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# Associations of Alzheimer Disease–Protective *APOE* Variants With Age-Related Macular Degeneration

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**IMPORTANCE** The association of major lipid genes with and their potential as drug targets for age-related macular degeneration (AMD) is unknown. These associations are important to study because AMD is the leading cause of irreversible late-onset blindness in high-income countries.

**OBJECTIVE** To determine whether the full range of structural genetic variation in apolipoprotein E (*APOE*), a master gene in peripheral and cerebral lipid metabolism, is associated with risk of AMD.

**DESIGN, SETTING, AND PARTICIPANTS** This cohort study used data from the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS) cohorts. Participants were followed from study inclusion at the time of blood sampling to occurrence of event, death, emigration, or December 7, 2018, whichever came first. For participants in CCHS, the *APOE* gene was sequenced, and 9 variants with a heterozygote frequency of at least 0.0002 were genotyped in the CGPS. Observers were masked to patient groupings. Data were analyzed from March to September 2021.

**EXPOSURES** The exposure was *APOE* status, and the direct gene product in plasma, apoE levels, was measured in all participants.

**MAIN OUTCOMES AND MEASURES** Cox regression was applied to estimate risk of AMD associated with *APOE* genotype.

**RESULTS** A total of 105 546 participants (mean [SD] age, 57.7 [13.4] years; 58 140 [55%] female participants) were included. Compared with participants with the common ε33 genotype, risk of AMD was lower in participants with ε44 (multifactorially adjusted hazard ratio [aHR], 0.66; 95% CI, 0.45-0.96) and ε43 (aHR, 0.80; 95% CI, 0.71-0.90) genotypes and higher in the ε32 (aHR, 1.15; 95% CI, 1.00-1.31) genotype. Compared with noncarriers, risk of AMD was higher for participants with Gly145Asp (aHR, 3.53; 95% CI, 1.14-10.96) and Arg154Cys (aHR, 4.52; 95% CI, 1-13-18.13) heterozygotes. Results were similar after further adjustment for lipid traits and after adjustment for the *APOE* ε2/ε3/ε4 variant. Combining all common and rare structural variants in a weighted allele score, risk of AMD per 1-mg/dL genetically higher plasma apoE was increased in the adjusted model (aHR, 1.12; 95% CI, 1.05-1.19), the adjusted model plus *APOE* ε2/ε3/ε4 status (aHR, 1.82; 95% CI, 1.20-2.76), and the adjusted model in individuals with the ε33 genotype only (aHR, 1.77; 95% CI, 1.14-2.75).

**CONCLUSIONS AND RELEVANCE** These findings highlight that structural variation in *APOE* beyond the ε2/ε3/ε4 variants may be important for risk of AMD in a population of European ancestry. Rare functional ε2-like variants in *APOE* have previously been reported to have protective associations for Alzheimer disease but the present findings suggest a simultaneous high risk of AMD. This would limit the drug target potential of mechanisms resembling these variants.

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Supplemental content

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Age-related macular degeneration (AMD) is the leading cause of irreversible late-onset blindness in high-income countries.[1] Apolipoprotein E (apoE) is a major apolipoprotein in peripheral lipid metabolism and in the central nervous system, and in AMD, apoE is present in drusen and basal laminar deposits in the macula.[2-4] Recently, a variation in *APOE* (OMIM 107741) was observed to be associated with protection against Alzheimer disease,[5] fueling the idea that drugs resembling the function of this variation could be a viable path to follow to treat or prevent Alzheimer disease. However, it is important to study potential adverse effects of such mechanisms before drawing conclusions on a putative drug development potential. Obvious areas to examine are other common diseases in which apoE plays a central role, such as AMD.

ApoE plays a central role in plasma clearance of lipoprotein particles by serving as a ligand for members of the low-density lipoprotein (LDL) receptor family.[6] Not only plasma levels of apoE, but also levels of other lipids and lipoproteins are affected by the well-known *APOE* variant, which is a combination of 2 genetic variants (rs429358 and rs7412), giving rise to 6 common *APOE* genotypes: ε22, ε32, ε33, ε42, ε43, and ε44. The ε2 allele encodes an LDL receptor binding defect protein isoform (apoE2), leading to high plasma levels of apoE and triglycerides and a propensity to develop the highly atherogenic dysbetalipoproteinemia.[6,7] The ε4 allele is associated with an atherogenic lipid profile with a moderate increase in both plasma LDL cholesterol and triglycerides and is also associated with low plasma levels of apoE.[8,9] Furthermore, apoE is an essential apolipoprotein in cerebral cholesterol metabolism: the ε4 allele is a well-known genetic risk factor for Alzheimer disease,[10] and ε4 also plays a role in vascular dementia[11-13] and cerebrovascular disease[11,14,15] and appears important for immune responses[16,17] and longevity.[18,19] Interestingly, the *APOE* ε2/ε3/ε4 variant has been reported to be associated with risk of AMD, whereas the ε4 allele has been suggested to be associated with reduced risk and the ε2 allele with increased risk in a few small studies.[2,20-23] The *APOE* region has also been identified as a genomic signal in genome-wide association studies of AMD.[24] In mice models, the ε2 allele is associated with an age- and stress-related accumulation of subretinal mononuclear phagocytes, retinal degeneration, and exacerbated choroidal neovascularization, whereas the ε4 allele shows opposite associations.[25]

The association of the entire spectrum of rare and common variation in the *APOE* gene with risk of AMD in the general population is not known. To address this question in White individuals of European ancestry, we used 2 large general population cohorts, the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS), and performed population-based sequencing in 10 369 individuals from the CCHS and further genotyped 9 variants with a frequency of at least 2 per 10 369 in 95 177 individuals from the CGPS.

