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The *APOE* E4 Allele Is Associated with Faster Rates of Neuroretinal Thinning in a Prospective Cohort Study of Suspect and Early Glaucoma

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Purpose: To investigate the association between the apolipoprotein E (*APOE*) E4 dementia-risk allele and prospective longitudinal retinal thinning in a cohort study of suspect and early manifest glaucoma.

Design: Retrospective analysis of prospective cohort data.

Participants: This study included all available eyes from participants recruited to the Progression Risk of Glaucoma: Relevant SNPs [single nucleotide polymorphisms] with Significant Association (PROGRESSA) study with genotyping data from which *APOE* genotypes could be determined.

Methods: Apolipoprotein E alleles and genotypes were determined in PROGRESSA, and their distributions were compared with an age-matched and ancestrally matched normative cohort, the Blue Mountains Eye Study. Structural parameters of neuroretinal atrophy measured using spectral-domain OCT were compared within the PROGRESSA cohort on the basis of *APOE* E4 allele status.

Main Outcome Measures: Longitudinal rates of thinning in the macular ganglion cell–inner plexiform layer (mGCIPL) complex and the peripapillary retinal nerve fiber layer (pRNFL).

Results: Rates of mGCIPL complex thinning were faster in participants harboring ≥1 copies of the *APOE* E4 allele ($\beta = -0.13$ μm/year; $P \leq 0.001$). This finding was strongest in eyes affected by normal-tension glaucoma (NTG; $\beta = -0.20$ μm/year; $P = 0.003$). Apolipoprotein E E4 allele carriers were also more likely to be lost to follow-up ($P = 0.01$) and to demonstrate a thinner average mGCIPL complex (70.9 μm vs. 71.9 μm; $P = 0.011$) and pRNFL (77.6 μm vs. 79.2 μm; $P = 0.045$) after a minimum of 3 years of monitoring.

Conclusions: The *APOE* E4 allele was associated with faster rates of mCGIPL complex thinning, particularly in eyes with NTG. These results suggest that the *APOE* E4 allele may be a risk factor for retinal ganglion cell degeneration in glaucoma. *Ophthalmology Science 2022;2:100159* © *2022 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

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Glaucoma describes a heterogeneous group of optic neuropathies characterized by specific patterns of retinal ganglion cell atrophy and permanent vision loss.[1] Primary open-angle glaucoma (POAG) is the most common subtype of glaucoma and is defined by the presence of glaucoma in an eye with an anatomically normal anterior chamber.[1] Although intraocular pressure (IOP) is the only recognized modifiable risk factor of POAG,[2] not all cases of POAG are characterized by elevated IOP. For example, normal-tension glaucoma (NTG), a common subphenotype of POAG, is characterized by disease progression in the presence of low to normal IOP. Therefore, POAG is a complex disease with hypothesized pathologic contributions from underlying neurologic or systemic disease processes.[3,4]

A body of evidence supports an association between POAG and dementia, with multiple studies demonstrating epidemiologic associations.[5–16] Of these, one large population-based registry study demonstrated a strong

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association between NTG and the incident diagnosis of dementia,[9] and another demonstrated a higher prevalence of impaired cognition in patients with NTG compared with those with high-tension glaucoma (HTG) sampled from a large multinational glaucoma registry.[17] Like POAG, dementing diseases, including Alzheimer's disease, frontotemporal dementia, and Lewy body disease, result from progressive loss of neurons within the central nervous system both generally[18] and within the retina.[19,20] Evidence also exists of a shared genetic architecture between POAG and these dementing phenomena, with genes such as *optineurin* and *tank-binding kinase 1* implicated in Mendelian forms of frontotemporal dementia and NTG.[21–23] Furthermore, genome-wide association studies have identified POAG risk variants in an intronic region of *membrane-associated protein tau* and *near amyloid precursor protein*, which are genes involved in Mendelian forms of Alzheimer's disease and frontotemporal dementia.[24–26] However, because of conflicting data from other studies investigating genetic associations between POAG and dementia,[27–30] the nature of this association remains unclear.

Several explanations exist for why these studies have not resulted in a consensus understanding of this association. First, because POAG[31,32] and dementia[33] are underdiagnosed disease phenomena, epidemiologic studies investigating their associations may be confounded by diagnostic mislabeling (information bias). Second, because dementia is associated with self-neglect, which commonly results in missed diagnosis of comorbid diseases,[34] such studies may be confounded by recruitment bias toward participants with normal cognition (selection bias).

Attempting to account for these potential biases, further studies have investigated associations between genetic risk parameters for dementia and POAG.[35–44] The most commonly investigated genetic parameter in these studies is the apolipoprotein E (*APOE*) genotype, the principal genetic determinant of Alzheimer's disease,[45] Lewy body disease,[46] and all-cause dementia.[47] The *APOE* gene encodes a lipid transport molecule with major functions within the central nervous system.[48] Three common *APOE* alleles (E2, E3, and E4) are determined by 2 single nucleotide polymorphisms (SNPs) in colocated protein-coding regions within the *APOE* gene. The *APOE* E3 (wild-type) allele is the most common *APOE* allele across ethnicities and is used in research to define the baseline risk of dementia developing. Relative to *APOE* E3, the *APOE* E2 allele is associated with decreased dementia risk, whereas *APOE* E4 is associated with increased dementia risk.[47] Because of its association with an increased prevalence of all-cause dementia by 80 years of age (E4 heterozygotes odds ratio, 1.9; E4 homozygotes odds ratio, 3.6),[47] the *APOE* E4 allele has been the most commonly studied of these alleles in POAG. Although several studies have demonstrated positive associations between *APOE* E4 and POAG prevalence,[38,41] others have demonstrated either no association[35–37,39,40] or depletion of *APOE* E4 within POAG cohorts,[42–44] highlighting the possibility that the *APOE* E4 may even be protective against POAG.[42]

This study sought to further mitigate any potential confounding factors complicating studies investigating the association between POAG and genetic risk of dementia by exploring associations between the *APOE* E4 allele and structural markers of neuroretinal thinning relevant to glaucoma progression within the Progression Risk of Glaucoma: Relevant SNPs with Significant Association (PROGRESSA) cohort, a prospectively monitored cohort of suspect and early manifest glaucoma.

Methods

Ethical Approval

Ethics approval was obtained through the Southern Adelaide Clinical Human Research Ethics Committee, and all participants were enrolled by written informed consent. This study adhered to the tenets of the Declaration of Helsinki and followed the National Health and Medical Research Council statement of ethical conduct in research involving humans.

Study Overview

This study investigated associations between the *APOE* E4 allele and prospective neuroretinal thinning in the PROGRESSA study, a closely monitored cohort of patients with suspect glaucoma and early manifest glaucoma. To determine baseline distribution of *APOE* genotype and *APOE* E4 allele prevalence within PROGRESSA, we made comparisons with the Blue Mountains Eye Study (BMES), an age-matched and ancestrally matched population acquisition cohort. To address potential confounding resulting from impaired survivorship associated with the *APOE* E4 allele,[49] we investigated associations between *APOE* genotype and study attrition. The primary outcome of neuroretinal thinning was assessed in eyes of participants characterized by their *APOE* E4 allele status by analyzing longitudinal change in spectral-domain (SD) OCT parameters, including average macular ganglion cell–inner plexiform layer (mGCIPL) complex thickness and average peripapillary retinal nerve fiber layer (pRNFL) thickness.

