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Original Article

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The effects of eight serum lipid biomarkers on age-related macular degeneration risk: a Mendelian randomization study

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Abstract

Background: Age-related macular degeneration (AMD) is a leading cause of vision loss. Whereas lipids have been studied extensively to understand their effects on cardiovascular diseases, their relationship with AMD remains unclear.

Methods: Two-sample Mendelian randomization (MR) analyses were performed to systematically evaluate the causal relationships between eight serum lipid biomarkers, consisting of apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), direct low-density lipoprotein cholesterol (LDL-C), lipoprotein A [Lp(a)], triglycerides (TG) and non-HDL cholesterol (non-HDL-C), and the risk of different AMD stages and subtypes. We derived 64–407 genetic instruments for eight serum lipid biomarkers in 419 649 participants of European descent from the UK Biobank cohort. We conducted genome-wide association studies (GWAS) for 12 711 advanced AMD cases [8544 choroidal neovascularization (CNV) and 2656 geographic atrophy (GA) specific AMD subtypes] and 5336 intermediate AMD cases with 14 590 controls of European descent from the International AMD Genomics Consortium.

Results: Higher genetically predicted HDL-C and ApoA1 levels increased the risk of all AMD subtypes. LDL-C, ApoB, CHOL and non-HDL-C levels were associated with decreased risk of intermediate and GA AMD but not with CNV. Genetically predicted TG levels were associated with decreased risk of different AMD subtypes. Sensitivity analyses revealed no evidence for directional pleiotropy effects. In our multivariable MR analyses, adjusting for the effects of correlated lipid biomarkers yielded similar results.

Conclusion: These results suggest the role of lipid metabolism in drusen formation and particularly in AMD development at the early and intermediate stages. Mechanistic studies are warranted to investigate the utility of lipid pathways for therapeutic treatment in preventing AMD.

Key words: Lipids, age-related macular degeneration, Mendelian randomization, causal effect, UK Biobank

Key Messages

- The association between lipids and age-related macular degeneration (AMD) is inconsistent and the relationships between lipid subfractions and different AMD stages and subtypes have not been well studied.
- In the largest study to date, we have sufficient power for a two-sample Mendelian randomization analysis to evaluate the causal relationship between different lipid subfractions and the risk for different AMD stages and subtypes.
- Our study provides genetic evidence that circulating high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A1 (ApoA1) levels increase the risk of all AMD subtypes, whereas low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), total cholesterol (CHOL) and non-HDL-C levels are particularly associated with decreased risk of intermediate and geographic atrophy AMD.
- These findings help us glean a better understanding of the role of lipid metabolism in drusen formation and particularly in AMD development at both the early and intermediate stages.

Introduction

Age-related macular degeneration (AMD) is a leading cause of vision loss among the elderly in Western countries.[1–3] The global prevalence of AMD is 8.7% among individuals aged ≥45 years, with a higher prevalence of 12.3% in Europeans.[2] The progression of AMD is classified as early, intermediate and late stage.[4,5] The clinical hallmark in the early stage of AMD is the presence of drusen, which are formed by deposits of extracellular debris between the retinal pigment epithelium and Bruch's membrane.[6] The initiation and formation of drusen are not yet well understood; histochemical studies support an 'oil spill' model, indicating lipid-rich extracellular lesions in drusen.[7–9] Approximately 40% of drusen content is comprised of lipids.[10] Intermediate AMD is characterized by extensive intermediate drusen or at least one large drusen.[11] AMD has two advanced types: (i) geographic atrophy (GA, dry) AMD, accounting for 90% of AMD, is characterized by drusen and retinal pigment epithelium degeneration (focal hyperpigmentation or atrophy); and (ii) choroidal neovascularization (CNV, wet) AMD, is characterized by abnormal vascular proliferation underneath the retina. Currently, anti-vascular endothelial growth factor therapies have been used to reduce the progression of CNV.[12] However, the treatment is not curative, and there are no effective medications for GA. Moreover, a better scenario is to treat AMD at an earlier stage before serious vision loss occurs. It is therefore important to find new pathogenesis pathways and intervention targets for AMD.