### Key Points

**Question** Are structural genetic variations in *APOE* associated with risk of age-related macular degeneration (AMD)?

**Findings** In this cohort study including 105 546 participants, *APOE* variants associated with high plasma apolipoprotein E levels were associated with increased risk of AMD.

**Meaning** The findings of this study suggest that *APOE* variants that have previously been reported to have a protective association against Alzheimer disease were also associated with a simultaneous high risk of AMD.

## Methods

The CCHS and CGPS studies were approved by institutional review boards and Danish ethical committees and were conducted according to the Declaration of Helsinki, with written informed consent from participants. All participants were White individuals of European ancestry, according to the Danish Civil Registration System. There was no overlap of individuals between studies. Race and ethnicity were assessed and only White individuals of European ancestry were included in the study, as the allelic frequency of *APOE* variations vary among populations. Participants did not receive a stipend or incentives to participate in the study. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies.

### Participants

We included individuals from 2 similar studies of the Danish general population: the CCHS and the CGPS. The populations were random samples of individuals living in the central part of Copenhagen (CCHS) and in the suburbs surrounding Copenhagen (CGPS). Individuals were randomly selected from the national Danish Civil Registration System to reflect the adult population aged 20 years or older. Both studies are prospective studies of the Danish general population, and examinations included a questionnaire, a physical examination, and blood sampling for biochemical and DNA analyses.

The CCHS was initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003.[26-28] Individuals who gave blood for biochemical and DNA analyses at the 1991 to 1994 or 2001 to 2003 examinations were included. The CGPS was initiated in 2003 with the first enrollment period from 2003 to 2015.[26-28]

### End Points

Information on AMD diagnoses was collected from the national Danish Patient Registry, with data on all patient contacts from all clinical hospital departments in Denmark since 1977, and from the national Danish Causes of Death Registry, with data on causes of all deaths in Denmark, as reported by hospitals and general practitioners, since 1977. The diagnosis of nonneovascular AMD included *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10)* codes DH353E and DH353L, neovascular AMD included *ICD-10* codes DH353C, DH353J, DH353K, and DH353M. All AMD included a diagnosis of neovascular AMD, nonneovascular AMD, or both, whichever came first. Individuals with a history of both nonneovascular and neovascular AMD were included in both end point analyses at the

Table. Baseline Characteristics of Individuals in the General Population According to the Plasma Apolipoprotein E–Weighted Allele Score

| Characteristic | Allele score, No. (%)[a] | | | *P* value[b] |
|---|---|---|---|---|
| | Low (n = 30 023) | ε33 (n = 58 402) | High (n = 17 121) | |
| Sex | | | | |
| Male | 13 416 (45) | 26 320 (45) | 7670 (45) | .53 |
| Female | 16 607 (55) | 32 082 (55) | 9451 (55) | |
| Age, mean (SE), y | 57.3 (0.1) | 57.9 (0.1) | 57.8 (0.1) | <.001 |
| BMI, mean (SE) | 26.0 (0.0) | 26.1 (0.0) | 26.2 (0.0) | <.001 |
| Hypertension[c] | 10 154 (59) | 34 840 (60) | 17 689 (59) | .10 |
| Diabetes[d] | 1093 (4) | 2378 (4) | 711 (4) | .003 |
| Smoking[e] | 6107 (20) | 12 191 (21) | 3535 (21) | .18 |
| High alcohol consumption[f] | 5129 (17) | 9984 (17) | 3018 (18) | .24 |
| Physical inactivity[g] | 14 966 (50) | 29 594 (51) | 8629 (50) | .07 |
| Postmenopausal[h] | 11 083 (67) | 21 644 (67) | 6299 (67) | .15 |
| Hormonal replacement therapy[h] | 1759 (16) | 3509 (16) | 1002 (16) | .68 |
| Lipid-lowering therapy | 3879 (13) | 6160 (11) | 1336 (8) | <.001 |
| Education <8 y | 3620 (12) | 7296 (12) | 2146 (13) | .14 |
| Laboratory values, mean (SD), mg/dL[i] | | | | |
| Total cholesterol | 224.9 (0.3) | 218.1 (0.2) | 204.8 (0.3) | <.001 |
| LDL cholesterol | 134.6 (0.2) | 127.8 (0.2) | 110.1 (0.3) | <.001 |
| Apolipoprotein B | 114.1 (0.2) | 108.1 (0.1) | 99.3 (0.3) | <.001 |
| HDL cholesterol | 61.0 (0.1) | 62.5 (0.1) | 64.6 (0.2) | <.001 |
| Apolipoprotein A1 | 157.8 (0.2) | 159.8 (0.1) | 164.7 (0.2) | <.001 |
| Triglycerides[d] | 130.2 (0.9) | 124.6 (0.6) | 133.6 (1.2) | <.001 |

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL, high-density lipoprotein; LDL, low-density lipoprotein.