Sampling

All eyes from participants recruited to the PROGRESSA study for whom genotyping data were available were included. The PROGRESSA study is a prospective longitudinal cohort study of >2000 individuals with suspect or early manifest glaucoma that was designed to investigate genetic and clinical associations with glaucoma progression (as previously described).[50–52] Participants within this cohort undergo 6-month routine ophthalmic examinations that include SD OCT imaging. A reference cohort, the BMES cohort,[53] was included as an age-matched and ancestrally matched cohort to determine baseline distribution of the *APOE* genotypes and alleles within PROGRESSA. In brief, the BMES is a population-based study that sampled participants $\geq$ 49 years of age from 2 postal codes in New South Wales, Australia. The initial cohort (BMES I; n = 3654) was sampled from 1992 through 1994 and captured 82.4% of noninstitutionalized residents. The subsequent extension cohort (BMES E) identified a further 1510 residents from the same postal codes as identified through the following national census. DNA microarray data were available for 2571 individuals from these 2 cohorts.

Genotyping

The 3 major *APOE* alleles (E2, E3, and E4) are defined by 2 SNPs in colocated protein coding regions within the *APOE* gene (rs429358 and rs7412; GRCh38 reference genome). Apolipoprotein E alleles and genotypes were determined for individuals from both PROGRESSA and BMES cohorts using a combination of DNA microarray and exome sequencing data. DNA microarray genotyping was generated using HumanCoreExome arrays (Illumina), and exome sequencing data was generated using the SureSelect Human All Exon version 5 enrichment platform on an Illumina (NovaSeq 6000) sequencer. Because neither of the relevant *APOE* SNPs were included within the DNA microarray panel, both were imputed in Minimac3 using Haplotype Reference Consortium r1.1 as a reference panel.[54] Individual *APOE* allele frequencies were calculated as proportions of total *APOE* alleles within each sample. Although allele discrepancies between methods would most often result from DNA microarray imputation errors (rs429358, $R^2 = 0.93$; rs7412, $R^2 = 0.92$), we opted a priori to exclude any participants with discordant imputations between the 2 sequencing methods.

OCT

Associations between the *APOE* E4 allele and structural markers of neuroretinal thinning were investigated using output data from serial longitudinal SD OCT scans performed in PROGRESSA participants. All structural data were derived from CIRRUS SD OCT images of the optic nerve head and macula (software version 9.5; Carl Zeiss Meditec). Structural parameters quantifying thickness of tissues constituting primary retinal neurons were analyzed, including the mGCIPL complex (generated using the macula 512 × 128 cube) and the pRNFL (generated using the optic disc 200 × 200 cube). Longitudinal changes in SD OCT thickness were analyzed using corresponding macular and optic nerve head guided progression analysis output data. These tools estimate rates of change in the average mGCIPL complex and pRNFL by determining the slope of a regression line plotted using the initial and most recent (up to 7) SD OCT scan measurements. Imaging was performed by an experienced operator using CIRRUS FastTrac eye-tracking technology with fixation adjustments to capture the optic disc or fovea. Image quality was subsequently assessed by an experienced clinician (H.M.) who examined each individual scan. Scans were included if they met the following criteria: signal strength of ≥ 6, no significant acquisition artefact, and no evidence of any grossly apparent nonglaucomatous retinal pathologic features resulting in either overestimation of cell layer thickness (epiretinal membrane with macular traction or macular edema) or impaired automated segmentation (macular edema or incident age-related macular degeneration). All genotyped individuals had been monitored for a minimum of 3 years. We performed longitudinal analyses using the CIRRUS-generated rates of change in the average thicknesses of the mGCIPL complex and pRNFL acquired using SD OCT guided progression analysis data generated at the time of the most recent measurement.

Clinical and Ophthalmic Parameters

Loss to follow-up data were identified from the PROGRESSA study database, where study attrition is qualified as deceased or "no more appointments." For the purpose of this study, we analyzed attrition as total attrition, which represented all-cause attrition, with subanalysis of deceased and "opt-out" (no more appointments) participants. Reasons for no more appointments were not routinely documented, and in accordance with the PROGRESSA ethics protocol, we were unable to recontact these participants retrospectively to clarify. For each eye, IOP, which is associated with neuroretinal thickness, was measured. Baseline IOP, defined as IOP at recruitment, was used for baseline analyses. All IOP measurements were obtained using Goldmann applanation tonometry. Mean longitudinal IOP across monitoring was used for longitudinal rate-of-change analyses.

Statistical Analysis

All statistical analyses were performed in R software version 4.0.3 (R Foundation for Statistical Computing). In accordance with statistical best practice in ophthalmology, we used mixed-effects models to account for potential confounding resulting from inter-eye correlation within individuals contributing 2 eyes to analyses.[55] Mixed-effect models were implemented using the lmer function from the lmerTest package version 3.1.3 in R. Statistical significance was defined by a P value threshold of 0.05. To account for missing ophthalmic data, we imputed missing guided progression analysis data using the Amelia II package version 1.8.0.[56] In brief, this package imputes missing values within a dataframe using an expectation-maximization with bootstrapping algorithm. An imputation data frame was generated by applying the amelia function using a single imputation (m = 1) to the study data matrix.

Because of strong intercorrelation between the SD OCT parameters of interest (mGCIPL complex and pRNFL thickness), no multiple testing corrections were made in their analyses. Post hoc pairwise comparisons of baseline features underwent multiple testing corrections using the Benjamini-Hochberg method. Outcome statistics from these analyses were represented as adjusted P values.

Genotype Distribution

Apolipoprotein E genotype and allele frequencies were compared between PROGRESSA and BMES studies using logistic regression models as previously described.[57] For the genotypic regression (formula 1), an indicator variable for each non-E3E3 genotype was included. For the allelic regression (formula 2), an indicator variable for E2 or E4 status (each defined by the presence or absence of the relevant allele) was included. Both models also included age and gender as covariates. Thus defined, a significant β_{e4e4} term in the genotypic regression would be interpreted as a significant difference in *APOE* E4E4 genotype frequencies between study cohorts.

$$\begin{aligned} \mathrm{logit}(study) = {} & \beta_0 + \beta_{e2e2}X_{e2e2} + \beta_{e2e3}X_{e2e3} + \beta_{e2e4}X_{e2e4} \\ & + \beta_{e3e4}X_{e3e4} + \beta_{e4e4}X_{e4e4} + \beta_{Age}Age \\ & + \beta_{Gender}Gender + e \end{aligned} \quad (1)$$

and

$$\mathrm{logit}(study) = \beta_0 + \beta_{e2}X_{e2} + \beta_{e4}X_{e4} + \beta_{Age}Age + \beta_{Gender}Gender + e \quad (2)$$

