes	AD	Yes
Paquet et al., 2007 [39]	pRNFL	Thinner, but no differences in MCI and AD	Yes	MCI and AD	Yes
Berisha et al., 2007 [40]	pRNFL	Thinner in the superior quadrant	Yes	AD	Yes
Iseri et al., 2006 [9]	pRNFL and macula	Thinner	Yes	AD	Yes
Parisi et al., 2001 [41]	pRNFL	Thinner	Yes	AD	Yes
Kergoat et al., 2001 [42]	pRNFL	No differences	Yes	AD	Yes
Hedges et al., 1996 [43]	pRNFL	Thinner in the superior quadrants	Yes	AD	Yes

Abbreviations: AD, Alzheimer's disease; GCL, ganglion cell layer; GC-IPL, ganglion cell–inner plexiform layer complex; INL, inner nuclear layer; IPL, inner plexiform layer; MCI, mild cognitive impairment; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer; RGCL (or GCC), retinal nerve fiber layer–GCL complex; RNFL, retinal nerve fiber layer.

current report, we followed up the same cohort of subjects at high risk for preclinical AD over 27 months to explore changes in all retinal neuronal layers and we relate these findings to PET imaging evidence of cortical amyloid aggregation in the same participants.

In our present study, we compared morphological changes in retina with cognitive performance. Previous studies examining this relationship have relied on the Mini–Mental State Examination (MMSE) as a screening measure of general cognitive function, and these studies

have led to conflicting results. Some studies have found no relationship between RNFL thinning and MMSE scores in AD [41,47] and MCI [39], whereas one study found a correlation between RNFL thickness and MMSE scores in MCI [29]. Another study reported a correlation between macular volume thinning (whole retina) and MMSE scores in patients with AD [9]. For individuals in the preclinical stage of disease severity, the MMSE would be a poor choice as a cognitive marker, due to several psychometric limitations for this test [48]. With respect to the MCI stage of disease severity, two prior investigators have reported surprisingly inverse relationships between performance on measures of verbal episodic memory and RNFL thickness [49,50]. Importantly, both of these studies reported cross-sectional data, and it is unclear as to whether this inverse relationship (i.e., relatively enhanced performance on verbal episodic memory tests associated with relatively diminished RNFL thicknesses) would persist if participants were followed up longitudinally and as the disease progresses.

Our intent was to explore this very question, that of the relationship between cognitive functioning and evidence of morphologic change in the retina, over the course of several years and within the very early, preclinical stage of the disease. Based on prior literature in this specific patient population, we chose the Groton Maze Learning Test (GMLT) as a measure of learning efficiency, problem solving, and working memory [51,52], and the International Shopping List Test (ISLT) to measure verbal episodic memory [53,54]. Finally, we administered an audiovisual McGurk task, a speech processing paradigm that relies on the integrity of white matter tracts that underlie corticocortical connectivity, as prior work has shown disruption of functional integration for posterior sensory regions in AD [55,56]. Because alterations in white matter integrity may occur in early stages of the disease [57–59], similar early changes in the RNFL, which comprised mostly myelinated axons from the cell bodies in the GCL, might be directly related to the loss of white matter tracts in the cortex and hence correlate with performance on this cognitive task that specifically assesses corticocortical connectivity and has recently been advanced as a potential biomarker of early-stage AD pathology [60].

2. Methods

2.1. Participants

A total of 56 adults aged between age 55 and 75 years (mean age = 65.36 years) with two well-established risk factors for AD, namely, a self-reported first-degree family history of the disease and self-identification of subjective memory concerns, were recruited using a selection process described previously [61]. All participants underwent a detailed medical screening interview. Exclusion criteria included a diagnosis of MCI or AD following National Institute on Aging - Alzheimer's Association diagnostic criteria [45,62], history of neurological or psychiatric disorder, any significant systemic illness or unstable medical condition (e.g., active cardiovascular disease), and current use of any medications known to affect cognition (e.g., use of sedative narcotics). Subjects with histories of cataract surgery, corneal Laser-Assisted In-Situ Keratomileusis (LASIK) surgery, age-related macular degeneration, or subjects with known ophthalmic pathology were excluded. Inclusion criteria included having an MMSE score of $\geq$27 and performance within normal limits on a battery of cognitive tests described previously (listed in Table 2) [63,64]. Because RNFL thinning is a characteristic of glaucoma, subjects with glaucoma were excluded. Participants were also excluded if they had a history of optic neuritis, intraocular surgery apart from cataract extraction, or a history of visual loss

Table 2
Demographic characteristics

		Full sample (n = 56)	Aβ+ (n = 15)	Aβ− (n = 41)		
	Main outcome	N (%)	N (%)	N (%)	*P*	
Sex	Number of females	35 (62.5)	11 (73.3)	24 (58.5)	.311	
APOE	Number of ε4 carriers	27 (4.2)	8 (53.3)	19 (46.3)	.643	
		Mean (SD)	Mean (SD)	Mean (SD)	*P*	Cohen's *d*
Age	Number of years	65.36 (5.55)	68.25 (5.81)	64.56 (5.26)	.06	0.68
Education	Number of years	17.31 (2.77)	17.75 (3.91)	17.19 (2.42)	.54	0.19
Florbetapir PET SUVr	SUVr	**1.02 (0.2)**	**1.32 (0.18)**	**0.94 (0.09)**	**.000**	**3.17**
GDS	Total score	1.4 (1.87)	0.91 (0.94)	1.52 (2.02)	.34	0.34
Body Mass Index	Body mass index	26.69	26.58 (4.36)	26.86 (6.05)	.879	0.35
MAC-Q	Total score	21.90 (3.08)	21.97 (2.81)	21.90 (3.08)	.527	0.23
MMSE	Total score	29.25 (1.29)	28.72 (1.85)	29.38 (1.10)	.132	0.49
ISLT–Total Recall	Total words recalled	26.07 (3.79)	26.00 (3.52)	26.09 (3.90)	.944	0.02
GMLT–Total Errors	Total number of errors	8.40 (5.31)	8.27 (3.53)	8.44 (5.71)	.92	0.03

Abbreviations: APOE, apolipoprotein E; GDS, Geriatric Depression Scale; GMLT, Groton Maze Learning Test; ISLT, International Shopping List Test; MAC-Q, Memory Complaint Questionnaire; MMSE, Mini–Mental State Examination; PET, positron emission tomography; SUVr, standardized uptake value ratio.

NOTE. Bold values denote significant group differences.

apart from refractive error as these conditions may affect the RNFL thickness [65–67]. In addition, individuals were excluded if the epiretinal membrane was observed using OCT imaging [68]. All participants live independently, most were engaged in full-time or part-time employment, and many were caretakers for a parent with AD. The amount of neocortical Aβ protein aggregation and apolipoprotein E (*APOE*) genotype were unknown at the time of assessment and were not used to determine enrollment. Although Aβ status and *APOE* genotyping were conducted as a part of the study protocol, researchers remained blinded to these results throughout testing.

From this larger sample, we identified 15 participants who were categorized as presenting with preclinical-stage disease based on evidence of both (1) elevated neocortical Aβ burden as determined by PET amyloid imaging [45,46] and (2) relative cognitive impairments in response to a challenge with a very low–dose muscarinic anticholinergics [59,63].

The study was approved by and complied with the regulations of Rhode Island Hospital's Institutional Review Board, and all participants provided written informed consent in accordance with the Declaration of Helsinki. The study complied with Health Insurance Portability and Accountability Act regulations.

2.2. Aβ PET imaging

To assess neocortical amyloid burden, all participants had an Aβ PET scan at baseline and another at 27-month visit. A 370 MBq (10 mCi 1/2 10%) bolus injection of 18F-florbetapir was administered intravenously. Approximately 50 minutes after injection, a 20-minute PET scan was performed with a head computed tomography scan for attenuation correction purposes. Images were obtained using a 128 × 128 matrix and reconstructed using iterative or row action maximization likelihood algorithms. PET standardized uptake value data were summed and normalized to the whole-cerebellum standardized uptake value, resulting in a region-to-cerebellum ratio termed standardized uptake value ratio (SUVr).

