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Increased High-Density Lipoprotein Levels Associated with Age-Related Macular Degeneration

Evidence from the EYE-RISK and European Eye Epidemiology Consortia

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Purpose: Genetic and epidemiologic studies have shown that lipid genes and high-density lipoproteins (HDLs) are implicated in age-related macular degeneration (AMD). We studied circulating lipid levels in relationship to AMD in a large European dataset.

Design: Pooled analysis of cross-sectional data.

Participants: Individuals (N = 30 953) aged 50 years or older participating in the European Eye Epidemiology (E3) consortium and 1530 individuals from the Rotterdam Study with lipid subfraction data.

Methods: AMD features were graded on fundus photographs using the Rotterdam classification. Routine blood lipid measurements, genetics, medication, and potential confounders were extracted from the E3 database. In a subgroup of the Rotterdam Study, lipid subfractions were identified by the Nightingale biomarker platform. Random-intercepts mixed-effects models incorporating confounders and study site as a random effect were used to estimate associations.

Main Outcome Measures: AMD features and stage; lipid measurements.

Results: HDL was associated with an increased risk of AMD (odds ratio [OR], 1.21 per 1-mmol/l increase; 95% confidence interval [CI], 1.14−1.29), whereas triglycerides were associated with a decreased risk (OR, 0.94 per 1-mmol/l increase; 95% CI, 0.91−0.97). Both were associated with drusen size. Higher HDL raised the odds of larger drusen, whereas higher triglycerides decreases the odds. LDL cholesterol reached statistical significance only in the association with early AMD ($P = 0.045$). Regarding lipid subfractions, the concentration of extra-large HDL particles showed the most prominent association with AMD (OR, 1.24; 95% CI, 1.10−1.40). The cholesteryl ester transfer protein risk variant (rs17231506) for AMD was in line with increased HDL levels ($P = 7.7 \times 10^{-7}$), but lipase C risk variants (rs2043085, rs2070895) were associated in an opposite way ($P = 1.0 \times 10^{-6}$ and $P = 1.6 \times 10^{-4}$).

Conclusions: Our study suggested that HDL cholesterol is associated with increased risk of AMD and that triglycerides are negatively associated. Both show the strongest association with early AMD and drusen. Extra-large HDL subfractions seem to be drivers in the relationship with AMD, and variants in lipid genes play a more ambiguous role in this association. Whether systemic lipids directly influence AMD or represent lipid metabolism in the retina remains to be answered. *Ophthalmology 2019;126:393-406 © 2018 by the American Academy of Ophthalmology*

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Age-related macular degeneration (AMD) is a leading cause of blindness in the developed world, with 10.4 million persons diagnosed worldwide in 2015.[1] It is a multifactorial disease affecting the elderly, which involves genetics and lifestyle factors. The diagnosis of AMD is based on imaging of the retina, with drusen as the hallmark of early disease. Chorioretinal neovascularization and atrophy of the retinal pigment epithelium (RPE) are indicative of late disease. The number of drusen and total drusen area are prominent predictors of progression of the early stages of AMD.[2,3]

Drusen are lipid-rich, protein-containing deposits that accumulate between the RPE and Bruch's membrane. The accumulation of drusen resembles the formation of atherosclerotic plaques[4] seen in cardiovascular disease, with a similar composition of proteins and protein complexes, such as apolipoprotein E, cholesterol esters, and complement proteins.[5,6] The lipid load in drusen is as high as 40%[7] and is thought to be derived partly from the systemic circulation. This triggered many studies evaluating the relationship between serum or plasma lipids and AMD.[8–12] Some found associations with various serum or plasma lipid levels and drusen or AMD,[11–18] but results mainly were weak and inconsistent. Because a biological explanation is lacking, the relationship remains unsettled yet intriguing.

Genetically, lipid metabolism also is involved in AMD. Genetic associations have been established for 4 genes encoding components of the high-density lipoprotein (HDL) metabolism: Adenosine triphosphate-binding cassette transporter A1 (*ABCA1*), cholesteryl ester transfer protein (*CETP*), apolipoprotein E (*APOE*), and lipase C, hepatic type (*LIPC*).[19–25] *ABCA1* encodes a cellular cholesterol efflux pump leading to formation of nascent HDL. Apolipoprotein E, encoded by the *APOE* gene, facilitates cholesterol uptake by HDL. CETP exchanges cholesteryl esters and triglycerides between HDL and other lipoproteins and thereby, influences HDL particle size.[26] Finally, hepatic lipase encoded by the *LIPC* gene hydrolyzes triglycerides and phospholipids in lipoproteins[27] and thereby, partly converts very low-density lipoproteins (VLDLs) and intermediate density lipoproteins to low-density lipoproteins (LDL)[20] and plays a role in altering the HDL contents.

The European Eye Epidemiology (E3) consortium within the European EYE-RISK project enabled us to investigate the relationships between systemic lipids levels, lipid genes, and AMD using a very large data set. With nuclear magnetic resonance (NMR) spectroscopy, we studied these relationships in greater detail to investigate which particles drive potential associations.

Methods

Study Population

Routine Blood Lipid Measurements. Fourteen studies from France, Germany, Italy, The Netherlands, Norway, Portugal, and the United Kingdom participating in the E3 consortium enrolled in the current study (Supplemental cohort descriptions, available at www.aaojournal.org). The E3 consists of European studies with epidemiologic data on common eye disorders; a detailed description on the studies included in the consortium has been published elsewhere.[28] All studies with gradable macular fundus photographs (n = 30 953 participants) 50 years of age and older contributed their data to the EYE-RISK database version 4.0. Studies were population-based cohort studies except for Creteil and the European Genetic Database (EUGENDA), which are clinic-based studies. Routine blood lipid measurements and AMD outcomes of the same visit were used for this analysis; for TwinsUK, the closest visit to capturing of the retinal fundus photographs was used. All studies were performed in accordance with the Declaration of Helsinki for research involving human subjects and the good epidemiologic practice guidelines. All participants gave fully informed consent and the study was approved by all local institutional review boards of each study site.

