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RESEARCH ARTICLE

Thicker macula in asymptomatic *APOE* ε4 middle-aged adults at high AD risk

Ygal Rotenstreich[1,2,3] | **Inbal Sharvit-Ginon**[4,5] | **Ifat Sher**[1,2] | **Ofira Zloto**[1,2] | **Ido Didi Fabian**[1,2] | **Amir Abd-Elkader**[1,2] | **Aron Weller**[4,6] | **Anthony Heymann**[2,7] | **Michal Schnaider Beeri**[5,8] | **Ramit Ravona-Springer**[2,5,9]

[1] Goldschleger Eye Institute, Sheba Medical Center, Tel Hashomer, Israel

[2] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

[3] Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

[4] Psychology Department, Bar Ilan University, Ramat-Gan, Israel

[5] The Joseph Sagol Neuroscience Center at the Sheba Medical Center, Tel Hashomer, Israel

[6] Gonda Brain Research Center, Bar Ilan University, Ramat-Gan, Israel

[7] Maccabi Healthcare Services, Tel Aviv, Israel

[8] Department of Psychiatry, The Icahn School of Medicine at Mount Sinai, New York, New York, USA

[9] Department of Psychiatry, Sheba Medical Center, Tel Hashomer, Israel

Correspondence
Ramit Ravona-Springer, Sheba Medical Center, Tel Hashomer 52621, Israel.
E-mail: ramit.ravona@sheba.health.gov.il

Abstract

Introduction: We compared retinal layers' thickness between apolipoprotein E (*APOE*) ε4 carriers and non-carriers in a cohort of cognitively normal middle-aged adults enriched for Alzheimer's disease (AD) risk.

Methods: Participants (N = 245) underwent spectral domain optical coherence tomography. Multivariate analyses of covariance adjusting for age, sex, education, and best corrected vision acuity was used to compare retinal thickness between *APOE* groups.

Results: Participants' mean age was 59.60 (standard deviation = 6.42) with 66.4% women and 32.2% *APOE* ε4 carriers. Greater macular full thickness was observed in *APOE* ε4 carriers compared to non-carriers ($P = .017$), reaching statistical significance for the inner and outer nasal ($P = .009$ and $P = .005$, respectively), inner superior ($P = .041$), and inner and outer inferior ($P = .013$ and $P = .033$, respectively) sectors. The differences between *APOE* groups were mainly driven by the ganglion cell layer ($P < .05$) and the inner plexiform layer ($P < .05$).

Discussion: A thicker macula is observed already in midlife asymptomatic *APOE* ε4 carriers at high AD risk.

KEYWORDS
Alzheimer's disease, apolipoprotein E ε4 genotype, high risk for Alzheimer's disease, offspring of Alzheimer's disease patients, optical coherence tomography, retinal biomarkers, retinal layers, retinal sectors

1 | INTRODUCTION

The number of people living with dementia is rapidly increasing, and is expected to reach 150 million worldwide by 2050.[1] Alzheimer's disease (AD) is considered to be the most prevalent type of dementia, encompassing approximately 60% of all cases.[1] Numerous drugs developed for the treatment of AD have failed in clinical trials. One of the leading hypotheses for the failures of multiple pharmacological treatments is that the latter were introduced in people with already ascertained dementia, when neuropathology is advanced and such interventions have limited benefits. The clinical expression of AD dementia is preceded by years, or even decades, of an asymptomatic progressive neuropathological process. The conceptualization of timely intervention has thus shifted from treatment initiation in people with frank dementia to earlier, asymptomatic stages of neuropathology, that is, midlife. This is also a period in life during which exposure to lifestyle, metabolic, and cardiovascular risk factors has been consistently associated with increased risk for dementia, stressing its importance as

a window of opportunities for introduction of dementia prevention strategies.[2] Effective establishment of such interventions requires valid methods for identification of asymptomatic individuals at early stages of the AD-related neuropathological process. Studies on cognitively normal populations enriched for AD risk provide an opportunity to validate such methods.

The apolipoprotein E (*APOE*) ε4 genotype is the most important genetic risk factor for sporadic AD, with a 2- to 3- and up to 15-fold increased risk for the disease compared to non-carriers in heterozygotes and homozygotes for this allele, respectively.[3] This genotype is associated with an earlier age of disease onset, increased rates of cognitive decline and brain atrophy, altered brain activity, lower cerebral blood flow, and higher brain amyloid load,[4] sometimes as early as young adulthood.[5] Cognitively normal *APOE* ε4 carriers thus offer an opportunity to investigate neuropathology and its trajectories in the asymptomatic stages of AD.

Currently available biomarkers for early AD, namely structural and functional brain imaging techniques or measurement of amyloid burden, using cerebrospinal fluid analysis or positron emission tomography imaging techniques, are not applicable as screening tools for large populations due to high costs, invasiveness, or limited accessibility, stressing the urgent need to validate other biomarkers for AD.

