



No Strong Association between the Apolipoprotein E E4 Allele and Glaucoma

A Multicohort Study

Sean Mullany, MD,^{1,*} Santiago Diaz-Torres, MPhil,^{2,*} Joshua M. Schmidt, PhD,¹ Daniel Thomson, PhD,¹ Ayub Qassim, MBBS, PhD,¹ Henry N. Marshall, MD,¹ Lachlan S.W. Knight, MOrth,¹ Ella C. Berry, MChD,¹ Antonia Kolovos, MD,¹ David Dimasi, PhD,¹ Stewart Lake, MBChB, PhD,¹ Richard A. Mills, MBBS, PhD,¹ John Landers, MBBS, PhD,¹ Paul Mitchell, MBBS, PhD,³ Paul R. Healey, MBBS, PhD,³ Toby Commerford, MBBS,⁴ Sonja Klebe, MD, PhD,⁵ Emmanuelle Souzeau, PhD,¹ Mark M. Hassall, MBBS, DPhil,¹ Stuart MacGregor, PhD,² Puya Gharahkhani, PhD,² Owen M. Siggs, MD, DPhil,^{1,6,†} Jamie E. Craig, MBBS, DPhil^{1,†}

Purpose: To elucidate a potential association between the apolipoprotein E (APOE) E4 allele and glaucoma prevalence in large cohorts.

Design: A cross-sectional analysis of baseline and prospectively collected cohort data.

Participants: UK Biobank (UKBB) participants of genetically determined European ancestry (n = 438 711). Replication analyses were performed using clinical and genotyping data collected from European participants recruited to the Canadian Longitudinal Study of Aging (CLSA; n = 18 199), the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG; n = 1970), and the Blue Mountains Eye Study (BMES; n = 2440).

Methods: Apolipoprotein E alleles and genotypes were determined, and their distributions were compared on the basis of glaucoma status. Similar analyses were performed using positive control outcomes associated with the APOE E4 allele (death, dementia, age-related macular degeneration) and negative control outcomes not associated with the APOE E4 allele (cataract, diabetic eye disease). Outcome phenotypes were also correlated with Alzheimer's dementia (AD), a clinical outcome highly associated with the APOE E4 allele.

Main Outcome Measures: Results of APOE E4 genotype-phenotype comparisons were reported as odds ratios (ORs) with 95% confidence intervals (CIs). Replication analyses investigated APOE E4 associations in 2 replication cohorts (CLSA and ANZRAG/BMES).

Results: The APOE E4 allele was inversely associated with glaucoma (OR, 0.96; 95% CI, 0.93–0.99; $P = 0.016$) and both negative controls (cataract: OR, 0.98; 95% CI, 0.96–0.99; $P = 0.015$; diabetic eye disease: OR, 0.92; 95% CI, 0.87–0.97; $P = 0.003$) in the UKBB cohort. A paradoxical positive association was observed between AD and both glaucoma (OR, 1.30; 95% CI, 1.08–1.54; $P < 0.01$) and cataract (OR, 1.15; 1.04–1.28; $P = 0.018$). No association between the APOE E4 allele and glaucoma was observed in either replication cohort (CLSA: OR, 1.03; 95% CI, 0.89–1.19; $P = 0.66$; ANZRAG/BMES: OR, 0.97; 95% CI, 0.84–1.12; $P = 0.65$).

Conclusions: A small negative association observed between APOE E4 and glaucoma within the UKBB was not evident in either replication cohort and may represent an artifact of glaucoma underdiagnosis in APOE E4 carriers.

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Glaucoma describes a heterogeneous group of optic neuropathies characterized by specific patterns of neuroretinal atrophy and irreversible vision loss.¹ Although there are many causes for glaucoma, primary open-angle glaucoma (POAG) represents the most common glaucoma sub-phenotype worldwide² and is defined by glaucomatous change in the presence of an anatomically normal anterior

chamber.¹ Although intraocular pressure (IOP) remains the single modifiable risk factor for POAG, and all current treatments target IOP lowering, the pathophysiology of POAG remains poorly understood.³ It has been hypothesized that systemic neurodegenerative processes may contribute to POAG.⁴ Consequently, neurodegeneration in glaucoma is an area of considerable

research interest. Several genes including optineurin and TANK-binding kinase 1 have been implicated in Mendelian forms of glaucoma, amyotrophic lateral sclerosis, and frontotemporal dementia.^{5–7} Associations have also been demonstrated between glaucoma and both Alzheimer's dementia (AD)^{8–14} and its common risk allele, apolipoprotein E (*APOE*) E4.^{15–19}

Apolipoprotein E is a major lipid transport protein within the central nervous system where it is involved in cholesterol transport and neuronal repair.²⁰ Three common *APOE* alleles (E2, E3, and E4), which are defined by permutations of 2 collocated single-nucleotide polymorphisms (SNPs) in coding regions of the *APOE* gene, have been identified as important covariates of human disease.²⁰ Despite variation across ethnicities, the E3 allele is the most common allele in Europeans (variant allele frequency: 78%), followed by E4 (14%) and E2 (8%).²¹ The E4 allele is a major risk factor for AD and other common dementia phenotypes including vascular dementia and Lewy body disease.^{22–24} It is also associated with hyperlipidemia,²⁵ ischemic heart disease,²⁵ and mortality,^{26,27} which was quantified in 1 study as a decrease in population frequency of the E4 allele frequency from 17.6% to 8.3% from age 60 to 90 years.²⁶ By contrast, the E2 allele is associated with other outcomes including peripheral vascular disease,²⁵ age-related macular degeneration (AMD),^{28,29} and increased survival²⁷ and may be protective against AD.^{22,30}

The *APOE* E4 allele has been investigated in multiple studies of glaucoma, with individual studies reporting positive,^{15,16} inverse,^{17–19} or absent associations.^{31–35} Similarly, several meta-analyses of data from these studies have generated variable results.^{36–38} Several factors which may account for variable results across these studies include important correlates of glaucoma prevalence (age, sex, and ethnicity) and *APOE* allele distribution (age, ethnicity, and the alternate minor [E2] allele). Furthermore, dementia and AD commonly result in executive dysfunction, with impaired attention and self-neglect, which are recognized factors contributing to underdiagnosis of common diseases of aging.³⁹ Consequently, sample stratification based on a powerful dementia risk allele may introduce mislabeling bias with different disease diagnosis rates between case and control cohorts. The current study sought to generate a model to understand and further investigate the association between the *APOE* E4 allele and glaucoma in large cohort studies.

Methods

Ethical Approval

UK Biobank and the Canadian Longitudinal Study of Aging. The UK Biobank (UKBB) received ethical approval through the North West Multi-centre Ethics Committee, and the Canadian Longitudinal Study of Aging (CLSA) received ethical approval from 13 local research ethics committees at various sites throughout Canada. All participants provided informed written consent, and study procedures were performed in accordance with the ethical principles of the World Medical Association Declaration of Helsinki.

Australian and New Zealand Registry of Advanced Glaucoma. Ethics approval for the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) was obtained through the Southern Adelaide Clinical Human Research Ethics Committee, and all participants were enrolled by informed written 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.

Blue Mountains Eye Study. The Blue Mountains Eye Study (BMES) was approved by the Western Sydney Area Health Service Human Ethics Committee and complied with the Declaration of Helsinki. All participants were provided with a full explanation of the nature of the study and read and signed informed consent before participation.

