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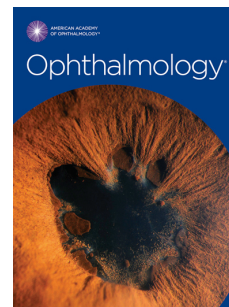
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Increased High Density Lipoprotein-levels associated with Age-related Macular degeneration. Evidence from the EYE-RISK and E3 Consortia

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1 **Increased High Density Lipoprotein-levels associated with Age-related Macular** 2 **degeneration. Evidence from the EYE-RISK and E3 Consortia**

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49

50 **Running head:** High HDL associated with AMD

51

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113 114 **ABBREVIATIONS**

115 **AMD** = Age-related macular degeneration; **ABCA1** = ATP-binding cassette (ABC) transporter A1; **(Apo)E** =
116 Apolipoprotein E; **CETP** = Cholesteryl transfer protein; **LIPC** = Lipase C; **E3** = European Eye Epidemiology;
117 **RPE** = retinal pigment epithelium; **VLDL** = very low density lipoproteins; **IDL** = intermediate density
118 lipoproteins; **LDL** = low density lipoproteins; **HDL**= high density lipoproteins; **NMR**= nuclear magnetic
119 resonance; **EPIC** = European Prospective Investigation into Cancer and Nutrition; **ALIENOR** =
120 Antioxydants, Lipids Essentiels, Nutrition et maladies OculaiRes Study; **POLA**= Pathologies Oculaires
121 Liées à l'Age Study, **EUGENDA** = European genetic database; **RS** = Rotterdam Study; **BMI** = body mass
122 index; **PAMDI** = Prevalence of Age-related Macular Degeneration in Italy; **EDTA** =
123 Ethylenediaminetetraacetic acid; **SNP**= single nucleotide polymorphism; **OR** = Odds Ratio; **HR** = Hazard
124 Ratio; **LCAT** = lecithin-cholesterol acyltransferase;

125
126 This article contains additional online-only material. The following should appear online-only: Figure 4 ,
127 Tables 1, 4-19 and cohort descriptions.

128 **ABSTRACT**

129 **Purpose:** Genetic and epidemiologic studies have shown that lipid genes and High Density Lipoproteins
130 (HDL) are implicated in age-related macular degeneration (AMD). We studied circulating lipid levels in
131 relation to AMD in a large European dataset, and investigated whether this relationship is driven by
132 certain sub fractions.

133 **Design:** (Pooled) analysis of cross-sectional data.

134 **Participants:** 30,953 individuals aged 50+ participating in the E3 consortium; and 1530 individuals from
135 the Rotterdam Study with lipid sub fraction data.

136 **Methods:** In E3, AMD features were graded per eye on fundus photographs using the Rotterdam
137 Classification. Routine blood lipid measurements were available from each participant. Data on genetics,
138 medication and confounders such as body mass index, were obtained from a common database. In a
139 subgroup of the Rotterdam Study, lipid sub fractions were identified by the Nightingale biomarker
140 platform. Random-intercepts mixed-effects models incorporating confounders and study site as a
141 random-effect were used to estimate the associations.

142 **Main Outcome Measures:** early, late or any AMD, phenotypic features of early AMD, lipid
143 measurements.

144 **Results:** HDL was associated with an increased risk of AMD, corrected for potential confounders (Odds
145 Ratio (OR) 1.21 per 1mmol/L increase (95% confidence interval[CI] 1.14-1.29); while triglycerides were
146 associated with a decreased risk (OR 0.94 per 1mmol/L increase [95%CI 0.91-0.97]). Both were
147 associated with drusen size, higher HDL raises the odds of larger drusen while higher triglycerides
148 decreases the odds. LDL-cholesterol only reached statistical significance in the association with early
149 AMD ($p=0.045$). Regarding lipid sub fractions: the concentration of extra-large HDL particles showed the
150 most prominent association with AMD (OR 1.24 [95%CI 1.10-1.40]). The *CETP* risk variant (rs17231506)
151 for AMD was in line with increased-HDL levels ($p=7.7 \times 10^{-7}$); but *LIPC* risk variants (rs2043085, rs2070895)
152 were associated in an opposite way ($p=1.0 \times 10^{-6}$ and 1.6×10^{-4}).

153 **Conclusions:** Our study suggests that HDL-cholesterol is associated with increased risk of AMD and
154 triglycerides negatively associated. Both show the strongest association with early AMD and drusen.
155 Extra-large HDL sub fractions seem to be drivers in the relation with AMD, variants in lipid genes play a
156 more ambiguous role in this association. Whether systemic lipids directly influence AMD or represent
157 lipid metabolism in the retina remains a question to be answered.

158

159 Word count: 350

160 **Keywords:** Age-related macular degeneration , lipids, high-density lipoproteins, cholesterol, E3
161 Consortium

162 **INTRODUCTION**

163 Age-related macular degeneration (AMD) is a leading cause of blindness in the developed world with
164 10.4 million sufferers worldwide in 2015¹. It is a multifactorial disease affecting the elderly involving
165 genetics and lifestyle factors. The diagnosis of AMD is based on imaging of the retina with drusen as the
166 hallmark of early disease, and chorioretinal neovascularization and atrophy of the retinal pigment
167 epithelium (RPE) are indicative of late disease. The number of drusen and total drusen area are
168 prominent predictors of progression of the early stages of AMD^{2,3}.

169 Drusen are lipid-rich, protein-containing deposits that accumulate between the RPE and Bruch's
170 membrane. The accumulation of drusen shows resemblance to the formation of atherosclerotic
171 plaques⁴ seen in cardiovascular disease, with a similar composition of proteins and protein complexes
172 such as apolipoprotein (Apo)E, cholesterol esters and complement proteins.^{5,6} The lipid load in drusen is
173 as high as 40%⁷, and is thought to be partly derived from the systemic circulation. This triggered many
174 studies to evaluate the relationship between serum or plasma lipids and AMD.⁸⁻¹² Some found
175 associations with various serum or plasma lipid levels and drusen or AMD¹¹⁻¹⁸, but results were mainly
176 weak and inconsistent. As a biological explanation is lacking, the relationship remains unsettled but
177 intriguing.

178 Genetically, lipid metabolism is also involved in AMD. Genetic associations have been
179 established for four genes encoding components of the HDL metabolism: *ABCA1*, *CETP*, *APOE* and *LIPC*¹⁹⁻
180 ²⁵. The ATP-binding cassette (ABC) transporter A1 (*ABCA1*) is a cellular cholesterol efflux pump leading to
181 formation of nascent HDL. ApoE, encoded by the *APOE* gene, facilitates cholesterol uptake by HDL.
182 Cholesteryl transfer protein (*CETP*) exchanges cholesteryl esters and triglycerides between HDL and
183 other lipoproteins, and thereby influences HDL particle size.²⁶ Lastly, hepatic lipase encoded by the *LIPC*
184 gene hydrolyzes triglycerides and phospholipids in lipoproteins²⁷ and thereby partly converts very low
185 density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) to low density lipoproteins
186 (LDL)²⁰ and plays a role in altering the HDL contents.

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

191

192

193 **METHODS**194 **Study population:**

195 Routine blood lipid measurements

196 Fourteen studies from France, Germany, Italy, the Netherlands, Norway, Portugal and United Kingdom
197 participating in the E3 consortium enrolled in the current study (cohort descriptions available at External
198 link <http://www.aaajournal.org>). E3 consists of European studies with epidemiologic data on common
199 eye disorders; a detailed description on the studies included in the consortium has been published
200 elsewhere.²⁸ All studies with gradable macular fundus photographs ($N=30,953$ participants) aged 50
201 years and over contributed their data to the EYE-RISK database version 4.0. Studies were population-
202 based cohort studies except for CRETEIL and EUGENDA which are clinic-based studies. Routine blood
203 lipid measurements and AMD outcomes of the same visit were used for this analysis; for TwinsUK the
204 closest visit to capturing of the retinal fundus photos was used. All studies were performed in
205 accordance with the Declaration of Helsinki for research involving human subjects and the good
206 epidemiological practice guideline.

207 Detailed lipid analyses

208 The population-based Rotterdam Study (RS) I provided data on lipid sub fractions which were
209 determined at visit 4. Descriptive statistics of this cohort are shown in **Supplementary Table 1** (available
210 at External link <http://www.aaajournal.org>).