Detailed Lipid Analyses. The population-based Rotterdam Study (RS) I provided data on lipid subfractions that were determined at visit 4. Descriptive statistics of this cohort are shown in Table S1 (available at www.aaojournal.org).

Clinical Examination

Age-related macular degeneration features were graded per eye on fundus photographs by experienced graders or clinicians; the most severe AMD grade classified the AMD status of the person. When needed, photographs were regraded by expert graders from Moorfields Eye Hospital and the RS to harmonize the outcome. Age-related macular degeneration status was determined for all included studies using the Rotterdam Classification as described previously.[29] In brief, grades 0 or 1 are considered no AMD; grades 2 and 3 with soft indistinct drusen, reticular drusen, or distinct drusen with pigmentary changes are considered as early AMD; and grade 4 with geographic atrophy or choroidal neovascularization is considered as late AMD. The area of the Early Treatment Diabetic Retinopathy Study grid covered by drusen was estimated in RS I visit 4 per grid circle and was calculated using previously defined harmonization criteria.[30] Medication use and lifestyle factors including smoking habits were assessed by questionnaire; lipid measurements and other clinical determinants such as hypertension, body mass index (BMI), and diabetes mellitus were examined at each individual research center (Supplemental cohort descriptions available at www.aaojournal.org). Fasting blood draws were obtained in all studies except for the EUGENDA study, Muenster aging and retina study (MARS), and the Tromsø Eye Study, which drew blood samples in a nonfasting scenario. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured in plasma (Pathologies Oculaires Liées à l'Age Study [POLA]), Prevalence of Age-Related Macular Degeneration in Italy (PAMDI study), Montrachet-3 city (3C) study, and Creteil) or in serum (remaining studies) using standard operating procedures. When LDL was not measured and triglycerides were less than 4.52 mmol/l, a proxy was calculated using the Friedewald formula[31]: LDL cholesterol = Total cholesterol − HDL cholesterol − (total triglyceride / 2.19); only positive values entered the analysis.

Nuclear Magnetic Resonance Metabolomics Analysis

Lipid subfractions were measured with the Nightingale's NMR-based biomarker platform in fasting ethylenediaminetetraacetic acid plasma samples (Nightingale Ltd., Helsinki, Finland). These measurements cover multiple metabolic pathways, including lipoprotein lipids and subclasses, fatty acids, amino acids, and glycolysis-related metabolites. The NMR-based metabolic profiling has been described previously in detail[32] and has been used in multiple large-scale epidemiologic and genetic studies.[33–36]

Table 2. Baseline Data and Results of Logistic Regression Analysis of the 14 European Studies

	Controls (n = 23 782)	Cases (n = 7171)	P Value	Odds Ratio	95% Confidence Interval
AMD		n = 4730			
Early					
Late		n = 2441			
Gender (% female)					
Early		61.7 (n = 2918)	**<0.0001**	1.21	1.13–1.29
Late		59.4 (n = 1449)	0.74	0.98	0.88–1.10
Any	57.5 (n = 13680)	60.9 (n = 4367)	**<0.0001**	1.15	1.09–1.23
Age (yrs)					
Early		72.7 (SD, 8.4)	**<0.0001**	1.06	1.06–1.06
Late		76.9 (SD, 8.1)	**<0.0001**	1.12	1.11–1.13
Any	68.1 (SD, 8.7)	74.1 (SD, 8.5)	**<0.0001**	1.08	1.07–1.08
BMI (kg/m^2)					
Early		26.6 (SD, 4.2)	**0.008**	0.99	0.98–1.00
Late		26.4 (SD, 4.0)	**<0.0001**	1.03	1.02–1.05
Any	27.0 (SD, 4.3)	26.5 (SD, 4.1)	0.543	1.00	0.99–1.05
Smoking (%)					
Early					
Former		40.4 (n = 1843)	0.77	1.01	0.94–1.09
Current		8.7 (n = 399)	0.14	1.10	0.97–1.24
Late					
Former		44.8 (n = 917)	**<0.0001**	1.51	1.31–1.75
Current		12.3 (n = 253)	**<0.0001**	3.29	2.66–4.07
Any					
Former	41.3 (n = 9530)	41.7 (n = 2760)	**0.02**	1.09	1.01–1.17
Current	12.8 (n = 2947)	9.9 (n = 652)	**<0.0001**	1.37	1.22–1.53
Hypertension (%)					
Early		48.7 (n = 2153)	0.30	1.04	0.97– 1.12
Late		45.3 (n = 977)	0.84	1.01	0.90–1.14
Any	49.0 (n = 11 010)	47.6 (n = 3130)	0.43	1.03	0.96–1.10
Diabetes (%)					
Early		9.7 (n = 435)	0.35	0.95	0.84–1.06
Late		13.2 (n = 284)	**0.002**	1.33	1.11–1.58
Any	10.7 (n = 2408)	10.8 (n = 719)	0.70	1.02	0.92–1.13
Lipid-lowering drugs (%)					
Early		24.7 (n = 1084)	**0.006**	0.89	0.83–0.97
Late		22.5 (n = 459)	0.86	0.99	0.85–1.14
Any	24.5 (n = 5492)	24.0 (n = 1543)	**0.004**	0.90	0.83–0.97

AMD = age-related macular degeneration; BMI = body mass index; SD = standard deviation.
Odds ratios are corrected for age, gender, and study site. Numbers in bold indicate statistically significant values.