The retina is an extension of the brain and shares many of its structural and functional features as well as its neuropathologies. Structural abnormalities of the retina have been demonstrated in several neuropsychiatric diseases including multiple sclerosis, Parkinson's disease, and AD.[6] Unlike the brain, due to the transparency of the eye, the retina is easily accessible for direct and noninvasive imaging with high resolution and sensitivity. Several retinal impairments have previously been demonstrated in patients already expressing clinical symptoms of AD. These include cell loss, retinal atrophy, and axonal degeneration.[7] Thinning of the macula and peripapillary retinal nerve fiber layer (pRNFL) have previously been demonstrated in patients with AD dementia and mild cognitive impairment compared to cognitively normal controls.[8] However, retinal findings in asymptomatic people are scarce and inconsistent, with some demonstrating an association between thinner retinal layers and worse cross-sectional and longitudinal cognitive outcomes[9–13] while others reporting the opposite, that is, an association of greater retinal layers' thickness with worse cognitive functioning[14] or greater brain burden of amyloid beta (Aβ).[15]

The aim of the present study is to examine the relationship of retinal layers' thickness with *APOE* genotype in cognitively normal middle-aged adults enriched for AD risk due to a parental family history, participating in the Israel Registry for Alzheimer's Prevention (IRAP).

HIGHLIGHTS

- Apolipoprotein E ε4 is associated with thicker maculae in asymptomatic middle-aged adults.
- This relationship is mainly driven by the inner plexiform layer and ganglion cell layer.
- Retinal alterations may be early biomarkers for Alzheimer's disease pathology.

RESEARCH IN CONTEXT

1. Systematic review: The authors searched the literature using traditional (e.g., PubMed) sources. Previous studies have demonstrated macular impairments in patients with clinically symptomatic Alzheimer's disease (AD), mostly atrophy and thinning. However, findings in asymptomatic people at risk for AD are relatively scarce and inconsistent. We compared macular layers' thickness between apolipoprotein E (*APOE*) ε4 carriers and non-carriers in a cohort of asymptomatic middle-aged people enriched for high AD risk due to family history. *APOE* ε4 carriers had increased full macular thickness in the nasal, superior, and inferior sectors. This relationship was mainly driven by thicker inner retinal layers (inner plexiform layer and ganglion cell layer).
2. Interpretation: These results support the role of retinal alterations as early biomarkers for AD pathology.
3. Future directions: Longitudinal investigations of cotemporaneous changes in retinal measures, AD-related neuropathologies, and cognition are warranted to establish retinal changes as a biomarker for AD.

2 | METHODS

The IRAP study is a collaboration between the Sheba Medical Center, Israel, and the Maccabi Healthcare Services (MHS), the second largest health maintenance organization in Israel. The study was approved by the Sheba Medical Center and MHS institutional review board committees and all participants signed an informed consent. The described research adhered to the tenets of the Declaration of Helsinki.

The IRAP study methods have been described in detail elsewhere.[16] Briefly, the study collects detailed cognitive, health-related, genetic, lifestyle, and brain imaging data, with follow-up visits every 3 years. The main source of participant recruitment is through advertisements on the home page of the MHS website and participants' word of mouth. Eligibility criteria are (1) age between 40 and 65, (2) MHS membership, and (3) fluency in Hebrew. After obtaining informed consent, each IRAP participant completes an entry assessment that includes anthropometric measurements, neuropsychological testing by trained neuropsychologists, laboratory testing, and a detailed health and lifestyle history. Assessments are performed at the Sheba Medical Center. The majority of IRAP participants (N = 409; 80.7%) have a parental family history of AD, making this sample, overall, enriched for AD risk. Of the 507 IRAP participants, 401 were randomly approached and offered to

take part in the retinal assessment. Of these, 301 were recruited, out of whom, 56 were ineligible due to ocular pathologies (such as glaucoma, retinal injury, blindness, or visual acuity of 20/50 or worse), leaving 245 participants in the present analysis (see study flowchart, Figure S1 in supporting information).

2.1 | Determination of parental AD status

Detailed methods are provided in Ravona-Springer et al.[16] Briefly, individuals who approach the study team undergo initial questioning about their age, MHS membership, and parental dementia. Then, medical records of parents of potential participants are provided to the study team and a dementia questionnaire (DQ) is administered telephonically prior to invitation of potential participants to the study site. The DQ is a validated, informant-based instrument, to determine the likely presence of AD in parents of potential study volunteers.[17] All the medical history and diagnostic workup available is reviewed together with the DQ by the study team to reach a probable AD diagnosis (according to National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria) or lack of, in parents of potential participants. Offspring of probands with partial information about dementia type or with dementia other than AD, are excluded from the study. Parents with an AD onset before the age of 55 are assumed to have familial early onset AD, and their offspring are excluded from the study. Siblings are excluded from the study as their data may substantively confound results, especially of analyses including genetic components. Thus, the first offspring who volunteers and is eligible is included in the study.

2.2 | *APOE* ε4 genotyping

Blood samples are collected for *APOE* genotype. DNA is extracted and frozen at −80°C. *APOE* status is determined based on rs429358 and rs7412 single nucleotide polymorphism genotypes[18] at LGC company, UK, using Kompetitive allele specific polymerase chain reaction (KASP) technology.

