Association of *APOE* Haplotypes With Common Age-Related Ocular Diseases in 412,171 Individuals

Perttu J. Liuska,[1,*] Joel T. Rämö,[2,3,*] Susanna Lemmelä,[2,4] Kai Kaarniranta,[5] Hannu Uusitalo,[6] Elisa Lahtela,[2] Mark J. Daly,[2,3] Mika Harju,[7] Aarno Palotie,[2,3] and Joni A. Turunen[1,7]; for the FinnGen Study[†]

[1]Eye Genetics Group, Folkhälsan Research Center, Biomedicum Helsinki, Haartmaninkatu 8, Helsinki, Finland
[2]Institute for Molecular Medicine Finland (FIMM), HiLIFE, PL 20, University of Helsinki, Finland
[3]The Broad Institute of MIT and Harvard, Stanley Building, Cambridge, Massachusetts, United States
[4]Finnish Institute for Health and Welfare, PL 30, Helsinki, Finland
[5]Department of Ophthalmology, Kuopio University Hospital and University of Eastern Finland, KYS, Finland
[6]TAYS Eye Center, Tampere University and Tampere University Hospital, PL 2000, Tampere, Finland
[7]Department of Ophthalmology, University of Helsinki and Helsinki University Hospital, Haartmaninkatu 4C, Helsinki, Finland

Correspondence: Perttu Liuska, Department of Ophthalmology, Helsinki University Hospital, Haartmaninkatu 4C, PL220, FI-00029 HUS, Helsinki, Finland; perttu.liuska@helsinki.fi.

[†]Related online manuscript file.

PJL and JTR contributed equally to the work.

Received: May 25, 2023
Accepted: October 27, 2023
Published: November 21, 2023

Citation: Liuska PJ, Rämö JT, Lemmelä S, et al. Association of *APOE* haplotypes with common age-related ocular diseases in 412,171 individuals. *Invest Ophthalmol Vis Sci.* 2023;64(14):33. https://doi.org/10.1167/iovs.64.14.33

Purpose. Apolipoprotein E4 (APOE4), a known risk factor for Alzheimer's disease, has controversially been associated with reduced risk of primary open-angle glaucoma (POAG) and age-related macular degeneration (AMD). Here, we sought to systematically quantify the associations of *APOE* haplotypes with age-related ocular diseases and to assess their scope and age-dependency.

Methods. We included genetic and registry data from 412,171 Finnish individuals in the FinnGen study. Disease endpoints were defined using nationwide registries. *APOE* genotypes were directly genotyped using Illumina and Affymetrix arrays or imputed using a custom Finnish reference panel. We evaluated the disease associations of *APOE* genotypes containing $\varepsilon 2$ (without $\varepsilon 4$) and $\varepsilon 4$ (without $\varepsilon 2$) compared with the $\varepsilon 3\varepsilon 3$ genotype using logistic regressions stratified by age.

Results. *APOE* $\varepsilon 4$ enriched haplotypes were inversely associated with overall glaucoma (odds ratio [OR] = 0.95, 95% confidence interval [CI] = 0.92–0.99, $P = 0.0047$), and its subtypes POAG (OR = 0.95, $P = 0.027$), normal-tension glaucoma (OR = 0.87, $P = 0.0058$), and suspected glaucoma (OR = 0.95, $P = 0.014$). Individuals with the $\varepsilon 4$ allele also had lower odds for AMD (OR = 0.80, 95% CI = 0.76–0.84, $P < 0.001$), seen both in dry and neovascular subgroups. A slight negative association was also detected in senile cataract, but this was not reproducible in age-group analyses.

Conclusions. Our results support prior evidence of the inverse association of *APOE* $\varepsilon 4$ with glaucoma, but the association was weaker than for AMD. We could not show an association with exfoliation glaucoma, supporting the hypothesis that APOE may be involved in regulating retinal ganglion cell degeneration rather than intraocular pressure.

Keywords: apolipoprotein E (APOE), glaucoma, age-related macular degeneration (AMD)

Apolipoprotein E (APOE), the major lipoprotein in the brain and a lipid transport protein, is strongly associated with late-onset Alzheimer's disease (AD) – the leading cause of dementia in the elderly – and cardiovascular disease.[1] It has also emerged as a potential regulator of age-related macular degeneration (AMD), and, more recently, glaucoma, which together account for up to 49.8% of blindness in developed, high-income countries.[1–3]

The *APOE* gene normally encodes a 299-aminoacid lipoprotein which regulates plasma lipid levels and acts as a ligand for low-density lipoprotein (LDL) receptors. *APOE* has three alleles ($\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$), defined by two single-nucleotide polymorphisms (SNPs), rs429358 and rs7412. Thus, six different genotypes are formed by the combination of these three alleles ($\varepsilon 2/\varepsilon 2$, $\varepsilon 3/\varepsilon 3$, $\varepsilon 4/\varepsilon 4$, $\varepsilon 2/\varepsilon 3$, $\varepsilon 3/\varepsilon 4$, and $\varepsilon 2/\varepsilon 4$), encoding different isoforms of the APOE protein, affecting lipid processing, transport, and clearance from the circulation.[4] The $\varepsilon 3$ allele is the most common and constitutes a baseline risk for AD, whereas $\varepsilon 4$ is a major risk allele and $\varepsilon 2$ is protective for AD in an allele-dose dependent manner.[5,6]

Primary open-angle glaucoma (POAG; MIM 137760), the leading cause of irreversible blindness in the world, is a group of neurodegenerative diseases characterized by the loss of retinal ganglion cells (RGCs) and their axons in the optic nerve. Intraocular pressure (IOP) remains the only modifiable risk factor for POAG and there are no neuroprotective therapies to promote RGC survival.[7] Interestingly, the