An SUVr threshold of 1.1 or greater was used to discriminate between Aβ+ and Aβ−. These SUVr calculations were performed using the MIMneuro software, with a normative database of 74 healthy normal individuals (48 males and 26 females), aged between the ages of 18–50 years, who all had negative amyloid scans on visual assessment [69]. For all cases, Aβ positivity was confirmed by consensus overread by two board-certified radiologists who were also board certified in nuclear medicine.

2.3. SD-OCT imaging

Participants were administered two drops of tropicamide (Mydriacyl 1%) per eye for pupil dilation before OCT imaging. The Heidelberg SPECTRALIS SD-OCT was used to acquire retinal OCT scans of the optic nerve head and the macula for the right and left eyes of all participants at baseline and 27 months. Heidelberg SPECTRALIS has automatic segmentation and quantification of retinal layers and uses the eye-tracking image alignment for repeated measures (TruTrack; Heidelberg Engineering, Inc). Outcome measures for the SD-OCT imaging, following the Anatomic Positioning System protocol sequences, included pRNFL, mRNFL, GCL, IPL, inner nuclear layer, outer plexiform layer, and outer nuclear layer (ONL). For each individual participant, the volume and thickness of all retinal neuronal layers were measured and averaged for both eyes.

The mean macular thickness of each retinal layer was measured within four sectors (superior, inferior, nasal, and temporal) leading to a macular volume measurement for each retinal layer (see Fig. 1). For the pRNFL, the mean thickness also was calculated in four sectors (superior, inferior, nasal, and temporal) centered in the optic nerve, and the thicknesses of all areas were averaged to result in the mean pRNFL thickness.

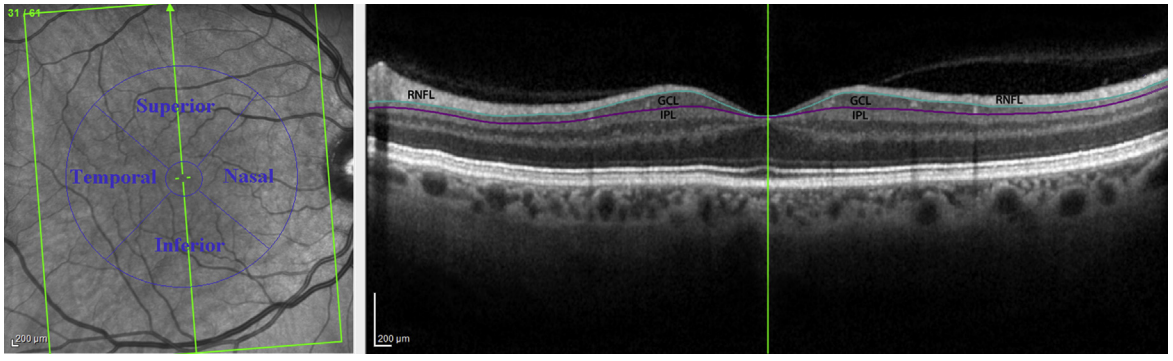


Fig. 1. Representative cross-sectional OCT image through macular region, with the fovea in the center (green line). Labels are shown for the RNFL, GCL, and IPL. The mean macular thickness of each retinal layer was measured within four sectors (superior, inferior, nasal, and temporal), extending 3.45 mm from the center of the fovea, leading to a macular volume measurement for each retinal layer. Mean volumes (mm^3) for each layer and thickness (μm) for each quadrant (right and left eyes averaged) were computed for both the baseline and the 27-month time points. Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; OCT, optical coherence tomography; RNFL, retinal nerve fiber layer.

The volumes for all layers were obtained separately, across the entire macular region extending 3.45 mm from the center of the fovea. Mean volumes (mm^3) for each layer and thickness (μm) for each quadrant (right and left eyes averaged) were computed for both the baseline and the 27-month time points. The difference between these two examination time points was obtained for each subject and for each layer. Multicolor imaging, which uses individual laser wavelengths [70] to characterize anatomic and pathologic detail at different retinal depths, served to identify and exclude individuals with background diabetic retinopathy and retinal microbleeds within the region of interest.

2.4. Cognitive assessment

The GMLT (http://www.cogstate.com) is a computer-administered hidden maze learning test, designed by one of the authors (P.J.S.) to measure spatial working memory and problem-solving functions [71]. The GMLT has been described previously both in terms of performance within the context of studies with healthy elderly adults [51,72,73] and in MCI [74].

In addition, all subjects completed the ISLT (www.cogstate.com) as a measure of verbal episodic memory [75], the MMSE as a measure of general cognitive function, and the Memory Complaint Questionnaire [76] as a measure of subjective memory complaints.

As noted previously, we also administered an audiovisual McGurk task that has recently been described as a potential new marker of early-stage AD [60]. The McGurk effect is a compelling misperception in which discrepant visual and auditory speech information presented simultaneously results in the listener hearing a fused audiovisual speech sound, rather than the veridical auditory sound (e.g.,/ba/auditory + /ga/visual is heard as/da/rather than/ba/) [77]. The integrity and efficiency of the audiovisual integration process can be measured by comparing accuracy and response times to identify the auditory information under congruent (matching audio and visual components) and incongruent (conflicting audio and visual components) conditions. For the purpose of this study, we focused on a measure of integration efficiency, defined as the proportional increase in response time from congruent to incongruent conditions for the weaker McGurk stimulus in this task. This stimulus condition was particularly sensitive at discriminating patients diagnosed with amnestic MCI from healthy elderly [60]. Higher efficiency scores reflect greater sensitivity to binding strength.

All cognitive measures were performed by trained staff supervised by a licensed clinical neuropsychologist.

2.5. Statistical methods

For the demographic variables, *t*-tests were performed for each variable between the preclinical AD and healthy control groups. For the layers RNFL (macular and peripapillary regions), GCL, ONL, outer plexiform layer, inner nuclear layer, and IPL, paired 1-sided significance level of 0.05 *t*-tests was performed, and the effect sizes for each comparison were computed with the Cohen's *d* statistic. For each layer, the differences between baseline and 27-month measurements and between Aβ+ and Aβ− subjects for the total volume and the thickness of the average and the quadrants (N3.45, T3.45, S3.45, and I3.45) were computed. Linear regression models were performed with neocortical PET imaging SUVr entered as a response variable, and retinal layer measures as the explanatory variables, and covarying for effects of normal aging. Although age was not significantly different between groups, it is a key predictor of volumetric loss over time in the macula. To assess whether a correlation exists between retinal layer thickness and any of the cognitive tests, linear regression analysis (Pearson's test) was adopted, a P value less than .05 was considered significant, and the magnitude of differences was quantified using Cohen's *d*.

3. Results

3.1. Sample demographics

Of the 56 participants enrolled, 35 were female and 11 of them were amyloid positive on PET imaging. There were no significant differences between Aβ+ and Aβ− groups with respect to sex ($P = .311$). Likewise, there were no group differences in the proportion of individuals with the APOE ε4 genetic risk marker for AD. The mean age for the total sample was 65.36 years, and this sample had an average of 17.31 years of education. All relevant demographic information, for both groups, is provided in Table 2. There were no group differences with respect to body mass index, subjective memory complaints, and cognitive performance at baseline examination (on any of the tests described previously). By definition, both groups significantly differed with respect to neocortical amyloid aggregation as measured via PET imaging ($P = .000$).

With respect to genetic risk for AD, eight of 15 individuals (53%) in the preclinical AD group (florbetapir PET SUVr scores $\geq$1.10) had at least one copy of the APOE ε4 allele, whereas 20 of 45 individuals (45%) in the control group (florbetapir PET SUVr scores $<$ 1.10) had at least one copy of the APOE ε4 allele. Hence, roughly half of the subjects in each group presented with this additional risk factor for AD, but due to small sample sizes, we were not able to further evaluate the specific effect of *APOE* genetic risk on retinal layer measurements. Subject demographic information, for both groups, is provided in Table 2.