Genetic Analyses

The Alienor-3 city (3C) study and Montrachet-3 city (3C) study participants were genotyped with the Illumina Human 610-Quad BeadChip (Illumina, Inc., San Diego, CA) and imputed with the 1000 Genomes Phase I integrated variant set (March 2012) using Shapeit software version 2.r727 (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) for pre-phasing and Impute2 software version 2.3 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) for imputation. The RS I, II, and III participants were genotyped using the Illumina 550K, 550k due/610K Illumina arrays (Illumina, Inc., San Diego, CA). The genotypes were imputed with the 1000 Genomes (phase 1 version 3) reference panel using the Markov chain haplotyping/minimac software.[37–39] The EUGENDA participants were genotyped with a custom-designed Illumina HumanCoreExome array (Illumina, Inc. San Diego, CA) within the International AMD Genetics Consortium. Details regarding the design of this array, as well as annotation, imputation, and quality control of the genotypic data, have been described previously.[19] All cohorts applied similar quality control procedures to genotype data before analysis, and imputation quality was $r^2 > 0.3$.

A total AMD genetic risk score was calculated using 33 of the 52 known AMD risk variants[19] available in the EYE-RISK database version 4.0 (Table S19, available at www.aaojournal.org). Genetic allele dosage was annotated as 0 for noncarriers, 1 for heterozygotes, and 2 for homozygotes. The genetic risk score was composed by calculating the sum of the βs of independent risk variants. The score was standardized and added as a covariate in a linear regression analysis with AMD as the dependent variable. The linear regression was corrected for age, gender, lipid-lowering drugs, and study site. The effect of individual lipid-related single-nucleotide polymorphisms on each lipid level or lipid subfraction was assessed in a mixed-effects regression correcting for age, gender, lipid-lowering drug use, plasma or serum, and fasting state and using study site as a random effects term. The *P* value threshold for these analyses was 0.05 / 60 = 0.00083 (8.3×10^{-4}) after Bonferroni correction.

Statistical Analysis

The outcome variable was presence of early or late AMD versus no AMD. Differences in baseline characteristics were evaluated with a

Table 3. Mixed-Effects Logistic Regression Associations of Blood Lipids with Age-Related Macular Degeneration

Lipid	Controls	Cases	Odds Ratio for 1-mmol/l Increase	95% Confidence Interval	*P* Value
Total cholesterol					
Early AMD	5.60 (4.90–6.30), n = 20 555	5.58 (4.80–6.30), n = 3907	0.98	0.95–1.01	0.24
Late AMD	5.60 (4.90–6.30) n = 20 234	5.66 (4.90–6.50), n = 1620	1.03	0.997–1.07	0.07
Any AMD	5.60 (4.90–6.30) n = 20 555	5.60 (4.80–6.34), n = 5538	1.00	0.97–1.02	0.67
HDL cholesterol					
Early AMD	1.40 (1.16–1.69), n = 19 931	1.50 (1.23–1.80), n = 3802	1.34	1.22–1.48	**6.48×10^{-10}**
Late AMD	1.40 (1.16–1.69) n = 19 662	1.47 (1.20–1.79), n = 1626	1.12	1.002–1.24	0.044
Any AMD	1.40 (1.16–1.69) n = 19 931	1.50 (1.22–1.80), n = 5439	1.21	1.14–1.29	**1.35×10^{-9}**
LDL cholesterol					
Early AMD	3.49 (2.84–4.14), n = 19 590	3.37 (2.71–4.02), n = 3746	0.96	0.92–0.999	0.045
Late AMD	3.49 (2.85–4.14), n = 19 334	3.45 (2.79–4.14), n = 1580	1.01	0.97–1.06	0.51
Any AMD	3.49 (2.84–4.14), n = 19 590	3.39 (2.74–4.07), n = 5337	0.98	0.95–1.01	0.13
Triglycerides					
Early AMD	1.34 (1.00–1.87), n = 19 539	1.30 (0.97–1.80), n = 3768	0.88	0.84–0.92	**2.44×10^{-7}**
Late AMD	1.34 (1.00–1.87) n = 19 474	1.43 (1.01–2.01), n = 1601	1.01	0.97–1.06	0.57
Any AMD	1.34 (1.00–1.87) n = 19 539	1.32 (0.98–1.86), n = 5374	0.94	0.91–0.97	**2.35×10^{-5}**

AMD = age-related macular degeneration; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Data are median (twenty-fifth–seventy-fifth percentile). Odds ratio estimates and 95% confidence intervals of lipid on early, late, or any AMD after adjusting for age, gender, lipid-lowering drug use, body mass index, smoking, plasma or serum, fasting state, and study site. Late AMD was also corrected for diabetes. $P = 0.0042$ is Bonferroni statistically significant. Numbers in boldface indicate statistically significant values.

Wald test using a logistic regression analysis, adjusting for age, gender, and study site. Analyses were conducted on complete data. Odds ratios (ORs) for the routine blood lipid measurements were calculated using random-intercepts mixed-effects logistic regression models, including study site as a random effect term to allow for variability between study sites. The study site–specific fixed-effects estimates were transformed to their marginal counterparts as described by Heagerty and Zeger.[40]

Association of HDL cholesterol with AMD characteristics (presence of various drusen sizes, hyperpigmentation, or hypopigmentation) was calculated in a univariate logistic regression analysis for the worse eye, defined as the eye with the most severe lesions of each AMD characteristic, correcting for age, gender, lipid-lowering drug use, and study site. The linear regression for HDL cholesterol and drusen area was calculated in the RS I visit 4 only.

For the analysis on lipid subfractions, all subfractions were +1 log transformed and scaled to make comparable measurements. Association magnitudes were reported in units of standard deviation or OR change per 1-standard deviation increase in each metabolite, as previously suggested by others.[34,35] To account for the correlation between lipid subfractions, the eigenvalues were calculated as proposed by Li and Ji[41] on the SNPSpD online interface.[42] Bonferroni correction was applied to correct for multiple testing using the eigenvalues to calculate the *P*-value threshold ($P = 0.001087$). To test for differences between AMD stage and the mean of the lipid subfractions, a Welch test was performed on the total of all age categories. The Welch test was chosen because homogeneity of variance was violated among the AMD severity classes. The post hoc Games–Howell test was used to investigate differences between the mean of the no-AMD and late-AMD groups.