In recent years, epidemiological and genetic studies have shown the potential role of lipids in AMD risk.[13–19] For instance, an observational meta-analysis reported that a higher level of high-density lipoprotein cholesterol (HDL-C) was associated with an increased risk of AMD, whereas higher levels of total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were associated with a decreased risk of AMD.[14] However, observational studies have shown inconsistent results with respect to the association between lipids and AMD risk,[14,17] and are susceptible to confounding factors or influenced by reverse causality.[13,14,18]Genome-wide association studies (GWAS) have identified > 50 variants associated with AMD, and some of them are also associated with lipid traits, such as *ABCA1*, *APOE*, *CETP*, *LIPC* and *VEGFA*.[20,21]

Mendelian randomization (MR) is an approach to investigate the causal relationships between risk factors and outcomes via the use of genetic variants as natural experiments. Compared with traditional observational studies, MR is less likely to be affected by confounding or reverse causation.[22,23] Two previous MR studies have shown a causal relationship between increased HDL-C levels and advanced AMD risk.[15,16] However, the relationship between HDL-C and different AMD subtypes remains unclear. More importantly, the associations between different lipid subfractions, such as apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB) and lipoprotein A [Lp(a)], and AMD risk have not been well studied. Elucidating these relationships might help us identify lipid-modifying therapeutic targets for AMD.

In this study, we systematically investigate the association between eight major serum lipid biomarkers [ApoA1, ApoB, CHOL, HDL-C, LDL-C, Lp(a), TG, and non-HDL-C] and the risk of different AMD subtypes using large scale genetic data from the UK Biobank and the International AMD Genomics Consortium via a two-sample MR

framework. To our knowledge, our study is the first to consider the effect of a wide range of lipid biomarkers [eight in total, including ApoA1, ApoB and Lp(a)] on the risk of AMD and its subtypes. This study would help us glean a better understanding of the role of lipids in different AMD stages and subtypes, and provide therapeutic implications for AMD.

Methods

We performed GWAS for each of the eight serum lipid biomarkers in the UK Biobank cohort to identify genetic instruments. We then conducted a series of GWAS analyses on AMD outcomes of interest (namely, for intermediate AMD, advanced AMD and its subtypes CNV and GA) using the individual-level data from the International AMD Genomics Consortium (independent samples from UK Biobank). Causal inferences can then be drawn via two-sample MR analysis to evaluate the potential causal relationships between each of the eight serum lipid biomarkers and different AMD subtypes using GWAS summary statistics.[24]

The UK Biobank study was approved by the National Research Ethics Service Committee North West—Haydock, all participants provided informed written consent, and all study procedures were performed in accordance with the World Medical Association Declaration of Helsinki ethical principles for medical research. In the International AMD Genomics Consortium, all groups collected data according to the Declaration of Helsinki principles. All study participants provided informed consent, and protocols were reviewed and approved by the local ethics committees.[20]

Serum lipid biomarkers in UK biobank

The UK Biobank is a prospective cohort study with deep genetic and phenotypic data collected on half a million people aged between 40 and 69 years across the UK.[25] The sample collection and quality control procedures for serum lipid biomarkers were described in detail elsewhere (see: http://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/biomarker_issues.pdf). We identified 438 870 individuals who were genetically similar to those of White British ancestry.[26] For lipid biomarker GWAS analyses, we only included participants of White British ancestry.[26] The serum lipid biomarkers ApoA1, ApoB, CHOL, HDL-C, direct LDL-C, Lp(a) and TG, were measured using standard procedures in a Beckman Coulter AU5800. We calculated non-HDL-C by subtracting HDL-C from total cholesterol.[27] The sample size and characteristics for each of the serum lipid biomarkers are presented in Table 1. The distributions of some serum lipid biomarkers were right-skewed [such as Lp(a) and TG, Supplementary Figure S1, available as Supplementary data at *IJE* online]. We applied a rank-based inverse-normal transformation to the concentration values for each lipid biomarker in order to interpret genetic estimates in standard deviation (SD) units.[28] We computed the phenotypic correlation between lipid biomarkers using the transformed concentration values (Supplementary Figure S2, available as Supplementary data at *IJE* online).

