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Longitudinal analysis of the retina and choroid in cognitively normal individuals at higher genetic risk for Alzheimer disease

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Abstract

Purpose: To assess baseline differences and longitudinal rate of change in retinal and choroidal imaging parameters between APOE ε4 carriers and non-carriers with normal cognition.

Design: Prospective study.

Subjects: 413 eyes of 218 individuals with normal cognition aged ≥55 years with known APOE status (98 ε4 carriers, 120 non-carriers). Exclusion criteria included diabetes mellitus, uncontrolled hypertension, glaucoma, and vitreoretinal or neurodegenerative disease.

Methods: Optical coherence tomography (OCT) and OCT angiography (OCTA) was performed at baseline and at 2 years [Zeiss Cirrus HD-OCT 5000 with AngioPlex (Zeiss Meditec, Dublin, CA)]. Groups were compared using sex- and age-adjusted generalized estimating equations.

Main Outcome Measures: OCT: retinal nerve fiber layer thickness, macular ganglion cell-inner plexiform layer thickness, central subfield thickness (CST), choroidal vascularity index.

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OCTA: foveal avascular zone area, perfusion density (PD), vessel density, peripapillary capillary perfusion density and capillary flux index (CFI). Rate of change per year was calculated.

Results: At baseline, ε4 carriers had lower CST (p=0.018), PD in the 6mm Early Treatment Diabetic Retinopathy Study (ETDRS) circle (p=0.049), and temporal CFI (p=0.047). Seventy-one ε4 carriers and 78 non-carriers returned at 2 years; at follow-up, the 6mm ETDRS circle (p=0.05) and outer ring (p=0.049) showed lower PD in ε4 carriers, with no differences in rates of change between groups (all p>0.05).

Conclusions: There was exploratory evidence of differences in CST, PD, and peripapillary CFI between APOE ε4 carriers and non-carriers, both with normal cognition. Larger and longer-term studies may further elucidate the potential value of these findings.

INTRODUCTION

Alzheimer disease (AD) is associated with comorbid medical conditions such as diabetes mellitus, hypertension, and cerebrovascular disease, among others.[1-3] However, genetics may contribute to about 70% of cases with late-onset AD,[4-6] which has onset after age 65 and is the most common form of AD.[2,7] The identification of an association between the apolipoprotein E (APOE) gene ε4 allele and late-onset AD has afforded the opportunity to better understand the heritability of AD and the potential to identify individuals at risk prior to symptom onset.[8-10] The presence of an APOE ε4 allele remains the strongest known genetic risk factor for late-onset AD.[11-13] The precise contribution of the ε4 allele to AD pathophysiology and its utility in clinical diagnosis and management however, are still evolving.[2,14] The less frequent APOE ε2 allele is potentially neuroprotective, whereas the most frequent allele, APOE ε3, is neither protective nor associated with increased risk.[11,15-17] Given that every person carries 2 alleles in the APOE gene, the higher risk spectrum of APOE genetic combinations is ε4/ε4 > ε4/ε3 > ε4/ε2 in comparison to the 'neutral' ε3/ε3 and lower risk ε3/ε2 and ε2/ε2 phenotypes.[17-19]

Validated diagnostic and prognostic tools such as positron emission tomography (PET) and structural and functional magnetic resonance imaging (MRI) have demonstrated some capability in differentiating ε4 carriers who have higher genetic risk for AD, and non-carriers who have lower genetic risk for AD.[20-23]. Previous investigations have demonstrated the increased presence of characteristic amyloid[24] and tau proteins[23,25], decreased metabolic activity on PET,[20,26-29] structural loss on MRI and abnormal functional MRI activation.[21,22] However, these methods are neither practical nor economical as screening tools in asymptomatic cognitively intact individuals, with their use often limited to those with high clinical suspicion.[29] The immediate clinical utility and impact of APOE testing and status disclosure are of uncertain value at this time, with some advocating that there may be unnecessary psychological burden related to disclosure of APOE status.[14,30] However, the Risk Evaluation and Education for Alzheimer's Disease (REVEAL) trial demonstrated some evidence that the benefits of disclosing pre-symptomatic APOE status may outweigh the potential psychological burden.[31] In individuals who have knowledge of potentially increased AD risk, studies have indicated an increased tendency towards making behavior and lifestyle modifications in an effort to minimize acquired risk and making more prudent decisions including purchasing of healthcare insurance and advanced care planning.[14,30,31]

Given the current limitations of the aforementioned imaging modalities, alternative modalities such as multimodal retinal imaging may be better suited to assess the potential changes associated with APOE genotype. However, little is currently known about retinal changes in cognitively normal APOE carriers. To our knowledge, only one prior study of the retinal microvasculature has included APOE status as the primary driver of the analysis, while others have only performed sub-analyses in other comparisons.[32-35]

In this study, we seek to identify alterations in the retinal and choroidal structure and microvasculature using OCT and OCTA in a large cohort of individuals with normal cognition and known APOE status, stratified into the higher risk ε4 carrier group (APOE ε4+) and lower risk ε4 non-carrier group (APOE ε4−). In addition to the baseline cross-sectional comparison, this cohort was further studied longitudinally over a 2-year period to characterize the differential rate of change in retinal and choroidal structural and microvascular parameters between the 2 groups.

METHODS

Protocol

This longitudinal study was approved by the Duke University School of Medicine Institutional Review Board (Pro00082598) and in compliance with the Declaration of Helsinki and the Health Insurance Portability and Accountability Act. Written informed consent was received from all subjects or designated legally authorized representative. This study is registered with the identifier NCT03233646 at clinicaltrials.gov.

Participants and Enrollment

Volunteer participants from the community aged 55 years or older were recruited from a subset of the Duke Alzheimer Disease Prevention Registry that participated in the PREPARE cohort. The participants did not have dementia or cognitive impairment based on prior screening with the Montreal Cognitive Assessment (MoCA), had contributed DNA for APOE genotyping as part of enrollment in the PREPARE cohort, and also agreed to participate in our study. The genetic status risk level (Group A = APOE ε4+; Group B = APOE ε4−) was masked for all investigators upon enrollment in the present study and during analysis. Unmasking was performed only upon completion of statistical analysis. Exclusion criteria included any of the following potentially confounding ocular or systemic comorbidities: diabetes mellitus, glaucoma, uncontrolled hypertension, demyelinating disorders, history of vitreoretinal or optic nerve disease, or corrected distance visual acuity (VA) worse than 20/40 on the day of image acquisition (as a surrogate for exclusion criterion of abnormal axial length greater than 6 diopters of magnitude in correction).[36] Family history of dementia (including, but not limited to AD) in any first-degree relative and years of education were collected.