Genotyping

DNA microarray genotyping was generated for the different cohorts using Axiom arrays (including the UK Biobank Lung Exome Variant Evaluation (BiLEVE) and UK Biobank arrays; ThermoFisher) for the UKBB, and a combination of Illumina SNP arrays (HumanCoreExome, Infinium OmniExpress, and omni1M; Illumina) for the ANZRAG. Human610-Quad arrays (Illumina) were used for the BMES, and UK Biobank Axiom arrays (ThermoFisher) were used for the CLSA. To account for ethnicity, which represents an independent covariate of both glaucoma prevalence and *APOE* allele distribution, analyses within this study were performed solely using individuals of European ancestry. Within the UKBB, European ethnicity was defined using a combination of self-reported ancestry and principal components analysis of genetic data following methods described by Bycroft et al⁴⁰ using the previously generated UKBB data.⁴¹ Principal components analyses were similarly used to determine genetic European ancestry within the ANZRAG, BMES, and CLSA cohorts. Apolipoprotein E alleles (E1, E2, E3, and E4) were determined from 2 relevant SNPs within the *APOE* gene (rs429358 and rs7412; GRCh38 reference genome). Because of the rarity of the E1 allele, rare E1 genotypes (E1E2 and E1E4) were excluded from analysis. In accordance with common practice, E1E3 genotypes, which are most likely to represent incorrect attribution of 2 variants to the same allele rather than alternate common variants on separate alleles, were relabeled as E2E4.²⁵ Apolipoprotein SNPs were measured directly from Axiom arrays. Because the relevant *APOE* SNPs were not included on the Illumina arrays, they were imputed in Minimac3 using Haplotype Reference Consortium r1.1 as a reference panel (rs429358 imputation $R^2 = 0.93$; rs7412 imputation $R^2 = 0.92$).⁴²

Samples

Analyses were performed in a primary cohort (UKBB) and 2 replication cohorts (CLSA and ANZRAG/BMES). These cohorts included all participants with genetically determined European ancestry for whom *APOE* alleles and genotypes could be determined or imputed. The primary and first replication cohorts investigated the association between the E4 allele and glaucoma in population cohorts (UKBB and CLSA). The second replication cohort investigated the association between the E4 allele and POAG using cases from a clinical glaucoma cohort (ANZRAG) and nonglaucoma controls from a population cohort with ocular phenotyping data (BMES).

Primary Study Cohort: UKBB. The UKBB is a longitudinal population-based clinical and genetic study of determinants of disease in aging, which commenced in 2006 and continues to monitor approximately 470 000 living participants recruited at age 40–69 y.⁴³ After a period of 12–16 years of follow-up, the mean

age of living participants has increased from 56.5 to 69.7 years. Because glaucoma prevalence increases with age, we choose to perform our analyses using current data acquired from the UKBB database at a single time point (February 2, 2022). Participant age (in whole years) was calculated as "age at the date of data acquisition." To avoid potential confounding resulting from E4-associated mortality, participants who had died during follow-up were included in analyses, calculating age as "age at death," representing each deceased participant's last observation carried forward. Glaucoma phenotypes were determined using a combination of self-reporting determined through completion of a touch screen self-reporting survey (datafield: 6148), and *International Classification of Diseases, Tenth Revision* (ICD10) coding data (datafield: 41270; code H40). These data included longitudinal data current to the time of data acquisition.

Replication Cohort 1: CLSA. The CLSA is longitudinal population-based study of healthy aging, comprising 51 338 Canadians aged 45–85 years who were recruited from 2010 to 2015 and for whom active follow-up was scheduled to be performed at 3-year intervals for 30 years.⁴⁴ In this study, we used data from 18 199 individuals with genetically European ancestry included in the August 2019 genotyping release.⁴⁵ Age was determined in whole years at the time of recruitment. Glaucoma status was defined using baseline self-reported data reported by the parameter 'ICQ_GLAUC_COM.' Individuals who did not provide baseline glaucoma data (n = 89) were excluded from analysis.

Replication Cohort 2: ANZRAG/BMES. Unlike the other cohorts, which were selected to investigate the association between the E4 allele and glaucoma, the second replication cohort sought specifically to investigate the association between E4 and POAG. This cohort was generated using known POAG cases sampled from ANZRAG, a large Australasian glaucoma registry, and known nonglaucoma controls recruited from BMES, an ancestrally matched population cohort. Primary open-angle glaucoma cases from ANZRAG were diagnosed by an ophthalmologist based on general disc appearance and corresponding visual field defects identified on a Humphrey 24-2 field, or in the absence of field testing, the loss of central acuity related to glaucoma. Participants with angle closure or secondary causes of glaucoma were excluded.^{46,47}

The BMES is a large population-based cohort that sampled 82.4% of noninstitutionalized individuals aged ≥ 49 years from within a region defined by 2 postal codes in regional New South Wales (Australia).⁴⁸ The purpose for conducting the BMES was to estimate the prevalence of eye disease within a population representing the general ethnic diversity of the Australian population at the time. Recruitment for the BMES occurred in 2 phases including primary (BMES-I) and extension (BMES-E) cohorts.⁴⁹ Because all participants underwent eye examination at the time of recruitment, BMES data could be filtered to determine a set of controls known not to have glaucoma. Insufficient data was available to determine which glaucoma cases had POAG, and therefore these individuals were excluded from analyses (n = 135). However, these glaucoma cases were used to determine the overall prevalence of glaucoma within the BMES. The current study included participants from both BMES phases for whom genotyping data were available. For both cohorts, age was defined as "age at recruitment". A subgroup analysis of normal-tension glaucoma (NTG) was performed in the second replication cohort through comparison of ANZRAG NTG participants to BMES no glaucoma controls. For the purpose of this analysis, NTG was defined as POAG with a highest pretreatment IOP of < 21 mmHg in either eye. This definition excluded all participants for whom pretreatment IOP was not explicitly recorded.

Statistical Analysis

All statistical analyses were performed using R (version 4.1.3, RCore Team) using publicly available packages. Baseline univariable comparisons of demographic parameters were performed using independent sample t tests and chi-square tests. Genotype comparisons were performed using generalized (binomial) linear models.

The primary analysis compared the *APOE* E4 allele to the outcome of glaucoma. For allelic regression, an indicator variable for E4 and E2 allele status (defined by the number [dosage] of relevant alleles [0–2]) was included (equation 1). Thus, the β_{e4} would represent the "per allele" effect on glaucoma prevalence. To determine whether any observed single allele effects were compounded in E4 homozygotes, this analysis was replicated comparing E4E4 to all other genotypes. For genotypic regression, an indicator variable for each variant genotype (i.e., non-E3E3) was included. Thus, the β_{e4e4} would represent the difference in glaucoma prevalence between E4E4 and E3E3 homozygotes after accounting for all other *APOE* genotypes (equation 2). Sex and age (squared) were included as covariates in all analyses. Outcome statistics for univariable analyses are presented as mean (standard deviation) and for multivariable analyses as odds ratios (OR) and 95% confidence intervals (CIs). The threshold for statistical significance (α) was set to $P < 0.05$.

$$\text{logit}(\text{Study}) = \beta_0 + \beta_{e2}X_{e2} + \beta_{e4}X_{e4} + \beta_{Age}Age + \beta_{Gender}Gender + \ell$$

(equation 1)

$$\text{logit}(\text{Study}) = \beta_0 + \beta_{e2e2}X_{e2e2} + \beta_{e2e3}X_{e2e3} + \beta_{e2e4}X_{e2e4} + \beta_{e3e4}X_{e3e4} + \beta_{e4e4}X_{e4e4} + \beta_{Age}Age + \beta_{Gender}Gender + \ell$$

(equation 2)

Model Generation

Associations between the *APOE* E4 allele and glaucoma determined from direct genotype-phenotype correlation include inherent assumptions that both exposure (genotype) and outcome (glaucoma) can be directly assayed. Within such models, the association between these 2 variables is therefore presumed not confounded by genotype-specific factors (Fig 1A). Because these models do not recognize that "diagnosed glaucoma" may not represent "true glaucoma" prevalence or that rates of "glaucoma diagnosis" may be confounded by AD, several additional assumptions were included to generate the current study's interim model (Fig 1B):

Assumption (1) Glaucoma prevalence is inferred in population-based studies such as the UKBB using documented "glaucoma diagnosis," which may be underestimated because of underdiagnosis or underreporting of disease.