Mixed-effects logistic regression models were performed with R package lme4,[43] and mixed-effects regression models were performed with nmle[44] (R Core Team, Vienna, Austria); Welch tests and genetic risk scores were carried out with SPSS software for Windows version 24.0 (IBM Corp., Armonk, NY). Graphical outputs were constructed with GraphPad Prism for Windows version 7 (GraphPad Software, La Jolla, CA).

Results

We identified a total of 4730 individuals with early AMD, 2441 with late AMD, and 23 782 nonaffected persons. The baseline characteristics of these participants are summarized in Table 2. Age-related macular degeneration patients and controls differed in age, gender, BMI, lipid-lowering drug use, and smoking, in accordance with the known AMD risk profile.

Routine Blood Lipid Measurements and Age-Related Macular Degeneration in the European Eye Epidemiology Consortium

Next, we examined lipid levels in the entire study population. Mean levels of all lipids were within physiologic limits: mean total cholesterol ranged between studies from 5.1 to 5.8 mmol/l, and mean HDL cholesterol ranged between studies from 1.4 to 1.9 mmol/l. Mean LDL cholesterol ranged from 3.0 to 3.8 mmol/l, and mean triglycerides ranged from 1.2 to 1.7 mmol/l. For one fifth of the study population, only nonfasting blood samples were collected; in these, the mean levels were similar but on the higher range of total cholesterol and triglycerides: 5.7 mmol/l for total

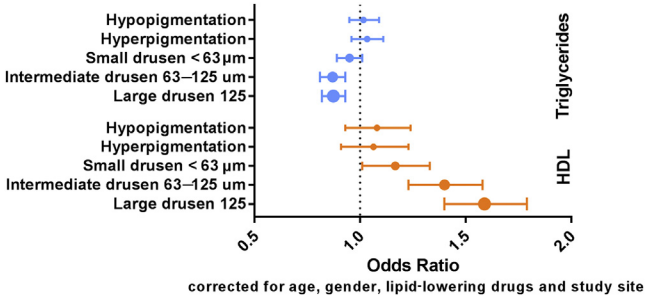

Figure 1. Graph showing the association of high-density lipoprotein (HDL) cholesterol and triglycerides with age-related macular degeneration characteristics.

cholesterol and 1.7 mmol/l for triglycerides. Mean HDL cholesterol was on the lower range with 1.5 mmol/l, and LDL cholesterol was very comparable with 3.5 mmol/l.

Table 3 shows the association between lipid levels and AMD adjusted for age, gender, lipid-lowering drug usage, BMI, smoking, plasma or serum, fasting state, and study site in the E3 consortium. Analyses for late AMD were also corrected for diabetes. Total cholesterol was not associated with any of the AMD outcomes. Higher HDL cholesterol was associated with an increased risk of any AMD, and risk estimates were slightly higher for early AMD (OR, 1.34 per 1-mmol/l increase) than for late AMD (OR, 1.12 per 1-mmol/l increase), but had overlapping confidence intervals (CIs). Low-density lipoprotein cholesterol and triglycerides were associated inversely with early AMD (OR, 0.96 and 0.88 per 1-mmol/l increase, respectively) and any AMD (OR, 0.98 and 0.94 per 1-mmol/l increase, respectively), but effect sizes were smaller than for HDL cholesterol. Sensitivity analysis on fasting and nonfasting sampling methods and gender showed no interaction, or it showed interaction but with similar point estimates of the ORs, which made sampling effect or confounding unlikely. However, a sensitivity analysis on plasma or serum sampling methods did show a change in direction of effect of triglycerides measured in plasma, although the change was not statistically significant (Tables S4–S10, available at www.aaojournal.org). To investigate whether observed associations were the result of the preferential survival of elderly persons without cardiovascular disease, we repeated the analyses in various age strata (Tables S11–S13, available at www.aaojournal.org). Even in those 65 years of age or younger with AMD, HDL cholesterol was associated significantly with increased risk of AMD (OR, 1.19 per 1-mmol/l increase; $P = 0.02$). Low-density lipoprotein cholesterol was associated inversely with AMD (OR, 0.93 per-1 mmol/l; $P = 0.02$); associations with triglycerides became insignificant.