2.3 | Neuropsychological assessment

A neuropsychological battery was administered to participants on the day of retinal assessment. The following tests were included: Rey Auditory Verbal Learning test–immediate and delayed recall and recognition,[19] Trail Making Tests A and B, the digit symbol substitution test,[20] and the digit span tests forward and backward.[21] A composite measure of all tests was calculated by converting each test score to a z-score; the mean of all z-scores comprised a measure of global cognition.

2.4 | Ophthalmic assessment

All participants underwent a complete ophthalmologic examination including determination of best-corrected visual acuity (BCVA), color vision (Farnsworth D15 test), and Humphrey perimetry (Swedish Interactive Threshold Algorithm standard protocol). After pupillary dilation, biomicroscopy including intraocular pressure measurement (Goldmann applanation tonometry) and spectral domain optical coherence tomography (SD-OCT) imaging were performed. Detection of ocular pathologies such as glaucoma or diabetic retinopathy, even in subtle, preclinical stages, led to exclusion of the participant from the study.

2.5 | Retinal macular layers thickness

SD-OCT imaging was done in both eyes after pupil dilation by three experienced OCT technicians certified for obtaining clinical trial images using a single Heidelberg SPECTRALIS SD-OCT device (Heidelberg Engineering) equipped with TruTrack technology that recognizes eye movements to overcome motion artifacts, and the SPECTRALIS Glaucoma Module Premium Edition software.

The scanner automatically detects retinal landmarks and aligns the scans relative to the participant's individual fovea-to- Bruch's membrane opening (BMO) center to improve accuracy and reproducibility of the measurements and to overcome measurement errors due to eye movement and head tilting.

To obtain perifoveal volumetric retinal scans, both eyes (except for n = 12 cases in which the left eye was excluded following the criteria indicated above) were examined using a fast macula protocol with automatic real time (ART) mean value of 9, acquiring 25 horizontal lines (6 × 6 mm area), each consisting of 512 A scans per line.[22] Retinal laters' thickness was determined by the automatic segmentation algorithms of the SPECTRALIS software. The SPECTRALIS SD-OCT software divides the macular scan into nine sectors, as defined by the Early Treatment Diabetic Retinopathy Study= scheme: a center circle of 1 mm diameter, inner (3 mm) and outer (6 mm) nasal, superior, temporal, and inferior sectors (Figure 1A[22]).

In this study we focused on full macular thickness (measured from the internal limiting membrane to the Bruch's membrane) and the thicknesses of inner retinal layers (from the internal limiting membrane [ILM] through to the external limiting membrane [ELM]) that include the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL; Figure 1B). Retinal layer thickness was determined automatically using the device software.[22]

The optic nerve head (ONH) analysis was performed using the BMO as the anatomical border of the rim. Peripapillary RNFL thickness was determined in three circle scans automatically centered on the individual fovea-to-BMO center axis to ensure accurate definition of each single sector independent of head position. Only the central circle was included in the analysis (Figure 1C[23]). The software automatically reports the mean global RNFL thickness and RNFL thickness in the temporal superior, nasal superior, nasal, nasal inferior, temporal inferior, and temporal sectors (Figure 1D, E).

Two ophthalmologists (YR and OZ) reviewed the OCT scans to exclude subjects with ocular pathologies and to verify correct layer