Assumption (2) Executive dysfunction resulting from AD may directly result in underdiagnosis of glaucoma. Because the *APOE* E4 allele is causally associated with AD, a cohort enriched for this allele is also likely to be enriched for AD and its potential effects on glaucoma underdiagnosis.

Because of this study's inability to quantify the effects of diagnostic biases arising from our hypothesized interaction between AD and "glaucoma diagnosis," a reference framework was created by performing parallel analyses of positive and negative controls. Positive controls were defined as outcomes known to be associated with the E4 allele, including AD and mortality, which are positively associated with E4.^{22,25–27} Alzheimer's dementia

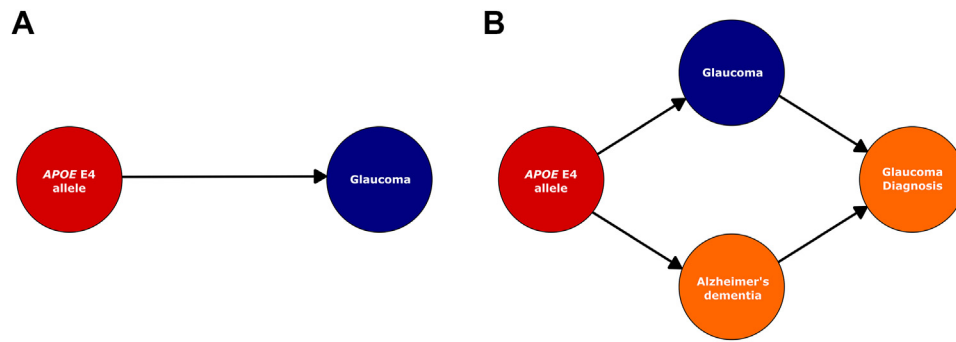


Figure 1. Preliminary causal frameworks. Causality diagrams were generated to demonstrate the basic and intermediate models to investigate associations between the apolipoprotein E (APOE) E4 allele and glaucoma. Within the simple model (A), the association between the APOE E4 allele and "true" glaucoma is assumed to be directly measurable and not confounded by external factors. The interim model proposed in the current study recognizes that rates of "glaucoma diagnosis" may be confounded by underdiagnosis resulting from executive dysfunction in Alzheimer's dementia (B). Arrows demonstrate; direction of causal effect; red = exposure; blue = outcome; orange = confounding factor.

was defined by a registered ICD10 diagnosis (ICD10 code: F00 [Dementia in AD]), and mortality was determined from death registry data (UKBB datafield: 40000 [Date of Death]). A set of ocular controls were selected on the basis of their nature as common diseases of aging and their similar reporting methods to glaucoma within the UKBB (i.e., combined self-reported touch screen questionnaire [datafield: 6148] and ICD10 encoded diagnosis [datafield: 41270]). These ocular controls included AMD (ICD10 code 35.3), which was defined as a positive control on the basis of its inverse association with E4.^{28,29} Cataract (ICD10: H25-H28) and diabetic eye disease (ICD10: 36.0 [diabetic retinopathy]) were selected as negative controls, which in this study were defined as outcomes without an established association with the E4 allele. Anticipating that a pathophysiological association between glaucoma and E4 would result in a corresponding association with AD, further analyses were performed comparing glaucoma prevalence to AD prevalence. Because diabetes is an established risk factor for AD,⁵⁰ diabetic eye disease was identified as a potentially confounded outcome for this analysis. A subanalysis investigating the temporal relationship between "AD diagnosis" and diagnosis of ocular outcomes included dates of first ICD10 code reporting (UKBB datafields: AD [130836]; glaucoma [131186]; AMD [131182]; cataract [131164, 131166, 131168, and 131170], and diabetic eye disease [131184]). Because all secondary analyses and control outcomes were analyzed to provide background reference data to support the primary outcome of E4 allele associations with glaucoma findings, multiple testing correction models were not considered appropriate.

Results

Sample Cohorts

Primary Study Cohort. Apolipoprotein E alleles and genotypes were determined for 438 730 UKBB participants with genetically determined European ethnicity (Fig 2). Because of the extreme rarity of the E1 allele, E1E3/E2E4 cases were relabeled as E2E4, and both E1E2 (n = 2) and E1E4 (n = 17) cases, which were most likely to have resulted from imputation error, were excluded from analysis. Within this cohort, 10 660 individuals had an ICD10 diagnosis of glaucoma, and 7170 had declared a self-reported diagnosis of glaucoma. Of these individuals,

3842 had both ICD10 and self-reported diagnoses of glaucoma. The final sample included 13 988 glaucoma cases (3.2% of total cohort) and 424 723 controls (i.e., individuals with no recorded glaucoma diagnosis). Death registry data identified 31 228 individuals (7.1%) from this sample who had died during the follow-up (Fig 2).

Replication Cohorts. The first replication cohort included 18 199 CLSA participants with European ancestry, 875 of whom had a self-reported diagnosis of glaucoma (4.8% of total cohort). Participants who had not responded to glaucoma self-reporting (n = 89) were excluded from this analysis. The second replication cohort included a case cohort of 1970 European ANZRAG POAG-affected participants, and after exclusion of 135 participants with glaucoma, a control cohort of 2440 European BMES nonglaucoma individuals.

Demographics

UK Biobank glaucoma cases were older than controls (74.2 [6.3] vs. 69.6 [8.0] years; $P < 0.001$), with a higher proportion of males (50.0% vs. 45.7%; $P < 0.001$), and higher mortality rates (9.2% vs. 7.0%; $P < 0.001$; Table 1). Glaucoma cases were older in both replication cohorts (CLSA: 70.0 [9.2] vs. 62.7 [10.1] years; $P < 0.001$; ANZRAG/BMES: 74.5 [10.6] vs. 62.9 [8.2] years; $P < 0.001$), but no difference in sex was observed ([% male] CLSA: 49.1% vs. 49.8%; $P = 0.71$; ANZRAG/BMES: 45.5% vs. 43.7%; $P = 0.22$). Glaucoma prevalence was 3.2% in the UKBB and 5.0% in the CLSA. Before the exclusion of the 135 glaucoma cases identified within the BMES cohort, glaucoma prevalence was 5.2% in the BMES.