APOE $\varepsilon 4$ allele has recently been associated with a reduced risk of POAG (odds ratio [OR] = 0.83, 95% confidence interval [CI] = 0.74–0.94, $P = 0.0022$) in an inverse association compared with AD.[1] This protective effect was found in both high-tension and normal-tension glaucoma (NTG) groups. Furthermore, in a Brazilian cohort, carrying the *APOE* $\varepsilon 2$ allele (OR = 1.516, $P = 0.04$) or the $\varepsilon 2\varepsilon 3$ genotype (OR = 1.655, $P = 0.02$) was associated with a greater risk for POAG when compared to $\varepsilon 3\varepsilon 3$ reference genotype.[8] A small negative association between *APOE* $\varepsilon 4$ and glaucoma was also observed in a recent study within the UK Biobank, but this was not reproducible in two other replication cohorts in the study set, and the authors suggested that the association might only reflect glaucoma underdiagnosis in the *APOE* $\varepsilon 4$ carriers.[9]

AMD (MIM 603075), identified by progressive degeneration of photoreceptors and the underlying retinal pigment epithelium (RPE) cells in the macula region of the retina, is the second leading cause of acquired visual impairment in the elderly population, and it is estimated to affect almost 30% of European individuals over 75 years of age.[2,10] It can be clinically classified into two major subtypes: the dry form, accounting for approximately 80% of the cases, and the wet form.[11] The early hallmark of AMD are inflammatory cellular debris called drusen in or under the RPE. The dry form of AMD is also characterized by pigment irregularities or geographic atrophy in the RPE (without angiogenesis), whereas the wet form is marked by choroidal neovascularization.[3] An inverse association between the *APOE* $\varepsilon 4$ allele and AMD was identified in early candidate gene studies,[12] and the *APOE* locus has since been identified in meta-analyses and genome-wide association studies of AMD with high statistical confidence.[3,13,14]

Despite increasing interest, the statistical evidence for a causal association of *APOE* with glaucoma remains weaker than for AMD.[9] Several studies have reported conflicting results regarding the risk profiles of different *APOE* haplotypes with glaucoma,[15–19] whereas others have detected no association.[20–25] Many previous reports are based on small to moderately sized case-control datasets and have not examined other diseases as negative controls to reveal possible bias in the study design. *APOE* $\varepsilon 4$ is a major determinant of cardiovascular disease and dementia, and a leading genetic cause of mortality and disability worldwide.[26,27] The association of *APOE* genotypes with life span and functional capacity in old age can bias associations of diseases primarily affecting the elderly population. Non-neurodegenerative diseases affecting a large proportion of the elderly population, such as senile age-related cataract,[28] can be analyzed as probable negative outcome controls to assess the likelihood of bias.

Here, we report the association and age dependency of *APOE* haplotypes with common age-related ocular diseases, glaucoma, AMD, and senile cataract in 412,171 Finnish individuals.

Methods

FinnGen (https://www.finngen.fi/en) is a public-private partnership research project combining genotype data of currently 429,209 individuals (Data Freeze 10) from Finnish biobanks, prospective epidemiological cohorts (initiated as far back as 1992), and disease-based cohorts with digital health record data from national health registries. The samples were linked by a unique national personal identification number assigned to all Finnish citizens and residents. Recruitment of samples to FinnGen was initiated in August 2017, and the data analysis was performed between December 2022 and April 2023.

The FINRISK study is a large Finnish population survey on risk factors for chronic, noncommunicable diseases. The survey was carried out for 40 years since 1972 every 5 years using independent, random, and representative population samples from different parts of Finland, as described earlier.[29] The Health 2000 Survey was carried out in the years 2000 to 2001, capturing a nationally representative sample of more than 8000 persons aged 30 years and over living in mainland Finland. The National FINRISK Study and Health 2000 Survey were joined to form a new population study, the National FinHealth Study. The data were transferred to THL Biobank in July 2015 and subsequently incorporated into FinnGen.

The FinnGen samples were genotyped using Illumina and Affymetrix arrays (Illumina Inc., San Diego, CA, USA, and Thermo Fisher Scientific, Santa Clara, CA, USA), as detailed previously.[30] Genotypes were imputed based on a population-specific SISu v4 imputation reference panel comprised of 8557 whole genomes. Non-Finnish outliers, twin, and duplicate samples were removed, as detailed previously,[30] and the remaining 412,171 participants were included in genotype analyses. The two SNPs forming the *APOE* alleles, rs429358 and rs7412, were identified either by direct genotyping or imputation.

The FinnGen study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Review Board of the Hospital District of Helsinki and Uusimaa (HUS/990/2017; Online-Only Supplement). Individuals gave written informed consent based on the Finnish Biobank Act or separate research cohort protocols. All DNA samples and data in this study were pseudonymized.

The disease endpoints were defined using nationwide registries for deaths, hospital discharges, outpatient specialist appointments, harmonizing over the International Classification of Diseases (ICD) revisions 8, 9, and 10, procedure codes (NOMESCO), Finnish-specific Social Insurance Institute (KELA) drug reimbursement codes and Anatomic Therapeutic Chemical (ATC) codes. Glaucoma-related operations included trabeculectomy and iridectomy, glaucoma shunt operation, non-penetrating glaucoma surgery, and other filtering operations. The definitions of clinical endpoints used in this study are presented in Supplementary Table S1 and control groups are provided in detail at https://www.finngen.fi/en/researchers/clinical-endpoints.

Statistical Analysis

APOE genotypes were identified from haplotypes of the two non-synonymous SNPs, rs429358 and rs7412, forming the corresponding alleles of $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$. Logistic regression in R (version 4.2.2; http://www.r-project.org) was used to analyze the association of genotypes with ocular disease, with covariates including genotypic sex, age at end of follow-up or death,[2] first 10 genotypic principal components (PCs), and genotyping array. All participants were included in the analyses, and the *APOE* $\varepsilon 3\varepsilon 3$ was used as the reference unless otherwise stated. A Wald test was used to evaluate the statistical significance of the coefficients. We additionally evaluated the disease associations of the rs429358 and rs7412 genotypes with 2405 binary endpoints and 3 quantitative endpoints using the additive model as implemented

Investigative Ophthalmology & Visual Science

in Regenie version 2.24, with genotypic sex, age at end of follow-up or death, first 10 PCs, genotyping array, and genotyping batch as fixed-effect covariates.[31] All tests were two-tailed. Because this study followed up previously reported associations, and the examined phenotypes and genotypes were correlated, multiple testing correction was not considered appropriate. The analyses were performed between December 2022 and April 2023.