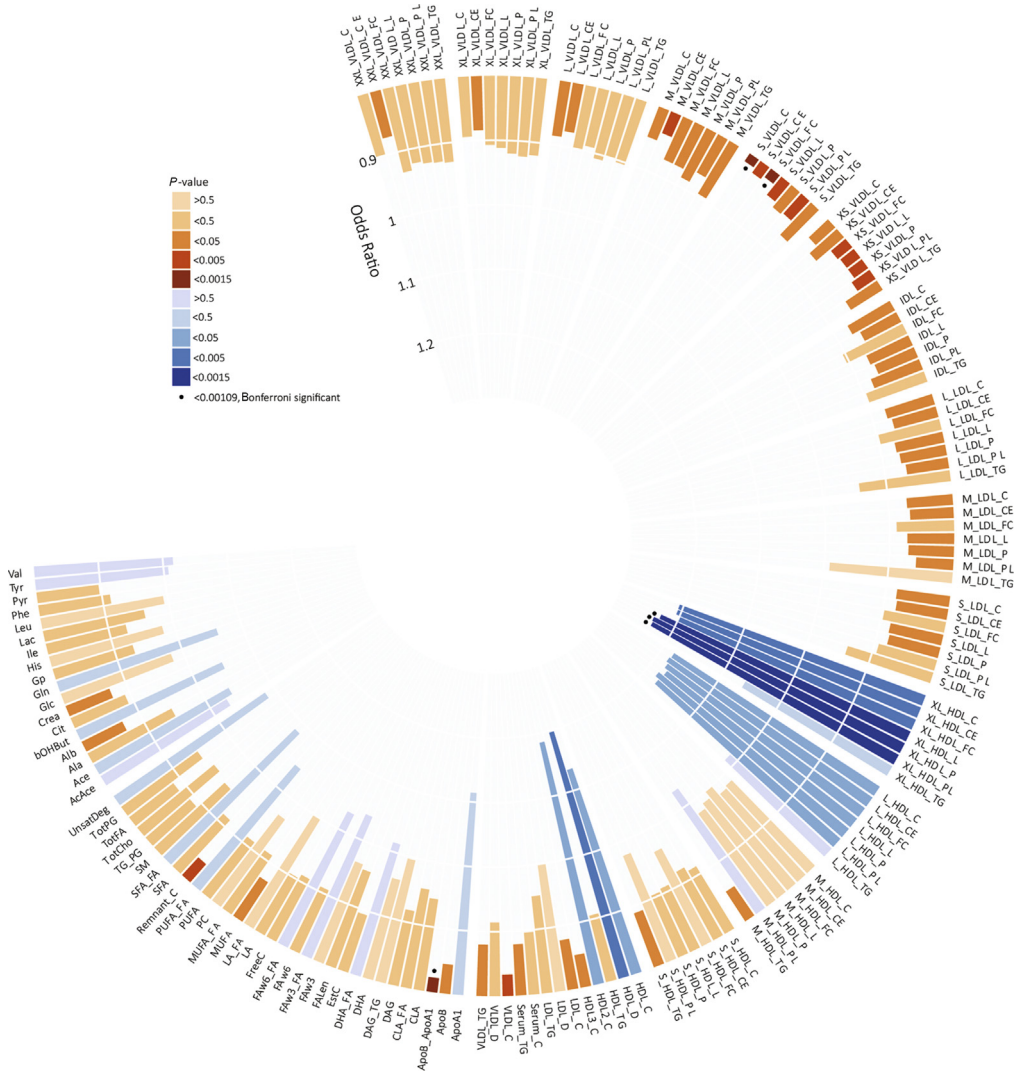

Figure 2. Graph showing the association of metabolic variables and age-related macular degeneration (AMD). Each bar represents the association with AMD, the size of the bar is the odds ratio, and color refers to effect direction and significance. Dots indicate Bonferroni statistically significant metabolic variables corrected for age, gender, and lipid-lowering drugs. Labels describe the properties measured in each lipid subfraction: C = total cholesterol; CE = cholesterol esters; FC = free cholesterol; L = total lipids; P = concentration of particles; PL = phospholipids; and TG = triglycerides. List of abbreviations available in the Supplementary materials, available online at www.aaojournal.org).

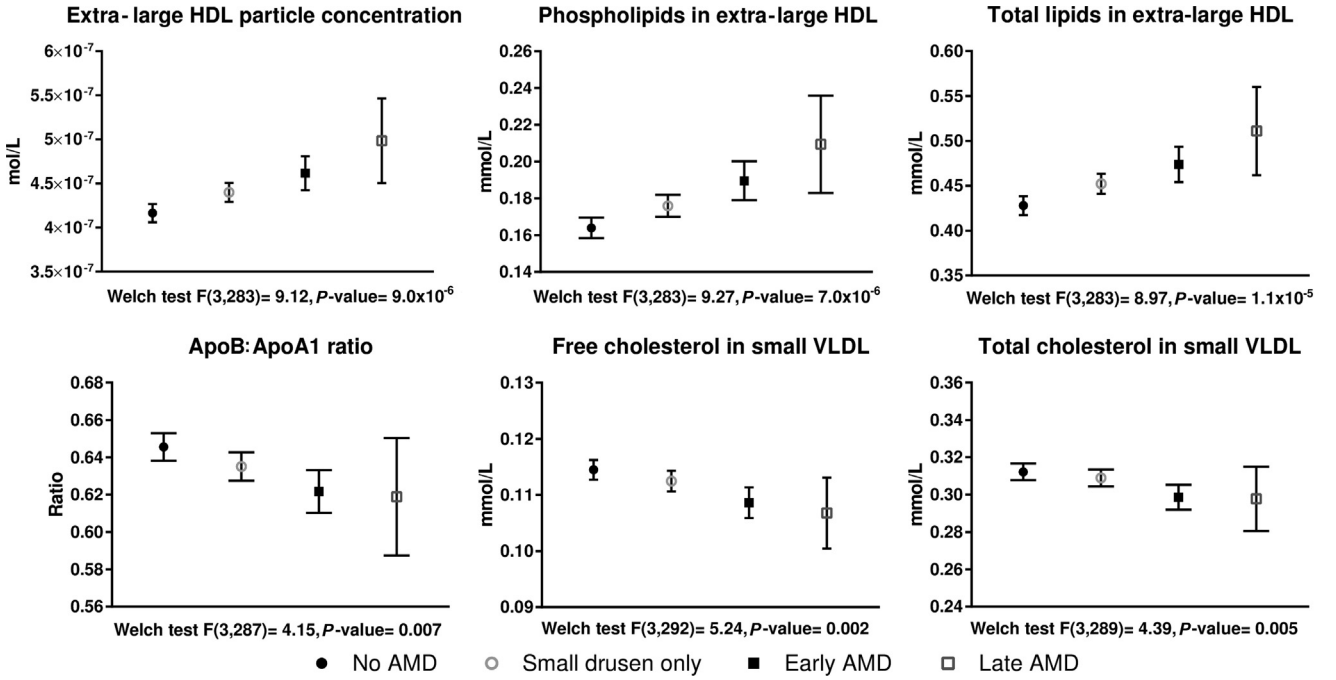

Figure 3. Box-and-whisker plots showing the stage-dependent relationship of the 6 associated lipid subfractions with age-related macular degeneration (AMD). Error bars indicate 95% confidence intervals of the mean. Apo = apolipoprotein; HDL = high-density lipoprotein; VLDL = very low-density lipoprotein.