Genetic instruments for serum lipid biomarkers

For the GWAS of serum lipid biomarkers, we constructed linear mixed models using BOLT-LMM software (version

Table 1 Serum lipid biomarkers in the UK Biobank[a]

Variables	*n*	Mean (SD)	Median (IQR[b])	*n* SNPs[c]	Variance explained
Sex	419 649	227 003 (54%)[d]	–	–	–
Age, years	419 649	56.83 (8.01)	58 (51 to 63)	–	–
ApoA1, g/L	382 867	1.54 (0.27)	1.52 (1.35 to 1.7)	407	0.11
ApoB, g/L	417 522	1.03 (0.24)	1.02 (0.87 to 1.18)	241	0.13
CHOL, mmol/L	419 516	5.71 (1.14)	5.67 (4.93 to 6.44)	231	0.09
HDL-C, mmol/L	384 986	1.45 (0.38)	1.4 (1.18 to 1.68)	488	0.12
Direct LDL-C), mmol/L	418 780	3.57 (0.87)	3.53 (2.96 to 4.13)	215	0.10
Lp(a), nmol/L	334 646	44.12 (49.49)	20.11 (9.33 to 60.1)	64	0.16
TG, mmol/L	419 185	1.76 (1.02)	1.49 (1.06 to 2.16)	394	0.11
non-HDL-C, mmol/L	384 915	4.26 (1.08)	4.2 (3.49 to 4.94)	207	0.09

[a]The biochemistry markers are described on http://biobank.ctsu.ox.ac.uk/crystal/label.cgi? id=17518.
[b]IQR, interquartile range.
[c]Number of genetic instruments.
[d]The frequency and percentage of females are presented.

2.3).[29] The models were adjusted for sex and age. The first ten principal components were also included as covariates to speed up the convergence of BOLT-LMM's mixed model computations. The genetic instruments for each of the serum lipid biomarkers were selected based on the following criteria: (i) *P*-value from GWAS $< 5 \times 10^{-8}$; (ii) linkage disequilibrium (LD) between single nucleotide polymorphisms (SNPs) $r^2 < 0.001$ within a clumping window of 10 000 kb;[30] and (iii) the SNPs being present in the AMD GWAS summary statistics (described below). We randomly selected 5000 UK Biobank White British ancestry individuals as the reference panel.[31] The LD-clumping procedure was performed using PLINK (version 1.9).[32]

AMD datasets

The International AMD Genomics Consortium has the largest collection of European AMD samples (16 144 advanced AMD cases and 17 832 controls, Supplementary Table S1, available as Supplementary data at *IJE* online).[20] A detailed description of the study design, AMD subtype definitions and genetic data were presented previously.[20] In brief, AMD samples were gathered from 26 studies with each including: (i) intermediate AMD cases with >5 macular drusen >63 μm in diameter or pigmentary changes in the retinal pigment epithelium and age at first diagnosis >50 years; (ii) advanced AMD cases with CNV and/or GA in at least one eye and age at first diagnosis >50 years; (iii) controls without known advanced or intermediate AMD.[20] The individual level AMD phenotypic and genetic data were obtained from the database of Genotypes and Phenotypes (dbGaP, study accession: phs001039.v1.p1).[20] The genetic imputation was based on the 1000 Genomes Project reference panel (1KGP Phase I, version 3) using Minimac.[20] The SNPs were filtered by imputation quality score (>0.3) and minor allele frequency (MAF > 0.01) for association analysis. In the association analysis, non-European ancestry participants were removed based on the first two principal components inferred ancestry.[20] For different AMD subtype GWASs, we included 5336 intermediate AMD cases, 8544 CNV cases, 2656 GA cases, 12 711 advanced AMD cases (CNV, GA cases, and 1511 mixed AMD cases with both CNV and GA) and 14 590 controls. The association analyses were implemented in PLINK software (version v2.00a1LM) adjusting for sex, age and the first ten principal components.

Statistical analysis

The R packages MendelianRandomization and TwoSampleMR were used for MR analyses.[33,34] All general analyses were performed with R (version 3.4.1). We used a two-sided alpha at 0.00625 (0.05/8) to account for the multiple testing of eight lipid-related traits, although given the high genetic correlation between the lipid-related traits the Bonferroni correction can be considered overly conservative.