Results

Study Characteristics

Altogether, 412,171 individuals from FinnGen passing genotype quality control were included (Table 1). Of those, 230,307 (55.9%) were female subjects, and 181,864 (44.1%) were male subjects. The mean age at the end of the follow-up period was 60.3 (SD = 17.9) years. The number of individuals with different disease endpoints are presented in Table 2.

The distribution of individuals with different *APOE* genotypes are tabulated in Table 1. The phenome-wide association study plots of the two *APOE* alleles forming the haplotypes, rs429358 and rs7412, are supplied in Supplementary Figure S1.

Table 1. Characteristics of Study Participants (n = 412,171) and Prevalence of the *APOE* Haplotypes

Characteristic	
Mean age, y (SD*)	60.3 (17.9)
Sex, *n* (%)	
Male	181,864 (44.1)
Female	230,307 (55.9)
Genotypes	
$\varepsilon2\varepsilon2$	1135 (0.3)
$\varepsilon2\varepsilon3$	33,597 (7.8)
$\varepsilon2\varepsilon4$	8040 (1.9)
$\varepsilon3\varepsilon3$	241,282 (56.2)
$\varepsilon3\varepsilon4$	114,836 (26.8)
$\varepsilon4\varepsilon4$	13,281 (3.1)

* Standard deviation.

Associations of the *APOE* Genotypes With AD and Ocular Diseases

First, we tested the known association of *APOE* genotypes with AD in our sample. The OR for genotypes with $\varepsilon4$ but without $\varepsilon2$ (i.e. $\varepsilon3\varepsilon4$ and $\varepsilon4\varepsilon4$) compared to the $\varepsilon3\varepsilon3$ reference genotype was 3.06 (95% CI = 2.93–3.20, $P < 0.001$) for AD. We subsequently investigated the association between the *APOE* genotypes with glaucoma endpoints and AMD. The *APOE* $\varepsilon4$ allele demonstrated an inverse association with all glaucoma endpoints (overall glaucoma, glaucoma suspects, POAG, NTG, and glaucoma operations) except XFG (see Table 2). The *APOE* $\varepsilon4$ allele also had a negative association with AMD with an OR of 0.80 (95% CI = 0.76–0.84, $P < 0.001$). To serve as a negative control, we also tested for associations among the *APOE* genotypes, myopia, and senile cataract. The *APOE* $\varepsilon4$ allele was not associated with myopia but there was an association with senile cataract (OR = 0.95, 95% CI = 0.93–0.97, $P < 0.001$).

The OR for genotypes with $\varepsilon4$ but without $\varepsilon2$ (i.e. $\varepsilon3\varepsilon4$ and $\varepsilon4\varepsilon4$) compared with all other genotypes was 0.93 (95% CI = 0.88–0.98, $P = 0.011$) for overall glaucoma; for POAG 0.86 (95% CI = 0.79–0.93, $P < 0.001$); for NTG 0.79 (95% CI = 0.68–0.93, $P = 0.0030$); and 0.92 (95% CI = 0.86–0.99, $P = 0.022$) for suspected glaucoma. The OR was 0.75 for AMD (95% CI = 0.69–0.81, $P < 0.001$); and 0.93 for senile cataract (95% CI = 0.89–0.96, $P < 0.001$). The associations of $\varepsilon3\varepsilon4$ and $\varepsilon4\varepsilon4$ genotypes with XFG (95% CI = 0.82–1.06, $P = 0.30$) or glaucoma-related surgery (95% CI = 0.73–1.08, $P = 0.23$) were not significant.

Table 2. Disease Prevalence and Association of the *APOE* $\varepsilon4$ Allele Containing Genotypes ($\varepsilon3\varepsilon4$ and $\varepsilon4\varepsilon4$ Genotypes) Compared With the Reference Genotype $\varepsilon3\varepsilon3$ With Different Types of Glaucoma, Age-Related Macular Degeneration, Myopia, Senile Cataract, and Alzheimer's Disease in the FinnGen Biobank Cohort With 412,171 Individuals

Phenotype	No. (%)* Controls	Odds Ratio (95% CI)	*P* Value†
Individuals after QC (n = 412,171)		NA	NA
Glaucoma (n = 20,905; 5.1%)	391,266	0.95 (0.92–0.99)	0.0047
POAG (n = 8530; 2.1%)	391,266	0.95 (0.90–0.99)	0.027
NTG (n = 2272; 0.58%)	391,266	0.87 (0.79–0.96)	0.0058
XFG (n = 3424; 0.87%)	391,266	1.03 (0.95–1.11)	0.52
Glaucoma suspects (n = 11,968; 3.0%)	391,266	0.95 (0.91–0.99)	0.014
Glaucoma operations (n = 1 486; 0.36%)	410,685	0.84 (0.75–0.95)	0.0049
AMD (n = 9721; 2.5%)	381,330	0.80 (0.76–0.84)	<0.001
Wet AMD (n = 5239; 1.9%)	273,912	0.76 (0.71–0.81)	<0.001
Dry AMD (n = 6651; 2.3%)	272,496	0.80 (0.75–0.84)	<0.001
Myopia (n = 4106, 1.0%)	394,019	0.94 (0.88–1.01)	0.089
Senile cataract (n = 65,235; 16.0%)	341,536	0.95 (0.93–0.97)	<0.001
Alzheimer's disease (n = 10,519; 2.6%)	401,652	3.06 (2.93–3.20)	<0.001

QC, quality control; POAG, primary open-angle glaucoma; NTG, normal-tension glaucoma; XFG, exfoliation glaucoma; AMD, age-related macular degeneration; NA, not applicable.

* Prevalence in cohort;

† Logistic regression, two-tailed, *P* values with Wald test.