Sensitivity Analysis

To account for potential confounding factors, we investigated correlations between *APOE* genetic status and common parameters associated with glaucoma progression and neuroretinal thickness. These comparisons were made according to (1) genotype and (2) the presence or absence of the *APOE* E4 allele. Polygenic glaucoma risk, which is independently associated with longitudinal retinal thinning in glaucoma, was determined using the summary statistics of a recent multitrait analysis of advanced glaucoma genome-wide association study.[24] Raw polygenic risk scores were calculated for each individual as previously described using the cumulative effect sizes at an optimal predetermined P value

threshold ($P < 0.001$).[24] These scores were normalized within the study sample and represented as z scores. Ophthalmic parameters including baseline IOP, mean longitudinal IOP, and baseline thickness of the mGCIPL complex and pRNFL were compared between eyes. Genotype comparisons demonstrating P values < 0.05 underwent post hoc pairwise analysis using paired independent sample t tests (continuous data) and chi-square tests (categorical data). Reported test statistics included mean ± standard error (SE) for continuous data, and total numbers, with proportions and P values for categorical data. Adjusted P values were reported for post hoc analyses.

Structural Progression

Structural progression was analyzed by comparing longitudinal change in mGCIPL complex and pRNFL thickness between participants categorized according to the presence or absence of the *APOE* E4 allele. Because of strong associations between various parameters of retinal thinning, multivariate linear models were implemented, including the additional covariates of mean longitudinal IOP, baseline thickness of the relevant layer (mGCIPL complex or pRNFL), and gender. Recognizing that glaucoma polygenic risk score strongly correlates with glaucomatous parameters including IOP and vertical cup-to-disc ratio (a proxy marker of pRNFL),[24] we chose to exclude polygenic risk scores from the structural progression models to avoid overcorrection. To minimize the potentially confounding effect of missing data from individuals who had left the study before obtaining sufficient SD OCT data to generate longitudinal rates of structural thinning, we included an imputation model in which missing data were imputed. To account for attrition, rates of longitudinal thinning of the average mGCIPL complex and pRNFL in the eyes of participants who had died or left the PROGRESSA study were imputed for an inclusive subanalysis. Results were reported as P values with β coefficients (continuous variables) or odds ratios with 95% confidence intervals (categorical variables). The latest average thicknesses of the mGCIPL complex and pRNFL, measured at the most recent appointment, were compared across genotypes using analyses of variance. To compare these measurements between participants characterized based on the presence or absence of the *APOE* E4 allele, we used mixed-effect logistic regression models with adjustment for covariates of age (at most recent appointment) and the single fixed effect of participant identification.

Subgroup analyses were performed to investigate associations between the *APOE* E4 allele and longitudinal rates of neuroretinal thinning in glaucomatous and nonglaucomatous eyes separately. Glaucomatous eyes were characterized by the presence of a reproducible glaucomatous visual field defect at any time during the monitoring period. Glaucomatous visual field defects were defined according to the PROGRESSA study criteria as abnormal glaucoma hemifield test results or pattern standard deviation with a probability value of $< 5\%$, accompanied by 3 contiguous Humphrey visual field (HVF) points with a pattern deviation probability value of $< 5\%$, that were reproducible in the same zone on 2 consecutive HVF tests. If the glaucoma hemifield test results and the pattern standard deviation were normal, then the 3 contiguous HVF locations were required to have a pattern deviation defect at $< 1\%$ probability value on 2 consecutive HVF tests. Eyes classified as having no glaucoma did not meet the criteria defining a glaucomatous visual field defect at any time during monitoring. However, in accordance with PROGRESSA recruitment criteria, all eyes were sampled from individuals with ≥ 1 eye exhibiting optic disc features suspicious for glaucoma (i.e., disc damage likelihood score, 1−2).[58]

Because the literature suggests that possible associations between glaucoma and dementia,[9,17] and glaucoma and the *APOE* E4 allele may be most relevant to NTG,[42] we further subclassified glaucomatous eyes according to their pressure subphenotypes. Normal-tension glaucoma eyes were defined by a highest pretreatment IOP of ≤ 21 mmHg, and HTG eyes were defined by a highest recorded IOP of > 21 mmHg. Eyes undergoing IOP-lowering therapy at the time of recruitment were allocated to the HTG group if they had a documented history of IOP of > 21 mmHg or to the NTG group if they had no documented history of IOP of > 21 mmHg. Rates of longitudinal change in the average mGCIPL complex and pRNFL were subsequently investigated according to *APOE* E4 allele status in participants classified as having NTG, HTG, or no glaucoma. Similar to the multivariate analysis above, these analyses involved linear regression models with adjustment for mean longitudinal IOP, baseline thickness of the relevant layer (mGCIPL complex or pRNFL), gender, and the single fixed effect of participant identification to account for intereye correlation.

Results

Samples

Complete genotyping data were available for 1162 PROGRESSA participants. Genotyping concordance between DNA microarray and whole exome sequencing data was high (44/45 individuals with imputations from both methods [97.8%]; Supplemental Fig S1). The single discordant *APOE* genotype imputation, which was excluded from further analyses, was likely the result of the margin of error associated with imputing these SNPs from DNA microarray data. Normative control data were generated using all participants from the BMES for whom age, gender, and genotyping data were available ($n = 2571$; Fig 1). The mean age in PROGRESSA was marginally higher than in BMES (mean ± SE, 65.0 ± 10.6 vs. mean ± standard deviation, 63.1 ± 8.3 years; $P < 0.001$). Gender was similar between cohorts (percentage of male participants, 44.1% [PROGRESSA] vs. 43.4% [BMES]; $P = 0.69$). The most common self-reported ethnicity within PROGRESSA was "Caucasian" (82.1%; Supplemental Table S1). No differences in *APOE* genotype distribution nor *APOE* E4 allele frequency were observed between the PROGRESSA and BMES cohorts (Fig 2).

After manually reviewing all SD OCT images, 127 eyes were excluded either because of segmentation errors (67 eyes), nonglaucomatous retinal pathologic features (50 eyes), or poor SD OCT signal strength (< 6; 10 eyes). Baseline SD OCT parameters were analyzed using the remaining 1124 participants (2170 eyes). Prospective analysis of SD OCT change was performed using participants who underwent ≥ 4 reliable SD OCT scans of the mGCIPL complex or pRNFL at 6-month intervals. A total of 532 and 724 eyes were excluded from longitudinal analysis of the pRNFL and mGCIPL complex, respectively, because of incomplete longitudinal SD OCT data. Longitudinal analysis of pRNFL change included 1446 eyes from 760 participants, and longitudinal analysis of the pRNFL included 1638 eyes from 853 participants (Fig 1).