At study entry and at follow-up 2 years later, a Mini-Mental State Examination (MMSE) was administered, and VA was measured in each eye. Participants underwent OCT and OCTA imaging performed by an experienced technician. Participants also underwent ultra-widefield fundus photography with scanning laser ophthalmoscopy (California, Optos,

Marlborough, MA). Review of fundus images served as a surrogate for a dilated fundus examination to screen for ocular pathology that would preclude participation.

OCT Acquisition

OCT images were acquired with a Zeiss Cirrus HD-OCT 5000, and retinal parameters were quantified by the native OCT software (Cirrus HD-OCT Review, version 11.5.0.40427). A 200x200 optic disc cube scan was obtained to measure average thickness of the retinal nerve fiber layer (RNFL), calculated within a circle of 3.46mm in diameter centered upon the optic disc. A 512×128 macular cube scan was used to measure average ganglion cell-inner plexiform layer (GC-IPL) thickness and central subfield thickness (CST) (neurosensory layer thickness between the inner limiting membrane and retinal pigment epithelium at the center of the fovea) within a standard fovea-centered elliptical annulus (14.13mm^2 area).

An HD 21-line scan with enhanced depth imaging was acquired and total choroidal area (TCA) and luminal area (LA) were measured using ImageJ (ImageJ, National Institutes of Health, Bethesda, MD); choroidal vascularity index (CVI) was calculated as the ratio of LA to TCA expressed as a percentage.[37,38] TCA was demarcated by a skilled reviewer using a polygon selection in ImageJ, capturing the available length of the visible choroid on each scan within anatomic boundaries and excluding non-choroidal tissue. The retinal pigmented epithelium marked as the upper boundary and the choroid scleral junction marked as the lower boundary. Lateral boundaries of this polygon were defined by the optic nerve head boundary and the most extreme edge of the image, essentially capturing the area of choroidal tissue over the maximal length of the image that was not obscured by the optic nerve head. This polygon was saved as the region of interest (ROI) using an ROI manager for subsequent analysis without additional changes, sub-selection, or cropping. Binary images were generated with Niblack's auto local threshold to give a contrast of dark and light pixels to distinguish areas of interest from the background of the un-cropped and original-sized image (Supplementary Figure 1A. All supplementary figures are available online at: https://www.ophthalmologyretina.org/).). The binarized image was then converted to red, green, blue (RGB) color to select a threshold color for the outline of dark and light pixels (Supplementary Figure 1B). The color selection was added to the ROI manager, and the area that intersected the TCA polygon was selected (Supplementary Figure 1C). This area was denoted as LA which was defined as the area of dark pixels within TCA. TCA and LA were calculated using the ROI manager. Supplementary Figure 2 shows an overlay of the ROI on the original OCT scan, indicating the luminal area (dark pixels) or total choroidal area subtracting the stromal area (light pixels). Poor quality images (defined as those with signal strength <7, motion artifact, shadow artifact, scan clipping, or poor resolution that impacts segmentation and automated measurements) were excluded.

OCTA Acquisition

OCTA imaging was also performed using the Zeiss Cirrus HD-OCT 5000 with AngioPlex (version 11.0.0.29946, Carl Zeiss, Meditec, Dublin, CA) with the intrinsic software providing algorithmic analysis of the retinal microvasculature. Fovea-centered 3×3mm and 6×6mm scans of the superficial capillary plexus slab, between the internal limiting membrane and the inner plexiform layer, were acquired in each eye. Images with low signal

strength (<7/10), poor centration, segmentation error, or shadow or motion artifact were excluded after manual review. Vessel density (VD), the total length of perfused vasculature per unit area, and perfusion density (PD), the total area occupied by perfused vasculature per unit area, were quantified in selected ETDRS subfields. Perfusion density is an area-based measurement wherein larger vessels have a greater influence on the measurement, whereas for vessel density, which is a length-based measurement, all vessels influence the measurement equally. Length-based measurements are more sensitive to changes in smaller capillaries. Measurement terminologies can differ across OCTA platforms, and it is important to recognize whether the measurements are area-based or length-based.[39] For 3×3mm scans, VD and PD were measured in the 3mm circle and 3mm ring (Supplementary Figure 3). The foveal avascular zone (FAZ) was automatically demarcated by the AngioPlex software, and its area quantified. FAZ boundaries were also manually reviewed; and if ungradable, those images were excluded from analysis. For 6×6mm scans, VD and PD were measured in the 6mm ETDRS circle and outer and inner rings (Supplementary Figures 3A-E). Peripapillary OCTA was also performed, with capillary perfusion density (CPD) and capillary flux index (CFI) extracted from 4.5×4.5mm optic disc-centered scans and reported by sector and as an area average (Supplementary Figure 3F). Both CPD and CFI were measured in the retinal peripapillary capillary plexus. Images were binarized at the pixel level by vessel or not-vessel status. The binarized image was also used to generate a skeletonized network of vessels 1 pixel in width. CPD was defined as the ratio of pixels in the binarized image map that also appear in the skeletonized vessel map expressed as a percent. CFI was calculated as the ratio of perfused vascular area per unit of image area weighted by vessel flow.[40,41]

Statistical Analysis

Statistical analysis of the masked groups was completed (S.S.S.) using SAS (SAS Institute, Cary, NC, Software Version 9.4). Demographic characteristics of participants in each group at each timepoint were compared using Fisher's exact test of difference between proportions for categorical variables, and the Wilcoxon rank sum test of difference between medians for continuous variables. OCT and OCTA retinal and choroidal imaging parameters were analyzed and compared between the APOE ε4+ and ε4− groups using multivariate generalized estimating equations (GEE) to adjust for age and sex differences between groups at baseline and follow-up. To characterize rate of change, the initial value of a given parameter was subtracted from the value at follow-up and divided by the time interval (years) between the visits; this value, rate of change, was compared between the APOE ε4+ and ε4− groups. An α significance level of 0.05 was used for all hypotheses. As this was a discovery study, statistics were not adjusted for multiple comparisons, as to not overlook any signal of potential differences.