Investigative Ophthalmology & Visual Science

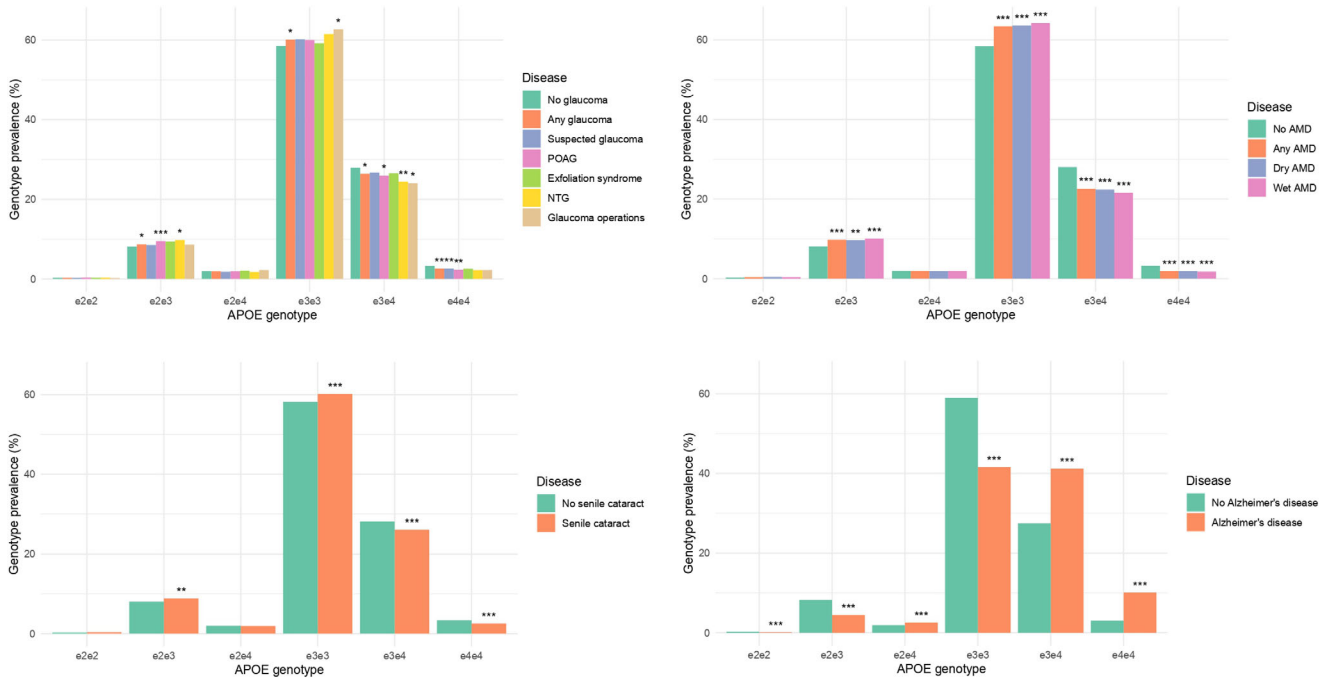

Figure. Prevalences of the *APOE* genotypes in different types of glaucoma, age-related macular degeneration, senile cataract, and Alzheimer's disease in FinnGen. The prevalence of each *APOE* genotype with respect to disease status was evaluated using logistic regression, with the respective *APOE* genotype as the outcome and disease status, genotypic sex, age at the end of follow-up or death[2], first 10 PCs, and genotyping array as independent predictors. A Wald test was used to evaluate the statistical significance (*** indicates $P < 0.001$, **$P < 0.01$, and *$P < 0.05$). Abbreviations: POAG = primary open-angle glaucoma, NTG = normal-tension glaucoma, AMD = age-related macular degeneration.

When comparing the less common $\varepsilon 2$ allele containing genotypes $\varepsilon 2\varepsilon 2$ and $\varepsilon 2\varepsilon 3$ with the $\varepsilon 3\varepsilon 3$ reference genotype, we observed a slight positive association with POAG (OR = 1.12, 95% CI = 1.03–1.20, $P = 0.0046$), but we did not observe statistically significant associations with any other glaucoma phenotypes (Supplementary Table S2).

Prevalences of Ocular Diseases by *APOE* Genotypes

The prevalence of glaucoma-related diseases among carriers of the *APOE* $\varepsilon 4\varepsilon 4$ and the reference $\varepsilon 3\varepsilon 3$ genotypes were 4.1% and 5.2%, respectively, for overall glaucoma; 1.5% and 2.1% for POAG; 0.66% and 0.84% for XFG; 0.38% and 0.58% for NTG; 2.3% and 3.0% for suspected glaucoma; and 0.25% and 0.39% for glaucoma-related surgery. The prevalence among carriers of the *APOE* $\varepsilon 4\varepsilon 4$ and the $\varepsilon 3\varepsilon 3$ genotypes were 1.4% and 2.6% for AMD; 0.71% and 1.4% for wet AMD; 0.95% and 1.8% for dry AMD; and 12.4% and 16.3% for senile cataract. The genotype prevalences in disease endpoints are presented in the Figure.

Age-Dependent Associations of *APOE* Genotypes With Ocular Diseases

We also evaluated associations of *APOE* haplotypes with the disease endpoints in different age groups by age at disease onset: <70 years, <60 years, and <50 years (i.e. all individuals diagnosed before the age limit; Table 3). The association of the $\varepsilon 4$ allele containing genotypes compared with the reference $\varepsilon 3\varepsilon 3$ genotype was statistically significant with POAG in all age groups (see Table 3). Similarly, we observed a significant association between *APOE* haplotypes and overall glaucoma in all age groups, and the association was more pronounced in the younger age groups (OR = 0.96, $P = 0.047$; and OR = 0.90, $P = 0.0098$ for individuals <70 and <50 years of age by disease onset, respectively; see Table 3). However, we could not repeat significant associations with AMD or senile cataract in any of these age groups. Myopia was not associated with *APOE* haplotypes in any age group. The results were similar in additional sensitivity analyses stratified by age at the end of the follow-up (Supplementary Table S3).