Power calculation for MR analysis

We first assessed the power of the MR analyses for different lipid biomarkers with different AMD subtypes. We calculated the phenotypic variance explained by genetic instruments for each biomarker using the formula $(2 \times \text{MAF} \times (1 - \text{MAF}) \times \text{beta}^2)/\text{var(biomarker)}$, where beta is the estimated effect size of each SNP and var(biomarker) is variance (typically very close to one) after the rank-based inverse-normal transformation.[35] We assumed different effect sizes of lipid biomarkers on AMD risk, and used the mRnd (http://cnsgenomics.com/shiny/mRnd/) method to calculate the power for MR analyses.[36]

Univariable MR analysis

For the two-sample MR analysis between each lipid biomarker and AMD risk, the univariable inverse-variance weighted (MR-IVW) method was used in the main analysis.[37,38]MR-IVW is a weighted linear regression method to regress the effects of genetic instruments on AMD (outcome) against their effects on lipid biomarker (exposure), with a forced intercept term at zero and weighted by inverse-variance.[37]

Sensitivity analysis

We then conducted various sensitivity analyses which allow violations of MR assumptions to assess the robustness of MR findings.[39] In particular, the weighted median MR method enables robust inference to be made providing >50% of the genetic variant weights are from valid instruments.[39] The MR-Egger method models an intercept term to detect and correct for bias due to directional pleiotropy.[34,39] Although pleiotropy is concerning, if the pleiotropic effects of genetic instruments average to zero (equally likely to be positive or negative, no directional pleiotropy), the overall estimate would be unbiased.[39] The intercept term from the MR-Egger method was used to assess evidence for directional pleiotropy (i.e. intercept close to zero and *P* value > 0.05).[34] We also applied the MR pleiotropy residual sum and outlier (MR-PRESSO) method to evaluate potential bias from outliers and assess the overall heterogeneity of our MR estimates.[40] The MR-PRESSO method can identify outlier variants and correct for their effects via outlier removal (MR-PRESSO outlier test). We

also implemented a leave-one-chromosome-out analysis by excluding genetic variants in each chromosome out in turn and re-computing the MR-IVW estimates, as a means to assess the influence of particular genes from the same chromosome on the overall MR findings.

Bi-directional MR analyses were used to estimate the potential effects of different AMD subtypes on serum lipid biomarker levels. In the reverse-directional analysis, the genetic instruments for different AMD subtypes were selected via similar criteria as was the case for serum lipid biomarkers as described earlier.

Multivariable MR analysis

We performed a regression-based multivariable MR (MVMR) analysis by selecting groups of exposures to avoid collinearity (Figure 1). In the multivariable MR-IVW analysis, the genetic instrumental variables associated with any of the included set of exposures were included.[41,42] The multivariable MR-Egger method is an extension of the univariable MR-Egger method to account for multiple lipid biomarkers, and at the same time models an intercept term to correct for both measured and unmeasured pleiotropy.[43]

We also used a recently developed MVMR approach based on Bayesian model averaging (MR-BMA) that scales to high-throughput data to detect true causal risk factors even when the candidate risk factors are highly correlated.[44,45] In the MR-BMA analysis, we included all genetic variants that were genome-wide significant for any lipid biomarker and selected 807 independent genetic variants as instrumental variables. The genetic correlation between lipid biomarkers was computed using the effect sizes of the independent genetic variants. The MR-BMA used a shotgun stochastic search algorithm to evaluate the posterior probability of all combinations of risk factors and then computed for each risk factor its marginal inclusion probability. More details are given in the Supplementary Material, available as Supplementary data at *IJE* online.