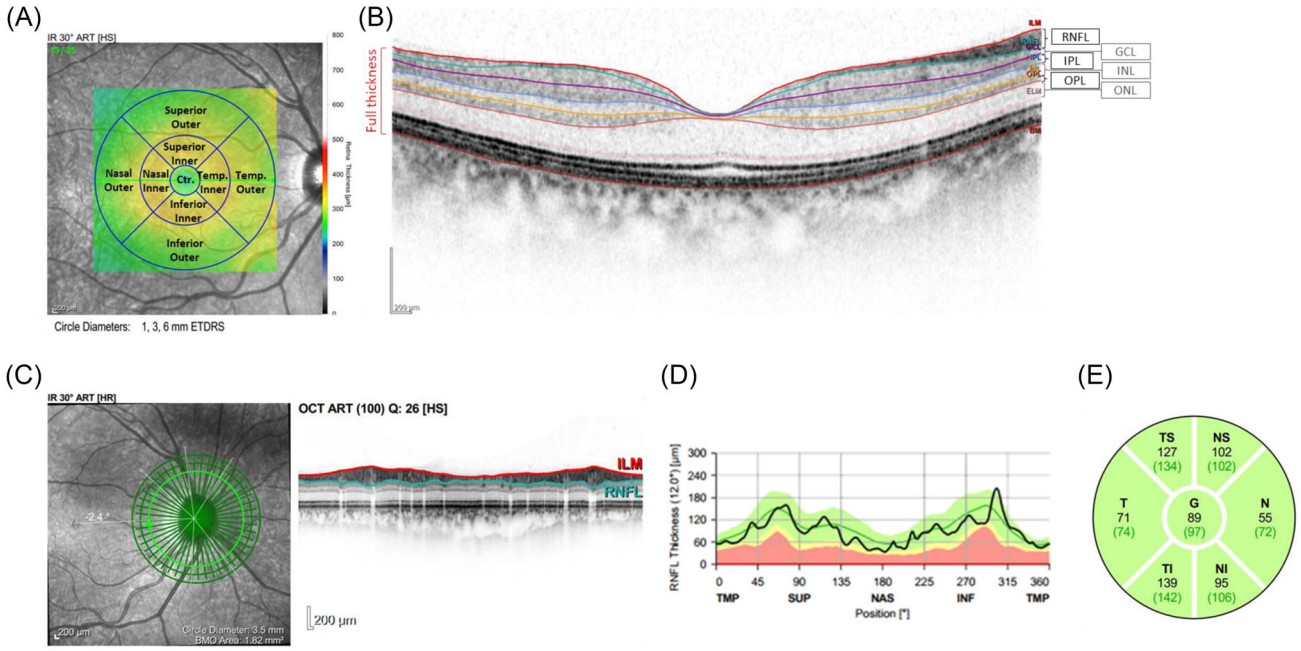

FIGURE 1 Spectral domain optical coherence tomography (OCT) imaging of the macular (A, B) and optic nerve head (C-E) areas. A, Representative macular scan depicting macular sectors (Ctr., center, nasal-inner, nasal-outer, superior-inner, superior-outer, temporal [temp.]-inner, temporal [temp.]-outer, inferior-inner, and inferior-outer) and automatic segmentation of macular layers by the Heidelberg OCT software (B). C, Optic nerve head scan. The inner circular scan was used for analysis. D, E, Automatic measurement of peripapillary retinal nerve fiber layer (RNFL) thickness in six sectors (T, temporal; TS, temporal superior; NS, nasal superior; N, nasal; NI, nasal inferior; TI, temporal inferior) and mean peripapillary thickness (G, global)

assignment. Data were automatically exported from the SPECTRALIS software and used for statistical analysis.

2.6 | Statistical analysis

For descriptive purposes, comparisons between *APOE* ε4 carriers and non-carriers were performed using χ^2 for categorical variables and independent *t*-tests for continuous variables. We compared retinal thickness between *APOE* ε4 carriers and non-carriers adjusting for age, sex, education, and BCVA in nine retinal subfields using multivariate analyses of covariance (MANCOVA). To assess whether some retinal layers are more susceptible than others to the potential effect of the *APOE* genotype on retinal thickness in each of the retinal subfields, we performed a secondary analysis; MANCOVA including the nine subfields was applied adjusting for the same co-variants for each retinal layer separately. *P*-value was set at .05. Analyses were performed using SPSS v. 21.

3 | RESULTS

Two hundred forty-five IRAP participants were included in the analysis, 245 right eyes (79 [32.24%] *APOE* ε4 carriers) and 233 left eyes (76 [32.6%] *APOE* ε4 carriers). Participants' mean age was 59.60 (standard deviation [SD] = 6.42) years, 66.4% were women, and the mean number of years of education was 16.73 (SD = 3.13). *APOE* ε4 carriers did not significantly differ from non-carriers in age, sex, education, BCVA, or global cognition (Table 1).

3.1 | Retinal thickness by *APOE* genotype

Compared to *APOE* ε4 non-carriers, significantly increased macular full thickness was observed in *APOE* ε4 carriers (F [9231] = 2.3, Wilks' $\lambda = 0.918$, $P = .017$). These differences reached statistical significance for the inner and outer nasal (mean difference: +5.83 μm; $P = .009$ and +5.41 μm $P = .005$; respectively), inner superior (+4.32 μm; $P = .041$), and inner and outer inferior (+4.82 μm; $P = .013$, and +3.72 μm; $P = .033$, respectively) sectors (Table 2, Figure 2A). Retinal thickness did not differ significantly by *APOE* status for the other subfields ($P > .05$). A similar trend was observed for the left eye, with differences reaching statistical significance in the inner nasal sector (+5.02 μm, $P = .022$) and approaching statistical significance in the outer nasal and inner inferior sectors (Table S2, Figure 2D).

3.2 | Retinal thickness in specific retinal layers by *APOE* ε4 genotype

In a secondary analysis, we examined whether the association of *APOE* ε4 genotype with retinal thickness is driven by differences in specific retinal layers. As shown in Table 3, in the inner ring, *APOE* ε4 carriers had thicker GCL and IPL layers in all sectors. Thus, mean

TABLE 1 Descriptive characteristics of study participants by *APOE* ε4 genotype

Demographic variable	*APOE* ε4 carriers (n = 79)	*APOE* ε4 non-carriers (n = 166)	*P* value
Age—mean (SD)	59.617 (6.87)	59.590 (6.21)	0.975
Sex (% female)	64.2%	67.5%	0.609
Years of education	16.864 (3.21)	16.663 (3.10)	0.636
Best corrected visual acuity	0.03 (0.08)	0.02 (0.03)	0.352
Global cognition	0.065 (0.61)	−0.086 (0.63)	0.192

Abbreviations: APOE, apolipoprotein E; SD, standard deviation.