Associations of *APOE* Genotypes With Ocular Diseases in Population Surveys

We performed an additional sensitivity analysis using the representative, randomly selected National FinHealth Study subpopulation in FinnGen with 35,599 individuals (Supplementary Table S4). The inverse association of the *APOE* $\varepsilon 4$ allele with different types of AMD remained significant. Although we did not detect statistically significant associations with glaucoma endpoints in this smaller sample, effect estimates were comparable to those observed in the entire FinnGen cohort (Supplementary Table S5).

Discussion

Here, we systematically evaluated the associations of *APOE* haplotypes with common age-related ocular diseases, with a particular focus on glaucoma subtypes and AMD. We

Investigative Ophthalmology & Visual Science

Table 3. Age-Related Odds Ratios (ORs) of *APOE* ε4 Allele (ε3ε4 and ε4ε4 Genotypes) Compared to the Reference ε3ε3 Genotype in Different Ophthalmic Diseases, and Alzheimer's Disease in FinnGen Data Freeze 10 (n = 412,171) by Age at Disease Onset

Age, Y	Cases (N)	OR (95% CI)	P Value*
Glaucoma			
<50 y	3334	0.90 (0.84–0.98)	**0.0098**
<60 y	7007	0.94 (0.89–0.99)	**0.020**
<70 y	13,177	0.96 (0.92–1.00)	**0.047**
POAG			
<50 y	671	0.82 (0.69–0.97)	**0.023**
<60 y	1922	0.86 (0.78–0.95)	**0.0037**
<70 y	4454	0.92 (0.86–0.99)	**0.019**
Glaucoma suspects			
<50 y	2215	0.95 (0.86–1.04)	0.28
<60 y	4566	0.96 (0.90–1.03)	0.26
<70 y	8138	0.97 (0.92–1.02)	0.20
Exfoliation glaucoma			
<50 y	129	1.08 (0.74–1.58)	0.69
<60 y	376	1.05 (0.84–1.32)	0.64
<70 y	1386	1.07 (0.95–1.21)	0.24
Normal-tension glaucoma			
<50 y	93	1.04 (0.67–1.62)	0.85
<60 y	372	0.83 (0.66–1.05)	0.12
<70 y	1047	0.90 (0.78–1.03)	0.11
Glaucoma-related surgery			
<50 y	131	0.89 (0.61–1.30)	0.54
<60 y	314	0.82 (0.64–1.06)	0.14
<70 y	712	0.76 (0.64–0.91)	**0.0020**
AMD			
<50 y	0	NA	NA
<60 y	541	1.02 (0.85–1.23)	0.80
<70 y	2494	0.92 (0.84–1.00)	0.058
Wet AMD			
<50 y	0	NA	NA
<60 y	240	1.11 (0.84–1.47)	0.45
<70 y	1234	0.88 (0.77–1.00)	0.051
Dry AMD			
<50 y	0	NA	NA
<60 y	340	0.98 (0.78–1.24)	0.89
<70 y	1604	0.92 (0.82–1.03)	0.14
Senile cataract			
<50 y	1166	0.90 (0.79–1.02)	0.11
<60 y	5903	0.96 (0.91–1.02)	0.17
<70 y	24,110	0.98 (0.95–1.01)	0.14
Myopia			
<50 y	2735	0.96 (0.89–1.05)	0.39
<60 y	3417	0.96 (0.89–1.03)	0.25
<70 y	3879	0.95 (0.88–1.02)	0.13
Alzheimer's disease			
<50 y	29	0.47 (0.19–1.16)	0.10
<60 y	217	1.92 (1.46–2.53)	**<0.001**
<70 y	1113	3.59 (3.16–4.08)	**<0.001**

POAG, primary open-angle glaucoma; AMD, age-related macular degeneration; OR, odds ratio; NA, not applicable.
* Logistic regression, two-tailed P values with Wald test.

build upon previous findings of a protective association of *APOE* ε4 with glaucoma by including genotype and registry data from 412,171 Finnish individuals. We demonstrate that the *APOE* ε4 enriched haplotypes were inversely associated with overall glaucoma, NTG, POAG, and suspected glaucoma. They were also inversely associated with both dry and neovascular AMD. Interestingly, a slight negative association was also shown in senile cataract.

In earlier studies, the most pronounced protective effect of *APOE* ε4 has been observed in NTG or populations with a high proportion of NTG.[1,16,17] Margeta et al. speculated that this is because *APOE* has a role in regulating RGC degeneration rather than IOP.[1] In our study, the effect was also most pronounced in the NTG group (OR = 0.79 compared with all other genotypes, P = 0.0030). We could not show an association with XFG, a relatively common cause of secondary glaucoma in the Nordics,[32] which is in line with this hypothesis. In exfoliation syndrome, extracellular fibrillary material can be discovered in the anterior segment of the eye supposedly hindering the outflow of the aqueous humor and increasing IOP. Thus, the pathogenesis of XFG differs from that of primary glaucoma.

Some other studies and meta-analyses have also reported contradictory results, suggesting either a lack of association of these alleles or a positive association of *APOE* ε4 with glaucoma.[9,33,34] A recent study within the UK Biobank, including 13,988 individuals with glaucoma, observed similar results to our analysis, but could not replicate the results in other cohorts and the authors proposed that the association may represent an artifact of glaucoma underdiagnosis in *APOE* ε4 carriers.[9] Considering the possible ethnic differences, the differences in the pathogenesis of different types of glaucoma, and false positive results when conducting targeted genetic association studies, the role of *APOE* with glaucoma has remained debatable. An additional confounding element arises from the observation that AD has an impact on the retina both molecularly and structurally.[35,36] The *APOE* ε4 allele has been associated with faster retinal nerve fiber layer thinning,[37] introducing a potential source of bias in imaging-based studies. Our results do not exclude a general role for *APOE* in glaucoma and support differential associations for glaucoma subtypes.