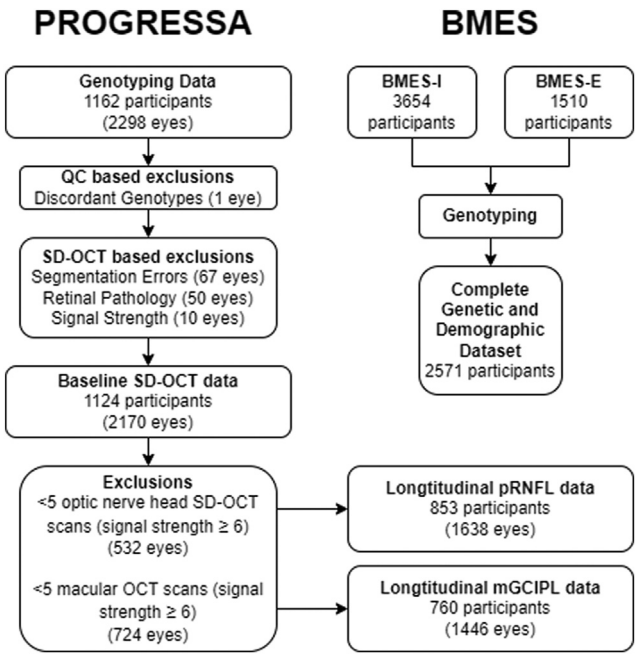

Figure 1. Sample flowchart. Sampling was performed using all genotyping data from the Progression Risk of Glaucoma: Relevant SNPs with Significant Association (PROGRESSA) and Blue Mountains Eye Study (BMES) cohorts. Sampling from PROGRESSA included all participants for whom genotyping data were available. One participant (contributing 1 eye to analysis) was excluded because of discordant genotyping results between DNA microarray and exome data. Analysis of genotype correlations with structural spectral-domain (SD) OCT parameters were made using all available baseline and longitudinal data after exclusions based on the presence of confounding nonglaucomatous retinal pathologic features or poor-quality images. Sampling from the BMES cohort included all participants from the baseline cohort (recruited 1992–1994; BMES-I) and extension cohort (recruited in 1999–2000; BMES-E) for whom demographic and genotyping data were available. Genotype comparisons between the 2 studies were performed using all participants from both studies. mGCIPL = macular ganglion cell–inner plexiform layer; QC = quality control; pRNFL = peripapillary retinal nerve fiber layer.

PROGRESSA Parameters

Baseline parameters were analyzed within the PROGRESSA cohort through comparisons made according to (1) *APOE* genotype and (2) the presence or absence of the *APOE* E4 allele. No observed differences were found in age, gender, or polygenic glaucoma risk scores across groups (Table 1). No differences in glaucoma or NTG prevalence were observed across groups. Baseline IOP differed across genotypes ($P = 0.049$), but not between participants categorized based on the presence or absence of the *APOE* E4 allele ($P = 0.80$). Mean longitudinal IOP also differed across genotypes ($P = 0.022$), but not between participants categorized based on the presence or absence of the *APOE* E4 allele ($P = 0.22$). Post hoc pairwise comparisons demonstrated no significant difference in baseline IOP between individual genotypes. However, mean longitudinal IOP was higher in eyes from E2E3 participants when compared with eyes from E3E3 participants (16.2 mmHg vs. 15.6 mmHg; $P = 0.049$) and E3E4 participants (16.2 mmHg vs. 15.5 mmHg; $P = 0.049$; Table 1; Supplemental Fig S2). No differences in baseline thicknesses of the mGCIPL complex or pRNFL were observed between eyes categorized according to participant genotype or the presence or absence of the *APOE* E4 allele.

Study Attrition

Total study attrition, defined as a registered exit from the PROGRESSA study because of death or opting out, varied according to *APOE* genotype ($P = 0.047$; Table 1) and was higher in participants harboring ≥ 1 copies of the *APOE* E4 allele ($P = 0.01$; Fig 3). Post hoc pairwise comparison of total study attrition according to genotype demonstrated higher attrition in the E4E4 group compared with the wild-type E3E3 group (15.0% vs. 3.0%), which survived multiple testing correction ($P = 0.035$; Supplemental Fig S3).

Structural Progression

Longitudinal rates of SD OCT thinning in the mGCIPL complex and pRNFL were compared between participants categorized by the presence or absence of the *APOE* E4 allele (Table 2). In our multivariate regression model, which adjusted for age, mean longitudinal IOP, baseline average mGCIPL complex thickness, gender, and the single fixed effect of participant identification (to account for intereye correlation), mean ± SE mGCIPL complex thickness changed at rate of -0.13 ± 0.04 μm/year faster in participants harboring the *APOE* E4 allele ($P < 0.001$). This association was also apparent in an imputation multivariate model that imputed average mGCIPL complex rate of change for eyes of participants who left the PROGRESSA study because of death or opting out (36 eyes of 19 individuals; $\beta = -0.14$ μm/year [SE, 0.04 μm/year]; $P < 0.001$).

Rates of change in the average pRNFL thickness were nominally but nonsignificantly lower in a similar multivariate model accounting for age, mean longitudinal IOP, baseline average pRFNL thickness, gender, and the single fixed effect of participant identification ($\beta = -0.07$ μm/year [SE = 0.05 μm/year]; $P = 0.17$). Similarly, no significant association between the prevalent *APOE* E4 allele and average pRNFL thinning was observed in an imputation model that included imputations for rate of change of the average pRNFL thickness for all eyes of participants who completed 3 years of longitudinal monitoring, but left the PROGRESSA study because of death or opting out (24 eyes from 13 individuals).

In a subanalysis of eyes classified as NTG, HTG, or no glaucoma, we observed that the association between the *APOE* E4 allele and rate of mGCIPL complex thinning was apparent in NTG eyes ($\beta = -0.20$ μm/year [SE, 0.07 μm/year]; $P = 0.003$), but not in HTG or no glaucoma eyes (HTG: $\beta = -0.14$ μm/year [SE, 0.09 μm/year]; $P = 0.15$; no glaucoma: $\beta = -0.08$ μm/year [SE, 0.05 μm/year]; $P = 0.08$). No associations were observed between *APOE* E4 allele status and rates of pRNFL thinning in NTG, HTG, or no glaucoma eyes (Table 3).