SI Conversion Factors: To convert cholesterol to millimoles per liter, multiply by 0.0259; triglycerides to millimoles per liter, multiply by 0.0113.

[a] Missing data on categorical and continuous covariates (<0.5%) were imputed from age and sex in each population separately.

[b] *P* for differences are by *P* for trend (for BMI, lipoproteins, and apolipoproteins), Kruskal-Wallis equality-of-populations rank test (for age and triglycerides) or by the Pearson $\chi^2$ test. The ε33 group was used as the reference.

[c] Hypertension was defined as use of antihypertensive medication, systolic blood pressure of 140 mm Hg or higher, and/or diastolic blood pressure of 90 mm Hg or higher.

[d] Diabetes was defined as self-reported disease, use of insulin or oral hypoglycemic agents, or nonfasting plasma glucose level greater than 198 mg/dL (to convert to millimoles per liter, multiply by 0.0555).

[e] Smoking was defined as current smoking.

[f] High alcohol consumption was defined as more than 14 U per week for female participants and more than 21 U per week for male participants (1 U = 12 g alcohol, equivalent to 1 glass of wine or spirit or 1 beer [33 cL]).

[g] Physical inactivity was defined as 4 hours per week or less of light physical activity in leisure time.

[h] Assessed in female participants only. Female participants reported menopausal status and use of hormonal replacement therapy (in female participants who were postmenopausal).

[i] Geometric means (SEs) of the mean are shown.

date of the first occurrence of the respective subtype. All diagnoses of AMD were given by physicians specializing in ophthalmology.

For Cox regression models for *APOE* variants and for the weighted score, follow-up began at study entry. Follow-up ended at occurrence of event, death, emigration, or on December 7, 2018 (last update of the registries), whichever came first.

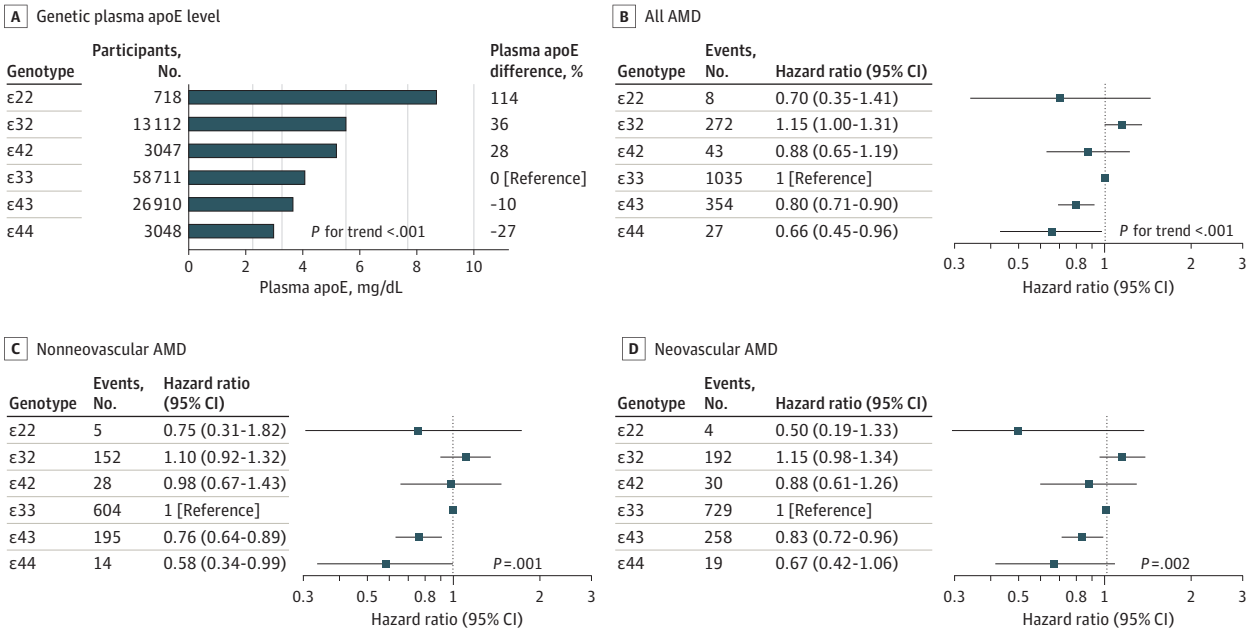
## Gene Screening and Genotyping

We screened the translated region of *APOE* in the CCHS as previously described.[9] An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) and Taqman-based assays were used to genotype for p.Cys130Arg (rs429358, formerly Cys112Arg, c.388T>C), defining the ε4 allele, and p.Arg176Cys (rs7412, formerly Arg158Cys, c.526C>T), defining the ε2 allele. Nine amino acid–changing rare variants with a heterozygote frequency of at least 2 per 10 369 population (allele frequency ≥0.01%) were further genotyped in the CGPS as previously described.[9] Biochemical analyses and other covariates are described in the eMethods in the Supplement.