Routine Blood Lipids Measurements and Early Age-Related Macular Degeneration Phenotype

Because the association between HDL cholesterol and AMD was most pronounced in those with early AMD, we performed more detailed analyses using the various early AMD features as outcomes. Effects of HDL cholesterol and triglycerides became larger with increasing drusen size (Fig 1). Likewise, higher HDL levels were associated with greater drusen area (β, 0.014; $P = 0.001$). Higher triglyceride levels were associated with smaller drusen area. Correcting for smoking did not change these results (data not shown). Lipids were not statistically significantly associated with pigmentary changes, and total cholesterol and LDL cholesterol were not associated with any early AMD characteristic (Tables S14 and S15, available at www.aaojournal.org).

Lipid Subfractions in the Rotterdam Study

To explore whether the association between HDL cholesterol, triglycerides, and AMD was driven by specific lipid subfractions, we examined lipid subfractions with NMR in RS I (Fig 2; Table S16, available at www.aaojournal.org). The concentration of extra-large HDL particles was associated most significantly with any AMD, particularly the subfractions of phospholipids and total lipids within extra-large HDL particles. These subfractions are highly correlated (Pearson correlation, >0.97). Next, total cholesterol and free cholesterol in small VLDLs were associated significantly, as well as the ratio of apolipoprotein B-to-apolipoprotein A1, with a Pearson correlation ranging between 0.93 and 0.87. No other metabolites were associated significantly with AMD. Correcting for smoking did not change these significant results (data not shown). The apolipoprotein B-to-apolipoprotein A1 ratio is a surrogate for the LDL-to-HDL ratio, with a small ratio suggesting a high level of HDLs compared with LDLs.

These associations show a dose-dependent relationship with AMD stages from the Rotterdam Classification (Fig 3). To test if the mean of the lipid subfractions per AMD stage differed statistically, we performed a Welch test, which was significant for each of the 6 subfractions. The subfractions related to HDL also showed a statistically significant difference in the Games-Howell post hoc test comparing the mean of those with no AMD to those with late AMD ($P = 0.01$ for the concentration of extra-large HDL, $P = 0.02$ for phospholipids in extra-large HDL, and $P = 0.01$ for total lipids in extra-large HDL). The Games−Howell post hoc test was not significant in the other 3 subfractions, likely because of the small group size and variance in late AMD. (Fig S4, available at www.aaojournal.org, shows dose dependency per age category).

We also performed the analyses stratified for lipid-lowering drug use, which was reported by fewer cases (17.0%) than controls (24.2%) in the RS ($P = 0.02$). Significance was found only in those not taking lipid-lowering drugs, and point estimates were highly similar to the overall group (Tables S17 and S18, available at www.aaojournal.org).

Lipid Genes, Lipid Subfractions, and Age-Related Macular Degeneration

Because genetic variants are an important cause of AMD, we investigated the relationship among genes, lipids, and AMD. First, we investigated whether a genetic risk score with 33 single nucleotide polymorphisms covering all major AMD genes influenced lipid levels in the E3-consortium and found that with increasing genetic risk also came an increase of HDL cholesterol ($P = 0.03$; Table S19, available at www.aaojournal.org). Subsequently, we focused on the individual AMD lipid genes. In the E3, the *CETP* variant rs17231506 was associated positively with HDL cholesterol levels and associated negatively with LDL cholesterol, whereas both *LIPC* variants

Table 20. Mixed-Effects Linear Regression Model Estimating the Effect of Single-Nucleotide Polymorphisms on Routine Lipid Measurements

Lipid	*CETP* rs17231506, Risk Allele T, Reference Allele C	*LIPC* rs2043085, Risk Allele C, Reference Allele T	*LIPC* rs2070895, Risk Allele G, Reference Allele A	*APOE* rs429358, Risk Allele T, Reference Allele C	*APOE* rs73036519, Risk Allele G, Reference Allele C	*ABCA1* rs2740488, Risk Allele A, Reference Allele C
Total cholesterol	0.03 ($P = 0.07$)	−0.019 ($P = 0.16$)	−0.04 ($P = 0.008$)	**−0.18 ($P < 0.0001$)**	0.01 ($P = 0.55$)	**0.06 ($P = 0.0002$)**
HDL cholesterol	**0.08 ($P < 0.0001$)**	**−0.04 ($P < 0.0001$)**	**−0.05 ($P < 0.0001$)**	**0.03 ($P < 0.0001$)**	−0.007 ($P = 0.24$)	**0.03 ($P = 0.0001$)**
LDL cholesterol	**−0.05 ($P = 0.0001$)**	0.02 ($P = 0.09$)	0.004 ($P = 0.77$)	**−0.19 ($P < 0.0001$)**	0.01 ($P = 0.67$)	0.04 ($P = 0.01$)
Triglycerides	−0.025 ($P = 0.04$)	0.001 ($P = 0.93$)	−0.006 ($P = 0.66$)	**−0.06 ($P = 0.0004$)**	0.03 ($P = 0.03$)	0.004 ($P = 0.78$)

HDL = high-density lipoprotein; LDL = low-density lipoprotein.
βs are corrected for age, gender, lipid-lowering drugs, plasma or serum, fasting state, and study site. βs indicate the effect of the risk allele versus the reference allele. Bonferroni: $0.05 / 60 = 0.00083$ (8.3×10^{-4}). Numbers in boldface indicate statistically significant values.

rs2043085 and rs2070895 were associated inversely with HDL cholesterol. In addition, the *APOE* variant rs429358 was associated with decreased levels of total cholesterol, triglycerides, and LDL cholesterol but with increased levels of HDL cholesterol. The *APOE* variant rs73036519 showed no significant effect on the routine lipid measurements or on the lipid subfractions. The *ABCA1* variant rs2740488 influenced only total cholesterol and HDL cholesterol (Table 20). When restricting the analysis to lipid subfractions in the RS (Table 21), we found similar results for the *CETP* variant and the *LIPC* variants with all extra-large HDL subfractions.