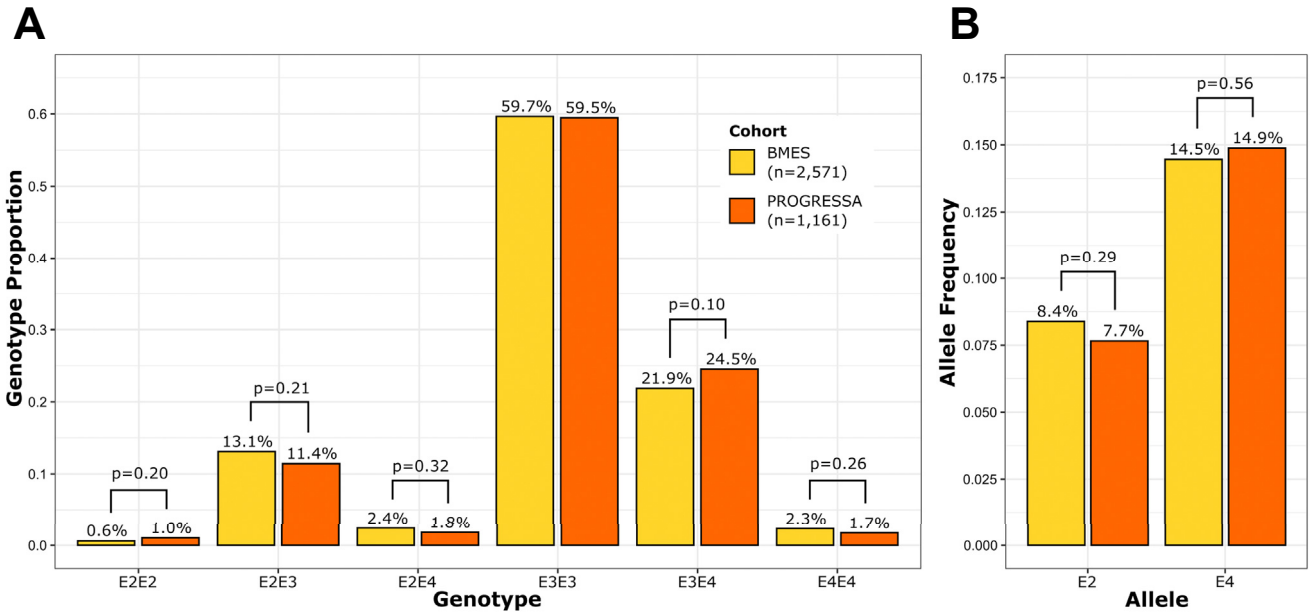

Figure 2. Bar graphs showing apolipoprotein E (*APOE*) genotype and allele proportions. **A**, Comparison of *APOE* genotype proportions between the Progression Risk of Glaucoma: Relevant SNPs with Significant Association (PROGRESSA; orange) and Blue Mountains Eye Study (BMES; yellow) cohorts. **B**, Comparison of *APOE* E4 allele frequency between the PROGRESSA (orange) and BMES (yellow) cohorts. No differences were observed in (**A**) proportions of each of the 6 *APOE* genotypes between the cohorts, nor in (**B**) the minor allele (i.e., *APOE* E2 and E4) frequencies between the 2 cohorts. *P* values were determined using logistic regression analysis with adjustment for age, gender, and genotype (genotype comparison) or the alternate minor allele (allele comparison).

Because of our observations that both the *APOE* E4 allele and advancing age were associated with faster rates of mGCIPL complex thinning, we reproduced our multivariate model while testing for interaction between the 2 variables (i.e., [*APOE* allele status × age]). However, this interaction term was not significantly associated with the outcome ($P = 0.82$), so was not included in our summary statistics. Structural progression was further investigated by comparing thickness of the average mGCIPL complex and pRNFL, which did not differ between participants characterized by the presence or absence of the *APOE* E4 allele at baseline (mean thickness: mGCIPL complex, 74.4 μm vs. 74.7 μm [$P = 0.44$]; pRNFL, 81.7 μm vs. 82.2 μm [$P = 0.40$]; Table 1). At the time of the most recent clinical visit,

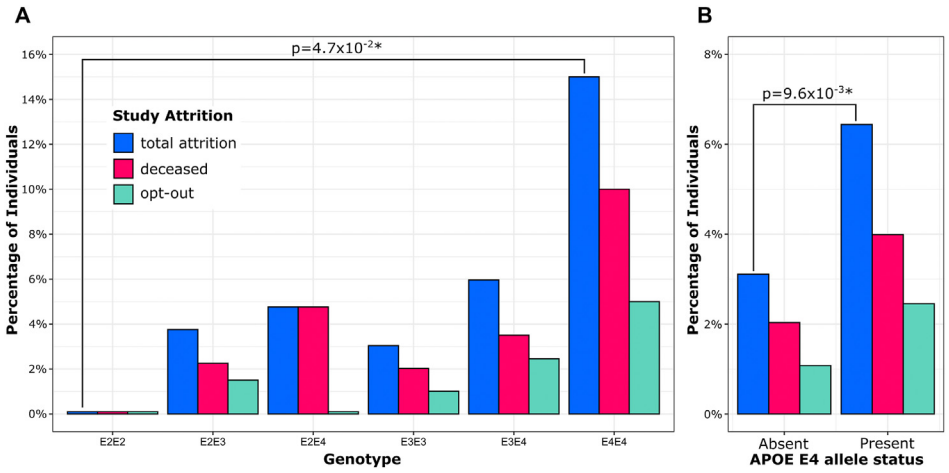

Figure 3. Bar graphs showing study attrition by apolipoprotein E (*APOE*) genotype. Comparisons of study attrition from the Progression Risk of Glaucoma: Relevant SNPs with Significant Association cohort were compared by (**A**) *APOE* genotype and (**B**) based on the presence or absence of the *APOE* E4 allele. Total attrition was defined as the combined total number of participants who had died over the monitoring period (deceased) or who declined follow-up (opt-out). Statistical analysis of attrition by *APOE* genotype was performed using chi-square tests comparing total attrition between groups. *P* values are reported for groupwise comparisons of total attrition. *$P < 0.05$.