## Statistical Analysis

The apoE weighted allele score has previously been described in detail.[9] In brief, the weighted allele score was calculated for each individual using a sum of weights for the 9 rare and 2 common structural *APOE* variants. The weights correspond to the sum of β coefficients for these variants for each individual, obtained from a linear regression for plasma levels of apoE measured directly in both cohorts, accounting for the effect of the 9 rare variants, *APOE* ε2/ε3/ε4, sex, age, and cohort (eTable 1 in the Supplement). By doing so, we ensured that the contributions from both the common *APOE* ε2/ε3/ε4 variant as well as the 9 rare variants were captured.

Figure 1. Risk of Age-Related Macular Degeneration (AMD) Associated With ε2/ε3/ε4 Genotype




Geometric mean (SEs) of the mean are given for plasma apolipoprotein E (apoE) levels for the 6 common *APOE* genotypes. Differences in plasma levels of apolipoprotein E are given in percentage change, with ε33 as the reference. Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status and hormonal replacement therapy (female participants only), and education. *P* for trend is from ε22 to ε32 to ε42 to ε33 to ε43 to ε44.

To test whether genetic variants in *APOE* were associated with risk of AMD, we used Cox regression models multifactorially adjusted for known biologically relevant risk factors and markers of lifestyle: age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status and hormonal replacement therapy (female participants only), and education. In sensitivity analyses, we further adjusted for total cholesterol, LDL cholesterol, apoB, high-density lipoprotein cholesterol, apoA1, triglycerides, and the *APOE* ε2/ε3/ε4 variant. Multifactorially adjusted Cox regression restricted cubic spine curves and Cox regression per 1-mg/dL genetically higher apoE were performed for the continuous apoE weighted allele score. To identify the isolated contribution to risk from the compiled impact of rare variants, these analyses were further adjusted for the common *APOE* ε2/ε3/ε4 variant and performed in ε33 individuals only. For the trend test across groups for the Cox regression models, we inserted the exposure (*APOE* genotype ordered from ε22 to ε32 to ε42 to ε33 to ε43 to ε44) as a continuous term, and then used the *P* value for the hazard ratio (HR) per genotype change as the test for trend. This assumes linearity for the per genotype change that appeared to be present for the common *APOE* genotypes (ε32, ε33, and ε43). Further details are provided in the eMethods in the Supplement.

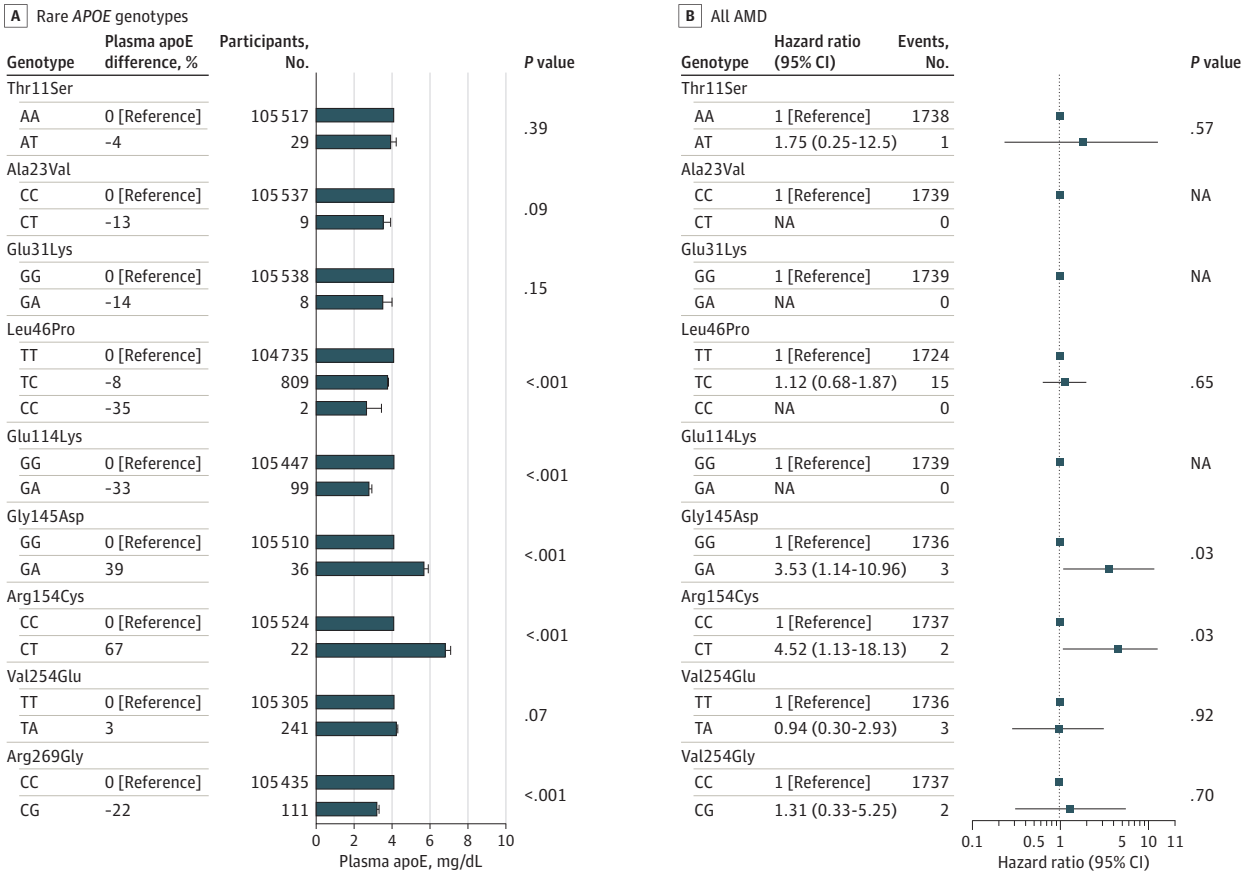
*P* values were 2-sided, and statistical significance was set at *P* = .05. Analyses were conducting using Stata SE version 13.1 (StataCorp). Data were analyzed from March to September 2021.