TABLE 2 Macular full thickness (right eye)

Sector	*APOE* ε4 carriers (n = 79)[a]	*APOE* ε4 non-carriers (n = 166)[a]	F	*P* value
Nasal inner	341.18 (15.99)	335.35 (15.97)	6.991	0.009**
Nasal outer	310.07 (14.02)	304.66 (14.00)	7.948	0.005**
Superior inner	338.35 (15.36)	334.03 (15.35)	4.229	0.041*
Superior outer	293.19 (12.98)	291.05 (12.97)	1.453	0.229
Temporal inner	325.29 (14.11)	321.89 (14.09)	3.108	0.079
Temporal outer	275.16 (11.92)	273.79 (11.91)	0.704	0.402
Inferior inner	336.55 (14.10)	331.73 (14.08)	6.238	0.013*
Inferior outer	282.95 (12.66)	279.23 (12.65)	4.609	0.033*
Center	224.67 (22.82)	225.17 (22.80)	0.025	0.875

[a]Mean thickness (SD) in μm; *$P < .05$ **$P < .01$.
Abbreviations: *APOE*, apolipoprotein E; SD, standard deviation

thickness of the GCL layer was higher in *APOE* ε4 carriers by a mean of 1.64 μm ($P = .011$), 1.06 μm ($P = .073$), 1.42 μm ($P = .034$), and 1.54 μm ($P = .012$) in the inner nasal, superior, temporal, and inferior sectors, respectively (Figure 2B). The mean thickness of the IPL layer was higher in *APOE* ε4 carriers by a mean of 1.52 μm ($P = .001$), 1.25 μm ($P = .009$), 1.42 μm ($P = .049$), and 0.84 μm ($P = .048$) in the inner nasal, superior, temporal, and inferior sectors, respectively (Figure 2C). In the outer ring, *APOE* ε4 carriers had thicker RNFL (+1.8 μm, $P = .033$) and thicker INL, approaching statistical significance (+0.74 μm, $P = .051$) in the nasal sector, and thicker RNFL, approaching statistical significance (+1.48 μm, $P = .053$) in the inferior sector (Table 3). *APOE* ε4 carriers did not differ from non-carriers, in outer retinal layers (Table 3). For the left eye, a similar trend was observed (Table S3 in supporting information, Figure 2D-F), with significantly increased thickness of the GCL (+1.39 μm, $P = .028$) and IPL (+0.93 μm, $P = .038$) in the inner nasal sector, as well as the IPL in the inferior sector (+0.84 μm, $P = .051$) and outer superior sector (+0.70 μm, $P = .050$). In the outer superior sector, the OPL was significantly thinner in *APOE* ε4 carriers (−1.5 μm, $P = .033$).

3.3 | Peripapillary RNFL thickness by *APOE* ε4 genotype

pRNFL thickness did not differ by *APOE* ε4 in any of the sectors (Tables S1 and S4 in supporting information).

4 | DISCUSSION

In a cohort of cognitively asymptomatic middle-aged people enriched for AD risk, individuals carrying the *APOE* ε4 genotype had increased full macular thickness in the nasal, superior, and inferior sectors. This relationship was mainly driven by thicker inner retinal layers (IPL and GCL) in the inner macular ring. Our study provides new evidence pointing to retinal thickness alterations, already in midlife, in yet cognitively normal individuals carrying the *APOE* ε4 genotype, the most robust genetic risk factor for sporadic AD. These findings support the potential of retinal measurements as early biomarkers for AD and for disease progression.

Previous studies examining early retinal biomarkers for AD examined populations at high risk due to family history, subjective cognitive complaints, brain amyloid positivity, or their combination. Some,[15,24] but not all,[25,26] reported an association of retinal thickness with AD pathology. In line with our findings, thickening of the inner nasal macular region has been reported in cognitively normal middle-aged and older adults with subjective cognitive complaints and brain amyloid positivity.[24] This finding remained significant after adjustment for *APOE* ε4 status, but the differential role of *APOE* ε4 genotype on retinal thickness was not examined. In cognitively normal offspring of AD patients with subjective cognitive complaints (mean age = 62.8 years), brain amyloid positivity was associated with greater IPL thickness. Moreover, the degree of amyloid burden in the brain was