RESULTS

At baseline, 186 eyes of 98 participants in the ε4+ group (carriers) and 227 eyes of 120 participants in the ε4− group (non-carriers) were enrolled and imaged (Table 1). In the ε4+ group, 89/98 (90.8%) participants had only one APOE ε4 allele, whereas 9/98 (9.2%) were homozygous for the APOE ε4 allele. Between groups, there were no significant differences

in mean age, mean number of years of education, proportion of female participants, or racial composition (all $p > 0.05$). In ε4 carriers, a significantly greater proportion of participants had a known family history of any type of dementia including AD (68% vs, 53%; $p = 0.027$). There was no difference in MMSE score between groups at baseline.

At baseline, there was a significantly lower CST in the ε4 carrier group (263.0 microns) vs the non-carrier group (268.8 microns) ($p = 0.018$). There was no significant difference in average GC-IPL thickness or RNFL thickness between groups (both $p > 0.05$) (Table 2). Total choroidal area, luminal area, and choroidal vascularity index did not differ between groups (all $p > 0.05$) (Table 2). At baseline, there were no differences in 3×3mm VD or PD in the 3mm ETDRS circle or ring or FAZ area (all $p > 0.05$) (Supplementary Figure 1). There was a slightly lower 6×6mm PD in the 6mm ETDRS circle ($p= 0.049$). However, there were no significant PD differences in the 6mm ETDRS inner or outer ring subfields. There were no differences in VD in the 6mm circle or either ring subfield. Among measurements of peripapillary OCTA, APOE ε4 carriers demonstrated slightly lower CFI in the temporal sector ($p = 0.047$) compared to non-carriers at baseline (Table 2). However, when baseline CFI in the temporal sector was examined in only those subjects who eventually returned for follow-up imaging, this trend of lower CFI in APOE ε4 carriers was not observed.

After an average interval of 25.6 months (range, 22.3 – 35.2 months) from baseline imaging, 71/98 subjects (72.4%) in the ε4 carrier group and 78/120 subjects (65.0%) in the non-carrier group returned for repeat OCT and OCTA imaging, and thus 134 eyes of 71 APOE ε4+ participants and 149 eyes of 78 in the APOE ε4− group were re-imaged. No subject presenting for follow-up achieved an abnormal MMSE score on the day of re-imaging that would suggest new-onset cognitive impairment or dementia during the follow-up interval. The delay of some participants was attributed to the SARS-CoV-2 (COVID-19) pandemic during which select clinical research protocols were temporarily paused. Of the 69/218 subjects (31.6%) who did not return for the 2-year follow-up visit, 35 (50.7%) declined participation due to concerns regarding the COVID-19 pandemic, and the other 34/69 subjects (49.3%) declined to return for repeat imaging, citing a combination of health, travel, or time-commitment reasons. Considering only subjects imaged longitudinally, the APOE ε4− cohort was significantly less racially diverse in comparison to the APOE ε4+ cohort, and there was no difference in family history of dementia. Otherwise, the demographics of subjects imaged longitudinally were similar to that of the total initial cohort including those who did not follow-up, with no significant differences in age, years of education, MMSE score at baseline or 2-year follow-up, or proportion of female subjects (Table 3). There may be a selection bias since only patients who returned to be imaged could be reported in the longitudinal arm of this study, potentially leading to selective differences in follow-up.

Within-group analysis of retinal imaging parameters for subjects imaged longitudinally is reported in Table 4. There were isolated signals of decrease in CST ($p = 0.015$) in the ε4− group after 2 years, increase in FAZ area ($p = 0.010$) and decrease in 6mm outer ring VD ($p = 0.039$) in the ε4+ group. Both the ε4− and ε4+ groups generally showed slight decline in CPD and CFI ($p < 0.05$) save for superior sector CPD (both $p> 0.05$) and inferior

sector CPD which showed slight increase in both groups. Notably, the standard deviation was larger than the mean difference after 2 years for all imaging parameters.

Between-group analysis of retinal and choroidal imaging parameters for the subjects imaged longitudinally is reported in Table 5. While there were no significant differences in any OCT or OCTA parameter between the APOE ε4+ and ε4− groups at baseline, ε4 carriers demonstrated slightly lower PD in the 6mm circle ($p = 0.050$) and ETDRS outer ring ($p = 0.045$) compared to ε4 non-carriers.

The rate of change in retinal and choroidal imaging parameters was calculated as units per year (Table 6). For OCT parameters, there were no significant differences in rate of change for CST, average GC-IPL thickness, and average RNFL thickness between the APOE ε4+ and ε4− cohorts (all $p > 0.05$). Of note, there was a negative rate of change for CST and RNFL thickness for both carrier and non-carrier risk groups, reflecting loss over time, as would be expected with normal aging. However, the average GC-IPL thickness demonstrated a negative rate of change in the APOE ε4+ group compared to no decline in the APOE ε4− group, reflecting stable measurements within the limits of measurement error or no significant decline in the ε4− group (Table 6). Choroidal parameters similarly did not show any significant differences in rates of change between groups. Both groups had similar slight negative rates of change in CVI measurements (Table 6). There were no significant differences in rates of change in OCTA parameters between groups (all $p > 0.05$). On 3x3mm OCTA images, both the ε4 carrier and non-carrier groups demonstrated no decline in FAZ area, or PD and VD in the 3mm circle and ring. PD and VD in the 6x6mm scans areas showed small negative rates of change, apart from PD in the 6mm inner ring which demonstrated no decline. There was no difference in rate of change in any peripapillary OCTA measure (Table 6).

Sub-analysis was performed comparing subjects in the APOE ε4+ risk group who also had a family history of dementia (129 eyes of 67 subjects at baseline) against those in the ε4− group who had no such family history (106 eyes of 56 subjects at baseline). At follow-up, there was a significant difference in superior sector CPD ($p = 0.036$). Yet, there were no other differences in any OCT or OCTA parameter between these groups at baseline or 2-year follow-up. Notably, the ε4+/family history+ group demonstrated a greater rate of decline, −0.157 microns/year, in GC-IPL thickness than the ε4−/family history- group ($p = 0.038$) (Supplementary Tables 1-5, available online at: https://www.ophthalmologyretina.org/).