## Results

A total of 105 546 participants (mean [SD] age, 57.7 [13.4] years; 58 140 [55%] female participants) were included. Among 10 369 individuals in the CCHS, 164 individuals developed any AMD, including 87 with nonneovascular AMD, and 123 with neovascular AMD, during follow-up. Among 95 177 individuals in the CGPS, 1575 developed any AMD, including 911 with nonneovascular AMD, and 1109 with neovascular AMD, during follow-up. Follow-up ended at occurrence of event (1739 individuals with any AMD), death (16 235 individuals), emigration (507 individuals), or on December 7, 2018 (last update of the registries), whichever came first. Median (range) follow-up was 10 (<1 to 27) years.

By sequencing the *APOE* gene in the CCHS, we identified 27 rare variants. The 9 amino acid–changing variants with frequencies of at least 2 per 10 369 were further genotyped in the CGPS (eFigure 1 in the Supplement).[9] Characteristics of study participants and details for the weighted allele score are given in the **Table** and eTable 1 in the Supplement. Neither the *APOE* ε2/ε3/ε4 variant nor the apoE-weighted allele score interacted with sex or cohort in estimating risk of any AMD, nonneovascular AMD, or neovascular AMD. Allele frequencies for the 9 rare variants ranged from 0.01% to 0.4%. All 9 rare variants and the 2 common variants (ε4 and ε2) were included in the weighted allele score (eTable 1 in the Supplement). For comparison, the allele frequencies in these and in publicly available resources are given in eTable 2 in the Supplement.

Figure 2. Risk of Age-Related Macular Degeneration (AMD) in Association With 9 Rare *APOE* Variants



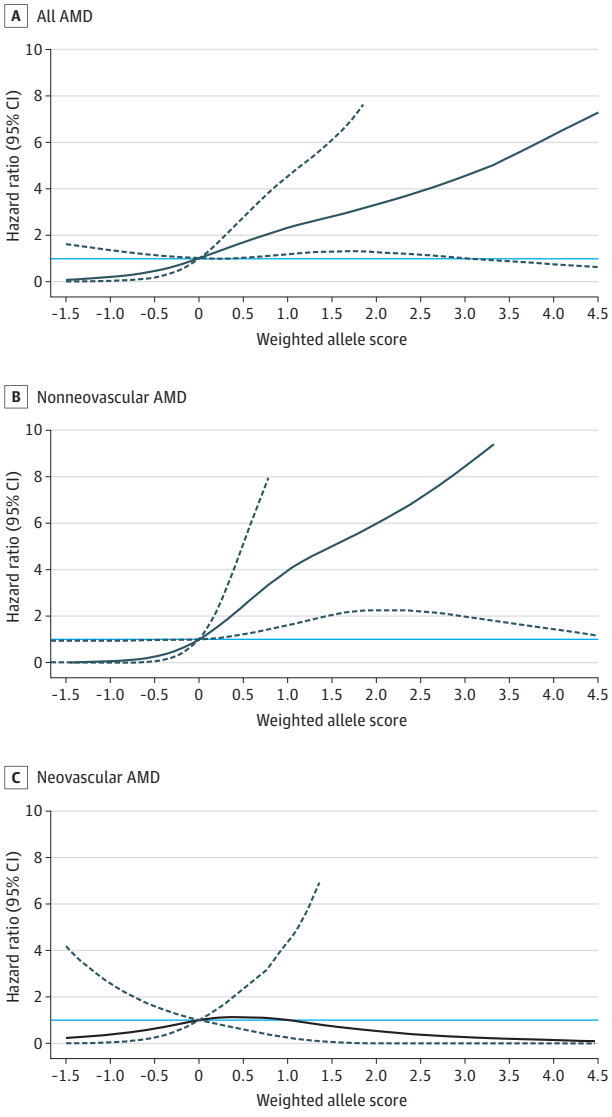

Geometric mean (SEs) of the mean are given for plasma apolipoprotein E (apoE) levels. Differences in plasma levels of apoE are given in percentage difference, with the wild type as the reference. Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status and hormonal replacement therapy (female participants only), and education. *P* for trend is given for Leu46Pro from wild type to heterozygotes to homozygotes, for the remaining 8 variants *P* for differences in plasma apoE were tested using Kruskal-Wallis equality-of-populations rank test. Associations of *APOE* with nonneovascular and neovascular AMD are presented in eFigure 4 in the Supplement).