211

212 **Clinical examination:**

213 AMD features were graded per eye on fundus photographs by experienced graders or clinicians; the
214 most severe AMD grade classified the AMD status of the person. When needed, photographs were
215 regraded by expert graders from Moorfields Eye Hospital and the Rotterdam Study to harmonize the
216 outcome. AMD status was determined for all included studies using the Rotterdam Classification as
217 previously described.²⁹ In brief, grade 0 or 1 are considered no AMD; grade 2 and 3 with soft indistinct
218 drusen, reticular drusen or distinct drusen with pigmentary changes as early AMD, and grade 4 with
219 Geographic Atrophy or Choroidal Neovascularization as late AMD. The area of the Early Treatment
220 Diabetic Retinopathy Study grid covered by drusen was estimated in RS I visit 4 per grid circle, and
221 calculated using previously defined harmonization criteria³⁰. Medication use and lifestyle factors

222 including smoking habits were assessed by questionnaire; lipid measurements and other clinical
223 determinants such as hypertension, body mass index (BMI), diabetes mellitus were examined at each
224 individual research center (**Supplement cohort descriptions** available at External link
225 <http://www.aaojournal.org>). Fasting blood draws were taken in all studies except for EUGENDA, MARS,
226 and the Tromsø Eye Study, which drew blood samples non-fasting. Total cholesterol, HDL-cholesterol,
227 LDL-cholesterol and triglycerides were measured in plasma (POLA, PAMDI, Montrachet-3C, CRETEIL) or
228 in serum (remaining studies) using standard operating procedures. When LDL was not measured and
229 triglycerides were below 4.52mmol/L, a proxy was calculated using the Friedewald formula³¹: LDL-
230 cholesterol = Total cholesterol – HDL cholesterol – (Total triglyceride ÷ 2.19); only positive values
231 entered the analysis.

232

233 **Nuclear magnetic resonance (NMR) metabolomics analysis**

234 Lipid sub fractions were measured with the Nightingale's NMR-based biomarker platform in fasting
235 Ethylenediaminetetraacetic acid (EDTA) plasma samples (Nightingale Ltd, Helsinki, Finland). These
236 measurements cover multiple metabolic pathways, including lipoprotein lipids and subclasses, fatty
237 acids, amino acids, and glycolysis-related metabolites. The NMR-based metabolic profiling has
238 previously been described in detail³² and has been used in multiple large-scale epidemiological and
239 genetic studies³³⁻³⁶.

240

241 **Genetic analyses**

242 The Alienor-3C study and Montrachet-3C study were genotyped with the Illumina Human 610-Quad
243 BeadChip and imputed with the 1000 Genomes Phase I integrated variant set (March2012) using Shapeit
244 v2.r727 (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) for pre-phasing and
245 Impute2 v2.3 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) for imputation. Rotterdam Study
246 I, II and III were genotyped using the Illumina 550K, 550k due/610K Illumina arrays. The genotypes were
247 imputed with the 1000 Genomes (Phase 1 version 3) reference panel using the Markov chain
248 Haplotyping (MaCH)/minimac software³⁷⁻³⁹. The EUGENDA study was genotyped with a custom-designed
249 Illumina HumanCoreExome array within the International AMD Genetics Consortium (IAMDGC). Details
250 regarding the design of this array, annotation, imputation and quality control of the genotypic data have

251 been previously described¹⁹. All cohorts applied similar quality control procedures to genotype data
252 prior to analysis, imputation quality was $r^2 > 0.3$.

253 A total AMD genetic risk score was calculated using 33 out of the 52 known AMD risk variants¹⁹ available
254 in the EYE-RISK database version 4.0, see also subscript **Supplementary Table 19** (available at External
255 link <http://www.aaojournal.org>). Genetic allele dosage was annotated as 0 for non-carriers, 1 for
256 heterozygotes, and 2 for homozygotes. The genetic risk score was composed by calculating the sum of
257 the betas of independent risk variants. The score was standardized and added as a covariate in a linear
258 regression analysis with AMD as the dependent variable. The linear regression was corrected for age,
259 sex, lipid lowering drugs and study site. The effect of individual lipid-related single nucleotide
260 polymorphisms (SNP) on each lipid level or lipid sub fraction was assessed in a mixed-effects regression
261 correcting for age, sex, lipid lowering drug usage, plasma or serum, fasting state and using study site as a
262 random effect term. The p-value threshold for these analyses were $0.05/60 = 0.00083$ (8.3×10^{-4}) after
263 Bonferroni correction.

264 **Statistical analysis**

265 The outcome variable was presence of early or late AMD versus no AMD. Differences in baseline
266 characteristics were evaluated with a Wald test using a logistic regression analysis, adjusting for age,
267 gender, and study site. Analyses were conducted on complete data. Odds ratios (OR) for the routine
268 blood lipid measurements were calculated using random-intercepts mixed-effects logistic regression
269 models, including study site as a random effect term to allow for variability between study sites. The
270 study site specific fixed effects estimates were transformed to their marginal counterparts as described
271 by Heagerty and Zeger⁴⁰.

272
273 Association of HDL-cholesterol with AMD characteristics (presence of various drusen sizes, hyper- or
274 hypopigmentation) were calculated in a univariate logistic regression analysis for the worse eye, defined
275 as the eye with the most severe lesions of each AMD characteristic, correcting for age, sex, lipid
276 lowering drugs usage and study site. The linear regression for HDL-cholesterol and drusen area was
277 calculated in the Rotterdam Study I visit 4 only.

278 For the analysis on lipid sub fractions, all sub fractions were +1 log transformed and scaled to make
279 comparable measurements. Association magnitudes were reported in units of standard deviation (SD) or
280 odds ratio (OR) change per 1-SD increase in each metabolite, as previously suggested by others^{34, 35}. To
281 account for the correlation between lipid sub fractions, the eigenvalues were calculated as proposed by

282 Li and Ji⁴¹ on the SNPSpD online interface⁴². Bonferroni correction was applied to correct for multiple
283 testing using the eigenvalues to calculate the p-value threshold (p-value=0.001087). To test for
284 differences between AMD stage and the mean of the lipid sub fractions, a Welch test was performed on
285 the total of all age categories. The Welch test was chosen since homogeneity of variance was violated
286 between the AMD severity classes. The post hoc Games-Howell test was used to investigate differences
287 between the mean of the No AMD and Late AMD groups.

288
289 Mixed-effects logistic regression models were performed with R package lme4⁴³ and mixed-effects
290 regression models with nml⁴⁴ (R Core Team (2016). R: A language and environment for statistical
291 computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>);
292 Welch-test and genetic risk scores with SPSS (IBM Corp. Released 2012 IBM SPSS Statistics for Windows,
293 Version 24.0 Amonk, NY: IBM Corp). Graphical outputs were constructed with GraphPad Prism 7
294 (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA,
295 www.graphpad.com”).

296

297 RESULTS

298 We identified a total of 4,730 individuals with early AMD; 2,441 with late AMD; and 23,782 non-affected
299 persons. The baseline characteristics of these participants are summarized in **Table 2**. AMD cases and
300 controls differed in age, sex, BMI, lipid lowering drug use, and smoking, in accordance with the known
301 AMD risk profile.

302 Routine blood lipid measurements and AMD in the E3 consortium

303 Next, we examined lipid levels in the entire study population. Mean levels of all lipids were within
304 physiologic limits: mean total cholesterol ranged between studies from 5.1 mmol/L to 5.8 mmol/L; mean
305 HDL-cholesterol from 1.4 mmol/L to 1.9 mmol/L. Mean LDL-cholesterol ranged from 3.0 mmol/L to 3.8
306 mmol/L; and mean triglycerides from 1.2 mmol/L to 1.7mmol/L. A fifth of the study population had
307 collected only non-fasting blood samples; in these, the mean levels were similar but on the higher range
308 of total cholesterol and triglycerides; 5.7 mmol/L for total cholesterol and 1.7mmol/L for triglycerides.
309 Mean HDL-cholesterol was on the lower range with 1.5mmol/L and LDL-cholesterol was very
310 comparable with 3.5 mmol/L.

311 **Table 3** shows the association between lipid levels and AMD adjusted for age, sex, lipid lowering
312 drug usage, body mass index, smoking, plasma or serum, fasting state and study site in the E3
313 consortium. Analyses for late AMD were also corrected for diabetes. Total cholesterol was not
314 associated with any of the AMD outcomes. Higher HDL-cholesterol was associated with an increased risk
315 of any AMD, and risk estimates were slightly higher for early AMD (OR 1.34 per 1 mmol/L increase), than
316 for late AMD (OR 1.12 per 1 mmol/L increase), but had overlapping confidence intervals. LDL-cholesterol
317 and triglycerides were inversely associated with early (OR 0.96 and OR 0.88 per 1 mmol/L increase,
318 respectively) and any AMD (OR 0.98 and OR 0.94 per 1 mmol/L increase, respectively), but effect sizes
319 were smaller than for HDL-cholesterol. Sensitivity analysis on fasting and non-fasting sampling methods
320 and sex showed no interaction or it showed interaction but with similar point estimates of the odds
321 ratios which made sampling effect or confounding unlikely. However, a sensitivity analysis on plasma or
322 serum sampling methods did show a change in direction of effect of triglycerides measured in plasma,
323 although not statistically significant (**Supplementary Table 4-10**, available at External link
324 <http://www.aaojournal.org>). To investigate whether observed associations were the result of
325 preferential survival of elderly without cardiovascular disease, we repeated the analyses in various age
326 strata (**Supplementary Table 11-13**, available at External link <http://www.aaojournal.org>). Even in those
327 aged 65 years or younger with AMD, HDL-cholesterol was significantly associated with increased risk of
328 AMD (OR 1.19 per 1 mmol/L increase, p-value 0.02). LDL-cholesterol was inversely associated with AMD
329 (OR 0.93 per 1 mmol/L, p-value 0.02), associations with triglycerides became insignificant.