Table 1. Demographic, Genetic, and Ophthalmic Characteristics by *APOE* E Status

	APOE Genotype							**APOE E4 Status**		
	E2E2	*E2E3*	*E2E4*	*E3E3*	*E3E4*	*E4E4*	P *Value*	*≥1 Copy*	*0 Copies*	P *Value*
Sample size, no.	12	133	21	691	285	20	NA	326	837	NA
Age at enrolment (yrs), mean ± SE	65.9 ± 14.2	66.2 ± 10.1	64.5 ± 10.5	64.9 ± 10.7	64.5 ± 10.5	67.1 ± 9.3	0.62	64.6 ± 10.5	65.1 ± 10.6	0.48
Sex, male:female (% male)	5:7 (41.7)	61:72 (45.9)	11:10 (52.4)	297:395 (43.0)	131:154 (46.0)	8:12 (40.0)	0.89	150:176 (46.0)	363:474 (43.4)	0.41
Polygenic risk score (*z* score*), (SE)	−0.11 (0.81)	−0.02 (0.96)	0.12 (1.36)	−0.02 (1.00)	0.08 (1.02)	−0.18 (0.79)	0.67	0.06 (1.03)	−0.03 (0.99)	0.18
Total attrition, no. (% of total)	0 (0.0)	5 (3.7)	1 (4.8)	21 (3.0)	17 (6.0)	3 (15.0)	0.047†	21 (6.4)	26 (3.1)	0.01†
Deceased	0 (0.0)	3 (2.3)	1 (4.8)	14 (2.0)	10 (3.5)	2 (10.0)	0.22	13 (4.0)	17 (2.0)	0.059
Opted out	0 (0.0)	2 (1.5)	0 (0.0)	7 (1.0)	7 (2.5)	1 (5.0)	0.39	8 (2.5)	9 (1.1)	0.078
Eyes, no.	23	249	34	1287	543	37	NA	614	1559	NA
Glaucoma diagnosis, no. (% affected)	14 (60.8)	101 (40.6)	10 (29.4)	519 (40.3)	233 (42.9)	15 (55.6)	0.25	258 (42.0)	634 (40.7)	0.58
NTG eyes, no. (% affected)	5 (21.7)	52 (20.9)	5 (14.7)	335 (26.0)	144 (26.5)	7 (18.9)	0.27	156 (25.4)	392 (25.1)	0.90
Baseline IOP (mmHg), mean ± SE	19.3 ± 7.1	18.4 ± 5.8	18.1 ± 9.0	17.4 ± 5.5	17.4 ± 5.4	18.1 ± 4.6	0.049†	17.5 ± 5.6	17.6 ± 5.6	0.80
Mean longitudinal IOP (mmHg), mean ± SE	16.2 ± 3.0	16.2 ± 3.5	15 ± 3.5	15.6 ± 3.3	15.5 ± 3.3	16.4 ± 3.4	0.022†	15.5 ± 3.3	15.7 ± 3.3	0.22
SE (diopters), mean ± SE	−0.9 ± 2.6	−0.2 ± 2.2	1.0 ± 0.9	−0.2 ± 2.2	−0.4 ± 2.6	−0.2 ± 1.6	0.19	−0.3 ± 2.5	−0.2 ± 2.2	0.58
Baseline mGCIPL complex thickness (μm), mean ± SE	76.7 ± 10.7	73.8 ± 7.8	75.9 ± 6.7	74.9 ± 8.1	74.5 ± 8.4	72.1 ± 6.9	0.17	74.4 ± 8.2	74.7 ± 8.1	0.44
Baseline pRNFL thickness (μm), mean ± SE	85.3 ± 14.7	81.5 ± 10.8	84.1 ± 8.9	82.4 ± 11.8	81.6 ± 11.8	81.6 ± 12.2	0.53	81.7 ± 11.6	82.2 ± 11.6	0.40

APOE = apolipoprotein E; IOP = intraocular pressure; mGCIPL = macular ganglion cell–inner plexiform layer; NA = not analyzed; NTG = normal-tension glaucoma; pRNFL = peripapillary retinal nerve fiber layer; SE = standard error.

Comparisons of baseline parameters between participants were performed on the basis of (1) genotype and (2) the presence or absence of the *APOE* E4 allele. The same criteria were used to categorize individual eyes investigated in analysis of relevant ophthalmic parameters. These analyses were performed using analysis of variance testing for continuous variables and chi-square tests for categorical variables. Comparisons between eyes from these groups were performed using mixed-effects linear regression with adjustment for the single fixed effect of participant identification (to account for intereye correlations in individuals contributing > 1 eye to the analysis). Boldface values indicate statistical significance.

*Glaucoma polygenic risk is represented as *z* scores based on normalization of the corresponding raw scores within the analyzed Progression Risk of Glaucoma: Relevant SNPs with Significant Association cohort.

†$P < 0.05$.

Table 2. Structural Rates of Change

	Multivariate Model		Imputation Model	
	β Coefficient	P Value	β Coefficient	P Value
Difference in mGCIPL complex rate of change (μm/year) in participants harboring the *APOE* E4 allele	−0.13 ± 0.04	<0.001*	−0.14 ± 0.04	<0.001*
Age (yrs)	$-6.9 \times 10^{-3} \pm 1.7 \times 10^{-3}$	<0.001*	$-6.1 \times 10^{-3} \pm 1.7 \times 10^{-3}$	<0.001*
Mean longitudinal IOP (mmHg)	$-2.8 \times 10^{-2} \pm 5.3 \times 10^{-3}$	<0.001*	$-2.8 \times 10^{-2} \pm 5.1 \times 10^{-3}$	<0.001*
mGCIPL complex baseline thickness (μm)	$2.9 \times 10^{-3} \pm 2.0 \times 10^{-3}$	0.14	$4.1 \times 10^{-3} \pm 2.0 \times 10^{-3}$	0.04†
Sex (male)‡	5.6×10^{-3} (-2.8×10^{-2} to 3.9×10^{-2})	0.87	6.3×10^{-3} (−0.03 to 0.04)	0.94
Difference in pRNFL rate of change (μm/yr) in participants harboring the *APOE* E4 allele	−0.08 ± 0.05	0.12	−0.06 ± 0.05	0.20
Age (yrs)	$-7.3 \times 10^{-3} \pm 2.2 \times 10^{-3}$	0.001§	$-7.7 \times 10^{-3} \pm 2.1 \times 10^{-3}$	<0.001*
Mean longitudinal IOP (mmHg)	$-4.5 \times 10^{-2} \pm 6.5 \times 10^{-3}$	<0.001*	$-4.5 \times 10^{-2} \pm 6.4 \times 10^{-3}$	<0.001*
pRNFL baseline thickness (μm)	$-6.0 \times 10^{-3} \pm 1.7 \times 10^{-3}$	<0.001*	$-5.4 \times 10^{-3} \pm 1.6 \times 10^{-3}$	0.001§
Sex (male)‡	1.2×10^{-2} (-3.2×10^{-2} to 5.6×10^{-2})	0.78	8.3×10^{-3} (-3.4×10^{-2} to 5.1×10^{-2})	0.85

APOE = apolipoprotein E; IOP = intraocular pressure; mGCIPL = macular ganglion cell−inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer.

Longitudinal rates of change in average thickness of the mGCIPL complex and pRNFL were compared between the eyes of participants categorized based on the presence or absence of the *APOE* E4 allele. Multivariate models were performed using mixed-effects linear regression adjusting for age, mean longitudinal IOP, baseline thickness of the corresponding spectral-domain OCT layer (mGCIPL or pRNFL), gender, and the single fixed effect of participant identification (to account for intereye correlations). Imputation models included additional mGCIPL or pRNFL longitudinal thickness data imputed for the eyes of participants who left the Progression Risk of Glaucoma: Relevant SNPs with Significant Association study before sufficient spectral-domain OCT scans were available to determine longitudinal rates of thinning. Data are presented as ± standard error, unless otherwise indicated. Boldface values indicated statistical significance.

*$P < 0.001$.

†$P < 0.05$.

‡Gender differences between groups are represented as odds ratios with 95% confidence intervals.

§$P < 0.01$.

both of these parameters were thinner in participants harboring ≥ 1 copy of the *APOE* E4 allele (mean thickness: mGCIPL complex, 70.9 μm vs. 71.9 μm [$P = 0.011$]; pRNFL, 77.6 μm vs. 79.2 μm; $P = 0.045$), despite younger age within the *APOE* E4-positive cohort (mean thickness: 69.2 μm vs.70.4 μm years [$P = 0.049$]; Table 4).