3.2. Retinal layer measurements

At baseline examination, no group differences were found with respect to the thicknesses of any macular quadrant for any neuronal layer, nor were there any group differences observed with respect to total volumes for each layer in the same region (Table 3).

Table 3
Baseline retinal measures by the analysis group

Retinal measures	Preclinical (n = 15)	Control (n = 41)	*P*-value
mRNFL			
Total volume (mm^3)	0.227 ± 0.021	0.231 ± 0.025	.283
Average thickness	28.58 ± 3.08	28.92 ± 3.57	.383
Inferior	33.04 ± 3.53	32.69 ± 4.39	.599
Nasal	28.87 ± 3.87	29.12 ± 4.70	.433
Superior	32.12 ± 4.42	32.74 ± 5.10	.353
Temporal	20.29 ± 1.20	21.12 ± 2.76	.157
pRNFL			
Average thickness	103.36 ± 5.36	101.48 ± 9.76	.726
Inferior	134.25 ± 8.15	129.05 ± 13.49	.882
Nasal	83.04 ± 17.36	85.3 ± 19.88	.368
Superior	127.45 ± 10.32	124.17 ± 13.72	.765
Temporal	67.86 ± 11.07	67.71 ± 11.68	.612
GCL			
Total volume (mm^3)	0.444 ± 0.018	0.428 ± 0.029	.942
Average thickness	51.15 ± 2.20	48.94 ± 3.61	.964
Nasal	53.60 ± 2.03	51.23 ± 3.70	.970
Temporal	50.90 ± 3.77	49.01 ± 4.41	.889
Superior	48.75 ± 4.01	48.08 ± 3.88	.683
Inferior	51.35 ± 1.94	47.42 ± 4.18	.997
IPL			
Total volume (mm^3)	0.353 ± 0.030	0.357 ± 0.027	.339
Average thickness	39.40 ± 3.67	39.18 ± 3.27	.578
Inferior	39.00 ± 4.92	37.54 ± 3.78	.862
Nasal	40.37 ± 3.37	40.66 ± 3.66	.403
Superior	37.50 ± 3.63	37.39 ± 3.68	.534
Temporal	40.71 ± 4.73	41.10 ± 3.28	.369
OPL			
Total volume (mm^3)	0.294 ± 0.373	0.301 ± 0.037	.031
Average thickness	31.32 ± 3.40	32.10 ± 3.89	.267
Inferior	30.91 ± 3.38	31.51 ± 4.68	.410
Nasal	33.87 ± 6.19	35.33 ± 7.42	.620
Superior	30.16 ± 3.36	30.65 ± 3.42	.442
Temporal	30.33 ± 3.54	30.88 ± 3.50	.483
INL			
Total volume (mm^3)	0.338 ± 0.020	0.348 ± 0.028	.124
Average thickness	38.08 ± 1.39	38.54 ± 3.13	.312
Inferior	37.54 ± 1.23	38.45 ± 3.04	.158
Nasal	39.70 ± 1.55	39.67 ± 4.66	.510
Superior	38.08 ± 1.81	38.70 ± 3.18	.645
Temporal	37.00 ± 2.83	37.34 ± 3.37	.372
ONL			
Total volume (mm^3)	0.679 ± 0.066	0.671 ± 0.067	.644
Average thickness	67.09 ± 7.02	66.48 ± 7.04	.604
Inferior	64.16 ± 7.38	64.00 ± 7.94	.529
Nasal	67.92 ± 8.66	64.51 ± 8.97	.877
Superior	68.08 ± 7.14	68.34 ± 6.63	.453
Temporal	68.20 ± 7.17	69.09 ± 7.68	.361

Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer.

NOTE. All measurements, for each layer, are acquired from all radial quadrants from the Early Treatment Diabetic Retinopathy Study circular grid, with a 3.45-mm diameter centered on the fovea. Data from both eyes are averaged. Values are mean ± SD. All values are in micrometer (μm), unless otherwise indicated.

Change over the 27-month follow-up period, for each of these measures, was calculated by subtracting the volumes (mm^3) and thickness (μm) obtained at the baseline visit from the same measurements obtained at the 27-month visit. As shown in Table 4, a significant group difference was found for the mRNFL (P = .050), ONL (P = .026), and IPL volumes (P = .020). In all cases, the preclinical AD group showed a larger reduction in volume over this time period, compared to the healthy control group, and in all cases these group differences were of a moderate effect size. The only significant changes observed in thickness after controlling for effects of age were in the inferior quadrant of the ONL (0.026) and IPL (P = .028), with a larger reduction in the preclinical group, and in the temporal quadrant of the outer plexiform layer (0.040), with a larger increase in the preclinical group compared to the control.

Considering each subject group separately, the change from baseline to 27 months in the total volume (mm^3) of the mRNFL was significantly decreased for the preclinical AD group (−0.032 ± 0.003, P = .002) and for the healthy control group (−0.0190 ± 0.003, P = .03). With respect to the pRNFL, although we found an overall nonsignificant difference in the magnitude of the thinning between the two groups, over the 27-month period, we observed substantially greater ranges of variance of measurements for both groups, as reflected by markedly larger standard deviations of measurement (Table 3). This region of the pRNFL is thought to have greater within-subject and between-subject variability because it contains a multitude of larger diameter blood vessels, particularly, with respect to vascular innervation within the regions of the superior and inferior arcuate bundles, individual differences in optic canal sizes, the presence or absence of space-occupying nerve head drusen, and other individual differences in this region [78].

3.3. Retinal layer change in relation to neocortical amyloid aggregation

A multivariate linear regression model, controlling for participants' age, with total neocortical PET amyloid ligand binding (SUVr) entered as the dependent measure was conducted. Macular RNFL volume change, over the 27-month study interval, accounted for 10% of the variance in PET amyloid neocortical binding at the end of the study (adjusted R^2 = 0.106, ρ = 0.017; Fig. 2). By comparison, change in the GCL over the same time was related to participants' age (ρ = 0.05), but not significantly related to PET amyloid SUVr (ρ = 0.78). For all neuronal cell layers, other than the mRNFL, the observed change over time was not related to neocortical PET amyloid SUVr.

3.4. Retinal layer change in relation to cognitive performance

There were no significant relationships found between volume reductions for any of the retinal layers over 27 months and performance on measures of spatial working memory and learning efficiency (GMLT) or on a measure of word-list learning and episodic verbal memory (ISLT) (P > .005).