Results

Serum lipid biomarkers, genetic instruments and statistical power

We included 419 649 participants with at least one lipid biomarker measured in the UK Biobank. The proportion of females was 54% and the mean age was 56.83 (SD 8.01) years (Table 1). We observed a high genetic correlation between ApoA1 and HDL-C, ApoB and LDL-C/CHOL/non-HDL-C levels (maximum genetic association of $|r| < 0.978$, Figure 1 and Supplementary Figure S3, available as Supplementary data at *IJE* online). For different serum lipid biomarkers, we identified 64–407 genome-wide significant independent variants as genetic instruments, and they collectively explained 9–16% of the phenotypic variance (Table 1). We calculated the MR analysis statistical power for different AMD subtypes; even with 9% variance explained, our power for intermediate, advanced AMD, GA and CNV AMD subtypes was 95, 100, 79 and 98%, respectively, assuming moderate effect

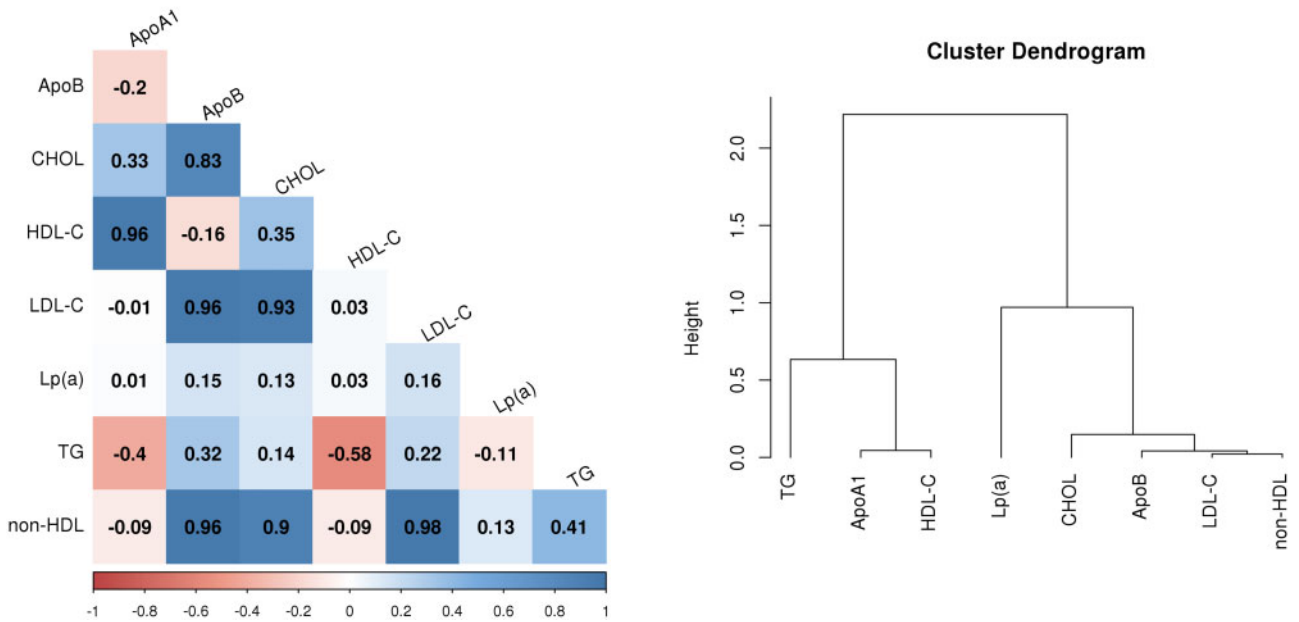

Figure 1 The genetic correlation and cluster of eight serum lipid biomarkers. The left panel displays genetic correlation between each pair of serum lipid biomarkers based on $n = 807$ independent genetic variants that were genome-wide significant for any lipid biomarker. The right panel shows the cluster of the eight serum lipid biomarkers

sizes [e.g. odds ratio (OR) 1.2, Supplementary Table S2, available as Supplementary data at *IJE* online).

The associations between eight serum lipid biomarkers and different AMD subtypes

In the univariable MR analysis, one SD higher HDL-C levels increased the risk of advanced AMD by 19% [MR-IVW OR 1.19, 95% confidence interval (CI) 1.07–1.33, P value 1.2×10^{-3}]. The association was consistent across different AMD subtypes and across different MR methods (weighted median, MR-Egger, Figure 2 and Supplementary Table S3, available as Supplementary data at *IJE* online). As expected given the high correlation with HDL-C, higher ApoA1 levels were also associated with increased risk of different AMD subtypes.