Preliminary Analysis and Model Building

Preliminary analyses within the primary cohort demonstrated strong associations between the APOE E4 allele and all positive control outcomes including AD (OR, 3.56; 95% CI, 3.33–3.81; $P < 0.001$), death (OR, 1.1; 95% CI, 1.10–1.15; $P < 0.001$), and AMD (OR, 0.91; 95% CI, 0.87–0.94; $P < 0.001$). Each of these associations was

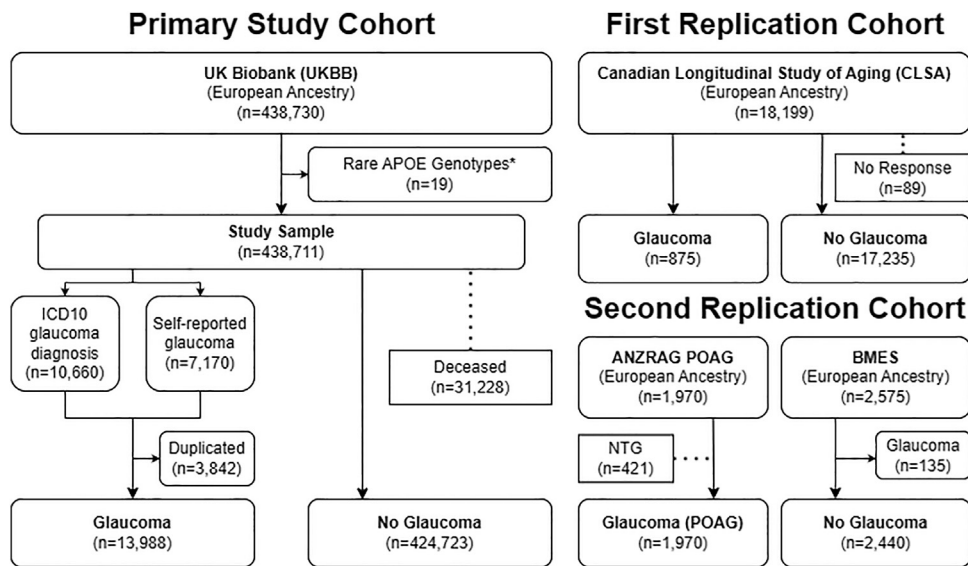


Figure 2. Cohort details. The primary study cohort included all European UK Biobank (UKBB) participants. Within this cohort, glaucoma was defined by a combination of self-reported and *International Classification of Diseases, Tenth Revision* (ICD10) "glaucoma diagnosis." Deceased participants were included in analyses with age determined from "age at death." The first replication cohort included all European individuals from the Canadian Longitudinal Study of Aging (CLSA). Within this cohort, glaucoma was defined by self-reported diagnosis at the time of recruitment. The second replication cohort pooled primary open-angle glaucoma (POAG) cases from the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) and nonglaucoma controls from the Blue Mountains Eye Study (BMES). *Participants with rare apolipoprotein E genotypes including E1E2 (n = 2) and E1E4 (n = 17) were excluded from the final sample.

compounded in E4E4 homozygotes (AD: OR, 6.7; 95% CI, 5.8–7.7; $P < 0.001$; death: OR, 1.43; 95% CI, 1.34–1.53; $P < 0.001$; AMD: OR, 0.76; 95% CI, 0.65–0.88; $P < 0.001$). Inverse associations were observed between E4 and glaucoma (OR, 0.96; 95% CI, 0.93–0.99; $P = 0.030$), and both negative controls, cataract (OR, 0.98; 95% CI, 0.96–0.99; $P = 0.024$) and diabetic eye disease (OR, 0.93; 95% CI, 0.88–0.99; $P = 0.003$). Positive associations were observed between AD and glaucoma (OR, 1.30; 95% CI, 1.08–1.54; $P = 0.004$), cataract (OR, 1.15; 95% CI, 1.04–1.28; $P = 0.008$), and diabetic eye disease (OR, 2.60; 95% CI, 2.01–3.29; $P < 0.001$). Because AD is associated with self-neglect, the associations observed between AD and both glaucoma and cataract were initially hypothesized

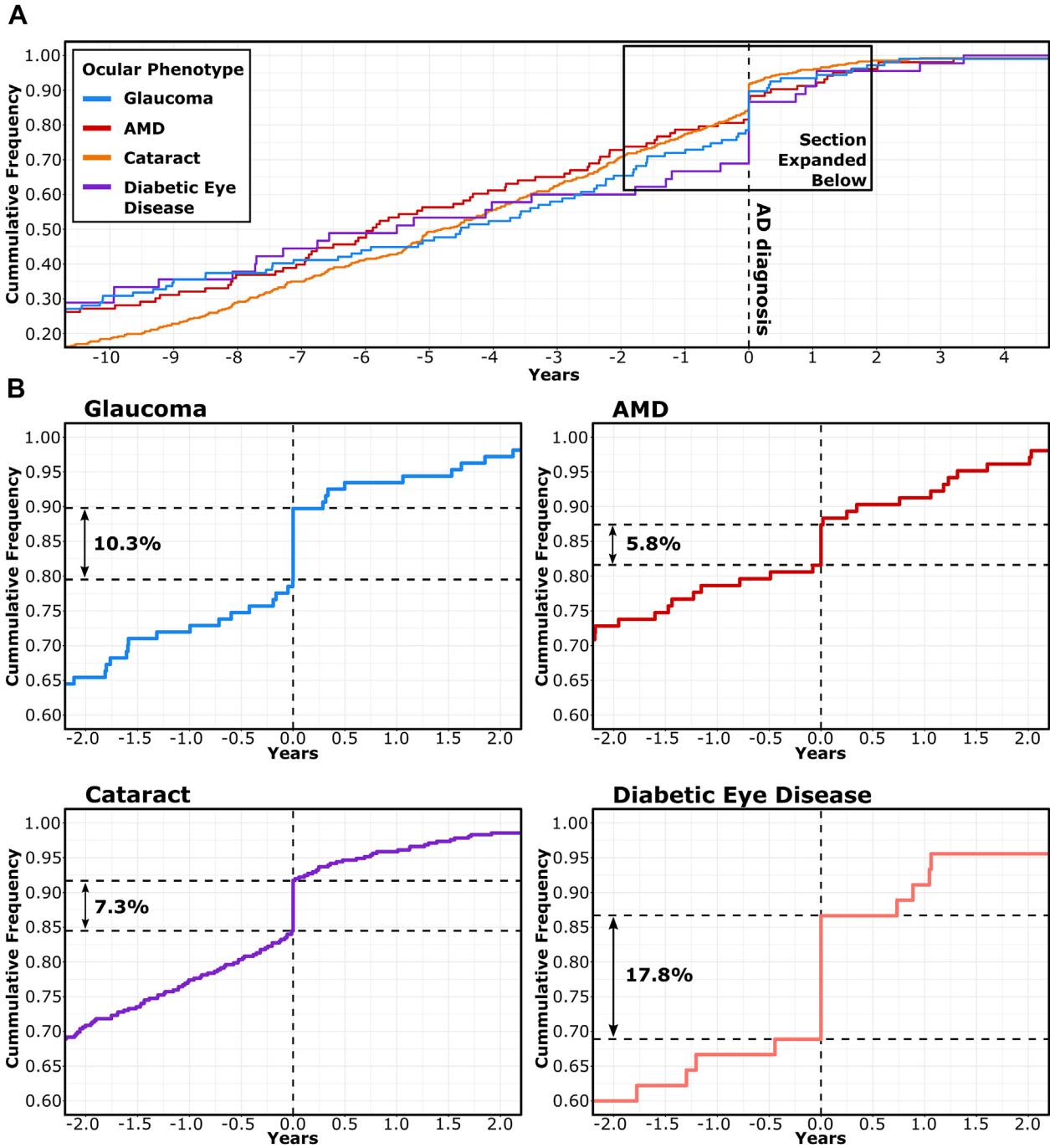
to be an effect of opportunistic diagnosis. This was further explored by investigating the temporal association between "AD diagnosis" and diagnosis of relevant ocular phenotypes, which demonstrated that ICD10 diagnosis of AD was associated with concurrent ICD10 diagnosis of each phenotype (Fig 3). Given this set of observations, the paradoxical associations between AD and ocular phenotypes were subsequently presumed to have resulted from opportunistic registration of ICD10 codes, which may have resulted from concurrent registration of previously unregistered diagnoses during hospital admissions.