330

331 **Routine blood lipids measurements and early AMD phenotype**

332 As the association between HDL-cholesterol and AMD was most pronounced in those with early AMD,
333 we performed more detailed analyses using the various early AMD features as outcomes. Effects of HDL-
334 cholesterol and triglycerides became larger with increasing drusen size, **Figure 1**. Likewise, higher HDL
335 levels were associated with greater drusen area (beta 0.014; P-value=0.001); higher triglyceride levels
336 were associated with lower drusen area. Correcting for smoking did not change these results (data not
337 shown). Lipids were not statistically significantly associated with pigmentary changes, and total
338 cholesterol and LDL-cholesterol were not associated with any early AMD characteristic (**Supplementary**
339 **table 15 and 16**, available at External link <http://www.aaojournal.org>).

340

341 Lipid sub fractions in the Rotterdam Study

342 To explore whether the association between HDL-cholesterol, triglycerides, and AMD was driven by
343 specific lipid sub fractions, we examined lipid sub fractions with NMR in the Rotterdam Study-I **Figure 2**
344 (**Supplementary table 14**, available at External link <http://www.aaojournal.org>). The concentration of
345 extra-large HDL particles (XL-HDL-P) was most significantly associated with any AMD, particularly the sub
346 fractions of phospholipids and total lipids within extra-large HDL particles. These sub fractions are highly
347 correlated (Pearson correlation >0.97). Next, total cholesterol and free cholesterol in the small VLDL
348 were significantly associated, as well as the ratio ApoB:ApoA1, with a Pearson correlation ranging
349 between 0.93 and 0.87. No other metabolites were significantly associated with AMD. Correcting for
350 smoking did not change these significant results (data not shown). The ApoB-ApoA1 ratio is a surrogate
351 for the LDL/HDL ratio with a small ratio suggesting a high level of HDL compared to LDL lipoproteins.
352 These associations show a dose dependent relation with AMD stages from the Rotterdam Classification
353 **Figure 3**. To test if the mean of the lipid sub fractions per AMD stage differed statistically, we performed
354 a Welch test, which was significant for each of the six sub fractions. The sub fractions related to HDL also
355 showed a statistically significant difference in the Games-Howell post hoc test comparing the mean of
356 those with no AMD with late AMD; p-value=0.01 for the concentration of extra-large HDL, p-value=0.02
357 for phospholipids in extra-large HDL and the p-value=0.01 for total lipids in extra-large HDL. The Games-
358 Howell post-hoc test was not significant in the other three sub fractions, likely due to the small group
359 size and variance in late AMD. (**Supplementary Figure 4** shows dose dependency per age category
360 available at External link <http://www.aaojournal.org>.)

361 We also performed the analyses stratified for lipid-lowering drug use, which was reported by
362 less cases (17.0%) than controls (24.2%) in the Rotterdam Study (p=0.02). Significance was found only in
363 those not taking lipid-lowering drugs, and point estimates were highly similar to the overall group.
364 (**Supplementary Table 17- 18**, available at External link <http://www.aaojournal.org>).

365

366 Lipid genes, lipid sub fractions and AMD

367 As genetic variants are an important cause of AMD, we investigated the relation between genes, lipids,
368 and AMD. First, we investigated whether a genetic risk score with 33 SNPs covering all major AMD genes
369 influenced lipid levels in the E3 consortium, and found with increasing genetic risk also an increase of
370 HDL-cholesterol (p=0.03) (**Supplementary Table 19** available at External link
371 <http://www.aaojournal.org>). Subsequently, we focused on the individual AMD lipid genes. In E3, the

372 CETP variant rs17231506 was positively associated with HDL-cholesterol levels and negatively associated
373 with LDL-cholesterol, while both LIPC variants rs2043085 and rs2070895 were inversely associated with
374 HDL-cholesterol. In addition, the APOE variant rs429358 was associated with decreased levels of total
375 cholesterol, triglycerides, and LDL-cholesterol, but with increased levels of HDL-cholesterol. APOE
376 variant rs73036519 had no significant effect on the routine lipid measurements or on the lipid sub
377 fractions. ABCA1 variant rs2740488 only influenced total cholesterol (**Table 20A**). When restricting the
378 analysis to lipid sub fractions in the Rotterdam Study (**Table 20B**), we found similar results for the CETP
379 variant and the LIPC variants with all extra-large HDL sub fractions.

380

381 **DISCUSSION**

382 **Routine blood lipid measurements**

383 Based on pooled data of 30,953 participants from Western Europe, we have shown that high circulating
384 HDL-cholesterol levels and low triglyceride levels are significantly associated with AMD. The magnitude
385 of the effect was higher for early than for late AMD, and associations were related to drusen size and
386 area. By focusing on lipid sub fractions, we revealed that extra-large HDL particles, small VLDL particles,
387 and the ApoB-ApoA1 ratio, a surrogate for the LDL/HDL ratio, were dose-dependent drivers of this
388 association. AMD risk variants in lipid genes did not provide a clear explanation, as in particular the
389 variants in *LIPC* which increase the risk of AMD, decreased HDL-cholesterol in the systemic circulation.

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

399 Many previous studies did not find a statistically significant association between lipids and AMD, but
400 studies with the larger sample sizes often found a positive association with HDL-cholesterol and an

401 inverse association with triglycerides¹³. The current pooled study showed that the levels of these lipids
402 were within physiological range in both cases and controls, and that absolute differences were small in
403 mmol/L. However, our data suggest that an increase of HDL from the 25th percentile to the 75th
404 percentile coincides with an AMD risk increase of about 20%. Selective survival does not appear to
405 explain our findings, as the association was already present in the youngest age group (≤ 65 yrs). The
406 exact clinical interpretation remains to be defined. Nevertheless, the findings contribute to the
407 understanding of AMD pathogenesis.

408 Animal research has provided some key insights in retinal lipid metabolism. Studies in rodents showed
409 that most lipids in the retina are synthesized locally and up to a quarter is derived from the systemic
410 circulation⁴⁵. Another study in mice showed that a high fat diet increases cholesterol in the retina, but
411 not as much as in the circulation. These results suggest that transport from the systemic circulation to
412 the retina does take place, albeit modestly. Although LDL delivers cholesterol most efficiently from the
413 systemic circulation to the retina, HDL-cholesterol, with ApoA-I as its major lipid component⁴⁶, does this
414 as well via scavenger receptors^{26, 47, 48}. The RPE processes the internalized lipids and subsequently
415 secretes them again on the apical side via ABCA1 transporters into the inter-photoreceptor matrix.
416 Thereafter, lecithin-cholesterol acyltransferase (LCAT), located at the surface of nascent HDL⁴⁹, converts
417 free cholesterol into esterified cholesterol,⁵⁰ which are present in nascent HDL. In this way LCAT
418 transforms nascent HDL into larger, mature HDL, while LIPC hydrolyzes phospholipids in the HDL
419 lipoprotein^{23, 51}. As suggested by Tserentsoodol *et al*²⁶, due to the absence of LDL in the retina, it is
420 possible that CETP has a role in transferring esterified cholesterol between lipoproteins or
421 photoreceptor membranes. In the inter-photoreceptor matrix, HDL functions as a transport vehicle
422 between the RPE and the photoreceptors supporting the high synthesis and degradation of the lipid-rich
423 photoreceptor disks²⁶. The RPE maintains the lipid balance by transporting lipoproteins back to Bruchs
424 membrane⁵². The lipid contents of these lipoproteins resemble that of LDL lipoproteins rather than HDL
425 as it has a high abundance of esterified cholesterol, but both ApoA and ApoB⁵³. It has been proposed
426 that this large amount of esterified cholesterol acts as a barrier for lipid transport through an aging
427 retina, thereby facilitating the formation of deposits⁵⁴. Another mechanism proposed to form deposits is
428 through the impairment of ABCA1 transporter of macrophages, which impairs the efflux of free
429 cholesterol out of the macrophage. This results in senescent macrophages with high levels of cholesterol
430 in the retina of mice⁵⁵.