Discussion

Based on pooled data of 30 953 participants from Western Europe, we showed that high circulating HDL cholesterol levels and low triglyceride levels are associated significantly with AMD. The magnitude of the effect was higher for early than for late AMD, and associations were related to drusen size and area. By focusing on lipid subfractions, we revealed that extra-large HDL particles, small VLDL particles, and the apolipoprotein B-to-apolipoprotein A1 ratio, a surrogate for the LDL-to-HDL ratio, were dose-dependent drivers of this association. Age-related macular degeneration risk variants in lipid genes did not provide a clear explanation; in particular, the variants in *LIPC* that increase the risk of AMD decreased HDL cholesterol in the systemic circulation.

Our results should be interpreted in light of the strengths and limitations of the study. The combined efforts of 2 European consortia enabled us to create a very large database providing the statistical power to resolve conflicting findings from previous studies. The detailed NMR lipid analysis in a subset created the opportunity to find the metabolic profile behind the lipid associations. A weakness of the consortia was the use of different protocols for blood sampling, definition of confounders, and AMD phenotyping. We addressed this issue by performing a stratified analysis on sampling methods and found that only the associations for triglycerides changed direction of effect for plasma, albeit nonsignificantly. We harmonized all confounders as well as the criteria for early and late AMD and corrected for study site in the mixed-effect models.

Many previous studies did not find a statistically significant association between lipids and AMD, but studies with the larger sample sizes often found a positive association with HDL cholesterol and an inverse association with triglycerides.[13] The current pooled study showed that the levels of these lipids were within physiologic range in both cases and controls and that absolute differences were small in millimoles per liter. However, our data suggest that an increase of HDL from the twenty-fifth percentile to the seventy-fifth percentile coincides with an AMD risk increase of approximately 20%. Selective survival does not seem to explain our findings because the association was present already in the youngest age group (≤65 years). The exact clinical interpretation remains to be defined. Nevertheless, the findings contribute to the understanding of AMD pathogenesis.

Animal research has provided some key insights in retinal lipid metabolism. Studies in rodents show that most lipids in the retina are synthesized locally and up to one quarter are

Table 21. Linear Regression Model Estimating the Effect of Single-Nucleotide Polymorphisms on Lipid Subfraction

Lipid	*CETP* rs17231506, Risk Allele T, Reference Allele C	*LIPC* rs2043085, Risk Allele C, Reference Allele T	*LIPC* rs2070895, Risk Allele G, Reference Allele A	*APOE* rs429358, Risk Allele T, Reference Allele C	*APOE* rs73036519, Risk Allele G, Reference Allele C	*ABCA1* rs2740488, Risk Allele A, Reference Allele C
Percentage extra-large HDL	**0.15 ($P = 7.68 \times 10^{-7}$)**	**−0.14 ($P = 1.00 \times 10^{-6}$)**	**−0.13 ($P = 1.55 \times 10^{-4}$)**	−0.07 ($P = 0.112$)	0.04 ($P = 0.167$)	0.05 ($P = 0.164$)
Phospholipids in extra-large HDL	**0.15 ($P = 3.51 \times 19^{-7}$)**	**−0.13 ($P = 4.0 \times 10^{-6}$)**	**−0.13 ($P = 1.04 \times 10^{-4}$)**	−0.03 ($P = 0.479$)	0.04 ($P = 0.233$)	0.03 ($P = 0.349$)
Total cholesterol in small VLDL	−0.09 ($P = 0.003$)	−0.09 ($P = 0.003$)	−0.08 ($P = 0.02$)	−0.05 ($P = 0.294$)	−0.02 ($P = 0.631$)	0.08 ($P = 0.027$)
Ratio ApoB-to-ApoA1	−0.10 ($P = 0.002$)	0.004 ($P = 0.897$)	−0.003 ($P = 0.936$)	−0.09 ($P = 0.037$)	−0.04 ($P = 0.250$)	0.02 ($P = 0.521$)
Total lipids in extra-large HDL	**0.15 ($P = 4.06 \times 10^{-7}$)**	**−0.14 ($P = 8.59 \times 10^{-7}$)**	**−0.12 ($P = 3.13 \times 10^{-4}$)**	−0.08 ($P = 0.056$)	0.04 ($P = 0.213$)	0.05 ($P = 0.138$)
Free cholesterol in small VLDL	−0.08 ($P = 0.01$)	−0.08 ($P = 0.005$)	−0.09 (0.009)	0.01 ($P = 0.784$)	−0.03 ($P = 0.434$)	0.07 ($P = 0.058$)

Apo = apolipoprotein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.
βs are corrected for age, gender, and lipid-lowering drug use; subfractions are log+1 and standardized. βs indicate the effect of the risk allele versus the reference allele. Numbers in boldface indicate statistically significant values.

derived from the systemic circulation.[45] Another study in mice shows that a high-fat diet increases cholesterol in the retina but not as much as in the circulation. These results suggest that transport from the systemic circulation to the retina does take place, albeit modestly. Although LDLs deliver cholesterol most efficiently from the systemic circulation to the retina, HDL cholesterol, with apolipoprotein A-I as its major lipid component,[46] does this as well via scavenger receptors.[26,47,48] The RPE processes the internalized lipids and subsequently secretes them again on the apical side via ABCA1 transporters into the interphotoreceptor matrix. Thereafter, lecithin−cholesterol acyltransferase, located at the surface of nascent HDL,[49] converts free cholesterol into esterified cholesterol,[50] which is present in nascent HDL. In this way, lecithin−cholesterol acyltransferase transforms nascent HDL into larger, mature HDL, whereas LIPC hydrolyzes phospholipids in the HDL lipoprotein.[23,51] As suggested by Tserentsoodol et al,[26] because of the absence of LDL in the retina, it is possible that CETP has a role in transferring esterified cholesterol between lipoproteins or photoreceptor membranes. In the interphotoreceptor matrix, HDL functions as a transport vehicle between the RPE and the photoreceptors supporting the high synthesis and degradation of the lipid-rich photoreceptor discs.[26] The RPE maintains the lipid balance by transporting lipoproteins back to Bruch's membrane.[52] The lipid contents of these lipoproteins resemble those of LDL lipoproteins rather than of HDL, because it has a high abundance of esterified cholesterol and both apolipoprotein A and B.[53] It has been proposed that this large amount of esterified cholesterol acts as a barrier for lipid transport through an aging retina, thereby facilitating the formation of deposits.[54] Another mechanism proposed to form deposits is through the impairment of ABCA1 transporter of macrophages, which impairs the efflux of free cholesterol out of the macrophage. This results in senescent macrophages with high levels of cholesterol in the retina of mice.[55]