Table 4
Change over 27 months by groups

Location	Preclinical AD (N = 15)	Healthy controls (N = 41)	*P*-value	Effect size (Cohen's *d*)
mRNFL				
Volume (mm^3)	**−0.032 ± 0.003**	**−0.019 ± 0.003**	**.050**	**0.550**
Average	−4.45 ± 3.68	−3.26 ± 3.94	.448	0.3072
Inferior	−4.45 ± 3.314	−3.09 ± 4.323	.656	0.3352
Superior	−5.10 ± 3.671	−4.50 ± 4.845	.348	0.1318
Nasal	−4.50 ± 3.801	−2.41 ± 4.416	.196	0.4917
Temporal	−3.75 ± 3.936	−3.04 ± 2.212	.77	0.2528
pRNFL				
Average	−3.389 ± 2.058	−1.181 ± 4.057	.060	0.605
Inferior	−1.39 ± 6.22	1.63 ± 9.10	.386	0.357
Superior	−3.33 ± 11.92	−1.41 ± 14.10	.631	0.141
Nasal	1.67 ± 5.97	3.81 ± 7.79	.381	0.290
Temporal	−1.04 ± 4.13	0.18 ± 4.51	.400	0.276
GCL				
Volume (mm^3)	−0.016 ± 0.016	−0.011 ± 0.019	.206	0.29
Average	−1.96 ± 2.59	−1.34 ± 3.03	.590	0.209
Inferior	−2.4 ± 2.97	−0.86 ± 3.38	.091	0.464
Superior	−1.0 ± 3.51	−1.43 ± 4.18	.614	0.107
Nasal	−0.65 ± 3.54	−0.05 ± 3.54	.321	0.166
Temporal	−3.8 ± 2.87	−3.02 ± 3.83	.590	0.210
OPL				
Volume (mm^3)	0.017 ± 0.04	−0.003 ± 0.032	.965	0.603
Average	0.843 ± −3.05	−0.605 ± 2.89	.932	0.495
Inferior	0.75 ± 3.51	−0.84 ± 2.59	.956	0.566
Superior	0.5 ± 3.37	−0.23 ± 3.57	.262	0.208
Nasal	−0.83 ± 4.88	−1.77 ± 6.85	.329	0.144
Temporal	**2.95 ± 4.31**	**0.43 ± 4.53**	**.040**	**0.562**
ONL				
Volume (mm^3)	**−0.029 ± 0.030**	**−0.007 ± 0.035**	**.026**	**0.646**
Average	−1.61 ± 2.86	−0.068 ± 3.39	.077	0.469
Inferior	**−2.25 ± 5.66**	**0.84 ± 4.54**	**.026**	**0.644**
Superior	−1.70 ± 2.60	−1.43 ± 3.73	.405	0.078
Nasal	0.87 ± 3.58	2.92 ± 7.27	.176	0.305
Temporal	−3.37 ± 7.62	−2.60 ± 5.52	.347	0.128
IPL				
Volume (mm^3)	**−0.014 ± 0.015**	**−0.006 ± 0.011**	**.020**	**0.686**
Average	−1.80 ± 1.91	−1.06 ± 1.66	.099	0.427
Inferior	**−2.41 ± 3.32**	**−0.84 ± 2.17**	**.028**	**0.638**
Superior	−1.91 ± 1.50	−1.06 ± 2.49	.133	0.368
Nasal	−0.95 ± 2.23	−1.07 ± 2.011	.567	0.055
Temporal	−1.91 ± 2.11	−1.29 ± 2.57	.22	0.250
INL				
Volume (mm^3)	0.004 ± 0.019	0.001 ± 0.022	.64	0.12
Average	2.04 ± 2.14	2.30 ± 5.50	.43	0.276
Inferior	2.04 ± 2.32	0.19 ± 2.67	.98	0.708
Superior	0.33 ± 1.91	0.011 ± 2.38	.665	0.140
Nasal	0 ± 1.97	0.47 ± 3.94	.34	0.131
Temporal	0.70 ± 2.75	0.39 ± 2.25	.655	0.131

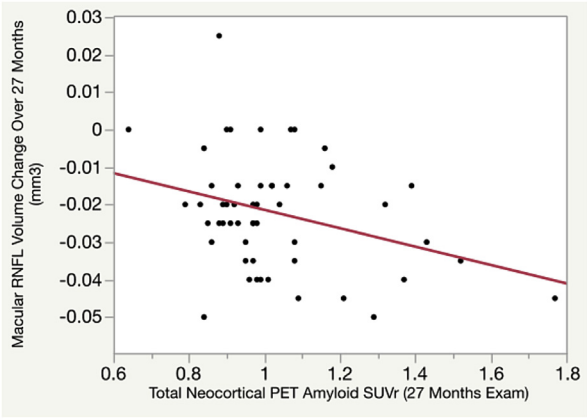
Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; pRNFL, peripapillary retinal nerve fiber layer.

NOTE. All measurements, for each layer, are acquired from all radial quadrants from the Early Treatment Diabetic Retinopathy Study circular grid, with a 3.45-mm diameter centered on the fovea. Data from both eyes are averaged. Values are mean ± SD, unless otherwise indicated. All values are in micrometer (μm), unless otherwise indicated. Bold values denote significant group differences.

However, an interesting correlation was found between the magnitude of mRNFL volume reductions and increasing difficulties on the McGurk task of efficiency for audiovisual integration. That is, individuals with greater volume reduction in mRNFL showed reduced sensitivity to the binding strength of the audiovisual stimulus ($\rho = 0.037$, 1-tailed; Fig. 3).

4. Discussion

The average RNFL thicknesses for both groups in our study are similar to the control groups reported by others [5,39] and to the measurements obtained by Golzan et al. in their preclinical AD [14]. Likewise, our OCT measurements

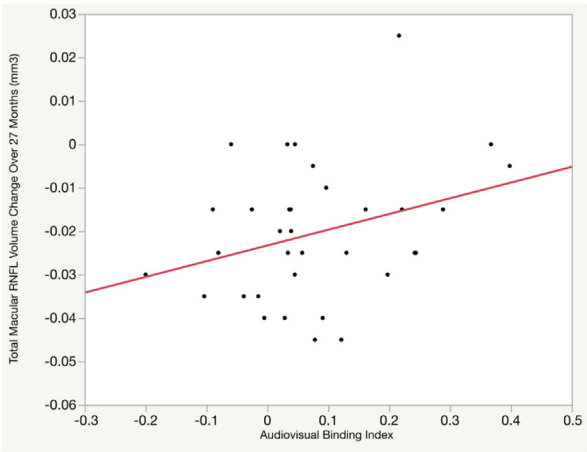


Linear Fit: Adj $R^2 = 0.106$, $\rho < 0.017$ (2-tailed)

Fig. 2. Relationship between macular RNFL volume change over 27 months and total neurocortical amyloid aggregation (^{18}F-florbetapir PET SUVr) at end of study. Abbreviations: PET, positron emission tomography; RNFL, retinal nerve fiber layer; SUVr, standard uptake value ratio.

of the GCL and IPL layers are quite similar to those reported by others [22]. Of the prior studies we reviewed and described previously, Garcia-Martin et al. reported retinal layer thickness values that differed substantially from both our measurements and from most other published reports [20].

Most published research on this topic has relied on cross-sectional study designs, with measurements of the thickness and volume of various retinal layers at a single point in time. Such studies are likely to be limited in their ability to identify retinal markers of the preclinical stage of AD, as such individuals are still relatively young and such between-groups comparisons have not generally accounted for a variety of confounding variables including, but not limited to, effects of sex differences, ethnicity, axial length, optic disc area, and refractive status on OCT measurements [44,79–81]. Hence, we performed this within-subjects longitudinal study over 27 months to measure individuals' structural parameters that can be reliably remeasured at different time points using the *Eye Tracker* software for SPECTRALIS SD-OCT [82]; we then calculated the differences for each of the measures collected (Table 4) for both the preclinical AD and the healthy control groups. To our knowledge, this is the first attempt both to explore within-subject changes in retinal neuronal layer structures, in the preclinical stage of AD, and to relate any such observed changes to performance on a cognitive assay that has been shown to be sensitive to detection of early-stage disease burden. Our findings suggest that a decrease in mRNFL volume is the earliest detectable structural retinal change associated with AD. Moreover, this change in mRNFL volume is related with neocortical Aβ accumulation in very early AD (Fig. 1), even after correcting for expected age-related decline [83] (Table 5). By comparison, the decrease in GCL volume observed over the same time period was principally related to the effects of aging ($\rho = 0.05$) rather than due to cortical Aβ aggregation ($\rho = 0.78$), at least during this earliest detectable stage of disease progression.



Linear Fit: Adj $R^2 = 0.098$, $\rho < 0.037$ (1-tailed)

Fig. 3. Relationship between macular RNFL volume change over 27 months and performance on a measure of audiovisual integration efficiency ("McGurk Task" [60]). Abbreviation: RNFL, retinal nerve fiber layer.

Table 5
Multivariate linear regression model of volume (mm^3), for different locations

Location	Coef. Neocortex SUVr	Neocortex SUVr *P* value	Coef. Age	Age *P* value
Volume				
mRNFL	**−0.228**	**0.041**	**0.00009**	**0.024**
GCL	−0.003	0.78	**−0.0008**	**0.05**
OPL	2.05	0.84	−0.034	0.691
ONL	−0.0267	0.423	0.00026	0.834
IPL	−0.014	0.144	−0.0034	0.196
INL	0.026	0.08	−0.005	0.26

Abbreviations: Coef., coefficients; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; mRNFL, macular retinal nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; PET, positron emission tomography; pRNFL, peripapillary retinal nerve fiber layer; SUVr, standardized uptake value ratio.