431 Interestingly, lipoproteins appear to be closely related to the complement system, the major pathway in
432 AMD pathogenesis. Proteomic studies have shown that HDL-lipoproteins can contain essential
433 complement components, such as C1, C2, C3, C5 and Factor B⁵⁶⁻⁵⁸. One study showed that CFH and
434 lipoproteins have competitive binding in the sub-RPE extracellular matrix, when CFH is low lipoproteins
435 can accumulate sub-RPE⁵⁹. By contrast, HDL can also carry complement regulators such as FH1, CFHR4
436 and CFHR5^{60, 61}. ApoA-I attached to HDL can bind clusterin, a complement lysis inhibitor that stops the
437 complement cascade just before the C5b-9 complex is inserted into the target⁶². These findings suggest
438 that HDL is involved in pro-inflammatory as well as complement inhibitory tasks. Higher HDL levels may
439 cause imbalance of the physiological homeostasis^{8, 63}. Taken together, this plethora of biological leads
440 supports the contention that HDL may play a role in the initiation of AMD. More comprehensive
441 research into lipid metabolism in the retina is warranted.

442 In our study, we found elevated levels of HDL-cholesterol in the circulation and decreased levels of
443 triglycerides in persons with AMD. In more detailed analysis, we observed a higher concentration of
444 extra-large HDL particles with a higher total lipid and phospholipid content, which are under genetic
445 control of CETP and LIPC. The high phospholipid content of extra-large HDL is very likely related to the
446 larger particle size, since phospholipids compose the outer shell of the lipoprotein. CETP may exert its
447 effect on AMD partly through systemic HDL, in line with previous Mendelian randomization studies^{24, 66}.
448 The opposing effects which we found for LIPC are less easily explained, but have been observed by
449 others^{23, 24}. This finding suggests that systemic HDL may be a biomarker rather than directly causally
450 related to AMD. In a larger study, Kettunen *et al*³³ found more genetic effects on lipid sub fractions;
451 variants in *CETP* and *APOE* also had a decreasing effect on the small VLDL sub fractions, while a variant
452 in *ABCA1* increased extra-large HDL. Our smaller sample size hampered us to replicate these findings.

453
454 Where do these lipid associations fit in the chronology of AMD development? The more pronounced risk
455 for early AMD and increasing odds ratios of HDL-cholesterol for the larger size drusen suggests that
456 lipids play an important role at the early phase of disease. Hypothetically, intervention at this phase
457 would be most promising in preventing blindness. We did not find statistical significance for any lipid
458 sub fraction in only those using lipid-lowering drugs, possibly because there is no effect, but probably
459 due to the lower power in this subgroup. Evidence from other studies indicate that statins increase HDL
460 levels slightly⁶⁷ but reduce extra-large HDL⁶⁸, and that HDL protein composition may change as well⁶⁹.
461 Most epidemiologic studies do not find any effect of lipid lowering drugs on AMD^{8, 70-72}; however, one
462 study observed a slower progression of AMD in persons with a certain complement factor H risk

463 variant⁷⁴. Large randomized controlled trials with long term follow-up are needed to clarify the relation
464 between lipid lowering drugs and AMD.

465
466 In conclusion, this study showed that HDL-cholesterol and triglycerides levels are particularly associated
467 with early AMD, mostly through the association with drusen. Extra-large HDL sub fractions seem to be
468 drivers of this association. Whether systemic lipids directly influence lipid metabolism in the retina or
469 whether these lipids mirror pathology in the retina is a question that remains to be answered.

470

471

472

473 **FIGURE LEGENDS**

474 **Figure 1.** Association of HDL-cholesterol and triglycerides with age-related macular degeneration
475 characteristics

476 **Figure 2.** The association of metabolic variables and AMD.
477 Each bar represents the association with AMD, the size of the bar is the odds ratio, coloring refers to
478 effect direction and significance. Dots indicate Bonferroni statistically significant metabolic variables
479 corrected for age, sex and lipid lowering drugs. Labels describe the properties measured in each lipid
480 sub fraction (*P*, concentration of particles; *L*, total lipids; *PL*, phospholipids; *C*, total cholesterol; *CE*,
481 cholesterol esters; *FC*, free cholesterol; *TG*, triglycerides). List of abbreviations is in the annex.

482

483 **Figure 3.** Stage dependent relationship of the six associated lipid sub fractions with AMD.

484 Error bars indicate 95% confidence intervals of the mean.

Précis: (max 35 words)

HDL-cholesterol is positively associated with AMD and triglycerides negatively. This is most prominently seen in drusen as early AMD features. This association seems to be driven by larger HDL sub fractions and HDL related genetics.

ACCEPTED MANUSCRIPT

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Annex

Abbreviation	Name
XXL_VLDL_C	Total cholesterol in extremely large VLDL
XXL_VLDL_CE	Cholesterol esters in extremely large VLDL
XXL_VLDL_FC	Free cholesterol in extremely large VLDL
XXL_VLDL_L	Total lipids in extremely large VLDL
XXL_VLDL_P	Concentration of extremely large VLDL
XXL_VLDL_PL	Phospholipids in extremely large VLDL
XXL_VLDL_TG	Triglycerides in extremely large VLDL
XL_VLDL_C	Total cholesterol in extra-large VLDL
XL_VLDL_CE	Cholesterol esters in extra-large VLDL
XL_VLDL_FC	Free cholesterol in extra-large VLDL
XL_VLDL_L	Total lipids in extra-large VLDL

XL_VLDL_P	Concentration of extra-large VLDL
XL_VLDL_PL	Phospholipids in extra-large VLDL
XL_VLDL_TG	Triglycerides in extra-large VLDL
L_VLDL_C	Total cholesterol in large VLDL
L_VLDL_CE	Cholesterol esters in large VLDL
L_VLDL_FC	Free cholesterol in large VLDL
L_VLDL_L	Total lipids in large VLDL
L_VLDL_P	Concentration of large VLDL
L_VLDL_PL	Phospholipids in large VLDL
L_VLDL_TG	Triglycerides in large VLDL
M_VLDL_C	Total cholesterol in medium VLDL
M_VLDL_CE	Cholesterol esters in medium VLDL
M_VLDL_FC	Free cholesterol in medium VLDL
M_VLDL_L	Total lipids in medium VLDL
M_VLDL_P	Concentration of medium VLDL
M_VLDL_PL	Phospholipids in medium VLDL
M_VLDL_TG	Triglycerides in medium VLDL
S_VLDL_CE	Cholesterol esters in small VLDL
S_VLDL_L	Total lipids in small VLDL
S_VLDL_P	Concentration of small VLDL
S_VLDL_PL	Phospholipids in small VLDL
S_VLDL_TG	Triglycerides in small VLDL
XS_VLDL_C	Total cholesterol in extra-small VLDL
XS_VLDL_CE	Cholesterol esters in extra-small VLDL
XS_VLDL_FC	Free cholesterol in extra-small VLDL
XS_VLDL_L	Total lipids in extra-small VLDL
XS_VLDL_P	Concentration in extra-small VLDL
XS_VLDL_PL	Phospholipids in extra-small VLDL
XS_VLDL_TG	Triglycerides in extra-small VLDL
IDL_C	Total cholesterol in IDL
IDL_CE	Cholesterol esters in IDL
IDL_FC	Free cholesterol in IDL
IDL_L	Total lipids in IDL
IDL_P	Concentration in IDL
IDL_PL	Phospholipids in IDL
IDL_TG	Triglycerides in IDL
L_LDL_C	Total cholesterol in large LDL
L_LDL_CE	Cholesterol esters in large LDL
L_LDL_FC	Free cholesterol in large LDL
L_LDL_L	Total lipids in large LDL
L_LDL_P	Concentration in large LDL
L_LDL_PL	Phospholipids in large LDL

L_LDL_TG	Triglycerides in large LDL
M_LDL_C	Total cholesterol in medium LDL
M_LDL_CE	Cholesterol esters in medium LDL
M_LDL_FC	Free cholesterol in medium LDL
M_LDL_L	Total lipids in medium LDL
M_LDL_P	Concentration of medium LDL
M_LDL_PL	Phospholipids in medium LDL
M_LDL_TG	Triglycerides in medium LDL
S_LDL_C	Total cholesterol in small LDL
S_LDL_CE	Cholesterol esters in small LDL
S_LDL_FC	Free cholesterol in small LDL
S_LDL_L	Total lipids in small LDL
S_LDL_P	Concentration of small LDL
S_LDL_PL	Phospholipids in small LDL
S_LDL_TG	Triglycerides in small LDL
XL_HDL_C	Total cholesterol in extra-large HDL
XL_HDL_CE	Cholesterol esters in extra-large HDL
XL_HDL_FC	Free cholesterol in extra-large HDL
XL_HDL_TG	Triglycerides in extra-large HDL
L_HDL_C	Total cholesterol in large HDL
L_HDL_CE	Cholesterol esters in large HDL
L_HDL_FC	Free cholesterol in large HDL
L_HDL_L	Total lipids in large HDL
L_HDL_P	Concentration of large HDL
L_HDL_PL	Phospholipids in large HDL
L_HDL_TG	Triglycerides in large HDL
M_HDL_C	Total cholesterol in medium HDL
M_HDL_CE	Cholesterol esters in medium HDL
M_HDL_FC	Free cholesterol in medium HDL
M_HDL_L	Total lipids in medium HDL
M_HDL_P	Concentration in medium HDL
M_HDL_PL	Phospholipids in medium HDL
M_HDL_TG	Triglycerides in medium HDL
S_HDL_C	Total cholesterol in small HDL
S_HDL_CE	Cholesterol esters in small HDL
S_HDL_FC	Free cholesterol in small HDL
S_HDL_L	Total lipids in small HDL
S_HDL_P	Concentration of small HDL
S_HDL_PL	Phospholipids in small HDL
S_HDL_TG	Triglycerides in small HDL
VLDL_C	Total cholesterol in VLDL
VLDL_D	VLDL diameter
VLDL_TG	Triglycerides in VLDL