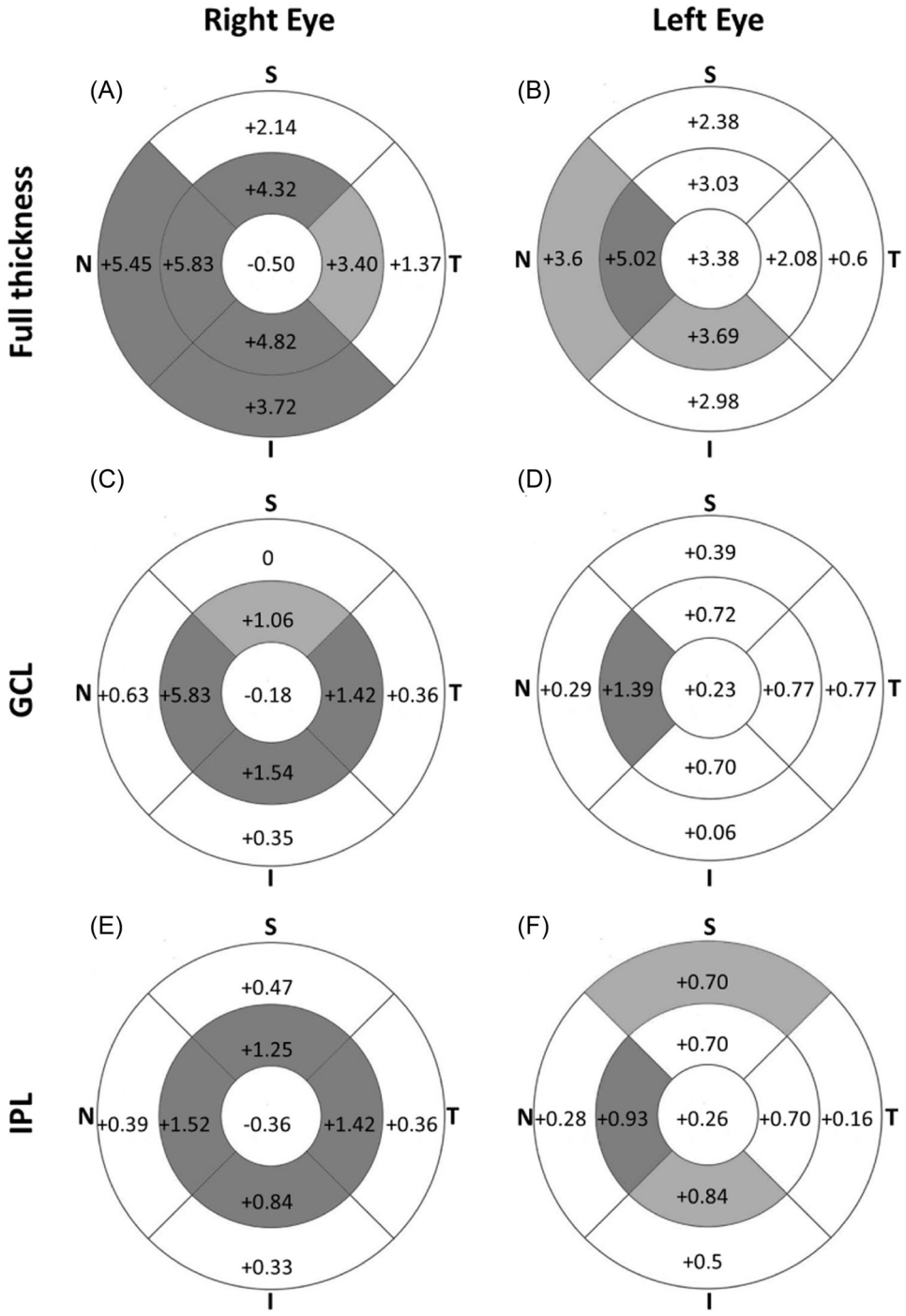

FIGURE 2 Schematic representation of multivariate analyses of covariance (MANCOVA) analysis of macular thickness. MANCOVA analysis identified areas in the macula that were statistically significantly thicker (dark gray) in full macular thickness (A, D), ganglion cell layer (GCL; B, E), and inner plexiform layer (IPL; C, F) layers in the right eye (A-C) and left eye (D-F) of apolipoprotein E (*APOE*) ε4 carriers compared to non-carriers. Sectors approaching statistical significance are shown in light gray. Numbers indicate the mean difference between *APOE* ε4 carriers and non-carriers (in μm)

associated with the surface area of retinal inclusion bodies, suspected to contain fibrillary forms of amyloid, and with IPL volume.[15] Interestingly, the inclusion bodies were observed within or in proximity to the IPL.[15] These findings are consistent with those of the present study, demonstrating IPL thickening in asymptomatic middle-aged high AD risk *APOE* ε4 carriers, pointing to the IPL as a highly sensitive retinal layer to early neurodegenerative alterations related to AD.

In contrast to our findings, AD biomarkers such as cerebrospinal fluid Aβ and tau ratio or brain amyloid positivity were not associated with retinal layers' thickness in the macula in cognitively normal older

TABLE 3 Thickness of macular layers (right eye)