DISCUSSION

While there is a growing body of literature on the retinal structure and microvasculature in AD and pre-clinical states, there is limited data evaluating the effect of APOE genetic status on the retina. In this prospective, longitudinal study, we observed exploratory evidence of lower CST, lower PD in the 6mm ETDRS circle, and lower peripapillary temporal CFI in APOE ε4+ individuals compared to those without an APOE ε4 allele. At the 2 year timepoint, we observed signs of lower PD in the 6mm ETDRS circle and outer ring. A sub-analysis of ε4 carriers with a family history of dementia and non-carriers without a family history found a greater rate of GC-IPL loss, with a similar but non-significant

trend observed in the main analysis as well. Such findings indicate that a thinner CST, lower PD, and possibly GC-IPL thickness could potentially represent early retinal findings attributed to a higher-risk APOE genetic status with larger or longer studies. However, as our results were not adjusted for multiple comparisons, we emphasize that there would be no p-values reaching the alpha significance level of 0.05 if an adjustment such as the Bonferroni correction was applied. Our analysis did not find evidence of differences in rates of change in any retinal or choroidal parameter measured at the 2-year follow-up, again suggesting that longer studies would be required to observe any meaningful differences that may develop over a lifetime in such individuals.

Retinal layer thickness, as measured by CST and RNFL, has been shown to be lower in individuals with AD,[42] and one study found that macular thinning may even be associated with greater disease severity.[43] Another study found no such differences, indicating that more investigation into these parameters is needed.[44] Our observation of lower CST in APOE ε4 carriers at baseline, relative to non-carriers, may be a signal of early changes in the retina of asymptomatic persons at higher genetic risk for AD. However, lack of this signal across the rate of change and between- arms of the groups' longitudinal analysis suggests limited value. Although retinal amyloid has been found in patients with AD, and amyloid deposits have been associated with RNFL loss, the precise mechanism of AD pathologic change in the retina is yet unknown.[45-47] Our findings of no significant difference in rate of change of CST and RNFL thickness over 2 years suggests that longer observation periods may be required to determine whether significant differences in rate of change exist. Longer study durations are an especially important component in future retinal imaging studies to determine timepoint thresholds for onset of retinal changes as the natural course of AD onset is often characterized by a long asymptomatic continuum period prior to progression.[48,49]

Other measures of retinal layer thickness such as GC-IPL have also been associated with AD and poor performance on neuropsychiatric cognitive testing.[50,51] We studied cognitively normal individuals with known APOE status and did not observe differences in GC-IPL thickness between the APOE ε4+ and ε4− groups. However, regarding rate of change, the APOE ε4+ group demonstrated GC-IPL thinning over the 2-year period compared to the APOE ε4− group which showed no decline over time. As the ganglion cells of the GC-IPL layer are closely associated with the optic nerve, central nervous system extension of AD-spectrum pathology could potentially affect this segment of the retina earlier in the course of disease development.[52] Any potential divergence in this measure may require a longer period of observation.[19,53,54] The APOE ε4 allele has been hypothesized to be associated with poor amyloid clearance, an important component of AD pathology, as well as more targeted abnormalities in neural tissue such as myelination, growth, and regeneration which are more closely related to the GC-IPL and other neurosensory components of the retina.[55-57] Sub-analysis of ε4+/dementia family history+ individuals vs ε4−/no family history subjects, comparing the patients at the theoretical extremes of potential for retinal or choroidal imaging differences, demonstrated a significant difference in GC-IPL rate of change with thinning in the ε4+/dementia family history+ individuals ($p = 0.038$). Notably, we observed a similar but non-significant trend in GC-IPL rate of change in the main cohorts as well ($p = 0.099$). Though small in magnitude, these subtle changes

suggest that GC-IPL may be a parameter of interest after a longer duration of study. We have previously described GC-IPL as a meaningful component of a convolutional neural network in identifying AD using retinal image inputs; GC-IPL thinning may be a sign of early APOE-mediated or neurodegeneration-associated disease, which may eventually result in a symptomatic phenotype.[58]

Measures of choroidal vascularity, in particular CVI, in asymptomatic individuals with known APOE status is a novel area of investigation. A previous study by our group has shown differences in TCA, LA, and CVI between individuals with normal cognition (and unknown APOE status) and those with mild cognitive impairment or AD.[59] As we did not observe any such changes in choroidal vascular parameters in this current analysis at baseline or at 2 years, it is possible that any difference in choroidal vascularity between these groups may be too subtle to detect in asymptomatic subjects over this duration despite known APOE status.

We observed significantly lower PD in the 6mm ETDRS circle in the total ε4 carrier group at baseline and at year 2, but no difference in rate of change for any OCTA parameter. PD in the 6mm outer ring was not initially significantly different, but was slightly lower in APOE ε4 carriers relative to non-carriers. That we did not observe any difference in the 6mm inner ring suggests that there may be a diffuse pattern of change better captured by a larger region. Though there was no significant difference in rate of change in the 6mm PD circle, our findings of some lower measures of 6mm PD in ε4 carriers at baseline, and at year 2, suggest that a longer duration of study may help differentiate these groups. A recent investigation by Elahi et al. found lower vessel area density and vessel skeleton density (most analogous to PD and VD, respectively) measured over the entire 3×3mm image area obtained using the same OCT as this study (Cirrus HD-OCT 5000) in a smaller cohort of 24 ε4 carriers compared to those without an ε4 allele and is the only study of cognitively normal ε4–differentiated subjects in the literature prior to our investigation.[35] While the entire 3×3mm image area is not precisely comparable to any single ETDRS subfield, we did not find any differences in PD or VD in the 3mm circle or ring. Furthermore, their measurements of vessel area density and vessel skeleton density were calculated using a different software algorithm.[60] Peripapillary changes observed at baseline (APOE ε4 carriers demonstrating lower temporal sector CFI) could potentially reflect subtle localized change in the peripapillary microvasculature attributable to APOE ε4; however, this finding was not observed in the sub-cohort of patients imaged longitudinally at either baseline or follow-up, or in any rate of change. Our within-group analysis findings of generally small and concordant decreases in CPD and CFI parameters in both groups combined with no observation of potentially differentiating between-group measures of peripapillary OCTA suggest that the observed within-group changes may be due to normal aging rather than differential progression of disease. In a small study of control and AD eyes, the temporal retina has been described as having a significantly higher amyloid burden, which could potentially manifest as early changes in CFI in APOE ε4 carriers, with which amyloid has been shown to correlate with a temporal retina distribution.[61,62]