## Common and Rare Variation in *APOE*, Plasma ApoE Levels, and Risk of AMD

Geometric mean values of plasma apoE levels for the 6 common *APOE* ε2/ε3/ε4 genotypes decreased from 8.6 mg/dL for ε22 to 5.5 mg/dL for ε32 to 5.1 mg/dL for ε42 to 4.0 mg/dL for ε33 to 3.6 mg/dL for ε43 to 2.9 mg/dL for ε44 (*P* for trend < .001) (**Figure 1**). An increased risk from ε4 to ε3 to ε2 was observed for any AMD, nonneovascular AMD, and neovascular AMD Compared with the common ε33 variant, risk of any AMD was lower in participants with ε44 (multifactorially adjusted HR [aHR], 0.66; 95% CI, 0.45-0.96) and ε43 (aHR, 0.80; 95% CI, 0.71-0.90) and higher in ε32 (aHR, 1.15; 95% CI, 1.00-1.31) genotypes (Figure 1). Similar risks were observed for nonneovascular AMD (ε44: aHR, 0.58; 95% CI, 0.34-0.99; ε43: aHR, 0.76; 95% CI, 0.64-0.89; ε32: aHR, 1.10; 95% CI, 0.92-1.32) and neovascular AMD (ε44: aHR, 0.67; 95% CI, 0.42-1.06; ε43: aHR, 0.83; 95% CI, 0.72-0.96; ε32: aHR, 1.15; 95% CI, 0.98-1.34) (*P* for trend < .001 from ε22 to ε32 to ε42 to ε33 to ε43 to ε44) (Figure 1). Results were similar after further adjustment for lipids, lipoproteins, and apolipoproteins (eFigure 2 in the Supplement) or for smoking status (eFigure 3 in the Supplement). Estimates for trend tests are given in eTable 3 in the Supplement.

Geometric mean values of plasma apoE levels, compared with noncarriers (4.1 mg/dL), were increased for participants with Gly145Asp (5.7 mg/dL; *P* < .001) and Arg154Cys (6.8 mg/dL; *P* < .001) and decreased for participants with Leu46Pro (1 minor allele: 3.8 mg/dL; 2 minor alleles: 2.7 mg/dL; *P* < .001), Glu114Lys (2.8 mg/dL; *P* < .001), and Arg269Gly (3.2 mg/dL; *P* < .001) as a function of the minor allele. For participants with Thr11Ser, Ala23Val, Glu31Lys, and Val254Glu, no statistically significant associations were observed (**Figure 2**). Participants heterozygous for Gly145Asp, compared with noncarriers, had increased risk of any AMD (aHR, 3.53; 95% CI, 1.14-10.96), nonneovascular AMD (aHR, 4.20; 95% CI, 1.05-16.85), and neovascular AMD (aHR, 1.46; 95% CI, 0.21-10.39). Similarly, participants heterozygous for Arg154Cys, compared with noncarriers, also had increased risk of any AMD (aHR, 4.52; 95% CI, 1.13-18.13) and nonneovascular AMD (aHR, 7.88; 95% CI, 1.96-31.67), but no events were observed for neovascular AMD (Figure 2; eFigure 4 in the Supplement). Results were similar

Figure 3. Risk of Age-Related Macular Degeneration (AMD) in Association With the Weighted Allele Score, With Adjustments for *APOE* ε2/ε3/ε4




Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status and hormonal replacement therapy (female participants only), education, and *APOE* ε2/ε3/ε4 genotype. The solid line indicates the hazard ratio, and the dashed lines indicate the 95% CI derived from restricted cubic splines with 3 knots and with the reference defined as a score of 0 (ε33 without rare variation).

after further adjustment for lipids, lipoproteins, and apolipoproteins; for smoking status; or after further adjustment for the *APOE* ε2/ε3/ε4 variant (eFigures 5-7 in the Supplement).

### ApoE-Weighted Allele Score and Risk of AMD

In Cox regression restricted cubic spline models with adjustment for the *APOE* ε2/ε3/ε4 variant, risk of all AMD and nonneovascular AMD increased with genetically higher plasma apoE (**Figure 3**). Risk for any AMD per 1-mg/dL genetic increase of plasma apoE were similar after multifactorial adjustment (aHR, 1.12; 95% CI, 1.05-1.19), further adjustment for *APOE* ε2/ε3/ε4 (aHR, 1.82; 95% CI, 1.20-2.76), and in a model for individuals with ε33 only (aHR, 1.77; 95% CI, 1.14-2.75) (**Figure 4**). For nonneovascular AMD, similar risks were found in the multifactorially adjusted model (aHR, 1.14; 95% CI, 1.05-1.23), further adjustment for *APOE* ε2/ε3/ε4 (aHR, 2.24; 95% CI, 1.47-3.42), and in individuals with ε33 only (aHR, 2.10; 95% CI, 1.13-3.38). For neovascular AMD, risk was significant in the multifactorially adjusted model (aHR, 1.09; 95% CI, 1.02-1.18), but not in the model further adjusted for APOE ε2/ε3/ε4 (aHR, 1.17; 95% CI, 0.53-2.57), or for the model for individuals with ε33 only (aHR, 1.13; 95% CI, 0.48-2.67).