LDL_C	Total cholesterol in LDL
LDL_D	LDL diameter
LDL_TG	Triglycerides in LDL
HDL_C	Total cholesterol in HDL
HDL_D	HDL diameter
HDL_TG	Triglycerides in HDL
HDL2_C	Total cholesterol in HDL2
HDL3_C	Total cholesterol in HDL3
Serum_C	Serum total cholesterol
Serum_TG	Serum total triglycerides
TotCho	Total cholines
Remnant_C	Remnant cholesterol (non_HDL, non_LDL_cholesterol)
ApoA1	Apolipoprotein A_I
ApoB	Apolipoprotein B
EstC	Esterfied cholesterol
FreeC	Free cholesterol
FAw3	Omega 3 fatty acids
FAw3_FA	Omega 3 fatty acids to total fatty acids ratio
FAw6	Omega 6 fatty acids
FAw6_FA	Omega 6 fatty acids to total fatty acids ratio
TotFA	Total fatty acids
FALen	Fatty acid length
UnsatDeg	Estimated degree of unsaturation
CLA	Conjugated linoleic acid
LA	Linoleic acid
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
DHA	Docosahexaenoic acid
TG_PG	Triglycerides to phosphoglycerides ratio
CLA_FA	Conjugated linoleic acid to total fatty acids ratio
DHA_FA	Docosahexaenoic acid to total fatty acids ratio
LA_FA	Linoleic acid to total fatty acids ratio
MUFA_FA	Monounsaturated fatty acids to total fatty acids ratio
PUFA_FA	Polyunsaturated fatty acids to total fatty acids ratio
SFA_FA	Saturated fatty acids to total fatty acids ratio
DAG	Diacylglycerol
DAG_TG	Diacylglycerol to triglycerides ratio
TotPG	Total phosphoglycerides
PC	Phosphatidylcholine and other cholines
SM	Sphingomyelins
AcAce	Acetoacetate
Ace	Acetate

Ala	Alanine
Alb	Albumin
bOHBut	3_hydroxybutyrate
Cit	Citrate
Crea	Creatinine
Glc	Glucose
Gln	Glutamine
Gp	Glycoprotein acetyls
His	Histidine
Ile	Isoleucine
Lac	Lactate
Leu	Leucine
Phe	Phenylalanine
Pyr	Pyruvate
Tyr	Tyrosine
Val	Valine

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Table 1. Descriptive statistics in Rotterdam Study I, visit 4.

Descriptive statistics	Controls N=1066	Cases N=464	p-value	OR	95% CI
Sex, % female	55.5% (N=592)	57.5% (N=267)	0.83	1.03	0.82 - 1.29
Age (years)	74.7 (SD 5.9)	78.1 (SD 6.6)	<0.0001	1.09	1.07 - 1.11
BMI	27.5 (SD 3.9)	27.0 (SD 3.9)	0.16	0.98	0.95 - 1.01
Smoking					
Former %	57.3 % (N=598)	54.3% (N=248)	0.69	0.94	0.71 - 1.25
Current %	14.5% (N=151)	15.5% (N=71)	0.26	1.24	0.85 - 1.81
Hypertension %	85.7% (N=911)	88.4% (N=410)	0.83	0.96	0.68 - 1.36
Diabetes	15.4% (N=152)	15.6% (N=68)	0.86	0.97	0.70 - 1.34
Lipid lowering drugs	24.2% (N=257)	17.0% (N=79)	0.02	0.72	0.54 - 0.96

Corrected for age and sex

Table 2. Baseline data and results of logistic regression analysis of the fourteen European studies.

		Controls N=23782	Cases N=7171	p-value	OR	95% CI
AMD						
Early AMD			N = 4730			
Late AMD			N = 2441			
Sex, % 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 (years)	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	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=11010)	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

Odds ratios are corrected for age, sex and study site. AMD = age-related macular degeneration, BMI= body mass index, OR = odds ratio

Table 3. Mixed-effects logistic regression associations of blood lipids with age-related macular degeneration

Lipid		Controls Median (25 th and 75 th percentile)	Cases Median (25 th and 75 th percentile)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.60 (4.90-6.30) N=20555	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=20234	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=20555	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=19931	1.50 (1.23-1.80) N=3802	1.34	1.22-1.48	6.48x10⁻¹⁰
	Late AMD	1.40 (1.16-1.69) N=19662	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=19931	1.50 (1.22-1.80) N=5439	1.21	1.14-1.29	1.35x10⁻⁹
LDL-cholesterol	Early AMD	3.49 (2.84-4.14) N=19590	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=19334	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=19590	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=19539	1.30 (0.97-1.80) N=3768	0.88	0.84-0.92	2.44x10⁻⁷
	Late AMD	1.34 (1.00-1.87) N=19474	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=19539	1.32 (0.98-1.86) N=5374	0.94	0.91-0.97	2.35 x10⁻⁵

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.

IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 4. Mixed-effects logistic regression for any age-related macular degeneration with interaction terms with fasting vs. non-fasting

Interaction term	OR	95% CI	p-value
Total Cholesterol *fasting/non-fasting	1.00	0.96 – 1.04	0.94
HDL-cholesterol*fasting/non-fasting	0.85	0.76 – 0.95	0.0054
LDL-cholesterol*fasting/non-fasting	1.01	0.96 – 1.06	0.77
Triglycerides *fasting/non-fasting	1.06	1.004- 1.13	0.038

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site, plasma or serum and fasting state. Non-fasting = 1, fasting =0.

Table 5. Mixed-effects logistic regression for any age-related macular degeneration in fasting samples

Lipid	Controls Median (IQR) N	Cases Median (IQR) N	OR	95% CI	p-value
Total Cholesterol	5.60 (1.40) N=17122	5.50 (1.40) N=3096	0.99	0.96 – 1.01	0.28
HDL-cholesterol	1.38 (0.51) N=16498	1.50 (0.58) N=2979	1.25	1.16 – 1.34	4.67x10⁻⁹
LDL-cholesterol	3.48 (1.28) N=16184	3.37 (1.29) N=2933	0.97	0.94 – 0.998	0.04
Triglycerides	1.32 (0.86) N=16106	1.23 (0.79) N=2942	0.93	0.89-0.96	3.63x10⁻⁵

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site and plasma or serum.

Table 6. Mixed-effects logistic regression for any age-related macular degeneration in non-fasting samples

Lipid	Controls Median (IQR) N	Cases Median (IQR) N	OR	95% CI	p-value
Total Cholesterol	5.70 (1.50) N=3433	5.70 (1.60) N=2442	1.03	0.97 – 1.08	0.34
HDL-cholesterol	1.50 (0.58) N=3433	1.49 (0.56) N=2460	1.21	1.06 – 1.40	0.006
LDL-cholesterol	3.50 (1.38) N=3406	3.40 (1.37) N=2404	1.01	0.95 – 1.07	0.74
Triglycerides	1.43 (0.95) N=3433	1.49 (0.97) N=2432	0.95	0.89 – 1.01	0.11

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site and plasma or serum.

Table 7. Mixed-effects logistic regression for any age-related macular degeneration GLMM with interaction terms for plasma or serum samples

Interaction term	OR	95% CI	p-value
Total Cholesterol * plasma serum	0.97	0.90 – 1.04	0.43
HDL-cholesterol *plasma serum	1.03	0.85 – 1.25	0.77
LDL-cholesterol *plasma serum	0.98	0.89 – 1.06	0.56
Triglycerides *plasma serum	0.90	0.81- 1.01	0.044

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum. Plasma serum is coded as; plasma = 0, serum =1.