A recent study of biomarker-positive (cerebrospinal fluid or neuroimaging) pre-clinical Alzheimer disease subjects imaged with a 3-year interval found an enlarged FAZ area in

biomarker-positive eyes compared to subjects that were biomarker-negative.[63] We did not observe any statistically significant differences in FAZ area between APOE ε4 carriers and non-carriers after a 2-year interval; however, differentiation of subjects by genetic status precludes a direct comparison. Both our study and that of O'Bryhim and colleagues observed no statistically difference in FAZ rate of change.[63] While we did observe a potentially meaningful increase in size of the FAZ in within-group analysis of the ε4+ group after 2 years, the magnitude of change, 0.006, was small in comparison to the standard deviation (0.023). Differences in between-group and rate of change analysis did not manifest similar changes, suggesting the utility of this measure may be somewhat limited in this context.

While the relative frequencies of APOE allele variants (ε3>ε4>ε2) have been found to be consistent across races,[64] Black individuals are more likely to carry at least one APOE ε4 allele[65,66] and Asians have demonstrated lower APOE ε4 frequencies.[67] Our study cohorts were not as racially diverse, especially in context of the aforementioned APOE ε4 allele racial distribution, lowering the generalizability of our results across racial groups. Our cohorts were disproportionately female, perhaps driven by self-selection bias due to perceived higher life-time risk of AD.[68,69] APOE ε4 frequency is higher in individuals with a family history of AD, with the co-occurrence of the ε4 allele and positive family AD history demonstrating a higher relative risk of developing AD as well as greater amyloid deposition on PET imaging.[70,71] As our study enrolled only 9 subjects who were homozygous for the APOE ε4 allele, larger studies may be able to better differentiate subtle differences between specific allelic combinations.

Several studies[50,72-78] have analyzed the role of APOE genotype indirectly, and our findings may be indirectly comparable to some of these prior studies. López-Cuenca et al. studied a pool of 64 subjects with known APOE status; however, participants were grouped as 35 ε4 carriers with family history of AD, compared to 29 age-matched ε4 non-carriers without family history.[32] While they observed decreases in macular RNFL thickness in the ε4 carrier/family history+ group, it is not possible to isolate the effects of ε4 genetic status alone as a single variable. Our analogous sub-analysis did not show differences in average RNFL thickness by ε4 status and family history of dementia.

Our study has several strengths, including a large number of subjects with strict inclusion and image quality criteria, imaged longitudinally over a 2-year period. With strict quality criteria, 96.1% of individual data parameters in the APOE ε4+ group and 94.1% of data in the APOE ε4− group were successfully obtained and of sufficient quality for analysis, indicating relatively small loss of data. The rigorous selection of patients against retina-altering medical comorbidities aided in minimizing confounding.

An important caveat to our study is its exploratory nature; the application of adjustment for multiple comparisons would render no significant findings. However, as a discovery study with a relatively novel longitudinal approach to this relatively large population, there may be some utility in identifying potential parameters of interest for more focused future investigation. Our study was also limited by the incomplete participant yield returning for the 2-year follow-up visit, with the APOE ε4+ group having slightly more participation.

However, the follow-up demographic characteristics were similar between groups, and the majority of participants lost to follow-up were attributed to the COVID-19 pandemic, a phenomenon which presumably affected both study groups to the same degree. Additionally, as the average age of our cohort at initial imaging was greater than age 65 (i.e., the earliest age at which individuals may be diagnosed with late-onset AD), the imaging of younger persons may offer more insight into any early changes in the retina although unlikely given our findings. This is an important consideration in the design of similar future studies. Stratifying our sample groups further into the 6 potential allele combinations, particularly for those homozygous for the APOE ε4 allele, may have better distinguished subtle changes in retinal imaging parameters; however, the sample sizes would have been underpowered for meaningful comparisons in this investigation. Considering the largely non-significant findings, future studies could require as many as 284 subjects per group to demonstrate significant differences in rate of change (calculated using data for CPD area average rate of change, testing the difference between two means with clustered data assuming equal sample sizes and two eyes per subject)[79] in addition to the aforementioned genotypic strata. Our investigation, which considers software-specific imaging parameters, may be difficult to apply directly to investigations carried out using devices produced by other manufacturers. As the potential impact of amyloid burden in asymptomatic controls in this study is uncertain, our study would have benefited from neuroimaging such as PET to better understand contributions of amyloid deposition to central and retinal pathology.

In conclusion, APOE ε4 allele carriers had lower CST, PD, and peripapillary temporal CFI compared to those without APOE ε4 demonstrating both retinal structural and microvascular alterations in APOE ε4 allele carriers with normal cognition. At 2 years, we observed lower values of some measures of 6mm ETDRS and outer ring PD in ε4 carriers; however, there were no significant differences in rate of change in any parameter between the 2 groups. The trend in thinning in GC-IPL in ε4 carriers in our longitudinal cohorts, and sub-analysis ε4 carriers with family history, suggests GC-IPL thinning may be a subtle subsequent change in the AD continuum. This study adds novel information to the literature on CVI in asymptomatic individuals with known APOE status. Studies with longer follow-up and larger cohorts may be needed to further evaluate the impact of APOE ε4 status on retinal and choroidal imaging parameters. Studies of individuals with varying APOE genetic status may facilitate the identification of early ocular imaging biomarkers of AD. Investigations of early differences in rate of change in the long continuum of potential AD development could distinguish subjects by rate of progression, thereby influencing individual advanced planning and lifestyle modifications as well as guide participant selection for future clinical trial entry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Demographic characteristics of all subjects at baseline

	APOE ε4+ N=98 patients	APOE ε4− N=120 patients	P-value[a]
Age, mean (SD)	70.1 (6.5)	71.0 (5.9)	0.322
Years of education, mean (SD)	16.9 (2.0)	17.1 (2.0)	0.592
Female sex, N (%)	74 (76)	92 (77)	0.874
Race, N (%)			
White	85 (87)	113 (94)	
Black	12 (12)	7 (6)	0.072
Asian	1 (1)	0	
Other/Not reported	0	0	
Family history of dementia, N (%)	67 (68)	64 (53)	**0.027**
MMSE score, mean (SD)	29.3 (1.0)	29.5 (0.8)	0.161

MMSE=Mini-Mental State Examination

[a]P-value for continuous variables based on Wilcoxon rank sum test of difference between medians. P-value for categorical variables based on Fisher's exact test of difference between proportions.