## Discussion

This large, prospective cohort study including gene sequencing of 10 369 individuals and genotyping of 9 selected variants in 95 177 additional individuals found that the *APOE* ε2/ε3/ε4 variant was associated with a significant linearly increasing trend for risk of AMD from ε4 to ε3 to ε2, and that rare variations associated with low plasma apoE, like ε4, were associated with a reduced risk, while rare variations associated with high plasma apoE, like ε2, were associated with increased risk of AMD. These findings highlight that structural variation in *APOE* beyond the ε2 and ε4 alleles are important for risk of AMD in a general population setting of White individuals of European ancestry.

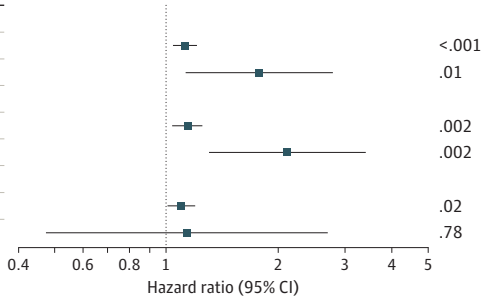
Our study demonstrated a clear protective association of ε4 against AMD, in line with previous studies reporting a reduced risk for the ε4 allele and a decreased frequency of ε4 in individuals with AMD vs control individuals.[2,20-22] We also found an increased risk of AMD for ε32 carriers, supporting previous observations of an increased frequency of the ε2 allele in individuals with AMD vs control individuals.[2,20-22] However, a recent phenome-wide association study did not detect an association of macular degeneration diagnoses with ε2/ε3/ε4 genotypes.[23] To our knowledge, this study is the first to describe the association of the entire range of structural variation in *APOE* with risk of AMD. The rare variations identified in this study are also rare in publicly available genomic resources.

The mechanisms underlying our findings are not well understood; however, existing hypotheses may support our findings. The retinal pigment epithelium constitutes the outer layer of the blood-retina barrier and is responsible for endocytosis of lipoproteins from the choriocapillaris via scavenger receptors (high-density lipoprotein cholesterol) and LDL receptors (LDL and very-low-density lipoprotein cholesterol) as well as for transport of lipids and cholesterol to and from photoreceptors.[29,30] The retinal pigment epithelium balances its lipid content by transporting lipoproteins back to the Bruch membrane, which forms the border to the choriocapillaris layer. These lipoproteins have a high amount of esterified cholesterol and contain both apoA1 and apoB, resembling the LDL cholesterol particle except for the content of apoA1.[29] A recent study of apoA1-containing lipoproteins isolated from Bruch membrane found a unique proteome with high concentrations of apoB and apoE.[31] The large amount of

Figure 4. Risk of Age-Related Macular Degeneration (AMD) per 1 mg/dL Genetically Higher Plasma Apolipoprotein E (apoE)

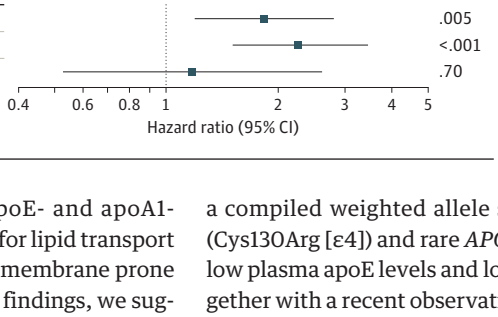
A Multifactorially adjusted

| Outcome | Hazard ratio (95% CI) | *P* value |
|---|---|---|
| All AMD | | |
| Weighted allele score | 1.12 (1.05-1.19) | <.001 |
| Weighted allele score, ε33 only | 1.77 (1.14-2.75) | .01 |
| Nonneovascular AMD | | |
| Weighted allele score | 1.14 (1.05-1.23) | .002 |
| Weighted allele score, ε33 only | 2.10 (1.31-3.38) | .002 |
| Neovascular AMD | | |
| Weighted allele score | 1.09 (1.02-1.18) | .02 |
| Weighted allele score, ε33 only | 1.13 (0.48-2.67) | .78 |




B Multifactorially adjusted + *APOE* ε2/ε3/ε4

| Outcome | Hazard ratio (95% CI) | *P* value |
|---|---|---|
| All AMD | 1.82 (1.20-2.76) | .005 |
| Nonneovascular AMD | 2.24 (1.47-3.42) | <.001 |
| Neovascular AMD | 1.17 (0.53-2.57) | .70 |




Cox regression models were adjusted for age (time scale), sex, body mass index, smoking, hypertension, diabetes, lipid-lowering therapy, alcohol consumption, physical inactivity, postmenopausal status and hormonal replacement therapy (female participants only), and education, and further for *APOE* ε2/ε3/ε4 genotype.

esterified cholesterol in these apoB-, apoE- and apoA1-containing lipoproteins may act as a barrier for lipid transport through an aging retina, making the Bruch membrane prone for lipid deposits and drusen. Based on our findings, we suggest that genetically determined high plasma apoE may mark or directly contribute to these cholesterol ester-rich lipoproteins in the retina. Whether this potential mechanism is through decreased affinity to members of the LDL receptor family, via association with cholesterol ester-rich lipoproteins, or through other unknown pathways warrants further investigation.