Irrespective of the mechanism by which "AD diagnosis" might affect the diagnosis of glaucoma (or other ocular phenotypes), "AD diagnosis" was observed to be a relevant

Table 1. Demographics of Study Cohorts

	Primary Cohort			First Replication Cohort			Second Replication Cohort		
	UKBB			CLSA			ANZRAG/BMES		
	Glaucoma	No Glaucoma	P	Glaucoma	No Glaucoma	P	Glaucoma	No Glaucoma	P
Total sample (n [% glaucoma])	13 988 [3.2]	424 723	NA	875 [4.8]	17 235	NA	1971	2440	NA
Age (yrs; mean [SD])	74.2 [6.3]	69.6 [8.0]	< 0.001	70.2 [9.2]	62.7 [10.1]	< 0.001	74.5 [10.6]	62.9 [8.2]	< 0.001
Sex (%male)	50.0	45.7	< 0.001	49.1	49.8	0.71	45.5	43.7	0.22
Deceased (%)	9.2	7.0	< 0.001	NA	NA	NA	NA	NA	NA

Current age, sex, and mortality rates were compared on the basis of glaucoma prevalence in all cohorts. The proportion of individuals diagnosed with glaucoma within the 2 population cohorts (UK Biobank [UKBB] and Canadian Longitudinal Study of Aging [CLSA]) are represented as percentages. Summary statistics were determined using independent *t* tests for continuous variables (mean [standard deviation]), and Chi-squared tests for categorical variables. ANZRAG = Australian and New Zealand Registry of Advanced Glaucoma; BMES = Blue Mountains Eye Study; NA = not applicable; SD = standard deviation.



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Figure 3. Cumulative frequency plot of ocular outcome diagnosis in Alzheimer's dementia (AD). UK Biobank Participants with a recorded date of *International Classification of Diseases, Tenth Revision* (ICD10) diagnosis of AD and recorded dates of ICD10 diagnosis of glaucoma, age-related macular degeneration (AMD), cataract, and diabetic eye disease were investigated to determine the temporal relationship between diagnoses (A). The time of "AD diagnosis" is represented as 0.0 years (vertical dashed line) on each x-axis. The cumulative prevalence of ocular outcome ICD10 diagnoses are represented as proportions on each y-axis. Horizontal dashed lines represent the upper and lower bounds of ocular outcomes reported concurrently with "AD diagnosis" (B). Percentages demonstrate the total number of incident ocular outcome diagnoses occurring concurrently with "AD diagnosis."

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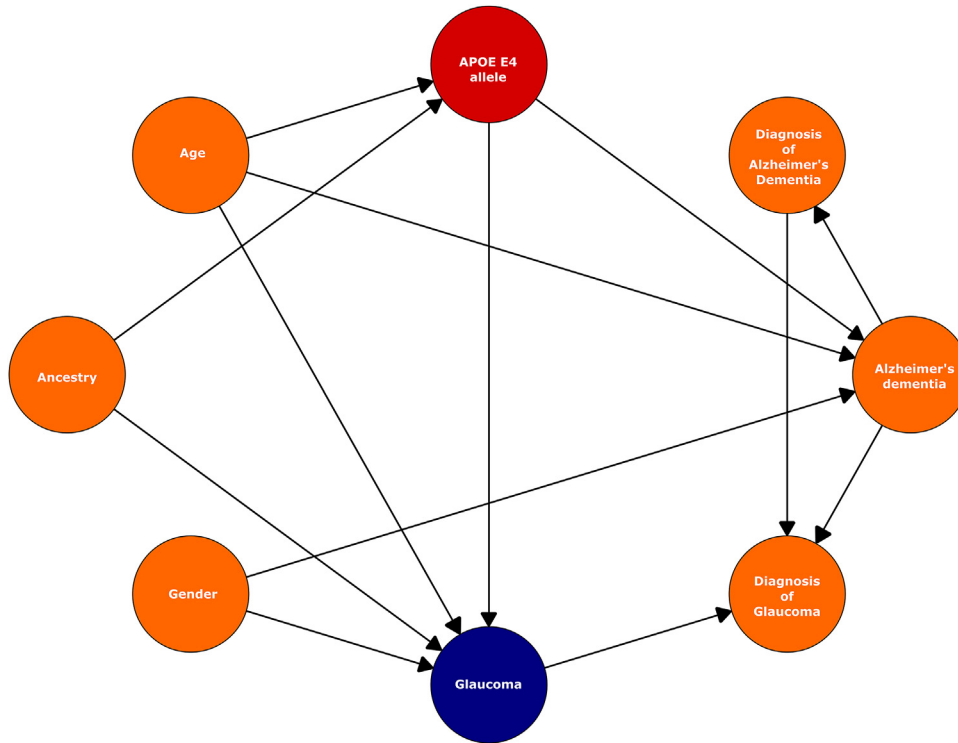


Figure 4. Causal framework. An amended causality framework was established to include additional potential confounders ("glaucoma diagnosis", Alzheimer's dementia [AD], and "AD diagnosis") and established confounders (age, ancestry/ethnicity, and sex) of the apolipoprotein E (APOE) E4 allele and glaucoma. Arrows indicate direction of causal effect. Red = exposure; blue = outcome; orange = confounders.

covariate of "glaucoma diagnosis" in the UKBB cohort. Consequently, a third assumption was included within our study model:

Assumption (3) Despite the theoretical confounding effects of AD resulting in underdiagnosis of glaucoma, "AD diagnosis" may have an opposite effect (observed though increased registration of glaucoma diagnosis concurrent with "AD diagnosis" in the UKBB cohort), thus representing an independent confounder of "glaucoma diagnosis".

A revised model used in all subsequent analyses (for which AD was an outcome) included "AD diagnosis" as a binary covariate of the outcome variable (Fig 4).

Definitive Analysis

Based on amendments made to the study model following preliminary analysis, including recognition of AD as a covariate of the ocular outcome diagnosis, a second analysis was performed. Within this analysis, the E4 allele was inversely associated with glaucoma in the UKBB (OR, 0.96; 95% CI, 0.93–0.99; $P = 0.016$; Table 2; Fig 5). Associations were also demonstrated between E4 and all controls including positive controls: AD (OR, 3.56; 95% CI, 3.33–3.81; $P < 0.001$), death (OR, 1.07; 95% CI, 1.05–1.10; $P < 0.001$), and AMD (OR, 0.91; 95% CI, 0.87–0.94; $P < 0.001$), and negative controls: cataract

Table 2. Apolipoprotein E4 Allele Prevalence by Outcome in the UK Biobank

Variable Type	Phenotype (n [% of total])	E4 Carrier (n = 126 082)	No E4 Allele (n = 312 629)	Odds Ratio [95% CI]	P
Primary outcome	Glaucoma	3867 [3.1%]	10 121 [3.2%]	0.96 [0.93,0.99]	0.016
Positive control	Alzheimer's dementia	1198 [1.0%]	1172 [0.2%]	3.56 [3.33,3.81]	< 0.001
	Death	9625 [7.6%]	21 603 [6.9%]	1.07 [1.05,1.10]	< 0.001
	AMD	2771 [2.2%]	7704 [2.5%]	0.91 [0.87,0.94]	< 0.001
Negative control	Cataract	15 983 [12.7%]	40 911 [13.1%]	0.98 [0.96,0.99]	0.015
	Diabetic eye disease	1316 [1.0%]	3518 [1.1%]	0.92 [0.87,0.97]	0.003

Correlation between the Apolipoprotein E4 allele and glaucoma in the UK Biobank. Alzheimer's dementia (AD), death, and age-related macular degeneration (AMD) are included as positive controls because of established associations with the E4 allele. Cataract and diabetic eye disease are included as negative controls. Odds ratios and P values have been generated using logistic regression to determine a per allele effect on the outcome variable. Adjustments for the E2 allele, age (squared), sex, and "AD diagnosis" were included in analysis. CI = confidence interval.