NOTE. Values are in cubic millimeter (mm^3) for the coefficients. Controlling for participants' age, with total neocortical PET amyloid ligand binding (SUVr) entered as the dependent measure. Bold values denote significant group differences.

The macular region of the retina is physiologically very active in healthy normal eyes [7], and this "hyperexcitation" might be diminishing in the preclinical stage of AD. In support of this hypothesis, postmortem histological studies have found prominent pathological alteration of retinal ganglion cells in the macular region in AD patients [26,84] and a preferential loss of larger axons, suggesting early involvement of the magnocellular retinal ganglion cells in AD as they contribute large caliber fibers to the optic nerve [85]. The RNFL is adjacent to the GCL, and it is composed largely of ganglion cell axons that are organized in superior and inferior arcuate bundles, and the papillomacular bundle, that lead to the optic nerve. Early in multiple sclerosis, there is demyelination without axonal loss. Likewise, the

mRNFL may be thinned due to demyelination, and possibly only when there is axonal loss, will we see loss of ganglion cells (GCL). Our results suggest early demyelination in the RNFL in the preclinical stage of AD, and our data confirm at least one other report of mRNFL changes occurring before pRNFL changes in AD [86]. Moreover, there is some suggestion that this might be most readily observed in the superior quadrant of the mRNFL [86], and this would make sense in light of at least one clinical report of preferential inferior visual field loss in AD [87]. However, in a meta-analysis of 17 studies comparing AD patients with healthy controls and five studies comparing individuals with MCI with controls, there were significant decreases in all four quadrants compared to controls, thus suggesting that the degenerative process affects the entire macular region [8].

There have been several prior reports of GCL thinning in the symptomatic stages of AD [5,15,22]. Moreover, Martin et al. [20] studied 150 patients with AD and 75 age-matched controls to model how changes in RNFL's and other neuronal cell layers' thicknesses are associated with disease duration and severity. This work suggests that there is axonal degeneration in the RNFL early in the disease followed by degenerative changes to the cell bodies in the GCL and then progression to deeper neuronal layers [20]. This progressive pattern of mRNFL loss before GCL loss is further confirmed by our results reported previously. In addition, we found that only loss of mRNFL tissue, and not loss in any other neuronal layer, was correlated with Aβ protein aggregation in the cerebral cortex. These results fit nicely with data from a large population-based epidemiological study (Rotterdam Study, 2007–2012) showing that having a thinner RNFL at a baseline examination was significantly associated with increased risk of later developing dementia [88].

4.1. Relationship of RNFL thinning to cognitive function in preclinical AD

Contrary to a few prior reports [49,50,89], we did not observe any correlation between any retinal layer changes and change in either episodic verbal memory (ISLT) or for performance on a working memory and reasoning task for visuospatial information (GMLT). However, because white matter degeneration is readily observable in the early stages of AD [90] and the cerebral white matter is principally composed of myelinated axons and glial cells—as is the retinal RNFL—we chose to administer a cognitive task that is putatively sensitive to functional disruption of corticocortical connections caused by disruption of white matter integrity [60]. Both prodromal and preclinical AD patients with high Aβ burden have been previously found to display changes in audiovisual integration efficiency that are likely due to AD-related disruptions in functional connectivity between posterior sensory regions. Using McGurk-like audiovisual speech stimuli (i.e., video clips of an individual mouthing a speech sound while the audio channel provides either consistent or inconsistent auditory speech information), we found that greater volume reduction in the mRNFL was significantly associated with reduced sensitivity to the binding strength of the audiovisual stimulus (Fig. 2). While healthy individuals typically show faster response times to consistent stimuli than inconsistent stimuli (i.e., response times are faster when the auditory and visual components provide the same compared to conflicting speech information), we found that this response time difference decreased with greater RNFL volume reduction. In fact, greater volume reduction in the mRNFL was significantly associated with reduced sensitivity to the binding strength of the audiovisual stimulus, suggesting that mRNFL volume reduction is related to white matter loss. Consistent with previous findings [60], this reduced sensitivity may reflect subtle disruptions of corticocortical projections within unimodal and heteromodal sensory cortices that indicate individual risk of AD.

4.2. Change in retinal neuronal layers, other than the RNFL, in preclinical AD

As noted previously, we observed significant changes in ONL ($P = .026$) and IPL volumes ($P = .002$), and inferior quadrant thicknesses for these same layers, respectively ($\rho = 0.026$; $\rho = 0.028$), in the preclinical AD group compared to the controls, over 27 months. However, neither of these changes was related to PET imaging severity of neocortical amyloidosis at the end of the 27-month interval (Table 5). We have previously reported, with the same cohort of subjects at their baseline examinations, an increase in the IPL volume in preclinical AD that may be related to either Aβ deposition or an inflammatory process [16]. Twenty-seven months later, we have now found evidence of tissue loss in the IPL, within our preclinical group relative to controls, suggesting that although there may be an initial early stage of IPL volume increase due to an inflammatory process, there is nonetheless some volume loss in this structure over continued disease progression.

Finally, the ONL consists of photoreceptor cell bodies, and thinning of this layer has previously been shown in patients with age-related macular degeneration in association with tears in the retinal pigment epithelium [91]. In the context of early AD, we believe that our observation of tissue loss in the ONL could suggest retrograde transsynaptic degeneration, but future exploration would be necessary to support this hypothesis.

5. Conclusion and future directions

To our knowledge, this is a first report of a within-subjects, prospective, longitudinal study of retinal anatomic changes in the preclinical stage of AD. *We have found that thinning of the mRNFL may be the earliest*

anatomic marker of retinal neuronal loss in the preclinical stage of AD, and such loss appears to account for 10% of the variance in observed PET imaging measurement of neocortical amyloidosis. Whereas most reports have relied on clinical examinations for determining disease burden, our study used the PET imaging biomarker of disease burden. Our study is limited by a small sample size, and so these findings require replication in a larger patient population. Second, we chose to recruit participants who were at high risk for the very early stages of the disease, that is, during a prodromal stage that is defined by a relative absence of readily identifiable cognitive and/or functional impairments, as well as by biomarker evidence of disease burden that is subtle and for which clear diagnostic criteria have not yet been established [46]. Third, retinal imaging is not only being studied to identify novel biomarkers for this neurodegenerative disease, but changes in GCL and RNFL thicknesses have also been reported in Parkinson's disease and multiple sclerosis [92–95].

Future work on this topic will require the recruitment of larger populations of participants, across the entire disease severity spectrum (from healthy controls to mild AD), and ideally such a large cohort should be followed up for an even longer time interval to better model the natural history of retinal anatomic changes over the entire course of disease progression. Additional imaging modalities should be included in such a study (e.g., OCT angiography), as retinal blood flow appears to be reduced in MCI and AD [19,40], and the disease has been associated with a retinal venular stenosis, reduced complexity of the branching pattern and geometry, and reduced and tortuous venules [96]. We also would recommend inclusion of scanning laser polarimetry methods to measure RNFL retardance in preclinical AD because, in glaucoma, RNFL retardance seems to occur before actual RNFL thinning [97]. The mechanisms of glaucomatous injury appear to be related to damage the integrity of axonal cytoskeletal ultrastructure before the point of RNFL thinning detectable by OCT [97–99]. The axonal cytoskeleton disruption that causes RNFL retardance [97] may possibly be similar to the effects of Aβ plaques and tau tangles in the AD brain.

These results point to the first retinal anatomic changes occurring in the early stages of AD. Characterizing retinal changes at this stage with a noninvasive method will facilitate the search for treatments that can delay or stop the progression of AD. This study also demonstrates that, although many different approaches have already been taken in this field, there is still much more to explore and much that we do not understand with respect to the effects of AD on the retina.