Interestingly, lipoproteins seem to be related closely to the complement system, the major pathway in AMD pathogenesis. Proteomic studies have shown that HDL lipoproteins can contain essential complement components, such as C1, C2, C3, C5, and factor B.[56–58] One study showed that complement factor H (CFH) and lipoproteins have competitive binding in the sub-RPE extracellular matrix, and when CFH is low, lipoproteins can accumulate under the RPE.[59] By contrast, HDL also can carry complement regulators such as FH1, CFHR4, and CFHR5.[60,61] Apolipoprotein A-I attached to HDL can bind clusterin, a complement lysis inhibitor that stops the complement cascade just before the C5b-9 complex is inserted into the target.[62] These findings suggest that HDL is involved in proinflammatory[63,64] as well as complement-inhibitory tasks. Higher HDL levels may cause imbalance of the physiologic homeostasis.[8,65] Taken together, this plethora of biological leads supports the contention that HDL may play a role in the initiation of AMD. More comprehensive research into lipid metabolism in the retina is warranted.

In our study, we found elevated levels of HDL cholesterol in the circulation and decreased levels of triglycerides in persons with AMD. In more detailed analysis, we observed a

higher concentration of extra-large HDL particles with higher total lipid and phospholipid contents, which are under genetic control of *CETP* and *LIPC*. The high phospholipid content of extra-large HDL very likely is related to the larger particle size, because phospholipids comprise the outer shell of the lipoprotein. *CETP* may exert its effect on AMD partly through systemic HDL, in line with previous Mendelian randomization studies.[24,66] The opposing effects that we found for *LIPC* are explained less easily, but have been observed by others.[23,24] This finding suggests that systemic HDL may be a biomarker rather than directly causally related to AMD. In a larger study, Kettunen et al[33] found more genetic effects on lipid subfractions; variants in *CETP* and *APOE* also had a decreasing effect on the small VLDL subfractions, whereas a variant in *ABCA1* increased extra-large HDL. Our smaller sample size hampered the replication of these findings.

Where do these lipid associations fit in the chronology of AMD development? The more pronounced risk for early AMD and increasing ORs of HDL cholesterol for the larger drusen suggest that lipids play an important role at the early phase of disease. Hypothetically, intervention at this phase would be most promising in preventing blindness. We did not find statistical significance for any lipid subfraction in only those using lipid-lowering drugs, possibly because there is no effect, but probably because of the lower power in this subgroup. Evidence from other studies indicates that statins increase HDL levels slightly[67] but reduce extra-large HDL[68] and that HDL protein composition may change as well.[69] Most epidemiologic studies do not find any effect of lipid-lowering drugs on AMD[8,70–72]; however, one study observed a slower progression of AMD in persons with a certain *CFH* risk variant.[73] Large randomized controlled trials with long-term follow-up are needed to clarify the relationship between lipid-lowering drugs and AMD.

In conclusion, this study showed that HDL cholesterol and triglycerides levels are particularly associated with early AMD, mostly through the association with drusen. Extra-large HDL subfractions seem to be drivers of this association. Whether systemic lipids directly influence lipid metabolism in the retina or whether these lipids mirror pathologic features in the retina is a question that remains to be answered.

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HUMAN SUBJECTS: Human subjects were included in this study. The recruitment and research protocols were reviewed and approved by the local institutional review boards (EUGENDA: The study was approved by the ethics committees in Cologne and Nijmegen; MARS: approved by the Institutional Review Board of the University of Muenster: Montrachet-3C: the Ethical Committee of the University Hospital of Kremlin-Bicêtre; PAMDI: approved by the IRB of the University of Padua; POLA: approved by the ethics committee of the University Hospital of Montpellier, France; Rotterdam Study I, II and III: The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC [registration number MEC 02.1015]; and by the Dutch Ministry of Health, Welfare and Sport [Population Screening Act WBO, license number 1071272-159521-PG]; Tromsø eye Study: approved by the Regional Committee for Medical and Health Research Ethics; TwinsUK: approved by by the St. Thomas' Hospital Local Research Ethics Committee [EC04/015]) and written informed consent was obtained from all study participants, in compliance with the Declaration of Helsinki.

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Abbreviations and Acronyms:
3C = 3 city; ***ABCA1*** = Adenosine triphosphate-binding cassette transporter A1; **ALIENOR** = Antioxydants, Lipids Essentiels, Nutrition et maladies OculaiRes Study; **AMD** = age-related macular degeneration; ***APOE*** = apolipoprotein E; **BMI** = body mass index; ***CETP*** = cholesterylester transfer protein; ***CFH*** = complement factor H; **CI** = confidence interval; **EPIC** = European Prospective Investigation into Cancer and Nutrition; **EUGENDA** = European Genetic Database; **E3** = European Eye Epidemiology; **HDL** = high-density lipoprotein; **HR** = hazard ratio; **IDL** = intermediate density lipoproteins; **LDL** = low-density lipoprotein; ***LIPC*** = lipase C, hepatic type; **MARS** = Muenster aging and retina study; **NMR** = nuclear magnetic resonance; **OR** = odds ratio; **RPE** = retinal pigment epithelium; **RS** = Rotterdam Study; **VLDL** = very low-density lipoprotein.

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