Table 2:

Retinal and choroidal imaging parameters of all subjects at baseline

Parameters	APOE ε4+ N=186 eyes (98 subjects)	APOE ε4− N=227 eyes (120 subjects)	P-value[a]
OCT parameters, mean (SD)			
Average CST, microns	263.0 (22.0)	268.8 (24.8)	**0.018**
Average GC-IPL thickness, microns	75.59 (7.35)	75.65 (6.93)	0.784
Average RNFL thickness, microns	88.42 (9.38)	87.70 (9.12)	0.806
Choroidal parameters, mean (SD)			
Total choroidal area, units^2 [b]	4.67 (1.02)	4.69 (1.10)	0.367
Luminal area, units^2 [b]	3.18 (0.67)	3.20 (0.73)	0.337
Choroidal vascularity index, %	0.68 (0.02)	0.68 (0.02)	0.847
OCTA parameters, mean (SD)			
FAZ area, mm^2	0.25 (0.10)	0.24 (0.10)	0.764
PD (3mm ETDRS circle)	0.36 (0.03)	0.36 (0.03)	0.252
PD (3mm ETDRS ring)	0.38 (0.03	0.38 (0.02)	0.341
VD (3mm ETDRS circle), mm^{-1}	19.91 (1.62)	20.06 (1.58)	0.228
VD (3mm ETDRS ring), mm^{-1}	20.99 (1.64)	21.10 (1.57)	0.301
PD (6mm ETDRS circle)	0.43 (0.03)	0.44 (0.03)	**0.049**
PD (6mm ETDRS outer ring)	0.44 (0.03)	0.45 (0.03)	0.099
PD (6mm ETDRS inner ring)	0.43 (0.04)	0.43 (0.03)	0.076
VD (6mm ETDRS circle), mm^{-1}	17.74 (1.28)	17.89 (1.11)	0.114
VD (6mm ETDRS outer ring), mm^{-1}	17.98 (1.28)	18.11 (1.20)	0.183
VD (6mm ETDRS inner ring), mm^{-1}	17.89 (1.40)	18.07 (1.14)	0.111
Peripapillary OCTA parameters, mean (SD)			
CPD (average), %	43.50 (2.09)	43.44 (2.04)	0.994
CPD (superior), %	42.05 (2.96)	42.26 (2.79)	0.444
CPD (nasal), %	42.14 (2.90)	42.31 (2.86)	0.506
CPD (inferior), %	44.34 (3.18)	43.96 (2.88)	0.514
CPD (temporal), %	45.57 (3.13)	45.39 (2.90)	0.657
CFI (average)	0.40 (0.04)	0.41 (0.04)	0.135
CFI (superior)	0.40 (0.04)	0.41 (0.03)	0.180
CFI (nasal)	0.40 (0.04)	0.41 (0.03)	0.224
CFI (inferior)	0.41 (0.04)	0.41 (0.03)	0.402
CFI (temporal)	0.39 (0.06)	0.41 (0.06)	**0.047**

CST=central subfield thickness, GC-IPL=ganglion cell-inner plexiform layer, RNFL=retinal nerve fiber layer, FAZ=foveal avascular zone, PD=perfusion density, VD=vessel density, ETDRS=Early Treatment Diabetic Retinopathy Study, CPD=capillary perfusion density, CFI=capillary flux index.

[a] P-values based on generalized estimating equations (GEE) model score statistic for testing difference among and between means. P-values from GEE model adjusted for sex and age.

[b] $\text{Units}^2 = 96 \times 96\ \text{pixels}^2$

Table 3:

Demographic characteristics of subjects imaged longitudinally

	APOE ε4+ N=71 patients (134 eyes)	**APOE ε4− N=78 patients (149 eyes)**	**P-value[a]**
Age at baseline, mean (SD)	69.5 (6.5)	70.4 (5.7)	0.341
Years of education, mean (SD)	17.1 (2.0)	17.1 (1.9)	0.896
Female sex, N (%)	56 (79)	56 (72)	0.348
Race, N (%)			
White	62 (88)	77 (99)	
Black	8 (11)	1 (1)	**<0.008**
Asian	1 (1)	0	
Other/Not reported	0	0	
Family history of dementia, N (%)	46 (68)	45 (58)	0.238
MMSE score at baseline, mean (SD)	29.4 (0.9)	29.5 (0.8)	0.577
MMSE score at follow-up, mean (SD)	29.6 (0.8)	29.7 (0.6)	0.633

MMSE=Mini-Mental State Examination

[a]P-value for continuous variables based on Wilcoxon rank sum test of difference between medians. P-value for categorical variables based on Fisher's exact test of difference between proportions.

Table 4:

Within-group retinal and choroidal imaging parameters of subjects imaged longitudinally