Table 3. Phenotype Prevalence According to APOE Genotype

Variable Type	Phenotype	UK Biobank APOE E Genotypes (n = 438 711)							Odds Ratio [95% CI]	P
		(n [% Total])	E4E4 (n = 10 525)	E2E2 (n = 2779)	E2E3 (n = 54 079)	E3E3 (n = 255 771)	E2E4 (n = 11 088)	E3E4 (n = 104 469)		
Primary outcome Positive control	Glaucoma	13 988 [3.2%]	319 [3.0%]	70 [2.5%]	1775 [3.3%]	8276 [3.2%]	345 [3.1%]	3203 [3.1%]	0.94 [0.84,1.06]	0.32
	AD	1864 [0.4%]	260 [2.5%]	11 [0.4%]	170 [0.3%]	99 [0.4%]	58 [0.5%]	887 [0.8%]	6.69 [5.81,7.67]	<0.001
	Deceased	31 228 [7.1%]	1016 [9.7%]	190 [6.8%]	3681 [6.8%]	17 732 [6.9%]	791 [7.1%]	7818 [7.5%]	1.27 [1.19,1.36]	<0.001
Negative control	AMD	10 475 [2.4%]	191 [1.8%]	73 [2.7%]	1353 [2.5%]	6278 [2.4%]	273 [2.5%]	2307 [2.2%]	0.75 [0.65,0.87]	<0.001
	Cataract	56 894 [13.0%]	1267 [12.0%]	330 [11.9%]	7074 [13.1%]	33 507 [13.1%]	1425 [12.9%]	13 291 [12.7%]	0.93 [0.88,0.99]	0.030
	Diabetic eye disease	4834 (1.1%)	104 [1.0%]	20 [0.7%]	595 [1.1%]	2903 [1.1%]	113 [1.0%]	1099 [1.1%]	0.83 [0.68,1.01]	0.07

Correlation between the Apolipoprotein E (APOE) E4E4 genotype and glaucoma (defined by the combination of self-reported glaucoma and current International Classification of Diseases, Tenth Revision diagnosis throughout follow-up) in the UK Biobank. Dementia and death are included as positive controls as both are known to be positively associated with the E4 allele. Similarly, age-related macular degeneration (AMD) is included as a positive control given the established protective association of the E4 allele and this phenotype. Diabetic eye disease and cataract are included as negative controls as neither are expected to be pathophysiologically associated with the E4 allele. Odds ratios and P values have been generated comparing phenotype prevalence between the E4 homozygotes (i.e., E4E4) against the reference of E3 homozygotes (i.e., E3E3). Adjustments for the 4 additional alternate genotypes, current age squared, and sex were included in the model. AD = Alzheimer's dementia; CI = confidence interval.

(OR, 0.98; 95% CI, 0.96–0.99; $P = 0.015$) and diabetic eye disease (OR, 0.92; 95% CI: 0.87–0.97; $P = 0.003$). Each of the odds ratios associated with ocular outcomes was more strongly negative with the inclusion of AD as a covariate. No compounding effect of the E4 allele in glaucoma was observed in E4E4 homozygotes (OR, 0.94; 95% CI, 0.84–1.06; $P = 0.43$; Table 3). Homozygous E4E4 was associated with positive control outcomes including AD (OR, 6.69; 95% CI, 5.81–7.67; $P < 0.001$), death (OR, 1.27; 95% CI, 1.19–1.36; $P < 0.001$), AMD (OR, 0.75; 95% CI, 0.65–0.87; $P < 0.001$), and the negative control, cataract (OR, 0.93; 95% CI, 0.88–0.99; $P = 0.03$). No homozygous E4E4 effect was observed in diabetic eye disease (OR, 0.83; 95% CI, 0.68–1.01; $P = 0.07$).

AD

Because of strong associations between the E4 allele and AD, outcome phenotypes were compared between UKBB participants with or without a diagnosis of AD. Alzheimer's dementia was positively correlated with glaucoma (OR, 1.30; 95% CI, 1.08–1.54; $P < 0.01$), death (OR, 27.1; 95% CI, 24.9–29.6; $P < 0.001$) and both negative controls (cataract: OR, 1.15; 95% CI, 1.04–1.28; $P < 0.01$; diabetic eye disease: OR, 2.60; 95% CI, 2.01–3.29; $P < 0.001$; Table 4). Age-related macular degeneration was not associated with AD (OR, 1.10; 95% CI, 0.88–1.35; $P = 0.41$).

Replication Cohorts

Replication analyses demonstrated no association between the E4 allele and glaucoma in the first replication cohort (CLSA: APOE E4 allele: OR, 1.03; 95% CI, 0.89–1.19; $P = 0.66$; E4E4 homozygotes: OR, 1.25; 95% CI, 0.77–1.94; $P = 0.36$), and no association between the E4 allele and POAG in the second replication cohort (ANZ-RAG/BMES: APOE E4 allele: OR, 0.97; 95% CI, 0.84–1.12; $P = 0.65$; APOE E4 homozygotes: OR, 0.70; 95% CI, 0.41–1.15; $P = 0.17$). Similarly, no association was seen between E4 and NTG in the second replication cohort (APOE E4 allele: OR, 0.98; 95% CI, 0.83–1.17; $P = 0.86$; APOE E4 homozygotes: OR, 0.69; 95% CI, 0.36–1.28; $P = 0.26$).

Discussion

To the best of our knowledge, this study represents the largest and most comprehensive analysis of the APOE E4 allele and glaucoma to date. After modeling and adjusting for multiple established and hypothetical confounders of glaucoma and the E4 allele, we demonstrated a small inverse association between the E4 allele and glaucoma within the UKBB. However, because of the identification of multiple potentially confounding parameters and failure to replicate this result in 2 separate replication cohorts, each of which may not be sufficiently powered to detect a small but potentially real effect, we suspect this association to be the result of inherent study biases rather than neuroprotection. By limiting our analysis to individuals with genetically determined European ancestry, we were able to mitigate