An important observation is the presence of a negative association between *APOE* ε4 allele-containing genotypes and senile cataract, a presumed negative control outcome. A similar association was also recently detected in the UK Biobank.[9] This association could reflect a genuine pathophysiological role of APOE in lens degeneration, or perhaps more likely, confounding. Because *APOE* ε4 is linked with greater disability and mortality, as well as with recruitment to biobank studies, this may distort the association with senile cataract in a large data set reliant on digital health registries for disease endpoints. Supporting this hypothesis, we observed that the association with cataract was no longer significant in age groups <70 years of age despite a considerable number of cases (see Table 3, Supplementary Table S3). In contrast, we observed significant associations of the ε4 allele containing genotypes with glaucoma in younger age groups.

We also carried out additional sensitivity analyses using representative and randomly selected population samples within the FinHealth Study subsample in FinnGen. In this subsample of 35,599 individuals, the associations of the *APOE* ε4 allele with AMD subtypes and AD remained significant, but we could not repeat significant associations with different types of glaucoma or senile cataract (see Supplementary Table S5). Nevertheless, the effect size of the *APOE* ε4 with respect to glaucoma endpoints remained consistent with the original analyses, and less robust associations may have been obscured by smaller sample sizes.

The exact mechanism by which APOE may affect the risk of glaucoma and AMD remains unclear. Although *APOE* is expressed widely in different cell types, APOE is also shown

Investigative Ophthalmology & Visual Science

to be synthesized in retinal Müller cells, secreted into the vitreous, and transported into the optic nerve by RGCs.[38] *APOE* is upregulated in mouse models of neurodegenerative diseases, such as AD, amyotrophic lateral sclerosis, and multiple sclerosis; and it seems to be a regulator of microglial neurodegeneration.[39,40] Microglia are the resident immune cells in the central nervous system that play a vital role in brain homeostasis but lose their homeostatic function in neurodegenerative diseases.[39] Margeta et al. showed in two mouse models that the microglial transition from homeostatic to neurodegenerative is characterized by upregulation of *APOE* and *LGALS3* (galectin-3), which were also upregulated in glaucomatous human retinas.[41] This transition to neurodegenerative microglia seems to be controlled by APOE and triggering receptor expressed on myeloid cells 2 (TREM2) signaling.[39] In human glaucoma, the activated microglia colocalize in the optic nerve with proinflammatory cytokines, including tumor necrosis factor α.[42] Furthermore, a recent study found that APOE and galectin-3 levels were significantly elevated in the aqueous humor of human eyes with glaucoma.[43] In mouse models, mice carrying the human *APOE4* allele or targeted deletion of *Apoe* were protected from RGC loss and did not upregulate neurodegeneration associated genes including *LGALS3*.[41] Thus, APOE4 may act as a loss-of-function isoform, preserving RGCs by impairing microglial activation.[41]

APOE also plays a significant role in neuroinflammatory response in AMD. AMD is associated with a breakdown of the subretinal immunosuppressive system and chronic accumulation of mononuclear phagocytes, which express high levels of APOE, interleukin-6 (IL-6) and CC chemokine ligand 2.[44,45] Moreover, APOE can activate the innate immunity receptor cluster and induce inflammatory cytokines including IL-6 and promote the survival of mononuclear phagocytes.[44] Notably, IL-6 levels are associated with AMD incidence.[46] The *APOE* $\varepsilon 4$ allele is associated with lower plasma and brain tissue APOE concentrations, whereas $\varepsilon 2$ associates with higher concentrations.[47] AMD is characterized by drusen, which contain considerable amounts of APOE and its cargo, and thus, a causal inverse association of APOE4 with AMD is plausible.[47]

Strengths and Limitations

A strength of the current study is the ability to evaluate multiple diseases, including subtypes of glaucoma and AMD, simultaneously within a large cohort. Glaucoma endpoints in FinnGen are based on both inpatient and outpatient diagnosis and operation codes reported by eye care specialists, which may decrease bias, compared with data from hospital admissions and self-report in cohorts such as the UK Biobank. Enrichment of the *APOE* $\varepsilon 4$ allele in the Nordics also increases the power to evaluate disease associations.[48] The two SNPs forming the *APOE* alleles, rs429358 and rs7412, both had very high-quality imputation INFO scores of 99.9% (ranges = 0.86-1.0 and 0.90-1.0, respectively).

Our study had several limitations. FinnGen is not a truly healthy population-based sample, as many biobanks collected consent from individuals during hospital visits, potentially inflating estimates of disease prevalence and presenting a challenge in determining accurate ORs. The study relied on reported codes in national health registries, which could result in undiagnosed or unreported individuals and overlapping diagnoses. It is also estimated that 50% of patients with glaucoma remain undiagnosed,[49,50] and we speculate that some diseases, such as senile cataract, might also be more likely diagnosed in the presence of other ophthalmic comorbidities. Finally, individuals with a high disease burden or disability may be less likely to participate in biobank studies compared with healthy individuals.

Conclusions

Our results corroborate prior evidence of an inverse association of the *APOE* $\varepsilon 4$ allele with glaucoma and its subtypes POAG, NTG, and suspected glaucoma. Furthermore, in age-stratified analyses, we were able to validate these results in younger age groups. We could not demonstrate an association with XFG, which supports the hypothesis that *APOE* may be involved in regulating RGC degeneration rather than IOP. We also observed a nominally significant association of the *APOE* $\varepsilon 2$ allele with POAG. However, the associations with different types of glaucoma appear to be markedly weaker than that observed with AMD, and the causality of these associations remains unresolved. Our study highlights the necessity to systematically assess the associations detected in genetic association studies considering false positive associations and the complex pleiotropy of many genetic loci.