Parameters	APOE ε4+ N=71 patients (134 eyes)		APOE ε4− N=78 patients (149 eyes)	
	Mean change (SD)	P-value[b]	Mean change (SD)	P-value[b]
OCT parameters				
Average CST, microns	−0.133 (5.635)	0.748	−1.384 (5.262)	**0.015**
Average GC-IPL thickness, microns	−0.292 (2.712)	0.265	0.224 (2.454)	0.413
Average RNFL thickness, microns	0.223 (3.221)	0.464	0.466 (3.701)	0.163
Choroidal parameters				
Total choroidal area, units^2 [c]	0.083 (0.544)	0.144	−0.025 (0.518)	0.696
Luminal area, units^2 [c]	0.048 (0.352)	0.176	−0.020 (0.328)	0.594
Choroidal vascularity index, %	−0.001 (0.013)	0.428	> −0.001 (0.013)	0.808
OCTA parameters				
FAZ area, mm^2	0.006 (0.023)	**0.010**	0.004 (0.031)	0.175
PD (3mm ETDRS circle)	0.002 (0.028)	0.746	0.004 (0.031)	0.204
PD (3mm ETDRS ring)	0.002 (0.027)	0.580	0.004 (0.031)	0.182
VD (3mm ETDRS circle), mm^{-1}	−0.122 (1.580)	0.353	−0.058 (1.825)	0.805
VD (3mm ETDRS ring), mm^{-1}	−0.105 (1.597)	0.415	−0.045 (1.884)	0.880
PD (6mm ETDRS circle)	−0.004 (0.036)	0.265	−0.002 (0.034)	0.614
PD (6mm ETDRS outer ring)	−0.005 (0.035)	0.117	−0.002 (0.035)	0.664
PD (6mm ETDRS inner ring)	> −0.001 (0.044)	0.926	> 0.001 (0.039)	0.961
VD (6mm ETDRS circle), mm^{-1}	−0.217 (1.344)	0.082	−0.162 (1.298)	0.221
VD (6mm ETDRS outer ring), mm^{-1}	−0.253 (1.304)	0.039	−0.163 (1.345)	0.246
VD (6mm ETDRS inner ring), mm^{-1}	−0.111 (1.682)	0.461	−0.135 (1.501)	0.329
Peripapillary OCTA parameters				
CPD (average), %	−0.454 (1.235)	**<0.001**	−0.284 (1.257)	**0.018**
CPD (superior), %	−0.016 (1.865)	0.935	−0.003 (1.788)	0.966
CPD (nasal), %	−0.880 (2.404)	**<0.001**	−0.516 (2.054)	**0.011**
CPD (inferior), %	0.354 (1.855)	**0.049**	0.435 (1.639)	**0.010**
CPD (temporal), %	−1.065 (1.860)	**<0.001**	−0.932 (2.060)	**<0.001**
CFI (average)	−0.031 (0.045)	**<0.001**	−0.020 (0.040)	**<0.001**
CFI (superior)	−0.015 (0.035)	**<0.001**	−0.010 (0.028)	**<0.001**
CFI (nasal)	−0.042 (0.061)	**<0.001**	−0.031 (0.056)	**<0.001**
CFI (inferior)	−0.014 (0.030)	**<0.001**	−0.006 (0.028)	**0.023**
CFI (temporal)	−0.051 (0.065)	**<0.001**	−0.031 (0.061)	**<0.001**

CST=central subfield thickness, GC-IPL=ganglion cell-inner plexiform layer, RNFL=retinal nerve fiber layer, FAZ=foveal avascular zone, PD=perfusion density, VD=vessel density, ETDRS=Early Treatment Diabetic Retinopathy Study, CPD=capillary perfusion density, CFI=capillary flux index.

[a] Note: baseline data only those for subjects were participated in 2-year follow-up imaging

[b] P-values based on generalized estimating equations (GEE) model score statistic for testing differences among and between means. P-values from GEE model adjusted for sex and age.

[c] $Unit^2 = 96 \times 96\ pixels^2$

Table 5:

Between-group analysis of retinal and choroidal imaging parameters of subjects imaged longitudinally

Parameters	Baseline[a]			2-year follow-up		
	APOE ε4+ N=71 patients (134 eyes)	APOE ε4− N=78 patients (149 eyes)	P-value[b]	APOE ε4+ N=71 patients (134 eyes)	APOE ε4− N=78 patients (149 eyes)	P-value[b]
OCT parameters, mean (SD)						
Average CST, microns	261.9 (22.08)	268.4 (21.13)	0.077	261.8 (22.02)	267.1 (20.47)	0.129
Average GC-IPL thickness, microns	75.90 (7.89)	76.29 (6.78)	0.599	75.61 (8.94)	76.51 (6.83)	0.419
Average RNFL thickness, microns	89.23 (9.61)	88.15 (8.91)	0.516	89.46 (9.70)	88.62 (9.11)	0.633
Choroidal parameters, mean (SD)						
Total choroidal area, units^2 [c]	4.79 (0.88)	4.70 (1.12)	0.875	4.88 (1.00)	4.67 (1.16)	0.451
Luminal area, units^2 [c]	3.27 (0.58)	3.20 (0.74)	0.815	3.32 (0.65)	3.18 (0.76)	0.401
Choroidal vascularity index, %	0.68 (0.02)	0.68 (0.02)	0.794	0.68 (0.02)	0.68 (0.02)	0.912
OCTA parameters, mean (SD)						
FAZ area, mm^2	0.24 (0.09)	0.23 (0.09)	0.643	0.25 (0.09)	0.24 (0.10)	0.635
PD (3mm ETDRS circle)	0.36 (0.03)	0.36 (0.03)	0.529	0.36 (0.03)	0.36 (0.02)	0.75
PD (3mm ETDRS ring)	0.38 (0.03)	0.38 (0.02)	0.563	0.38 (0.03)	0.38 (0.02)	0.229
VD (3mm ETDRS circle), mm^{-1}	20.04 (1.65)	20.14 (1.62)	0.437	19.92 (1.53)	20.08 (1.37)	0.230
VD (3mm ETDRS ring), mm^{-1}	21.10 (1.65)	21.17 (1.61)	0.464	21.00 (1.58)	21.13 (1.39)	0.271
PD (6mm ETDRS circle)	0.44 (0.03)	0.44 (0.03)	0.144	0.43 (0.04)	0.44 (0.03)	**0.050**
PD (6mm ETDRS outer ring)	0.45 (0.03)	0.45 (0.03)	0.257	0.44 (0.04)	0.45 (0.03)	**0.047**
PD (6mm ETDRS inner ring)	0.43 (0.04)	0.43 (0.03)	0.135	0.43 (0.04)	0.43 (0.03)	0.112
VD (6mm ETDRS circle), mm^{-1}	17.82 (1.29)	17.95 (1.04)	0.284	17.61 (1.33)	17.78 (1.03)	0.116
VD (6mm ETDRS outer ring), mm^{-1}	18.07 (1.27)	18.16 (1.08)	0.390	17.82 (1.39)	17.99 (1.07)	0.118
VD (6mm ETDRS inner ring), mm^{-1}	17.94 (1.49)	18.13 (1.18)	0.186	17.83 (1.36)	17.99 (1.23)	0.216
Peripapillary OCTA parameters, mean (SD)						
CPD (average), %	43.64 (2.02)	43.57 (1.51)	0.918	44.09 (1.95)	43.86 (1.45)	0.734
CPD (superior), %	42.08 (2.99)	42.36 (2.42)	0.340	42.10 (2.72)	42.36 (2.14)	0.201
CPD (nasal), %	42.44 (2.87)	42.46 (2.55)	0.889	43.33 (2.43)	42.97 (2.06)	0.498
CPD (inferior), %	44.65 (3.08)	44.23 (2.59)	0.506	44.30 (2.70)	43.80 (2.33)	0.388
CPD (temporal), %	45.52 (2.56)	45.27 (2.46)	0.794	46.59 (2.58)	46.20 (2.19)	0.474
CFI (average)	0.40 (0.04)	0.41 (0.04)	0.201	0.43 (0.03)	0.43 (0.03)	0.972
CFI (superior)	0.41 (0.03)	0.41 (0.03)	0.262	0.42 (0.03)	0.42 (0.03)	0.665
CFI (nasal)	0.39 (0.06)	0.40 (0.06)	0.425	0.43 (0.04)	0.43 (0.04)	0.958
CFI (inferior)	0.41 (0.03)	0.42 (0.03)	0.587	0.43 (0.03)	0.42 (0.03)	0.402
CFI (temporal)	0.40 (0.06)	0.42 (0.05)	0.051	0.45 (0.04)	0.45 (0.04)	0.678