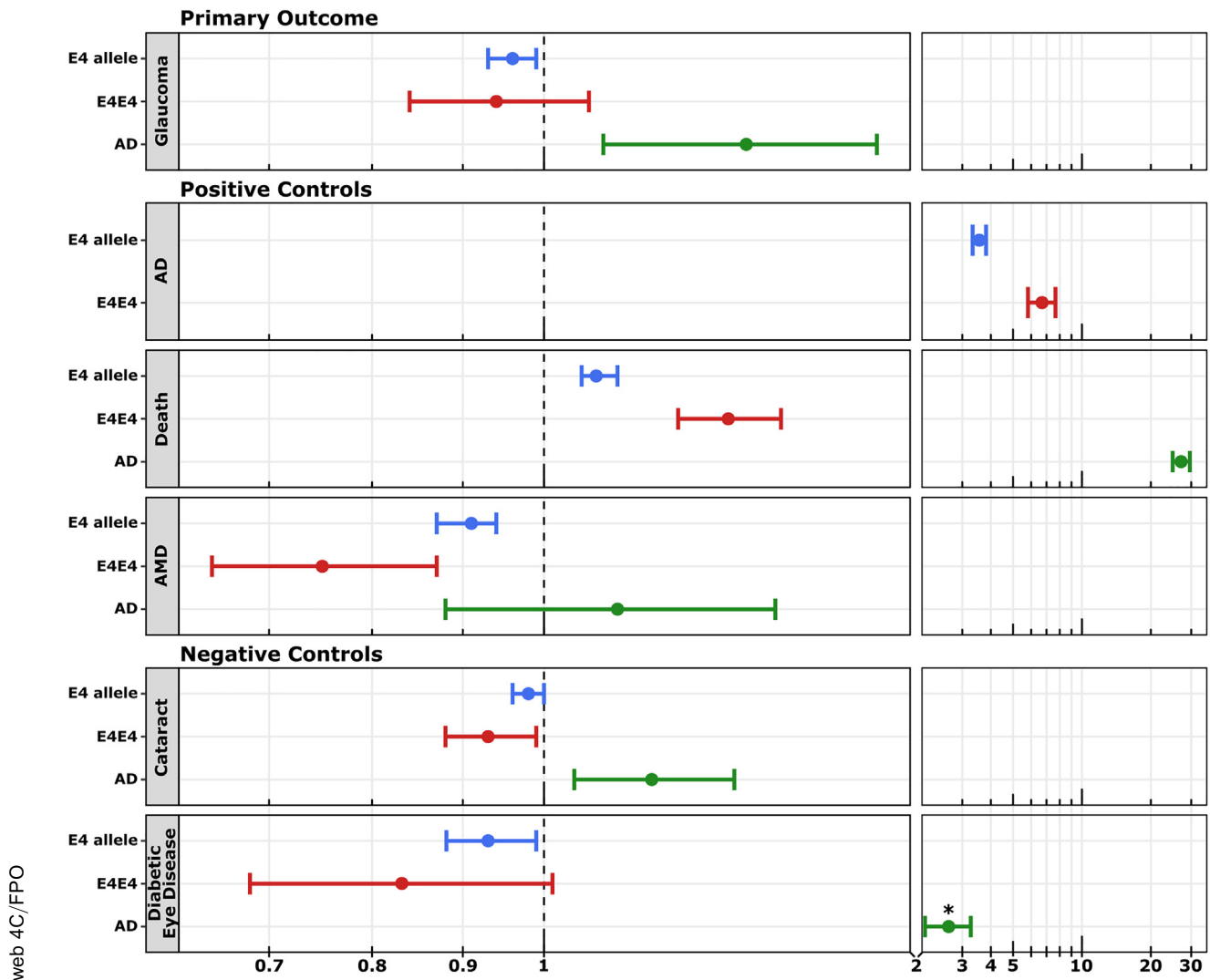


Figure 5. Forest plot. Associations of apolipoprotein E E4 allele (blue), genotype (E4E4; red), and Alzheimer's dementia (AD; green) with outcome phenotypes generated using UK Biobank data, represented as odds ratios with 95% confidence intervals. Individual phenotype comparisons represented include the primary outcome (glaucoma), positive controls (AD, death, age-related macular degeneration [AMD]), and negative controls (cataract and diabetic eye disease). Odds ratios are represented on a logarithmic scale with compression of the x-axis at higher limits. *Because diabetes is a risk factor for AD, diabetic eye disease does not represent a true negative control. Summary statistics were generated through logistic regression with adjustment for the alternate minor E2 allele (allelic regression) or alternate genotypes (genotype regression), age (squared), sex, and "AD diagnosis" (excluded from the analysis in which AD was the outcome).

potential confounding resulting from ethnicity, an important covariate of glaucoma and E4 allele prevalence.²¹ Our analysis also accounted for established associations between age and both glaucoma and prevalence of individual APOE alleles (survivorship effect) through adjustment for age (squared) and the alternate minor alleles/genotypes. Finally, we were able to indirectly investigate hidden effects of potential mislabeling resulting from underdiagnosis of glaucoma and dementia in the UKBB cohort.

Underdiagnosis and late diagnosis are important factors contributing to individual and societal glaucoma disease burden.⁵¹ Population studies of glaucoma estimate that the true prevalence of glaucoma in Europeans ranges from 6.0% at age 60–69 years to 17.3% at age 80+ years and

that approximately 50% of these cases remain undiagnosed.^{52,53} Glaucoma prevalence in the UKBB (3.2%) was lower than this estimate and similarly lower than was observed in both the CLSA (5.0%) and BMES cohorts (5.2%). This observation may have been a consequence of underreporting of glaucoma through data collection methods. One of the diagnostic parameters we included involved self-reporting, a recognized source of information bias.⁵⁴ Furthermore, only 44% of UKBB participants completed the eyesight touch screen survey. The second diagnostic parameter which involved medical coding (ICD10) data required individuals to undergo an ophthalmic examination to achieve a diagnosis. Given the recognized E4 allelic associations with AD, executive dysfunction, and underdiagnosis of common diseases of

Table 4. Correlations between Alzheimer's Dementia and Study Outcomes

Variable Type	Phenotype Associations (n [% Total])	AD (n = 1864 [0.4%])	No AD (n = 436 847 [99.6%])	Odds Ratio [95% CI]	P
Outcome variable	Glaucoma	123 [6.6%]	13,865 [3.2%]	1.30 [1.08,1.54]	< 0.01
Positive control	Death	1,019 [54.7%]	30,383 [7.0%]	27.1 [24.9,29.6]	< 0.001
	AMD	82 [4.4%]	10,393 [2.4%]	1.10 [0.88,1.35]	0.41
Negative control	Cataract	455 [24.4%]	56,439 [12.9%]	1.15 [1.04,1.28]	0.018
Unknown*	Diabetic eye disease	68 [3.6%]	4,766 [1.1%]	2.60 [2.01,3.29]	< 0.001

Phenotype-phenotype comparisons were made for study outcomes on the basis of Alzheimer's dementia (AD) diagnosis. Outcome statistics represented as odds ratios and 95% confidence intervals (CIs) were generated through logistic regression including adjustment for age (squared), sex, and "AD diagnosis." AMD = age-related macular degeneration.

*Because of established associations between diabetes and AD, diabetic eye disease was reclassified as an "unknown outcome" for this analysis.

aging, E4 carriers within the UKBB may have had higher rates of glaucoma underdiagnosis. If true, this phenomenon may explain why the same association was not observed in the first replication cohort (CLSA), which used a self-reported vision assessment tool in all participants at baseline, or the second replication cohort, which determined "glaucoma diagnosis" through ophthalmic examination of all individuals.

Epidemiological association studies of *APOE* may also be confounded by dementia, an underdiagnosed set of clinical syndromes that are not easily accounted for in statistical models.^{55–57} Despite population data estimating the prevalence of all-cause dementia to be 6.8% in Western European individuals aged ≥ 60 years,⁵⁸ ICD10-encoded diagnosis of AD, which is estimated to represent 60% to 80% of all dementia,⁵⁹ was reported in only 0.4% of UKBB participants. This could be an effect of recognized healthy volunteer bias within the UKBB.⁶⁰ However, because of the long duration of study follow-up, it is possible that low prevalence of "diagnosed AD" is the consequence of factors such as underdiagnosis and underreporting. Improving dementia diagnosis rates has been recognized as a health care priority for several reasons, one of which being that dementia diagnosis may facilitate other diagnoses of coexisting medical conditions frequently missed because of executive dysfunction and consequent self-neglect.^{59,61} Although we are unaware of data investigating the effect of AD on diagnosis of common eye diseases, our results, which demonstrated depletion of the E4 allele in cohorts characterized by pathophysiologically unrelated phenotypes (cataract and diabetic eye disease), suggest confounding from dementia-associated executive dysfunction. This assumption was supported by one subanalysis that demonstrated large percentages of ocular outcome ICD10 diagnosis were reported concurrently with AD ICD10 diagnosis. As expected, adjustment for "AD diagnosis" as an independent covariate of "glaucoma diagnosis" increased the strength of association between the E4 allele and each of the ocular outcomes. Unfortunately, this potential confounding could not be fully accounted for within this study because of our inability to measure undiagnosed AD.