Table 8. Mixed-effects logistic regression associations of **PLASMA** lipids with age-related macular degeneration

Lipid		Controls Median (IQR) N	Cases Median (IQR) N	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.68 (5.03-6.38) N=2769	5.75 (5.07-6.46) N=359	1.05	0.94-1.17	0.393
	Late AMD	5.71 (5.06-6.40) N=2539	5.66 (5.03-6.31) N=348	1.00	0.95-1.06	0.88
	Any AMD	5.68 (5.03-6.38) N=2769	5.69 (5.06-6.38) N=713	1.01	0.97-1.05	0.53
HDL-cholesterol	Early AMD	1.42 (1.17-1.68) N=2711	1.50 (1.24-1.80) N=358	1.26	0.92-1.71	0.133
	Late AMD	1.41 (1.17-1.68) N=2532	1.68 (1.33-2.07) N=347	1.09	0.91-1.29	0.35
	Any AMD	1.42 (1.17-1.68) N=2711	1.57 (1.28-1.93) N=711	1.09	0.97-1.21	0.15
LDL-cholesterol	Early AMD	3.69 (3.08-4.30) N=2687	3.62 (3.04-4.36) N=355	1.01	0.90-1.14	0.814
	Late AMD	3.71 (3.11-4.31) N=2520	3.64 (3.09-4.31) N=347	0.98	0.91-1.05	0.55
	Any AMD	3.69 (3.08-4.30) N=2687	3.63 (3.07-4.34) N=708	1.00	0.96-1.04	0.96
Triglycerides	Early AMD	1.07 (0.79-1.48) N=2546	1.03 (0.78-1.35) N=336	1.04	0.87-1.21	0.671
	Late AMD	1.07 (0.79-1.48) N=2539	1.01 (0.75-1.41) N=347	1.04	0.95-1.09	0.30
	Any AMD	1.07 (0.79-1.48) N=2546	1.02 (0.76-1.39) N=683	1.02	0.97-1.07	0.41

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.

IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 9. Mixed-effects logistic regression associations of **SERUM** lipids with age-related macular degeneration

Lipid		Controls Median (IQR) N	Cases Median (IQR) N	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.60 (4.88-6.30) N=17786	5.53 (4.80-6.30) N=3548	0.97	0.93-1.01	0.09
	Late AMD	5.60 (4.88-6.30) N=17695	5.66 (4.80-6.50) N=1272	1.07	1.00-1.14	0.03
	Any AMD	5.60 (4.88-6.30) N=17786	5.60 (4.80-6.31) N=4825	0.99	0.95-1.02	0.43
HDL-cholesterol	Early AMD	1.40 (1.16-1.69) N=17220	1.50 (1.23-1.80) N=3444	1.36	1.23-1.50	1.11x10⁻⁹
	Late AMD	1.40 (1.16-1.69) N=17130	1.42 (1.19-1.70) N=1279	1.19	0.99-1.42	0.06
	Any AMD	1.40 (1.16-1.69) N=17220	1.48 (1.22-1.78) N=4728	1.31	1.20-1.44	4.12x10⁻⁹
LDL-cholesterol	Early AMD	3.45 (2.80-4.11) N=16903	3.34 (2.69-3.98) N=3391	0.95	0.91-0.99	0.014
	Late AMD	3.45 (2.80-4.11) N=16814	3.39 (2.74-4.11) N=1233	1.04	0.97-1.12	0.26
	Any AMD	3.45 (2.80-4.11) N=16903	3.35 (2.70-4.02) N=4629	0.96	0.93-1.003	0.07
Triglycerides	Early AMD	1.40 (1.02-1.91) N=16993	1.32 (1.00-1.80) N=3432	0.87	0.83-0.91	4.49x10⁻⁸
	Late AMD	1.40 (1.02-1.92) N=16925	1.56 (1.12-2.17) N=1254	1.01	0.94-1.09	0.72
	Any AMD	1.40 (1.02-1.91) N=16993	1.40 (1.00-1.90) N=4691	0.90	0.86-0.94	5.07 x10⁻⁶

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.

IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 10. Mixed-effects logistic regression for any age-related macular degeneration GLMM with interaction terms for gender

Interaction terms	OR	95% CI	p-value
Total Cholesterol *gender	0.96	0.92-1.01	0.11
HDL-cholesterol *gender	1.08	0.95-1.22	0.24
LDL-cholesterol *gender	0.95	0.91-1.001	0.053
Triglycerides *gender	0.96	0.91-1.02	0.19

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum. gender is coded: 0= male, 1 = female.

Table 11. Mixed-effects logistic regression for any age-related macular degeneration for participants aged ≤ 65

Lipid	Controls Median (IQR) N	Cases Late AMD Median (IQR) N	OR	95% CI	p-value
Total Cholesterol	5.62 (1.36) N=8606	5.60 (1.48) N=955	0.96	0.91 – 1.01	0.09
HDL-cholesterol	1.39 (0.53) N=8310	1.48 (0.59) N=924	1.19	1.02– 1.38	0.02
LDL-cholesterol	3.50 (1.27) N=8188	3.40 (1.30) N=907	0.93	0.87 – 0.99	0.02
Triglycerides	1.34 (0.91) N=8139	1.34 (0.88) N=918	0.95	0.89- 1.02	0.15

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 12. Mixed-effects logistic regression for any age-related macular degeneration for participants aged > 65 & ≤ 80

Lipid	Controls Median (IQR) N	Cases Late AMD Median (IQR) N	OR	95% CI	p-value
Total Cholesterol	5.59 (1.44) N=10030	5.60 (1.51) N=3258	1.01	0.98– 1.04	0.60
HDL-cholesterol	1.40 (0.52) N=9775	1.50 (0.56) N=3199	1.25	1.15– 1.36	1.62x10⁻⁰⁷
LDL-cholesterol	3.48 (1.33) N=9587	3.40 (1.34) N=3146	1.00	0.96 – 1.03	0.80
Triglycerides	1.37 (0.90) N=9570	1.35 (0.89) N=3160	0.92	0.89- 0.96	6.22x10⁻⁰⁵

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 13. Mixed-effects logistic regression for any age-related macular degeneration for participants aged > 80

Lipid	Controls Median (IQR) N	Cases Median (IQR) N	OR	95% CI	p-value
Total Cholesterol	5.56 (1.45) N=1919	5.50 (1.59) N=1325	1.00	0.94 – 1.06	0.93
HDL-cholesterol	1.44 (0.56) N=1846	1.47 (0.55) N=1316	1.24	1.04 – 1.47	0.02
LDL-cholesterol	3.43 (1.30) N=1815	3.38 (1.34) N=1284	0.98	0.91 – 1.05	0.62
Triglycerides	1.24 (0.76) N=1830	1.30 (0.84) N=1296	0.96	0.87-1.06	0.41

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 14. Univariable logistic regression for AMD for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value

Lipid sub fraction	p-value	OR	95%CI
Concentration extra-large HDL	4.06x10⁻⁴	1.24	1.10-1.40
Phospholipids in extra-large HDL	4.32x10⁻⁴	1.24	1.10-1.40
Total cholesterol in small VLDL	7.36x10⁻⁴	0.81	0.72-0.92
Ratio ApoB ApoA1	7.65x10⁻⁴	0.82	0.730-0.92
Total lipids in extra-large HDL	7.77x10⁻⁴	1.23	1.10-1.39
Free cholesterol in small VLDL	7.80x10⁻⁴	0.81	0.72-0.92
Total cholesterol in extra-large HDL	1.40x10 ⁻³	1.22	1.08-1.37
Cholesterol esters in extra-large HDL	1.50x10 ⁻³	1.22	1.08-1.37
Cholesterol esters in small VLDL	1.56x10 ⁻³	0.82	0.73-0.93
Total cholesterol in VLDL	1.74x10 ⁻³	0.82	0.73-0.93

Bold p-values are Bonferroni significant, OR is for 1-SD. Lowest 10 p-values are shown.

Table 15. Association of LDL with age-related macular degeneration characteristics

AMD characteristic	Not present LDL Mean (+/-SD) N	Present LDL Mean (+/-SD) N	OR	95% CI	p-value
Small drusen <63um	3.25 (0.97) N=7079	3.59 (0.93) N=3295	0.99	0.93-1.05	0.78
Intermediate drusen >63um and <125um	3.55 (0.94) N=5651	3.62 (0.95) N=3254	0.95	0.90-1.00	0.07
Large drusen >125um	3.44 (0.98) N=11883	3.43 (0.99) N= 2066	0.99	0.94-1.05	0.74
Hyper pigmentation	3.59 (0.95) N=12982	3.48 (0.94) N=1578	0.95	0.88-1.01	0.09
Hypo pigmentation	3.58 (0.94) N=13027	3.52 (0.95) N=1531	0.98	0.92-1.05	0.56

Corrected for age, sex, lipid lowering drugs and study site

Table 16. Association of **total cholesterol** with age-related macular degeneration characteristics

AMD characteristic	Not present	Present	OR	95% CI	p-value
	Total cholesterol Mean (+/-SD) N	Total cholesterol Mean (+/-SD) N			
Small drusen <63um	5.40 (1.09) N=7890	5.73 (1.01) N=3450	0.99	0.94-1.04	0.67
Intermediate drusen >63um and <125um	5.56 (1.03) N=6408	5.72 (1.04) N=3412	0.97	0.93-1.02	0.24
Large drusen >125um	5.55 (1.08) N=12733	5.58 (1.08) N= 2205	1.02	0.98-1.07	0.32
Hyper pigmentation	5.63 (1.04) N=13950	5.61 (1.04) N=1646	0.97	0.91-1.02	0.24
Hypo pigmentation	5.63 (1.04) N=14004	5.64 (1.06) N=1590	1.00	0.95-1.06	0.98

Corrected for age, sex, lipid lowering drugs and study site

Table 17. Univariable logistic regression for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value only participants using lipid lowering drugs.