Discussion

This study investigated longitudinal associations between the *APOE* E4 allele and parameters of neuroretinal atrophy in a cohort comprising patients with suspect and early manifest glaucoma. Our principal finding of faster rates of mGCIPL complex thinning in participants harboring ≥ 1 copy of the *APOE* E4 allele provides novel evidence that *APOE* genotype may be a significant risk factor for retinal ganglion cell loss. Furthermore, because the same structural parameters are relevant to glaucomatous disease progression, our findings may suggest a common pathophysiologic link between POAG and dementia.

Our primary finding of faster rates of mGCIPL complex thinning in participants harboring the *APOE* E4 allele supports previous data demonstrating associations between mGCIPL complex and retinal thickness and dementia.[59] However, the mechanisms by which mGCIPL complex thinning may occur in individuals harboring the *APOE* E4 allele remain unclear. Apolipoprotein E is an important lipid transport protein within the central nervous system that seems to result in pathologic sequelae through an impaired ability of the APOE E4 isoform to clear toxic metabolic fragments, including β-amyloid and tau protein.[60] In addition to its role in dementia, the *APOE* E4 allele is associated with other features of neuronal vulnerability, including poor outcomes after traumatic brain injury.[61] Accordingly, *APOE* E4-associated mCGIPL thinning may be a consequence of retinal ganglion cell sensitivity to pathologic processes such as increased IOP. Our observation that this association was strongest in NTG-affected eyes provides a suggestion that this neurodegenerative allele may be relevant to glaucoma progression, even in the absence of raised IOP.

The PROGRESSA study differs from most other glaucoma cohort studies through inclusion of patients with suspect glaucoma and those with mild disease at enrollment. Consequently, the PROGRESSA participants are relatively young at recruitment, and glaucoma has not developed in all yet. We anticipated that performing this study in PROGRESSA would mitigate the survivorship effect of the *APOE* E4 allele and would minimize any possible selection bias associated with underdiagnosis of glaucoma in individuals with dementia. We accordingly observed similar distributions of the *APOE* genotypes and *APOE* E4 allele between the PROGRESSA and BMES cohorts. This observation seems consistent with multiple cohort studies

Table 3. Structural Rates of Change and Glaucoma Status

			NTG		HTG	No Glaucoma
No. of eyes			548		344	1279
Complete longitudinal mGCIPL complex data, no. (% total)			398 (72.6)		307 (89.2)	731 (57.2)
Complete longitudinal pRNFL data, no. (% total)			420 (76.6)		317 (92.2)	835 (65.3)
	β Coefficient	***P* Value**	**β Coefficient**	***P* Value**	**β Coefficient**	***P* Value**
Difference in mGCIPL complex rate of change (μm/yr) in participants harboring the *APOE* E4 allele	−0.20 (0.07)	0.003*	−0.14 (0.09)	0.15	−0.08 (0.05)	0.08
Age (yrs)	-1.9×10^{-3} (3.4×10^{-3})	0.58	-5.4×10^{-3} (4.6×10^{-3})	0.24	-6.4×10^{-3} (2.0×10^{-3})	**0.001***
Mean longitudinal IOP (mmHg)	-1.9×10^{-2} (1.3×10^{-3})	0.14	-3.7×10^{-2} (1.4×10^{-2})	**0.009***	-2.4×10^{-2} (6.4×10^{-3})	**<0.001†**
mGCIPL complex baseline thickness (μm)	-4.1×10^{-3} (3.7×10^{-3})	0.27	7.3×10^{-3} (4.7×10^{-3})	0.12	-1.6×10^{-3} (2.7×10^{-3})	0.55
Sex (male)‡	5.5×10^{-2} (-3.4×10^{-3} to 0.11)	0.35	6.2×10^{-2} (-2.4×10^{-2} to 0.15)	0.47	-2.0×10^{-2} (-6.1×10^{-2} to 2.0×10^{-2})	0.62
Difference in pRNFL rate of change (μm/yr) in participants harboring the *APOE* E4 allele	−0.10 (0.15)	0.49	−0.16 (0.13)	0.22	0.10 (0.15)	0.49
Age (yrs)	-1.5×10^{-3} (7.3×10^{-3})	0.83	-4.3×10^{-3} (6.8×10^{-3})	0.53	-5.6×10^{-4} (6.3×10^{-3})	0.92
Mean longitudinal IOP (mmHg)	-5.9×10^{-2} (2.5×10^{-2})	0.02§	-2.7×10^{-2} (2.1×10^{-2})	0.18	-5.6×10^{-2} (1.7×10^{-2})	**<0.001†**
pRNFL baseline thickness (μm)	1.0×10^{-3} (4.1×10^{-3})	0.81	-1.6×10^{-2} (4.7×10^{-3})	**<0.001†**	-1.3×10^{-2} (4.6×10^{-3})	**0.005***
Sex (male)‡	8.9×10^{-2} (-4.1×10^{-2} to 0.22)	0.49	8.3×10^{-3} (-2.4×10^{-2} to 2.2×10^{-3})	0.41	1.4×10^{-2} (−0.11 to 0.15)	0.92

APOE = apolipoprotein E; HTG = high-tension glaucoma; IOP = intraocular pressure; mGCIPL = macular ganglion cell–inner plexiform layer; NTG = normal-tension glaucoma; pRNFL = peripapillary retinal nerve fiber layer.
Longitudinal rates of change in average thickness of the mGCIPL complex and pRNFL were compared between the eyes of participants categorized based on the presence or absence of the *APOE* E4 allele. Eyes were classified further as glaucomatous in the presence of a reproducible glaucomatous defect. The subphenotypes of normal-tension glaucoma (NTG) and high-tension glaucoma (HTG) were defined by highest pretreatment pressures of 21 mmHg or less (NTG) or more than 22 mmHg (HTG), respectively. Glaucomatous eyes with no recorded pretreatment pressure were defined as having HTG. Multivariate models were performed using mixed-effects linear regression adjusting for age, mean longitudinal IOP, baseline thickness of the corresponding spectral-domain OCT layer (mGCIPL or pRNFL), gender, and the single fixed effect of participant identification (to account for intereye correlations). Data are presented as ± SE, unless otherwise indicated. Boldface values indicate statistical significance.
*$P < 0.01$.
†$P < 0.001$.
‡Gender differences between groups are presented as odds ratios (95% confidence intervals) and associated p-values.
§$P < 0.05$.

investigating *APOE* E4 allele frequency in glaucoma.[35–40,62] However, several other epidemiologic studies have demonstrated either enrichment or depletion of the *APOE* E4 allele in glaucoma cohorts.[41–44] Research into the population distribution of the *APOE* E4 allele has elucidated several reasons why genotype–phenotype studies of the *APOE* E4 allele may be confounded. First, the *APOE* E4 allele is associated with impaired survivorship in the elderly,[49,63] with one study demonstrating a decrease in E4 allele frequency from 17.6% to 8.3% from 60 to 90 years of age.[64] Second, ethnicity is associated with significant variation in *APOE* E4 allele frequency, which ranges from 8.5% to 40.7% in Africans, from 5.2% to 31.0% in Europeans, and from 7.1% to 24.0% in Asians.[65] Finally, the *APOE* E4 allele is strongly associated with both prevalent and incident dementia in elderly populations from which glaucoma studies are sampled.[66] Dementia is recognized to be associated with self-neglect and missed diagnosis of other chronic diseases and may be depleted from samples selecting individuals on the basis of having a diagnosis of glaucoma.[34] Accordingly, we observed high attrition in E4 homozygotes when compared with E3 homozygotes (15.0% vs. 3.0% total attrition) and in participants harboring ≥ 1 copy of the *APOE* E4 allele (6.4% vs. 3.1% total attrition). One study that demonstrated enrichment of the *APOE* E4 allele in an older POAG cohort suggested that this allele may confer neuroprotective benefits within the glaucomatous retina.[42] Our results suggest an alternative explanation, which is that *APOE* E4 allele depletion within glaucoma cohorts could be an artefact of these selection biases, which may be inherent to aging glaucoma cohorts.