Several additional factors contributed to our suspicion that the inverse association observed between the E4 allele and glaucoma was the result of confounding. Primarily, no compounded association was observed in E4E4

homozygotes, an anticipated effect which was observed for all positive control outcomes (AD, death, and AMD). Secondly, seemingly contradictory positive associations were observed between AD and both glaucoma and cataract. Because of the strength of association between E4 and both AD and mortality, we consider it unlikely that any potential unidentified parameter of AD risk could contribute sufficiently to result in higher AD prevalence in an E4-depleted cohort.

The second replication cohort allowed a different analysis through selective comparison of the E4 allele using carefully phenotyped POAG cases and nonglaucoma controls. The absence of association between the E4 allele and POAG within this cohort contradicted results from a recent study of the combined NEI Glaucoma Human genetics collaBORation (NEIGHBOR)-Massachusetts Eye and Ear Infirmary (MEEI) cohorts, which demonstrated an inverse association between E4 and POAG in 2606 POAG cases and controls (E4 "per allele" association: OR, 0.83; 95% CI, 0.74–0.94; $P = 0.0022$).¹⁹ Likewise, their observation of a stronger association between E4 and NTG (OR, 0.71; 95% CI, 0.58–0.87; $P = 0.0014$) was not replicated in a similar NTG subanalysis in the current study (OR, 0.98; 95% CI, 0.83–1.17; $P = 0.86$). Although the rationale for conflicting results between these 2 sets of analyses is unclear, we anticipate the potential for biases arising from different sampling methodologies. Further complicating the current understanding of association between glaucoma and the E4 allele, a recent study performed in the Predicting Risk Of Glaucoma: RElevant SNPs of Strong Association (PROGRESSA) cohort, a prospective study of early glaucoma, demonstrated faster rates of thinning in the macular ganglion cell/inner plexiform layer complex of participants harboring the E4 allele.⁶² Although faster macular ganglion cell/inner plexiform layer thinning does not necessarily constitute glaucomatous change, this result suggests the E4 allele may contribute to retinal degeneration.

We recognize a number of limitations in the current study, most important of which was our inability to account for mislabeling biases resulting from underdiagnosis of glaucoma and AD. The ideal sample in which to perform this analysis would be a large population-based cohort with a complete set of ocular phenotyping data. Accordingly, our 2 replication cohorts may have been underpowered to detect the E4 effects observed in the considerably larger UKBB

cohort. Given the sample size required to adjust for essential covariates of *APOE* and glaucoma, this phenomenon would more easily be studied using other models with objectively measurable parameters (i.e., OCT) in carefully phenotyped cohorts. Unfortunately, even such studies are likely to be inherently confounded through selection biases associated with identifying cases and controls. The inclusion of deceased participants within our UKBB cohort introduced selection bias. This step, which was undertaken to mitigate survivorship bias resulting from *APOE*-associated mortality, did not affect observed outcomes as was demonstrated in repeated glaucoma analyses excluding deceased participants. Despite POAG representing the most common glaucoma phenotype in Europeans, glaucoma cohorts identified in these cohorts are likely to have included other glaucoma phenotypes, which may have confounded results. This limitation was partly mitigated through our second replication cohort that solely investigated the association between E4 and POAG. Finally, attempting to account for the ethnic variation in glaucoma prevalence and *APOE* allele distribution, the current study limited its analysis to individuals with genetically European ancestry, which represented the predominant ethnic group within each study cohort. We were consequently unable to infer associations within other ethnic groups or identify potential ethnic variation, which may be relevant to the association between the E4 allele and glaucoma.

This study demonstrated a small inverse association between the *APOE* E4 allele and diagnosis of glaucoma in the UKBB, which was not reproducible in 2 replication cohorts. Although the E4 allele may contribute to glaucomatous neurodegenerative consequences, this study highlights the

complicated interactions between an allele associated with increased dementia, mortality risk, and underdiagnosed ocular phenotypes. Our result suggests this to be a complex association with multiple confounding parameters, which should be studied using different approaches involving objectively measurable outcomes.

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Footnotes and Disclosures

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¹ Department of Ophthalmology, Flinders Health and Medical Research Institute, Flinders University, Adelaide, South Australia.

² Department of Genetics and Computational Biology, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia.

³ Centre for Vision Research, University of Sydney, Sydney, Australia.

⁴ Department of Geriatric Medicine, Royal Adelaide Hospital, Adelaide, South Australia.

⁵ Department of Pathology, Flinders Health and Medical Research Institute, Flinders University, Adelaide, South Australia.

⁶ Garvan Institute of Medical Research, Darlinghurst, New South Wales.

*S.M. and S.D. contributed equally as first authors.

†O.M.S. and J.E.C. contributed equally as co-senior authors

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HUMAN SUBJECTS: Human subjects were included in this study. The UK Biobank (UKBB) has received ethical approval through the North West Multi-centre Ethics Committee (MREC), and the Canadian Longitudinal Study of Aging (CLSA) has received ethical approval from 13 local research ethics committees at various sites throughout Canada. All participants provided informed written consent, and study procedures were performed in accordance with the ethical principles of the World Medical Association Declaration of Helsinki. Ethics approval for the Australian and New Zealand Registry of Advanced Glaucoma (ANZRAG) was obtained through the Southern Adelaide Clinical Human Research Ethics Committee, and all participants were enrolled by informed written 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. The Blue Mountains Eye Study (BMES) has been approved by the Western Sydney Area Health Service Human Ethics Committee and complied with the Declaration of Helsinki. All participants were provided with a full explanation of the nature of the study and read and signed informed consent before participation.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Mullany, Diaz-Torres, Schmidt, Thomson, Qassim, Marshall, Knight, Berry, Kolovos, Dimasi, Lake, Mills, Landers, Mitchell, Healey, Commerford, Klebe, Souzeau, Hassall, MacGregor, Gharahkhani, Siggs, Craig

Data collection: Mullany, Diaz-Torres, Schmidt, Thomson, Qassim, Marshall, Knight, Berry, Kolovos, Dimasi, Lake, Mills, Landers, Mitchell, Healey, Commerford, Klebe, Souzeau, Hassall, MacGregor, Gharahkhani, Siggs, Craig

Analysis and interpretation: Mullany, Diaz-Torres, Schmidt, Thomson, Qassim, Marshall, Knight, Berry, Kolovos, Dimasi, Lake, Mills, Landers, Mitchell, Healey, Commerford, Klebe, Souzeau, Hassall, MacGregor, Gharahkhani, Siggs, Craig

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Overall responsibility: Sean Mullany

Abbreviations and Acronyms:

AD = Alzheimer's dementia; **AMD** = age-related macular degeneration; **ANZRAG** = Australian and New Zealand Registry of Advanced Glaucoma; **APOE** = apolipoprotein E; **BMES** = Blue Mountains Eye Study; **CI** = confidence interval; **CLSA** = Canadian Longitudinal Study of Aging; **ICD10** = International Classification of Diseases, Tenth Revision; **IOP** = intraocular pressure; **NTG** = normal-tension glaucoma; **OR** = odds ratio; **POAG** = primary open-angle glaucoma; **SNP** = single-nucleotide polymorphism; **UKBB** = UK Biobank.

Keywords:

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Correspondence:

Sean Mullany, Department of Ophthalmology, Flinders Health and Medical Research Institute, Flinders University, Flinders Drive, Bedford Park, Adelaide, SA 5042. E-mail: sean.mullany@flinders.edu.au.

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