Raised LDL-C levels were nominally associated with decreased risk of advanced AMD (OR 0.87, 95%

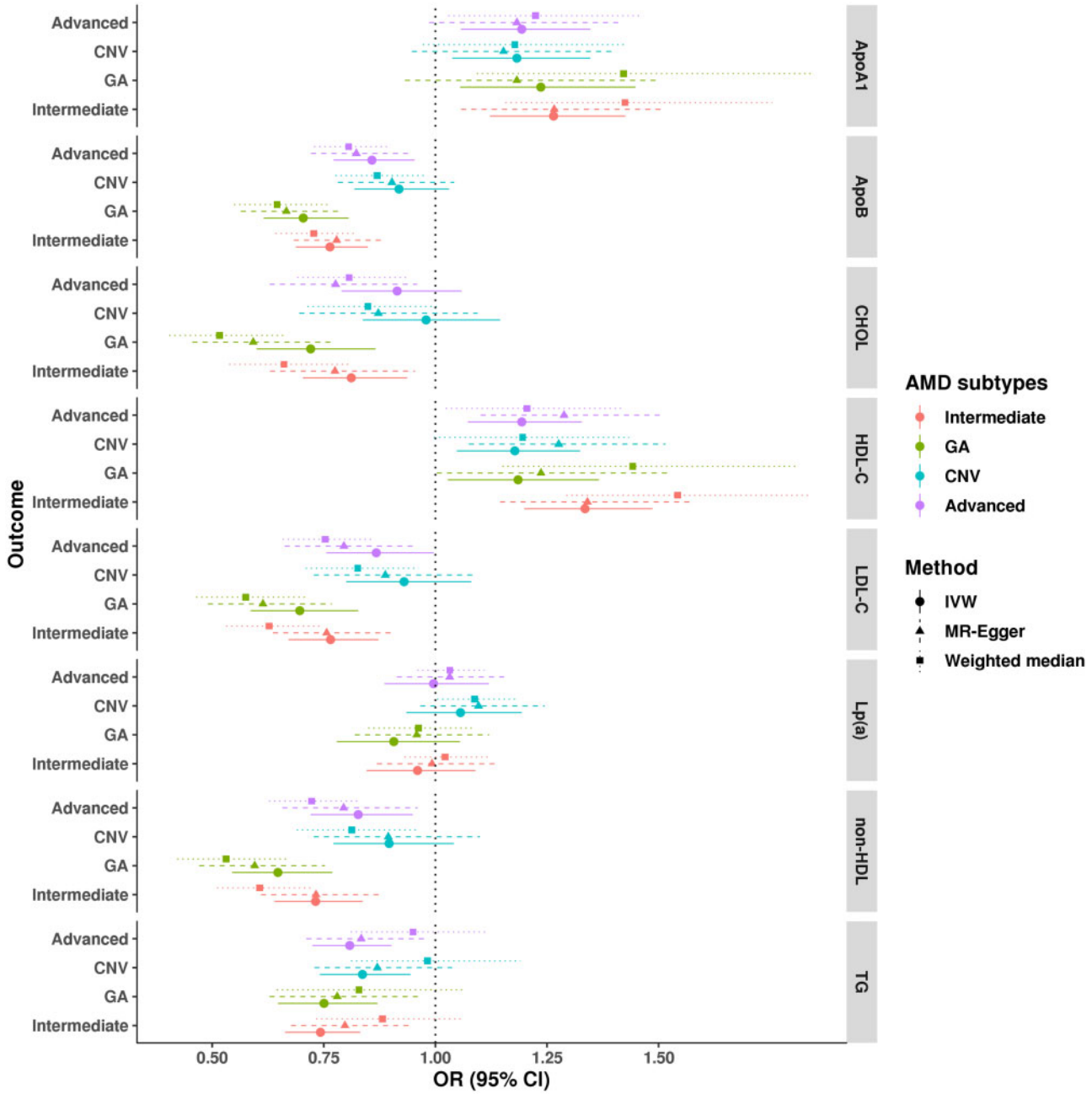

Figure 2 Univariable MR estimates of the associations between eight serum lipid biomarkers and different AMD subtypes. The *x*-axis is the OR of the effects of lipid biomarkers on AMD subtypes. The vertical dashed line is the reference at OR = 1. The *y*-axis presents different AMD subtypes, highlighted in different colours. Different MR methods are displayed with different line types (MR-IVW, solid line; MR-Egger, dashed line; weighted median, dotted line)