Retinal sector	Retinal layer	*APOE* ε4 carriers (n = 79) [a]	*APOE* ε4 non-carriers (n = 166) [a]	F	*P* value
Nasal inner	Total			1.902	0.081
	RNFL	21.78 (2.94)	21.16 (2.93)	2.332	0.128
	GCL	51.39 (4.68)	49.75 (4.67)	6.565	0.011*
	IPL	43.08 (3.36)	41.56 (3.36)	10.588	0.001**
	INL	41.49 (4.13)	40.88 (4.31)	1.163	0.282
	OPL	30.93 (5.59)	31.47 (8.10)	0.239	0.626
	ONL	71.38 (10.40)	69.52 (10.39)	1.697	0.194
Nasal outer	Total			1.603	0.147
	RNFL	50.78 (6.15)	48.98 (6.15)	4.577	0.033*
	GCL	37.37 (3.65)	36.74 (3.64)	1.571	0.211
	IPL	28.98 (2.71)	28.59 (2.71)	1.136	0.288
	INL	34.09 (2.78)	33.35 (2.78)	3.853	0.051
	OPL	27.25 (3.68)	27.53 (3.68)	0.300	0.584
	ONL	52.90 (7.37)	51.99 (7.36)	0.813	0.368
Superior inner	Total			1.695	0.123
	RNFL	24.92 (3.66)	24.16 (3.65)	2.284	0.132
	GCL	51.84 (4.31)	50.78 (4.31)	3.232	0.073
	IPL	41.77 (3.33)	40.52 (3.32)	6.942	0.009**
	INL	41.81 (4.03)	41.60 (4.03)	0.143	0.706
	OPL	30.84 (7.25)	31.78 (7.25)	0.895	0.345
	ONL	66.64 (10.26)	64.94 (10.25)	1.463	0.228
Superior outer	Total			1.057	0.389
	RNFL	39.00 (5.61)	38.58 (5.61)	0.296	0.587
	GCL	33.94 (3.12)	33.94 (3.11)	0.009	0.926
	IPL	28.08 (2.44)	27.61 (2.44)	1.888	0.171
	INL	31.55 (2.64)	31.32 (2.64)	0.376	0.540
	OPL	25.51 (2.55)	25.79 (2.55)	0.655	0.419
	ONL	56.34 (6.75)	55.70 (6.75)	0.480	0.489
Temporal inner	Total			1.008	0.421
	RNFL	17.27 (1.70)	17.39 (1.70)	0.278	0.599
	GCL	47.70 (4.86)	46.28 (4.85)	4.530	0.034*
	IPL	41.68 (3.25)	40.76 (3.24)	3.919	0.049*
	INL	38.66 (3.97)	38.01 (3.96)	1.413	0.236
	OPL	28.76 (3.79)	28.90 (3.78)	0.068	0.794
	ONL	70.55 (7.51)	70.12 (7.49)	0.182	0.670
Temporal outer	Total			0.850	0.532
	RNFL	19.10 (1.67)	18.98 (1.67)	0.251	0.617
	GCL	35.02 (3.75)	34.66 (3.74)	0.497	0.482
	IPL	31.34 (2.57)	30.98 (2.58)	0.978	0.324
	INL	32.89 (2.46)	32.39 (2.46)	2.216	0.138
	OPL	25.35 (1.96)	25.73 (1.95)	2.042	0.154
	ONL	53.77 (5.85)	53.47 (5.84)	0.142	0.707

(Continues)

TABLE 3 (Continued)

Retinal sector	Retinal layer	*APOE* ε4 carriers (n = 79) [a]	*APOE* ε4 non-carriers (n = 166) [a]	F	*P* value
Inferior inner	Total			1.140	0.340
	RNFL	26.18 (3.43)	25.68 (3.44)	1.149	0.285
	GCL	52.44 (4.43)	50.90 (4.43)	6.464	0.012*
	IPL	41.16 (2.95)	40.32 (2.94)	3.937	0.048*
	INL	42.26 (4.55)	41.36 (4.54)	2.096	0.149
	OPL	30.18 (5.63)	30.34 (5.63)	0.041	0.839
	ONL	65.03 (8.84)	64.15 (8.82)	0.525	0.469
Inferior outer	Total			0.909	0.489
	RNFL	41.14 (5.56)	39.66 (5.55)	3.787	0.053
	GCL	32.44 (3.31)	32.09 (3.31)	0.603	0.438
	IPL	26.69 (2.54)	26.36 (2.54)	0.945	0.332
	INL	30.82 (2.51)	30.38 (2.51)	1.662	0.199
	OPL	25.58 (2.55)	25.53 (2.55)	0.021	0.885
	ONL	49.32 (5.64)	48.97 (5.64)	0.206	0.650
Center	Total			0.295	0.939
	RNFL	3.41 (3.67)	3.44 (3.67)	0.002	0.967
	GCL	3.45 (4.15)	3.63 (4.16)	0.088	0.767
	IPL	7.58 (4.54)	7.94 (4.53)	0.341	0.560
	INL	4.67 (5.49)	5.20 (5.48)	0.494	0.483
	OPL	7.37 (7.50)	7.90 (7.49)	0.291	0.590
	ONL	105.94 (16.38)	103.38 (16.36)	1.313	0.253

Abbreviations: *APOE*, apolipoprotein E; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; RNFL, retinal nerve fiber layer; SD, standard deviation.

[a] Mean thickness (SD) in μm; *$P < .05$ **$P < .01$.