Our study had several limitations, including our inability to fully account for missing data. As part of the PROGRESSA protocol, participants undergo 6-month SD OCT scans of the optic nerve head and macula. Factors such as

Table 4. SD OCT Parameters at the Time of the Most Recent Clinical Assessment

	APOE Genotype							APOE E4 Status		
	E2E2	*E2E3*	*E2E4*	*E3E3*	*E3E4*	*E4E4*	P *Value*	*Positive*	*Negative*	P *Value*
Age at latest follow-up (yrs)	69.8 ± 17.3	71.6 ± 9.9	68.2 ± 6.8	70.1 ± 9.8	69.0 ± 10.8	72.2 ± 8.1	0.15	69.2 ± 10.5	70.4 ± 10.0	0.049*
Latest mGCIPL complex thickness (μm)	73.5 ± 12.1	70.9 ± 9.1	70.4 ± 7.9	72.1 ± 9.1	70.8 ± 9.6	73.0 ± 10.3	0.14	70.9 ± 9.6	71.9 ± 9.1	0.011*
Latest pRNFL thickness (μm)	81.3 ± 15.1	78.8 ± 11.7	78.9 ± 10.6	79.2 ± 12.0	77.7 ± 12.4	76.3 ± 12.9	0.28	77.6 ± 12.4	79.2 ± 12.0	0.045*

APOE = apolipoprotein E; mGCIPL = macular ganglion cell–inner plexiform layer; pRNFL = peripapillary retinal nerve fiber layer; SD OCT = spectral-domain OCT. Boldface indicates statistical significance.
Thickness of the average mGCIPL complex and average pRNFL measured from the most recent SD OCT scans were compared between participants categorized by (1) *APOE* genotype and (2) according the presence or absence of the *APOE* E4 allele. Comparisons based on genotype were performed using analysis of variance tests, and comparisons based on the presence or absence of the *APOE* E4 allele were performed using mixed-effects linear regression with adjustment for age and the single fixed effect of participant identification (to account for individuals contributing >1 eye to analysis). All comparisons of structural parameters (mGCIPL and pRNFL) included adjustment for age. Data are presented as mean ± standard error, unless otherwise indicated.
*$P < 0.05$.

poor attention, cognitive impairment, or attrition may lead to suboptimal or absent scans, thus leading to incomplete longitudinal data. In our imputation analysis, we attempted to account for missing data through imputation and found a persisting association between the *APOE* E4 allele and mGCIPL complex thinning. Although imputed data are subject to information biases associated with unidentified confounding variables,[67] we are confident for several reasons that our reported results are accurate. First, the associations between the *APOE* E4 allele and dementia[45–47] and the association between dementia and a thinner mGCIPL complex[68] are both well established. Second, our observation of high attrition among participants harboring the *APOE* E4 allele, which was associated with neuroretinal thinning in the participants who completed follow-up, informs our suspicion that these were most likely underestimated. The PROGRESSA cohort also differs from other ethnically similar glaucoma cohorts through its high proportion of patients with NTG. We recognize this phenomenon to be the result of the inclusion of optic nerve head morphologic features into PROGRESSA recruitment criteria. As a result, PROGRESSA may also represent other unidentified genetic and epidemiologic features distinguishing it from other glaucoma cohort studies. We recognize the importance of ethnicity as a covariate of *APOE* genotype. Unfortunately, because of our observations of poor self-reporting of ethnicity in PROGRESSA and because of previous data demonstrating the unreliability of self-reported ethnicity data,[69] we were unable to include ethnicity as a covariate of *APOE* E4 allele prevalence within our analyses. Future studies with nested cohorts may mitigate this limitation by using principal components analysis to stratify according to ethnicity.

Finally, the current study investigated associations between the *APOE* E4 allele and structural parameters that are associated with, but do not necessarily constitute, POAG. Further confounding this observation, *APOE* variants have not been implicated in genome-wide association studies of POAG. Although this finding suggests that *APOE* E4-associated neuroretinal thinning may not be relevant to glaucoma, it may also result from the same survivorship and selection biases within POAG genome-wide association study studies, which typically compare disease cohorts with younger control groups.[24] Consequently, it remains unclear how the observed *APOE* E4-associated mGCIPL complex thinning corresponds with glaucomatous visual outcomes. Likewise, the current study does not include longitudinal SD OCT data from a normative population cohort. Although we were able to demonstrate that faster rates of *APOE* E4-associated mGCIPL complex thinning seen in NTG eyes were not seen in control participants without glaucoma, these eyes were contributed by individuals recruited as having a diagnosis of suspect glaucoma. Consequently, rate of change comparisons could not be referenced to normative population data. Although such outcomes were beyond the scope of the current study, validation of this association would be best addressed through investigation of glaucomatous visual field progression in patients with glaucoma categorized by the presence or absence of the *APOE* E4 allele.

This study demonstrated an association between the *APOE* E4 allele and faster rates of retinal thinning in a cohort with early glaucoma, thus quantifying a genetic association with structural parameters of POAG. However, because genome-wide association study data has not identified glaucoma-associated risk variants within the *APOE* gene, the relevance of *APOE* E4-associated mCGIPL thinning to glaucoma and its visual outcome remains uncertain. Subsequent replication studies within separate cohorts will help to validate and elucidate the relationship between the *APOE* E4 allele and glaucoma.

Footnotes and Disclosures

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Abbreviations and Acronyms:
APOE = apolipoprotein E; **BMES** = Blue Mountains Eye Study; **HTG** = high-tension glaucoma; **HVF** = Humphrey visual field; **IOP** = intraocular pressure; **mGCIPL** = macular ganglion cell–inner plexiform layer; **NTG** = normal-tension glaucoma; **POAG** = primary open-angle glaucoma; **pRNFL** = peripapillary retinal nerve fiber layer; **PROGRESSA** = Progression Risk of Glaucoma: Relevant SNPs with Significant Association; **SD OCT** = spectral-domain OCT; **SE** = standard error; **SNP** = single nucleotide polymorphism.

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