Variable	p-value	OR	95%CI
Pyruvate(mmol/l)	0.005835	0.66	0.49-0.8
Albumin(signal area)	0.00821	0.69	0.52-0.90
Alanine(mmol/l)	0.013313	0.70	0.52-0.92
Tyrosine(mmol/l)	0.04998	0.76	0.57-0.99
Total lipids in small HDL	0.058837	0.76	0.57-1.01
Concentration of small HDL particles	0.060846	0.76	0.57-1.01
Diacylglycerol(mmol/l)	0.126999	0.80	0.60-1.05
Lactate(mmol/l)	0.136759	0.81	0.61-1.06
Triglycerides in extra large HDL	0.139265	1.22	0.94-1.60
Total cholesterol in small HDL	0.153029	0.82	0.62-1.08

Table 18. Univariable logistic regression for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value only in participants not using lipid lowering drugs

Variable	p-value	OR	95%CI
Total cholesterol in small VLDL	0.000806	0.796	0.695-0.908
ApoB - ApoA1 Ratio	0.000808	0.802	0.705-0.912
Phospholipids in extra-large HDL	0.001003	1.246	1.093-1.422
Concentration of extra- large HDL	0.001074	1.243	1.091-1.416
Total lipids in extra small VLDL	0.00109	0.801	0.700-0.914
Free cholesterol in small VLDL	0.001098	0.802	0.701-0.914
Total cholesterol in extra small VLDL	0.001122	0.803	0.703-0.915
Concentration of extra small VLDL	0.001232	0.803	0.702-0.916
Cholesterol esters in small VLDL	0.001387	0.804	0.702-0.918
Total cholesterol in VLDL	0.001516	0.806	0.704-0.919

Table 19 Univariable analysis of the genetic risk score for each routine lipid measurement. .

Lipid sub fractions	Estimate per 1 SD	Std. Error	p-value
Total cholesterol	-0.019	0.015	0.188
HDL-cholesterol	0.012	0.005	0.031
LDL-cholesterol	-0.025	0.013	0.056
Triglycerides	-0.011	0.012	0.365

$AMD_Risk_score = (CFH_rs10922109 * 0.673344553) + (CFH_rs570618 * 0.553885113) + (CFH_rs148553336 * 1.171182982) + (CFH_rs187328863 * 0.385262401) + (CFH_rs35292876 * 0.431782416) + (CFH_rs191281603 * 0.891598119) + (COL4A3_rs11884770 * 0.083381609) + (ADAMTS9_AS2_rs62247658 * 0.131028262) + (COL8A1_rs140647181 * 0.615185639) + (COL8A1_rs55975637 * 0.148420005) + (CFI_rs10033900 * 0.139761942) + (C9_rs62358361 * 0.512823626) + (C2_CFB_SKIV2L_rs181705462 * 0.444685821) + (C2_CFB_rs943080 * 0.139262067) + (KMT2E_SRPK2_rs1142 * 0.131028262) + (PILRB_PILRA_rs7803454 * 0.139761942) + (MIR6130_RORB_rs10781182 * 0.104360015) + (TGFB1_rs1626340 * 0.127833372) + (ARHGAP21_rs12357257 * 0.113328685) + (B3GALT1_rs9564692 * 0.105360516) + (RAD51B_rs61985136 * 0.127833372) + (RAD51B_rs2842339 * 0.165514438) + (LIPC_rs2043085 * 0.139761942) + (C2_CFB_SKIV2L_rs144629244 * 1.026041596) + (LIPC_rs2070895 * 0.15082289) + (CETP_rs17231506 * 0.104360015) + (TMEM97_VTN_rs11080055 * 0.083381609) + (APOE_rs429358 * 0.400477567) + (APOE_rs73036519 * 0.094310679) + (SYN3_TIMP3_rs5754227 * 0.235722334) + (SLC16A8_rs8135665 * 0.131028262) + (ABCA1_rs2740488 * 0.116533816) + (ARMS2_HTRA1_rs3750846 * 1.075002423).$

The risk score is standardized and estimates are corrected for age, sex, lipid lowering drugs, serum or plasma, fasting state and study site.

Table 20A. Mixed-effects linear regression model estimating the effect of SNPs on routine lipid measurements.

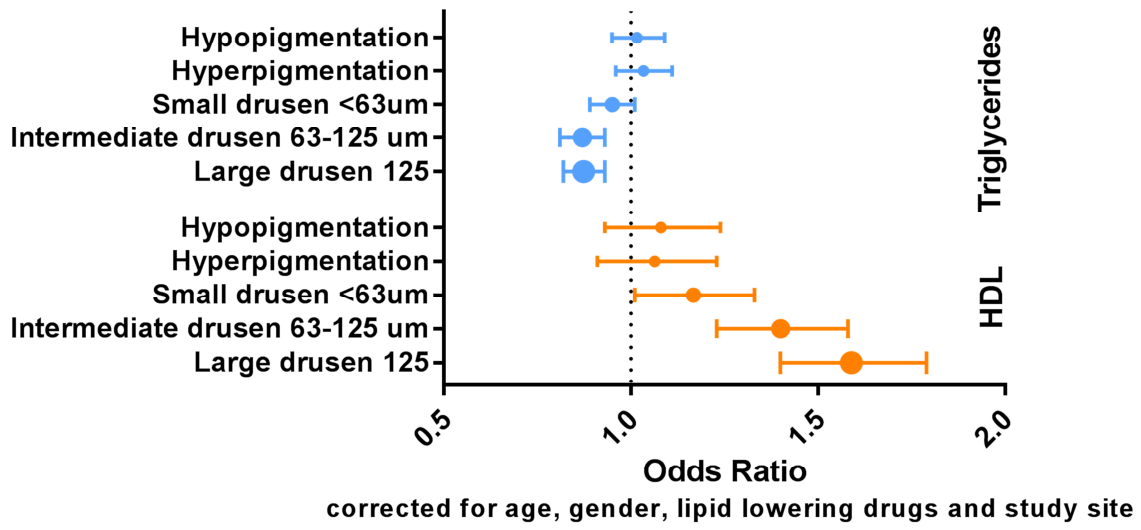
Lipid	<i>CETP</i> rs17231506 Risk allele T, reference allele C	<i>LIPC</i> rs2043085 Risk allele C, reference allele T	<i>LIPC</i> rs2070895 Risk allele G, reference allele A	<i>APOE</i> rs429358 Risk allele T, reference allele C	<i>APOE</i> rs73036519 Risk allele G, reference allele C	<i>ABCA1</i> rs2740488 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)