adults (mean age 70),[25] or were associated cross-sectionally[27,28] with retinal thinning rather than thickening. The associations of Aβ burden with longitudinal trends in macular thickness are inconsistent, with some studies demonstrating greater,[26] while others show less,[29] macular thinning over time. In those studies, the role of *APOE* ε4 genotype was not examined. High AD risk was associated with retinal layers' thinning is a small study comparing retinal thickness between middle-aged individuals at opposite "extremes" of AD risk: offspring of AD patients, carriers of the *APOE* ε4 genotype (n = 35) versus individuals without a parental history of AD who were *APOE* ε4 non-carriers (n = 29).[30] There were no statistically significant differences in total retinal thickness. The contradicting findings may be only apparent. Retinal involvement in AD pathology has been suggested to follow a similar course to that of the brain,[31] including thickening of the RNFL at its earliest stages, potentially reflecting gliotic reactive changes,[32] then followed by neurodegeneration and atrophy.[33] Thus, initial retinal thickening may result from neuroinflammation in response to tissue damage related to amyloid and tau deposition.[34] In contrast to the optic nerve head, which is composed of retinal cells' axons, the macula mostly contains the cell somas; thus, edema secondary to glial cell activation in the macula could account for the selective thickening in this retinal region. The lack of association of AD proneness with retinal thickening[25] or its association with retinal thinning[26,28] was most often reported in older cohorts, in which the degree of neuropathology—and the resulting effect on retinal thickness—may be more advanced, thus surpassing the early hypertrophic phase and reaching the atrophic phase, even in the presence of normal cognition. An additional factor potentially affecting the observations reported in older individuals is that irrespective of AD pathology, with advancing age, the retina undergoes atrophic changes, which may mask the effects of early AD-related neurodegeneration on the retina.[35]

Macular layers' thickening in *APOE* ε4 carriers participating in the IRAP study was most consistently detected in the IPL and GCL layers. The IPL may be more susceptible to amyloid deposition compared to other retinal layers.[15] This retinal layer is rich in cholinergic activity.[36] Brain cholinergic activity is disrupted early in the AD pathological process. Hence, thickening of the IPL may represent early brain cholinergic disruption. Of note, the *APOE* ε4 genotype has been associated with an accentuated reduction in age-related brain cholinergic activity,[37] suggesting that this gene may also affect retinal cholinergic activity.

The susceptibility of the GCL layer, containing the retinal neuronal cell bodies, to AD pathology, has also been previously demonstrated as reflected in cell loss and thinning[38] of this layer in AD patients compared to healthy controls. The GCL has been hypothesized to undergo processes that are parallel to neurons in the brain in response to AD neuropathology, that is, initial hypertrophy followed by degeneration and atrophy.[31]

The present findings could potentially be attributed to retinal vascular abnormalities, which have previously been demonstrated to be associated with brain amyloid load,[39] and to be affected by the *APOE* ε4 genotype.[40]

Finally, the *APOE* ε4 genotype has been demonstrated to affect retinal synapses, similarly to its effect on the brain synapses, even in very young mice carrying the *APOE* ε4 genotype.[41] This effect was observed in the IPL and OPL layers.[41] Additionally, in response to injury, more pronounced neovascularization, inflammation, and activation of Müller cells in the choroid and retina were observed in mice carrying the *APOE* ε4 genotype compared to those carrying the *APOE* ε3 genotype,[42] further supporting observations of initial retinal thickening in response to AD pathology.

In the IRAP cohort, the relationships of *APOE* ε4 genotype with macular thickness were most prominent in the inner macular ring. Consistent with our findings, others demonstrated a positive association between degree of brain amyloid positivity and total retinal thickness in the inner ring in cognitively healthy people aged ≥ 60.[25] Though this finding did not withstand correction for multiple comparisons, it may provide some evidence regarding the vulnerability of this macular region to the AD pathological process. As previously discussed, the effect of AD pathology on retinal layers' thickness may change over the course of the disease and with age, possibly explaining negative associations between brain amyloid positivity and retinal thickness in the inner ring in some studies.[27,38]

Strengths of the present study include the assessment of retinal characteristics in a relatively large sample of middle-aged asymptomatic individuals enriched for high AD risk, for whom *APOE* genotype was available. A thorough ophthalmological examination was conducted to exclude ocular pathologies that could have affected the results, such as glaucoma or macular edema. Study limitations include lack of amyloid or tau imaging in the retina and the brain, precluding examination of whether these pathologies are underlying mechanisms for the *APOE*–retinal thickness link. Inflammatory markers were not collected in the study, preventing assessment of the role of inflammation to the results observed. Finally, the IRAP study primarily comprises White individuals with relatively high education and may differ in important ways from middle-aged persons from different ethnicities and socioeconomic status. A similar trend of the relationships between macular thickness and *APOE* genotype were observed in both eyes. However, the differences between *APOE* ε4 carriers and non-carriers were more prominent in the right eye. These differences may have resulted from exclusion of 12 left eyes from the analysis; however, a similar predominance of the right eye in the associations between AD risk and retinal findings has previously been reported[28]

In conclusion, we provide new evidence demonstrating that the *APOE* ε4 genotype is related to macular changes already in midlife in asymptomatic individuals. We also show that the IPL and the GCL may be layers with particular susceptibility to these changes. Overall, our results support the growing recognition that retinal alterations represent early AD pathology and may assist in its very early detection. Longitudinal investigations of cotemporaneous changes in retinal measures, including retinal vasculature, AD-related neuropathologies, and cognition, are warranted to establish retinal changes as a biomarker for AD.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Ygal Rotenstreich, Inbal Sharvit-Ginon, Ifat Sher, Michal Schnaider Beeri, and Ramit Ravona-Springer: study design and initiation, data collection, data analysis, and manuscript preparation. Michal Schnaider Beeri, Aron Weller, and Anthony Heymann: manuscript final review. Ofira Zloto, Ido Didi Fabian, and Amir Abd-Ekader: data collection.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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