CST=central subfield thickness, GC-IPL=ganglion cell-inner plexiform layer, RNFL=retinal nerve fiber layer, FAZ=foveal avascular zone, PD=perfusion density, VD=vessel density, ETDRS=Early Treatment Diabetic Retinopathy Study, CPD=capillary perfusion density, CFI=capillary flux index.

[a] Note: baseline data only those for subjects were participated in 2-year follow-up imaging

[b] P-values based on generalized estimating equations (GEE) model score statistic for testing differences among and between means. P-values from GEE model adjusted for sex and age.

[c] $Unit^2 = 96 \times 96\ pixels^2$

Table 6:

Rate of change in retinal and choroidal imaging parameters

	APOE ε4+ N=134 eyes (71 subjects)		APOE ε4− N=149 eyes (78 subjects)		P-value[a]
	Mean change per year (SD)	Equivalent % change/year	Mean change per year (SD)	Equivalent % change/year	
OCT parameters					
Average CST, microns	−0.138 (3.107)	−0.05%	−0.657 (2.585)	−0.24%	0.325
Average GC-IPL thickness, microns	−0.158 (1.332)	−0.21%	0.128 (1.219)	0.17%	0.099
Average RNFL thickness, microns	0.104 (1.571)	0.12%	0.250 (1.815)	0.29%	0.420
Choroidal parameters					
Total choroidal area, units^2 [b]	0.023 (0.254)	0.48%	−0.024 (0.250)	−0.51%	0.303
Luminal area, units^2 [b]	0.013 (0.164)	0.40%	−0.017 (0.159)	−0.53%	0.287
Choroidal vascularity index, %	< −0.0005 (0.006)	< −0.07%	< −0.0005 (0.007)	< −0.09%	0.874
OCTA parameters					
FAZ area, mm^2	0.003 (0.016)	1.25%	0.002 (0.164)	0.87%	0.722
PD (3mm ETDRS circle)	0.002 (0.016)	0.56%	0.002 (0.015)	0.56%	0.916
PD (3mm ETDRS ring)	0.002 (0.016)	0.53%	0.002 (0.015)	0.53%	0.955
VD (3mm ETDRS circle), mm^{-1}	0.012 (0.910)	0.06%	0.006 (0.876)	0.03%	0.907
VD (3mm ETDRS ring), mm^{-1}	0.023 (0.939)	0.11%	0.014 (0.903)	0.07%	0.907
PD (6mm ETDRS circle)	−0.001 (0.018)	−0.23%	< −0.0005 (0.015)	< −0.11%	0.723
PD (6mm ETDRS outer ring)	−0.002 (0.017)	−0.44%	< −0.0005 (0.016)	< −0.11%	0.406
PD (6mm ETDRS inner ring)	0.001 (0.024)	0.23%	0.001 (0.018)	0.23%	0.784
VD (6mm ETDRS circle), mm^{-1}	−0.070 (0.677)	−0.39%	−0.054 (0.599)	−0.30%	0.782
VD (6mm ETDRS outer ring), mm^{-1}	−0.095 (0.637)	−0.53%	−0.054 (0.625)	−0.30%	0.525
VD (6mm ETDRS inner ring), mm^{-1}	−0.002 (0.919)	−0.01%	−0.048 (0.704)	−0.26%	0.644
Peripapillary OCTA parameters, mean (SD)					
CPD (average), %	−0.193 (0.679)	−0.44%	−0.149 (0.593)	−0.34%	0.855
CPD (superior), %	0.028 (1.026)	0.07%	0.002 (0.833)	0.00%	0.505
CPD (nasal), %	−0.390 (1.162)	−0.92%	−0.277 (0.993)	−0.65%	0.637
CPD (inferior), %	0.176 (0.913)	0.39%	0.211 (0.797)	0.48%	0.758
CPD (temporal), %	−0.486 (0.881)	−1.07%	−0.473 (0.986)	−1.04%	0.884
CFI (average)	−0.015 (0.021)	−3.75%	−0.010 (0.020)	−2.44%	0.305
CFI (superior)	−0.007 (0.016)	−1.71%	−0.005 (0.014)	−1.22%	0.573
CFI (nasal)	−0.020 (0.028)	−5.13%	−0.015 (0.027)	−3.75%	0.552
CFI (inferior)	−0.007 (0.014)	−1.71%	−0.003 (0.013)	−0.71%	0.211
CFI (temporal)	−0.024 (0.030)	−6.00%	−0.015 (0.030)	−3.57%	0.161

CST=central subfield thickness, GC-IPL=ganglion cell-inner plexiform layer, RNFL=retinal nerve fiber layer, FAZ=foveal avascular zone, PD=perfusion density, VD=vessel density, ETDRS=Early Treatment Diabetic Retinopathy Study, CPD=capillary perfusion density, CFI=capillary flux index.

[a] P-values based on generalized estimating equations (GEE) model score statistic for testing differences among and between means. P-values from GEE model adjusted for sex and age.

[b] $\text{Unit}^2 = 96 \times 96\ \text{pixels}^2$