Acknowledgments

Supported by grants from the Glaukooma Tukisäätiö Lux Foundation, the Mary and Georg C. Ehrnrooth Foundation, the Evald and Hilda Nissi Foundation, and the Finnish Medical Foundation. We want to acknowledge the participants and investigators of the FinnGen study. The FinnGen project is funded by two grants from Business Finland (HUS 4685/31/2016 and UH 4386/31/2016) and the following industry partners: AbbVie Inc., AstraZeneca UK Ltd, Biogen MA Inc., Bristol Myers Squibb (and Celgene Corporation & Celgene International II Sàrl), Genentech Inc., Merck Sharp & Dohme LCC, Pfizer Inc., GlaxoSmithKline Intellectual Property Development Ltd., Sanofi US Services Inc., Maze Therapeutics Inc., Janssen Biotech Inc, Novartis AG, and Boehringer Ingelheim International GmbH. The following biobanks are acknowledged for delivering biobank samples to FinnGen: Auria Biobank (www.auria.fi/biopankki), THL Biobank (www.thl.fi/biobank), Helsinki Biobank (www.helsinginbiopankki.fi), Biobank Borealis of Northern Finland (https://www.ppshp.fi/Tutkimus-ja-opetus/Biopankki/Pages/Biobank-Borealis-briefly-in-English.aspx), Finnish Clinical Biobank Tampere (www.tays.fi/en-US/Research_and_development/Finnish_Clinical_Biobank_Tampere), Biobank of Eastern Finland (www.ita-suomenbiopankki.fi/en), Central Finland Biobank (www.ksshp.fi/fi-FI/Potilaalle/Biopankki), Finnish Red Cross Blood Service Biobank (www.veripalvelu.fi/verenluovutus/biopankkitoiminta), Terveystalo Biobank (www.terveystalo.com/fi/Yritystietoa/Terveystalo-Biopankki/Biopankki/), and Arctic Biobank (https://www.oulu.fi/en/university/faculties-and-units/faculty-medicine/northern-finland-birth-cohorts-and-arctic-biobank). All Finnish Biobanks are members of BBMRI.fi infrastructure (www.bbmri.fi). Finnish Biobank Cooperative -FINBB (https://finbb.fi/) is the coordinator of BBMRI-ERIC operations in Finland. The Finnish biobank data can be accessed through the Fingenious services (https://site.fingenious.fi/en/) managed by FINBB.

The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Perttu Liuska, Folkhälsan Research Center; and Joel Rämö, The Broad Institute of MIT and Harvard, conducted and are responsible for the data analysis.

Perttu Liuska and Joel Rämö had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Data Availability: Summary-level genome-wide association study data from FinnGen is subjected to a one-year embargo and thereafter made available online (https://www.finngen.fi/en/access_results). Any researcher can apply for health register data from the Finnish Data Authority Findata and individual-level genotype data via the Fingenious portal (https://site.fingenious.fi/en/) hosted by the Finnish Biobank Cooperative FinBB (https://finbb.fi/en/).

Disclosure: **P.J. Liuska**, None; **J.T. Rämö**, None; **S. Lemmelä**, None; **K. Kaarniranta**, None; **H. Uusitalo**, None; **E. Lahtela**, None; **M.J. Daly**, None; **M. Harju**, None; **A. Palotie**, None; **J.A. Turunen**, None

References

1. Margeta MA, Letcher SM, Igo RP, Jr., et al. Association of APOE with primary open-angle glaucoma suggests a protective effect for APOE ε4. *Invest Ophthalmol Vis Sci*. 2020;61:3.
2. Steinmetz JD, Bourne RRA, Briant PS, et al. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the Right to Sight: an analysis for the Global Burden of Disease Study. *Lancet Glob Health*. 2021;9:e144–e160.
3. Fritsche LG, Igl W, Bailey JNC, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*. 2015;48:134–143.
4. Huang Y. Mechanisms linking apolipoprotein E isoforms with cardiovascular and neurological diseases. *Curr Opin Lipidol*. 2010;21:337–345.
5. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*. 1993;261:921–923.
6. Corder EH, Saunders AM, Risch NJ, et al. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet*. 1994;7:180–184.
7. Almasieh M, Levin LA. Neuroprotection in glaucoma: animal models and clinical trials. *Annu Rev Vis Sci*. 2017;3:91–120.
8. Occhiutto ML, de Melo MB, Cabral de Vasconcellos JP, et al. Association of APOE gene polymorphisms with primary open angle glaucoma in Brazilian patients. *Ophthalmic Genet*. 2021;42:53–61.
9. Mullany S, Diaz-Torres S, Schmidt JM, et al. No strong association between the apolipoprotein E E4 allele and glaucoma: a multicohort study. *Ophthalmol Sci*. 2023;3:100287.
10. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health*. 2014;2:e106–e116.
11. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science*. 1997;277:1805–1807.
12. Klaver CCW, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. *Am J Hum Genet*. 1998;63:200–206.
13. McKay GJ, Patterson CC, Chakravarthy U, et al. Evidence of association of APOE with age-related macular degeneration: a pooled analysis of 15 studies. *Hum Mutat*. 2011;32:1407–1416.
14. Xiying M, Wenbo W, Wangyi F, Qinghuai L. Association of apolipoprotein E polymorphisms with age-related macular degeneration subtypes: an updated systematic review and meta-analysis. *Arch Med Res*. 2017;48:370–377.
15. Al-Dabbagh NM, Al-Dohayan N, Arfin M, Tariq M. Apolipoprotein E polymorphisms and primary glaucoma in Saudis. *Mol Vis*. 2009;15:912–919.
16. Lam C, Fan B, Wang D, et al. Association of apolipoprotein E polymorphisms with normal tension glaucoma in a Chinese population. *J Glaucoma*. 2006;15:218–222.
17. Mabuchi F, Tang S, Ando D, et al. The apolipoprotein E gene polymorphism is associated with open angle glaucoma in the Japanese population. *Mol Vis*. 2005;11:609–612.
18. Vickers JC, Craig JE, Stankovich J, et al. The apolipoprotein ε4 gene is associated with elevated risk of normal tension glaucoma. *Mol Vis*. 2002;14:389–393.
19. Chiras D, Tzika K, Kokotas H, et al. Development of novel LOXL1 genotyping method and evaluation of LOXL1, APOE and MTHFR polymorphisms in exfoliation syndrome/glaucoma in a Greek population. *Mol Vis*. 2013;6:1006–1016.
20. Krumbiegel M, Pasutto F, Mardin CY, et al. Apolipoprotein E genotypes in pseudoexfoliation syndrome and pseudoexfoliation glaucoma. *J Glaucoma*. 2010;19:561–565.
21. Saglar E, Yucel D, Bozkurt B, Ozgul RK, Irkec M, Ogus A. Association of polymorphisms in *APOE*, p53, and p21 with primary open-angle glaucoma in Turkish patients. *Mol Vis*. 2009;15:1270–1276.
22. Jia L-Y, Tam PO-S, Chiang SW-Y, et al. Multiple gene polymorphisms analysis revealed a different profile of genetic polymorphisms of primary open-angle glaucoma in northern Chinese. *Mol Vis*. 2009;15:89–98.
23. Zetterberg M, Tasa G, Palmer MS, et al. Apolipoprotein E polymorphisms in patients with primary open-angle glaucoma. *Am J Ophthalmol*. 2007;143:1059–1060.
24. Lake S, Liverani E, Desai M, et al. Normal tension glaucoma is not associated with the common apolipoprotein E gene polymorphisms. *Br J Ophthalmol*. 2004;88:491–493.
25. Ressiniotis T, Griffiths PG, Birch M, Keers S, Chinnery PF. The role of apolipoprotein E gene polymorphisms in primary open-angle glaucoma. *Arch Ophthalmol*. 2004;122:258–261.
26. Ashiq S, Ashiq K. The association of apolipoprotein-E (APOE) gene polymorphisms with coronary artery disease: a systematic review and meta-analysis. *Egypt J Med Hum Genet*. 2021;22.
27. Jukarainen S, Kiiskinen T, Kuitunen S, et al. Genetic risk factors have a substantial impact on healthy life years. *Nat Med*. 2022;28:1893–1901.
28. Chua J, Koh JY, Tan AG, et al. Ancestry, socioeconomic status, and age-related cataract in Asians: the Singapore Epidemiology of Eye Diseases Study. *Ophthalmology*. 2015;122:2169–2178.
29. Mars N, Koskela JT, Ripatti P, et al. Polygenic and clinical risk scores and their impact on age at onset and prediction of cardiometabolic diseases and common cancers. *Nat Med*. 2020;26:549–557.
30. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;613:508–518.
31. Mbatchou J, Barnard L, Backman J, et al. Computationally efficient whole-genome regression for quantitative and binary traits. *Nat Genet*. 2021;53:1097–1103.
32. Hirvelä H, Tuulonen A, Laatikainen L. Intraocular pressure and prevalence of glaucoma in elderly people in Finland: a population-based study. *Int Ophthalmol*. 1994;18:299–307.
33. Wang W, Zhou M, Huang W, Chen S, Zhang X. Lack of association of apolipoprotein E (Apo E) ε2/ε3/ε4 polymorphisms with primary open-angle glaucoma: a meta-