We observed increased risk for all variants in *APOE* located in the LDL receptor binding site (ie, Gly145Asp or Arg154Cys) or in an amino acid residue known to interact directly with the binding site (ie, Arg176Cys [ε2]).[6] The 3 variants were also associated with increased plasma apoE levels, consistent with a reduced receptor affinity. Like Arg176Cys (ε2), Arg154Cys is associated with dysbetalipoproteinemia.[32] The similarity of Arg154Cys with Arg176Cys is supported by a recent report on Arg154Ser (*APOE3* Christchurch, formerly *Arg136Ser*): Arg154Ser has been suggested to have a protective association for Alzheimer disease and also with dysbetalipoproteinemia owing to its reduced affinity to the LDL receptor.[5,32] Taken together, *APOE* variations in the LDL receptor binding site may be protective for Alzheimer disease, probably owing to less neuronal tau pathology caused by the low receptor binding,[5] but at the same time, *APOE* variations are associated with severe adverse reactions, such as dysbetalipoproteinemia and AMD. A safer path to follow for drug development is probably to mimic mechanisms expressed by the Alzheimer disease Val254Glu variation (*APOE3* Jacksonville, formerly *Val236Glu*), in which the variant is more ε3-like, with intact LDL receptor binding and an open structure that facilitates lipid binding, and less aggregability.[33] Finally, in this study, a compiled weighted allele score including both common (Cys130Arg [ε4]) and rare *APOE* variants was associated with low plasma apoE levels and low AMD risk. These findings, together with a recent observation reporting a protective effect of ε4 for risk of primary open angle glaucoma,[34] suggest a beneficial role of ε4 in neuroretinal metabolism.

Besides the involvement in peripheral and tissue-specific lipid metabolism, apoE also plays a role in inflammation and immune responses.[16,17,35] Activation of the complement system is involved in AMD,[36] and several studies have shown interactions between APOE ε4 and complement pathways in Alzheimer disease.[37-39] Whereas an impaired cerebral innate immune response may confer susceptibility for Alzheimer disease, as observed in individuals with the ε44 genotype and low levels of complement C3,[39] an impaired immune response in the context of the ε4 allele may play a protective role for AMD, as overactivation of the complement system is destructive for the retina.

A strength of the study is the large prospective population cohort design with no losses to follow-up, a full cohort sequencing, and measuring the direct gene product in plasma individually in more than 100 000 individuals, enabling an estimation of the functional association of the identified genetic variation in *APOE*. The size of the study and the availability of both rare and common variations in *APOE* make it possible to relate estimates for rare variants to the common *APOE* ε2/ε3/ε4 genotype.

## Limitations

This study has some limitations. A potential limitation is the generalizability of our study: we studied a homogeneous population of White individuals of European ancestry only, and although in most populations, the ε33 genotype is the most common, allelic frequencies vary among populations.[6] Since this

large prospective cohort is the only study with data on both plasma apoE and *APOE* variants, to our knowledge, the weights for the variants were generated in the sample to which they were applied. Therefore, risk of overfitting is a potential limitation, and as the score is generated in a very homogeneous sample, it may be less likely to perform as well in other populations. That said, since we used *APOE* genotypes and plasma apoE levels among 105 546 individuals selected at random from the Danish general population to calculate the *APOE* allele score, this theoretical limitation is unlikely to influence our results to a large extent. Since receptor-binding deficient genetic variants unweighted and individually were associated with both high plasma apoE concentrations, and high AMD risk, the weighted allele score does not seem to introduce a severe bias. The availability and completeness of the diagnostic information is also a potential limitation. Milder cases of AMD with no further progression, as well as the prehospital part of follow-up, might not be captured. However, the national Danish Patient Registry includes all hospital visits, as well as outpatient visits, so all individuals requiring hospital evaluation and intravitral injections are included. All cases of neovascular AMD are referred for hospital evaluation in Denmark, and all diagnoses of AMD were given by physicians specialized in ophthalmology. Furthermore, a response rate of 45% may limit the interpretation of our results due to the well-established fact that nonresponders display higher morbidity and mortality than responders. However, this response rate is similar or high compared with other contemporary prospective cohorts (eg, the UK Biobank has a response rate of approximately 5%).[40] Additionally, the temporality of plasma apoE deserves discussion. Plasma apoE concentrations can be affected by different covariates and potentially also by the proximity in time to the AMD diagnosis. As we use plasma apoE concentrations as a functional proxy for the effects of genetic variants in *APOE*, and as the association between the common *APOE* variant and apoE concentrations and lipids and lipoproteins are well established,[8,9] it is likely that the plasma apoE measurements are not seriously affected by potential confounders and time to AMD diagnosis.

## Conclusions

This cohort study found that the *APOE* ε2/ε3/ε4 variant was associated with a significant linearly increasing trend for risk of AMD from ε4 to ε3 to ε2. Similarly, rare variations associated with low apoE, like ε4, were associated with a reduced risk of AMD, and rare variations associated with high apoE, like ε2, were associated with increased risk of AMD. These findings highlight that structural variations in *APOE* beyond the ε2/ε3/ε4 variant are important for risk of AMD in a general population study of White individuals of European ancestry. The high AMD risk observed for plasma apoE–increasing genetic variants may have been caused by a disrupted binding to the LDL receptor, which likely played roles in nutrient transport over Bruch membrane. Importantly, these *APOE* variants have previously been reported to have protective associations for Alzheimer disease, but our findings show a simultaneous high risk of AMD. This limits the drug target potential of mechanisms resembling these variants.

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