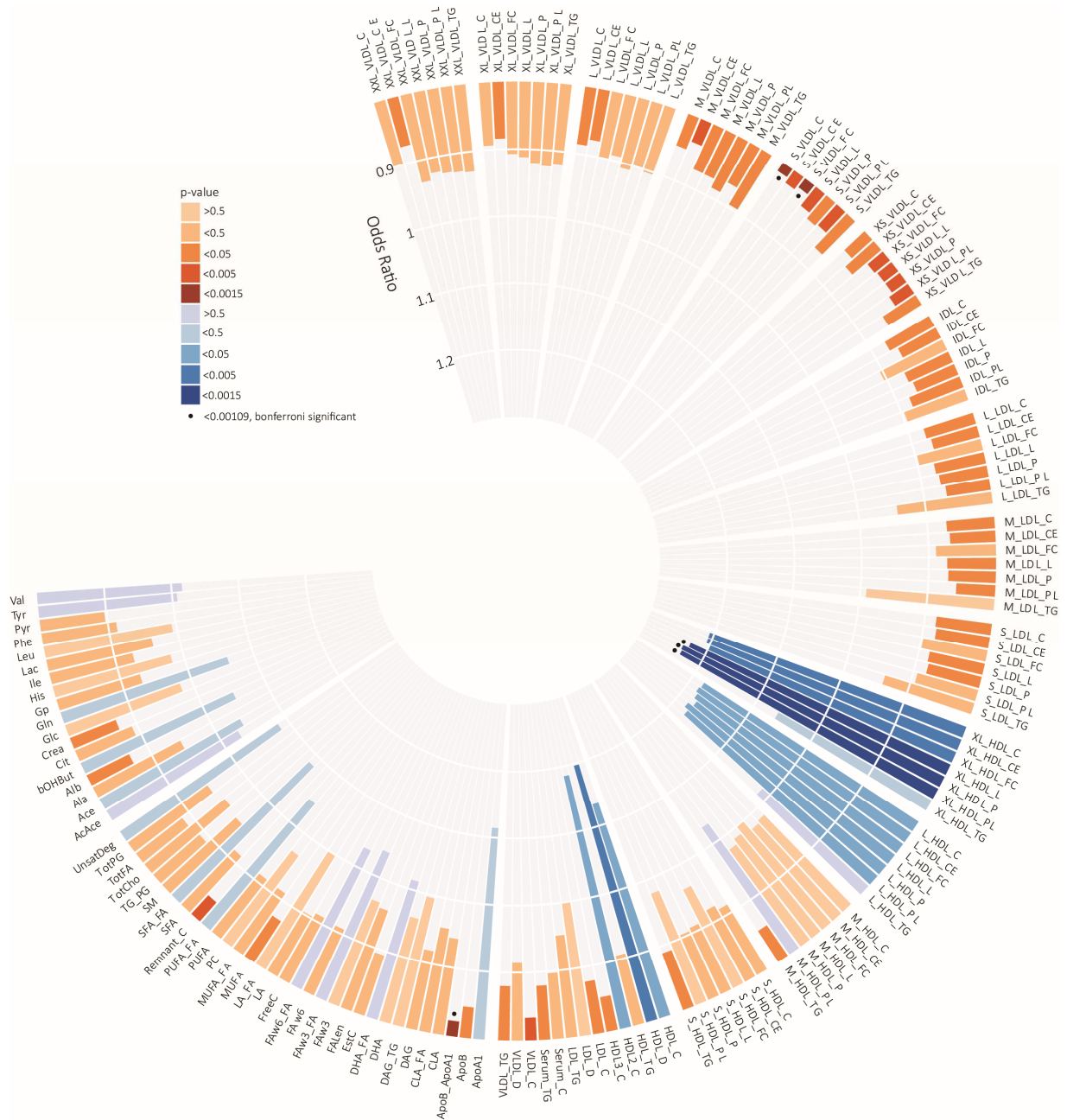
Betas are corrected for age, gender, lipid lowering drugs, plasma or serum, fasting state and study site. Betas indicate the effect of the risk allele versus the reference allele. Bonferroni: $0.05/60 = 0.00083$ (8.3×10^{-4}). Red is negative effect size, blue is positive effect size.

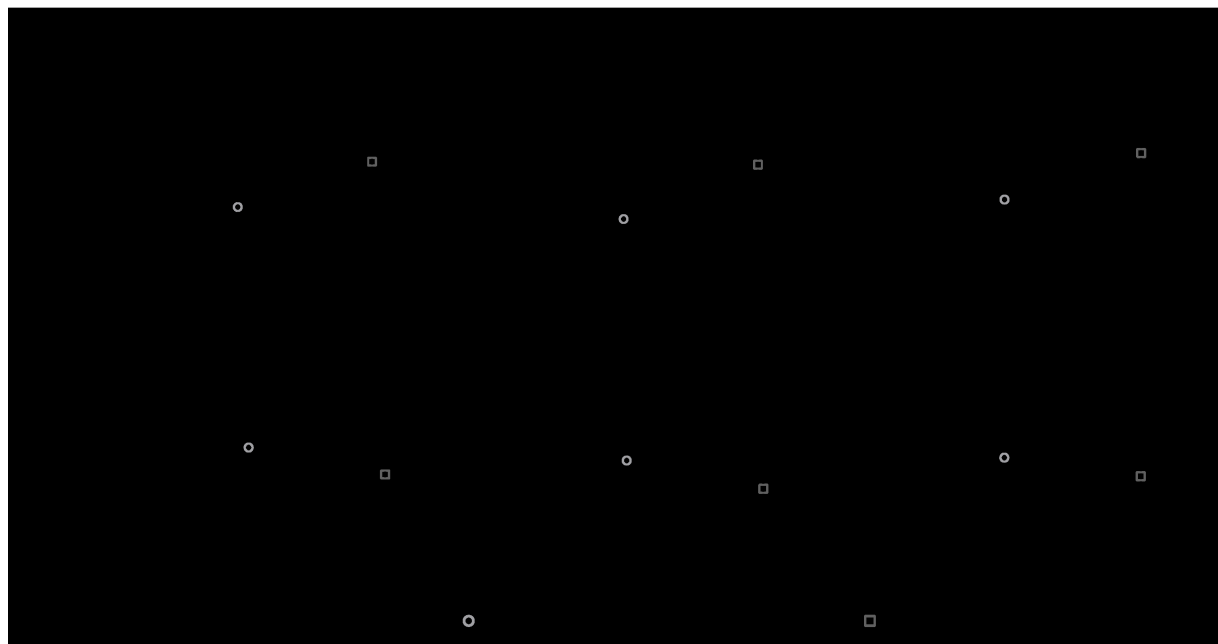
Table 20B. Linear regression model estimating the effect of SNPs on lipid sub fraction.

Lipid	<i>CETP</i> rs17231506 Risk allele T, reference allele C	<i>LIPC</i> rs2043085 Risk allele C, reference allele T	<i>LIPC</i> rs2070895 Risk allele G, reference allele A	<i>APOE</i> rs429358 Risk allele T, reference allele C	<i>APOE</i> rs73036519 Risk allele G, reference allele C	<i>ABCA1</i> rs2740488 Risk allele A, reference allele C
Percentage extra- large HDL	0.15 (p= 7.68×10^{-7})	-0.14 (p= 1.00×10^{-6})	-0.13 (p= 1.55×10^{-4})	-0.07 (p= 0.112)	0.04 (p=0.167)	0.05 (p=0.164)
Phospholipids in extra-large HDL	0.15 (p= 3.51×10^{-7})	-0.13 (p= 4.0×10^{-6})	-0.13 (p= 1.04×10^{-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 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= 4.06×10^{-7})	-0.14 (p= 8.59×10^{-7})	-0.12 (p= 3.13×10^{-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)

Betas are corrected for age, gender, lipid lowering drug usage, sub fractions are log+1 and standardized. Betas indicate the effect of the risk allele versus the reference allele.

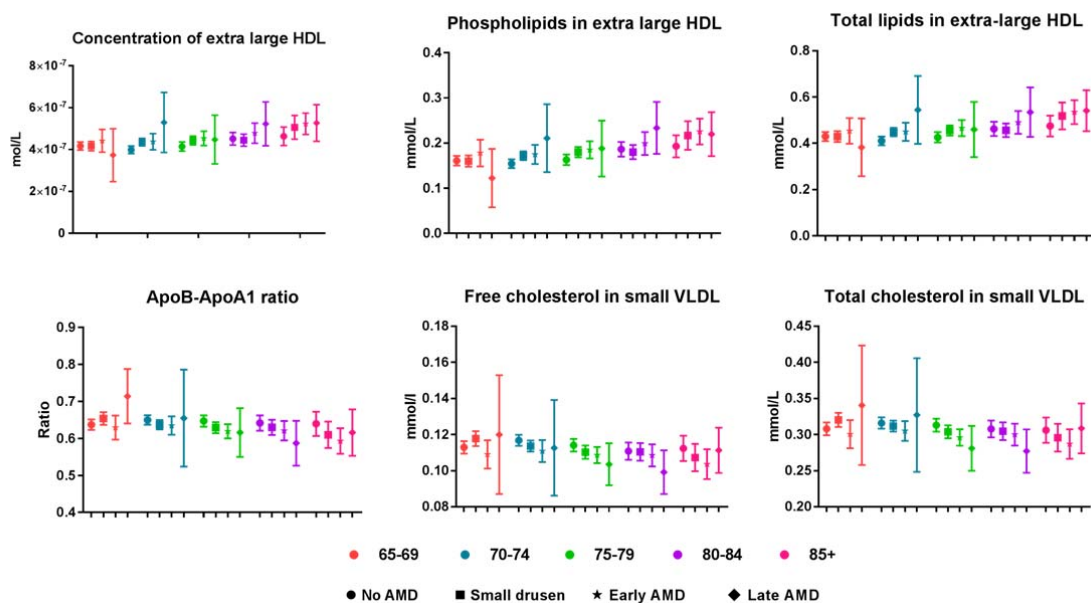






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Figure 4. Dose dependent relationship of the six associated lipid sub fractions with AMD per age category. Error bars indicate 95% confidence intervals of the mean.



Précis:

HDL-cholesterol is positively associated with AMD and triglycerides negatively. This is most prominently seen in drusen as early AMD features. This association seems to be driven by larger HDL sub fractions and HDL related genetics.

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