analysis from 1916 cases and 1756 controls. *PLoS One*. 2013;8:e72644.

34. Wang Y, Zhou Y, Zhao B, Gu Z, Li S. Apolipoprotein E gene $\varepsilon 4\varepsilon 4$ is associated with elevated risk of primary open angle glaucoma in Asians: a meta-analysis. *BMC Med Genet*. 2014;15:60.
35. Ge YJ, Xu W, Ou YN, et al. Retinal biomarkers in Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. *Ageing Res Rev*. 2021;69:101361.
36. Shi H, Koronyo Y, Rentsendorj A, et al. Identification of early pericyte loss and vascular amyloidosis in Alzheimer's disease retina. *Acta Neuropathol*. 2020;139:813–836.
37. Mullany S, Marshall H, Diaz-Torres S, et al. The APOE E4 allele is associated with faster rates of neuroretinal thinning in a prospective cohort study of suspect and early glaucoma. *Ophthalmol Sci*. 2022;2:100159.
38. Amaratunga A, Abraham CR, Edwards RB, Sandell JH, Schreiber BM, Fine RE. Apolipoprotein E is synthesized in the retina by Muller glial cells, secreted into the vitreous, and rapidly transported into the optic nerve by retinal ganglion cells. *J Biol Chem*. 1996;271:5628–5632.
39. Krasemann S, Madore C, Cialic R, et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity*. 2017;47:566–581.e9.
40. Keren-Shaul H, Spinrad A, Weiner A, et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell*. 2017;169:1276–1290.e17.
41. Margeta MA, Yin Z, Madore C, et al. Apolipoprotein E4 impairs the response of neurodegenerative retinal microglia and prevents neuronal loss in glaucoma. *Immunity*. 2022;55:1627–1644.e7.
42. Yuan L, Neufeld AH. Activated microglia in the human glaucomatous optic nerve head. *J Neurosci Res*. 2001;64:523–532.
43. Pitts KM, Neeson CE, Hall NE, et al. Neurodegeneration markers galectin-3 and apolipoprotein E are elevated in the aqueous humor of eyes with glaucoma. *Transl Vis Sci Technol*. 2022;11:1.
44. Levy O, Calippe B, Lavalette S, et al. Apolipoprotein E promotes subretinal mononuclear phagocyte survival and chronic inflammation in age-related macular degeneration. *EMBO Mol Med*. 2015;7:211–226.
45. Levy O, Lavalette S, Hu SJ, et al. APOE isoforms control pathogenic subretinal inflammation in age-related macular degeneration. *J Neurosci*. 2015;35:13568–13576.
46. Klein R, Knudtson MD, Klein BE, et al. Inflammation, complement factor h, and age-related macular degeneration: the Multi-ethnic Study of Atherosclerosis. *Ophthalmology*. 2008;115:1742–1749.
47. Riddell DR, Zhou H, Atchison K, et al. Impact of apolipoprotein E (ApoE) polymorphism on brain ApoE levels. *J Neurosci*. 2008;28:11445–11453.
48. Corbo RM, Scacchi R. Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? *Ann Hum Genet*. 1999;63:301–310.
49. Wensor MD, McCarty CA, Stanislavsky YL, Livingston PM, Taylor HR. The prevalence of glaucoma in the Melbourne visual impairment project. *Ophthalmology*. 1998;105:733–739.
50. Mitchell P, Smith W, Attebo K, Healey PR. Prevalence of open-angle glaucoma in Australia: the Blue Mountains Eye Study. *Ophthalmology*. 1996;103:1661–1669.