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RESEARCH IN CONTEXT

1. Systematic review: We reviewed 15+ years of literature on the relationship between the morphology of retinal neuronal layers and Alzheimer's disease (AD), and the evidence of disease-related retinal change over the progression of AD. This literature is mixed, with inconsistent findings, and it consists mostly of cross-sectional studies in patients with symptomatic disease. We were unable to identify prior publications that have sought to explore within-subjects longitudinal change in retinal morphology during the preclinical stage of AD for all retinal neuronal layers.
2. Interpretation: After controlling for expected effects of normal aging, we observed a decrease in the macular retinal nerve fiber layer that is moderately correlated with positron emission tomography imaging evidence of amyloid aggregation. Volume loss in the macular retinal nerve fiber layer, during the preclinical stage, is not related to performance on measures of episodic memory or problem solving but is modestly related to relative decrements in performance on a measure of visual-auditory integration of new information. This may be a first report of neuronal retinal layer volumetric changes in a within-subjects, prospective, longitudinal study of preclinical AD.
3. Future directions: Larger populations of participants, across the entire disease severity spectrum (from healthy controls to mild AD), should be followed up for an even longer time interval to better model the natural history of retinal anatomic changes over the entire course of disease progression. Additional imaging methods could improve our understanding of the mechanisms of disease-related retinal change, such as optical coherence tomography angiography and scanning polarimetry.

References

[1] Lamb TD, Collin SP, Pugh EN. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. Nat Rev Neurosci 2007;8:960–76.
[2] Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, et al. Optical coherence tomography. Science 1991;254:1178–81.
[3] Mahajan D, Votruba M. Can the retina be used to diagnose and plot the progression of Alzheimer's disease? Acta Ophthalmol 2017;95:S259.
[4] Koronyo-Hamaoui M, Koronyo Y, Ljubimov AV, Miller CA, Ko MK, Schwartz M, et al. Identification of amyloid plaques in retinas from Alzheimer's patients and noninvasive in vivo optical imaging of retinal plaques in mouse model. NeuroImage 2011;54:S204–17.

[5] Cunha LP, Lopes LC, Costa-Cunha LV, Costa CF, Pires LA, Almeida AL, et al. Macular thickness measurements with frequency domain-OCT for quantification of retinal neural loss and its correlation with cognitive impairment in Alzheimer's disease. PLoS One 2016; 11:e0153830.
[6] Danesh-Meyer HV, Birch H, Ku JYF, Carroll S, Gamble G. Reduction of optic nerve fibers in patients with Alzheimer disease identified by laser imaging. Neurology 2006;67:1852–4.
[7] Blanks JC, Schmidt SY, Torigoe Y, Porrello KV, Hinton DR, Blanks RHI. Retinal pathology in Alzheimer's disease. II. Regional neuron loss and glial changes in GCL. Neurobiol Aging 1996; 13:385–95.
[8] Thomson KL, Yeo JM, Waddel B, Cameron JR, Paj S. A systematic review and meta-analysis of retinal nerve fiber layer change in dementia, using optical coherence tomography. Alzheimers Dement (Amst) 2015;1:136–43.
[9] Iseri PK, Altinaş O, Tokay T, Yüksel NJ. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in Alzheimer disease. Neuroophthalmol 2006;26:18–24.
[10] Lu Y, Li Z, Zhang X, Ming B, Jia J, Wang R, et al. Retinal nerve fiber layer structure abnormalities in early Alzheimer's disease: evidence in optical coherence tomography. Neurosci Lett 2010;480:69–72.
[11] Jindahra P, Hedges TR, Mendoza-Santiesteban CE, Plant GT. Optical coherence tomography of the retina: applications in neurology. Curr Opin Neurol 2010;18:1864–9.
[12] Valenti DA. Alzheimer's disease and glaucoma: imaging the biomarkers of neurodegenerative disease. Int J Alzheimer's Dis 2010;2010:9.
[13] Cunha JP, Proença R, Dias-Santos A, Almeida R, Águas H, Alves M, et al. OCT in Alzheimer's disease: thinning of the RNFL and superior hemiretina. Graefes Arch Clin Exp Ophthalmol 2017;255:1827–35.
[14] Golzan SM, Goozee K, Georgevsky D, Avolio A, Chatterjee P, Shen K, et al. Retinal vascular and structural changes are associated with amyloid burden in the elderly: ophthalmic biomarkers of preclinical Alzheimer's disease. Alzheimers Res Ther 2017;9:13.
[15] Ferrari L, Huang SC, Magnani G, Ambrosi A, Comi G, Leocani L. Optical coherence tomography reveals retinal neuroaxonal thinning in frontotemporal dementia as in Alzheimer's disease. J Alzheimers Dis 2017;56:1101–7.
[16] Snyder PJ, Johnson LN, Lim YY, Santos CY, Alber J, Maruff P, et al. Nonvascular retinal imaging markers of preclinical Alzheimer's disease. Alzheimers Dement (Amst) 2016;4:169–78.
[17] Choi SH, Park SJ, Kim NR. Macular ganglion cell -inner plexiform layer thickness is associated with clinical progression in mild cognitive impairment and Alzheimer's disease. PLoS One 2016;11:e0162202.
[18] Trebbastoni A, D'Antonio F, Bruscolini A, Marcelli M, Cecere M, Campanelli A, et al. Retinal nerve fibre layer thickness changes in Alzheimer's disease: results from a 12-month prospective case series. Neurosci Lett 2016;629:165–70.
[19] Feke GT, Hyman BT, Stern RA, Pasquale LR. Retinal blood flow in mild cognitive impairment and Alzheimer's disease. Alzheimers Dement (Amst) 2015;1:144–51.
[20] Garcia-Martin E, Bambo MP, Marques ML, Satue M, Otin S, Larrosa J, et al. Ganglion cell layer measurements correlate with disease severity in patients with Alzheimer's disease. Acta Ophthalmol 2016;94:e454–9.
[21] Pillai JA, Bermel R, Bonner-Jackson A, Rae-Grant A, Fernandez H, Bena J, et al. Retinal nerve fiber layer thinning in Alzheimer's disease a case–control study in comparison to normal aging, Parkinson's disease, and non-Alzheimer's dementia. Am J Alzheimers Dis Other Demen 2016;31:430–6.
[22] Eraslan M, Çerman E, Çekiç O, Balci S, Dericioğlu V, Sahin Ö, et al. Neurodegeneration in ocular and central nervous systems: optical coherence tomography study in normal-tension glaucoma and Alzheimer disease. Turk J Med Sci 2015;45:1106–14.
[23] Güneş A, Demirci S, Tök L, Tök Ö, Demirci S. Evaluation of retinal nerve fiber layer thickness in Alzheimer disease using spectral-domain optical coherence tomography. Turk J Med Sci 2015; 45:1094–7.
[24] Cesareo M, Martucci A, Ciuffoletti E, Mancino R, Cerulli A, Sorge RP, et al. Association between Alzheimer's disease and glaucoma: a study based on Heidelberg retinal tomography and frequency doubling technology perimetry. Front Neurosci 2015;9:479.
[25] Salobrar-Garcia E, Hoyas I, Leal M, de Hoz R, Rojas B, Ramirez AI, et al. Analysis of retinal peripapillary segmentation in early Alzheimer's disease patients. Biomed Res Int 2015;2015:636548.
[26] La Morgia C, Ross-Cisneros FN, Koronyo Y, Hannibal J, Gallassi R, Cantalupo G, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease. Ann Neurol 2016;79:90–109.
[27] Shi Z, Zhu Y, Wang M, Wu Y, Cao J, Li C, et al. The utilization of retinal nerve fiber layer thickness to predict cognitive deterioration. J Alzheimers Dis 2016;49:399–405.
[28] Liu S, Ong YT, Hilal S, Loke YM, Wong TY, Chen CLH, et al. The association between retinal neuronal layer and brain structure is disrupted in patients with cognitive impairment and Alzheimer's disease. J Alzheimers Dis 2016;54:585–95.
[29] Oktem EO, Derle E, Kibaroglu S, Oktem C, Akkoyun I, Can U. The relationship between the degree of cognitive impairment and retinal nerve fiber layer thickness. Neurol Sci 2015;36:1141–6.
[30] Gao L, Liu Y, Li X, Bai Q, Liu P. Abnormal retinal nerve fiber layer thickness and macula lutea in patients with mild cognitive impairment and Alzheimer's disease. Arch Gerontol Geriatr 2015;60:162–7.
[31] Cheung CY, Ong YT, Hilal S, Ikram MK, Low S, Ong YL, et al. Retinal ganglion cell analysis using high-definition optical coherence tomography in patients with mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis 2015;45:45–56.
[32] Kromer R, Serbecic N, Hausner L, Froelich L, Aboul-Enein F, Beutelspacher SC. Detection of retinal nerve fiber layer defects in Alzheimer's disease using SD-OCT. Front Psychiatry 2014;25:22.
[33] Bambo MP, Garcia-Martin E, Pinilla J, Herrero R, Satue M, Otin S, et al. Detection of retinal nerve fiber layer degeneration in patients with Alzheimer's disease using optical coherence tomography: searching new biomarkers. Acta Ophthalmol 2014; 92:e581–2.
[34] Marziani E, Pomati S, Ramolfo P, Cigada M, Giani A, Mariani C, et al. Evaluation of retinal nerve fiber layer and ganglion cell layer thickness in Alzheimer's disease using spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci 2013;54:5953–8.
[35] Kirbas S, Turkyilmaz K, Anlar O, Tufekci A, Durmus MJ. Retinal nerve fiber layer thickness in patients with Alzheimer disease. Neuroophthalmol 2013;33:58–61.
[36] Moschos MM, Markopoulos I, Chatziralli I, Rouvas A, Papageorgiou SG, Ladas I, et al. Structural and functional impairment of the retina and optic nerve in Alzheimer's disease. Curr Alzheimer Res 2012;9:782–8.
[37] Kesler A, Vakhapova V, Korczyn AD, Naftaliev E, Neudorfer M. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Clin Neurol Neurosurg 2011;113:523–6.
[38] Chi Y, Wang YH, Yang L. The investigation of retinal nerve fiber loss in Alzheimer's disease. Zhonghua Yan Ke Za Zhi 2010;46:134–9.
[39] Paquet C, Boissonnot M, Roger F, Dighiero P, Gil R, Hugon J. Abnormal retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Neurosci Lett 2007;420:97–9.
[40] Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. Invest Ophthalmol Vis Sci 2007;48:2285–9.
[41] Parisi V, Restuccia R, Fattapposta F, Mina C, Bucci MG, Pierelli F. Morphological and functional retinal impairment in Alzheimer's disease patients. Clin Neurophysiol 2001;112:1860–7.
[42] Kergoat H, Kergoat MJ, Justino L, Chertkow H, Robillard A, Bergman H. An evaluation of the retinal nerve fiber layer thickness by scanning laser polarimetry in individuals with dementia of the Alzheimer type. Acta Ophthalmol Scand 2001;79:187–91.

[43] Hedges TR 3rd, Perez Galves R, Speigelman D, Barbas NR, Peli E, Yardley CJ. Retinal nerve fiber layer abnormalities in Alzheimer's disease. Acta Ophthalmol Scand 1996;74:271–5.
[44] Budenz DL, Anderson DR, Varma R, Schuman J, Cantor L, Savell J, et al. Determinants of normal retinal nerve fiber layer thickness measured by stratus OCT. Ophthalmology 2007;114:1046–52.
[45] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:270–9.
[46] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:280–92.
[47] Moreno RT, Benito LJ, Villarejo A, Bermejo FP. Retinal nerve fiber layer thinning in dementia associated with Parkinson's disease, dementia with Lewy bodies, and Alzheimer's disease. J Alzheimers Dis 2013;34:659–64.
[48] Spencer RJ, Wendell CR, Giggey PP, Katzel LI, Lefkowitz DM, Siegel EL, et al. Psychometric limitations of the Mini-Mental State Examination among nondemented older adults: an evaluation of neurocognitive and magnetic resonance imaging correlates. Exp Aging Res 2013;39:382–97.
[49] Knoll B, Simonett J, Vole NJ, Farsiu S, Ward M, Rademaker A, et al. Retinal nerve fiber layer thickness in amnestic mild cognitive impairment: Case-control study and meta-analysis. Alzheimers Dement (Amst) 2016;4:85–93.
[50] Shen Y, Liu L, Cheng Y, Feng W, Shi Z, Zhu Y, et al. Retinal nerve fiber layer thickness is associated with episodic memory deficit in mild cognitive impairment patients. Curr Alzheimer Res 2014;11:259–66.
[51] Snyder PJ, Bednar MM, Cromer JR, Maruff P. Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. Alzheimers Dement 2005;1:126–35.
[52] Fredrickson A, Snyder PJ, Cromer J, Thomas E, Lewis M, Maruff P. The use of effect sizes to characterize the nature of cognitive change in psychopharmacological studies: an example with scopolamine. Hum Psychopharmacol 2008;23:1–11.
[53] Thompson TA, Wilson PH, Snyder PJ, Pietrzak RH, Darby D, Maruff P, et al. Sensitivity and test-retest reliability of the international shopping list test in assessing verbal learning and memory in mild Alzheimer's disease. Arch Clin Neuropsychol 2011;26:412–24.
[54] Ames D, Ellis KA, Harrington K, Lachovitzki R, Lim YY, Maruff P, et al. Short term stability of verbal memory impairment in mild cognitive impairment and Alzheimer's disease measured using the International Shopping List Test. J Clin Exp Neuropsychol 2012;34:853–63.
[55] Delbeuck X, Van der Linden M, Collette F. Alzheimer's disease as a disconnection syndrome? Neuropsychol Rev 2003;13:79–92.
[56] Morrison J, Hof P, Bouras C. An anatomic substrate for visual disconnection in Alzheimer's disease. Ann N Y Acad Sci 1991;640:36–43.
[57] Gold BT, Johnson NF, Powell DK, Smith CD. White matter integrity and vulnerability to Alzheimer's disease: preliminary findings and future directions. Biochim Biophys Acta 2012;1822:416–22.
[58] Zhuang L, Sachdev PS, Trollor JN, Kochan NA, Reppermund S, Brodaty H, et al. Microstructural white matter changes in cognitively normal individuals at risk of amnestic MCI. Neurology 2012;79:748–54.
[59] Nir TM, Jahanshad N, Toga AW, Bernstein MA, Jack CR Jr, Weiner MW, et al. Connectivity network measures predict volumetric atrophy in mild cognitive impairment. Neurobiol Aging 2015;36(Suppl 1):S113–20.
[60] Festa E, Katz AP, Ott BR, Tremont G, Heindel WC. Dissociable effects of aging and mild cognitive impairment on bottom-up audiovisual integration. J Alzheimers Dis 2017;59:155–67.
[61] Snyder PJ, Lim YY, Schindler R, Ott BR, Salloway S, Daiello L, et al. Microdosing of scopolamine as a 'cognitive stress test': rationale and test of a very low dose in an at-risk cohort of older adults. Alzheimers Dement 2014;10:262–7.
[62] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–9.
[63] Lim YY, Maruff P, Schindler R, Ott BR, Salloway S, Yoo DC, et al. Disruption of cholinergic neurotransmission exacerbates Aβ-related cognitive impairment in preclinical Alzheimer's disease. Neurobiol Aging 2015;36:2709–15.
[64] Maruff P, Lim YY, Darby D, Ellis KA, Pietrzak RH, Snyder PJ, et al. Clinical utility of the Cogstate Brief Battery in identifying cognitive impairment in mild cognitive impairment and Alzheimer's disease. BMC Psychol 2013;1:1–11.
[65] Peng C, Wang W, Xu Q, Yang M, Zhou H, Zhao S, et al. Thickness of macular inner retinal layers and peripapillary retinal nerve fibre layer in neuromyelitis optica spectrum optic neuritis and isolated optic neuritis with one episode. Acta Ophthalmol 2017;95:583–90.
[66] Hong D, Bosc C, Chiambaretta F. Progression of nerve fiber layer defects in retrobulbar optic neuritis by the macular ganglion cell complex. J Fr Ophtalmol 2017;40:777–87.
[67] Lim HB, Lee MW, Kwak BS, Jo YJ, Kim JY. Longitudinal changes in thicknesses of the macula, ganglion cell-inner plexiform layer, and retinal nerve fiber layer after vitrectomy: a 12-Month observational study. Retina 2018;38:155–62.
[68] Cacciamani A, Cosimi P, Di Nicola M, Di Martino G, Ripandelli G, Scarinci F. Correlation between outer retinal thickening and retinal function impairment in patients with idiopathic epiretinal membranes [published online ahead of print November 28, 2017]. Retina 2017. https://doi.org/10.1097/IAE.0000000000001971.
[69] Clark CM, Schneider JA, Bedell BB, Beach TG, Bilker WB, Mintun MA, et al. Use of florbetapir-PET for imaging amyloid-β pathology. JAMA 2011;305:275–83.
[70] Tan ACS, Fleckenstein M, Schmitz-Valckenberg S, Hols FG. Clinical application of multicolor imaging technology. Ophthalmologica 2016;236:8–18.
[71] Thomas E, Snyder PJ, Pietrzak RH, Maruff P. Behavior at the choice point: decision making in hidden pathway maze learning. Neuropsychol Rev 2014;24:514–36.
[72] Thomas E, Snyder PJ, Pietrzak RH, Jackson CE, Bednar M, Maruff P. Specific impairments in visual spatial working memory following low dose scopolamine challenge in healthy older adults. Neuropsychology 2008;46:2476–84.
[73] Pietrzak RH, Cohen H, Snyder PJ. Spatial learning efficiency and error monitoring in normal aging: an investigation using a novel hidden maze learning test. Arch Clin Neuropsychol 2007;22:235–45.
[74] Papp KS, Snyder PJ, Maruff P, Bartkowiak J, Pietrzak RH. Detecting subtle changes in visuospatial executive function and learning in the amnestic variant of mild cognitive impairment. PLoS One 2011;6:e21688.
[75] Lim YY, Prang KH, Cysique L, Pietrzak RH, Snyder PJ, Maruff P. A method for cross-cultural adaptation of a verbal memory assessment. Behav Res Methods 2009;41:1190–200.
[76] Crook TH, Feher EP, Larrabee GJ. Assessment of memory complaint in age-associated memory impairment: the MAC-Q. Int Psychogeriatr 1992;4:165–76.
[77] McGurk H, MacDonald J. Hearing lips and seeing voices. Nature 1976;264:746–8.
[78] Hood DC, Fortune B, Arthur SN, Xing D, Salant JA, Ritch R, et al. Blood vessel contributions to retinal nerve fiber layer thickness profiles measured with optical coherence tomography. J Glaucoma 2010;17:519–28.
[79] Leung CK, Medeiros FA, Zangwill LM, Sample PA, Bowd C, Ng D, et al. American Chinese glaucoma imaging study: a comparison of the optic disc and retinal nerve fiber layer in detecting glaucomatous damage. Invest Ophthalmol Vis Sci 2007;48:2644–52.

[80] Duan XR, Liang YB, Friedman DS, Sun LP, Wong TY, Tao QS, et al. Normal macular thickness measurements using optical coherence tomography in healthy eyes of adult Chinese persons: the Handan Eye Study. Ophthalmology 2010;117:1585–94.
[81] Kim NR, Lim HL, Kim JH, Rho SS, Seong GJ, Kin CY. Factors associated with false positives in retinal nerve fiber layer color codes from spectral-domain optical coherence tomography. Ophthalmology 2011; 118:1774–81.
[82] Langenegger SJ, Funk J, Toteberg-Harms M. Reproducibility of retinal nerve fiber layer thickness measurements using the Eye Tracker and the retest function of SPECTRALIS SD-OCT in glaucomatous and healthy control eyes. IOVS 2011;52:3338–44.
[83] Leung CKS, Yu M, Weinreb RN, Ye C, Liu S, Lai G, et al. Retinal nerve fiber layer imaging with spectral-domain optical coherence tomography. A prospective analysis of age-related loss. Ophthalmology 2012;119:731–7.
[84] Blanks JC, Torigoe Y, Hilton DR, Blanks RH. Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. Neurobiol Aging 1996;17:377–84.
[85] Sadun AA, Bassi CJ. Optic nerve damage in Alzheimer's disease. Ophtalmology 1990;97:9–17.
[86] Garcia-Martin ES, Rojas B, Ramirez AI, de Hoz R, Salazar JJ, Yubero R, et al. Macular thickness as a potential biomarker of mild Alzheimer's disease. Ophthalmology 2014;121:1149–51.
[87] Trick GL, Trick LR, Morris P, Wolf M. Visual field loss in senile dementia of the Alzheimer's type. Neurology 1995;45:68–74.
[88] Mutlu U, Colijn JM, Licher S, Wolters FJ, Pieter WM, Caroline CW, et al. Retinal neurodegeneration optical coherence tomography and risk of dementia and stroke. Alzheimers Assoc 2017;13:1014–5.
[89] Ko F, Gallacher J, Muthy Z, Khaw K, Reisman CA, Yang Q, et al. Retinal nerve fiber layer thinning associated with poor cognitive function among a large cohort, the UK biobank. Alzheimers Dement 2016; 12:317–8.
[90] de la Monte SM. Quantitation of cerebral atrophy in preclinical and end-stage Alzheimer's disease. Ann Neurol 1989;25:450–9.
[91] Oishi A, Fang PP, Thiele S, Holz FG, Krone TU. Longitudinal change of outer nuclear layer after retinal pigment epithelial tear secondary to age-related macular degeneration [published online ahead of print May 10, 2017]. Retina 2017. https://doi.org/10.1097/IAE.0000000000001688.
[92] Pilat A, McLean RJ, Proudlock FA, Maconachie GD, Sheth V, Rajabally YA, et al. In vivo morphology of the optic nerve and retina in patients with Parkinson's disease. Invest Ophthalmol Vis Sci 2016; 57:4420–7.
[93] Petzold A, Balcer LJ, Calabresi PA, Costello F, Frohman TC, Frohman EM, et al. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol 2017;16:797–812.
[94] Pisa M, Guerrieri S, Di Maggio G, Medaglini S, Moiola L, Martinelli V, et al. No evidence of disease activity is associated with reduced rate of axonal retinal atrophy in MS. Neurology 2017;89:2469–75.
[95] Satue M, Obis J, Rodrigo MJ, Otin S, Fuertes MI, Vilades E, et al. Optical coherence tomography as a biomarker for diagnosis, progression, and prognosis of neurodegenerative diseases. J Ophthalmol 2016; 2016:8503859.
[96] Heaton G, Benjamin M, Lisa A, Cordeiro M. Ocular biomarkers of Alzheimer disease. Cent Nerv Syst Agents Med Chem 2015;5:117–25.
[97] Fortunate B, Burgoyne CF, Reynayd J, Wang L. Onset and progression of peripapillary retinal nerve fiber layer (RNFL) retardance changes occur earlier than RNFL thickness changes in experimental glaucoma. IOVS 2013;54:5653–61.
[98] Fortunate B, Cull G, Reynaud J, Burgoyne CF. Relating retinal ganglion cell function and retinal nerve fiber layer (RNFL) retardance to progressive loss of RNFL thickness and optic nerve axons in experimental glaucoma. IOVS 2015;56:3936–44.
[99] Zhou Q, Knighton RW. Light scattering and form birefringence of parallel cylindrical arrays that represent cellular organelles of the retinal nerve fiber layer. Appl Opt 1997;36:2273–85.