CI 0.76–1.00, *P* value 0.04). However, when split by AMD subtype, the association was primarily with GA (OR 0.70, 95% CI 0.59–0.83, *P* value 3.8×10^{-5}) and intermediate (OR 0.77, 95% CI 0.67–0.87, *P* value 6.5×10^{-5}) AMD; there was no strong evidence for association with CNV AMD (OR 0.93, 95% CI 0.80–1.08, *P* value 0.34). Similarly, for the correlated traits ApoB, CHOL and non-HDL-C, all were not associated with CNV AMD, but were associated with GA and intermediate AMD.

Higher levels of TG were associated with decreased risk of different AMD subtypes, and the estimates were broadly consistent across different AMD subtypes (intermediate AMD OR 0.74, 95% CI 0.66–0.83, *P* value 2.5×10^{-7}; advanced AMD OR 0.81, 95% CI 0.72–0.90, *P* value 1.4×10^{-4}). Lp(a) levels were not associated with any of the AMD subtypes (intermediate AMD OR 0.96, 95% CI 0.85–1.09, *P* value 0.53; advanced AMD OR 1.00, 95% CI 0.89–1.12, *P* value 0.94) even though the variance explained by the genetic instruments for Lp(a) was higher than for any other lipid biomarkers.

Sensitivity analysis

We applied MR median-weighted and MR-Egger methods to validate the MR-IVW estimates (Figure 2, Supplementary Table S3, available as Supplementary data at *IJE* online); their estimates were broadly consistent with the MR-IVW method with overlapping CIs. The MR-Egger intercepts showed no evidence of directional pleiotropy effects (intercepts were ~0, $P > 0.05$).

We conducted MR-PRESSO outlier-corrected tests, and found that most of the MR analyses were not meaningfully changed after removing outlier variants except the effects of HDL-C, ApoA1 and TG on CNV AMD risk (Supplementary Figure S4 and Supplementary Table S4,available as Supplementary data at *IJE* online). The removed outlier SNPs were mainly from genes *CETP*, *LIPC*, *APOE* and *ABCA1*. Given the strong associations between variants in these genes and both lipid biomarkers and AMD risk, removing these variants would affect the estimated effect sizes in MR analyses.[20,46] To further investigate the robustness of the MR results, we applied a leave-one-chromosome-out analysis by leaving genetic variants in each chromosome out in turn for the MR analyses (Supplementary Figure S5, available as Supplementary data at *IJE* online). We found a striking difference in the results for Lp(a) depending on chromosome 6. However, most of the variance in Lp(a) is controlled by variants in *LPA* (96.9%, in chromosome 6). We found no association between the SNP rs10455872 [the top SNP in the *LPA* region associated with Lp(a) levels] and AMD risk (OR = 1.03, $P = 0.40$ for advanced AMD; OR = 0.97, $P = 0.61$ for GA AMD).

We found weak evidence of liability towards AMD on lipid traits via reverse-direction MR analyses (Supplementary Figure S6, available as Supplementary data at *IJE* online). To investigate the influence of lipid-related drugs on our MR results, we identified 87 904 participants taking statins (data coding C10AA) in the UK Biobank.[47] We also found 6030 participants with self-reported or medical electronic health records of macular degeneration. We removed both statin users and AMD cases in the UK Biobank to re-select the genetic instruments for serum lipid biomarkers from GWAS. The MR results were unchanged (Supplementary Figure S7, available as Supplementary data at *IJE* online).

Multivariable MR

We conducted multivariable MR analyses (MVMR-IVW method) to estimate the direct effects of serum lipid biomarkers on AMD risk conditional on other serum lipid biomarkers. We selected groups of exposures to avoid collinearity. In the classic trio (HDL-C, LDL-C and TG), we included $n = 700$ independent SNPs associated with any of the three biomarkers as instrumental variables. The associations of HDL-C and LDL-C with AMD risk were essentially unchanged in multivariable MR analyses compared with univariable MR analysis (first column in Figure 3). We further replaced HDL-C with ApoA1 in the trio (i.e. ApoA1, LDL-C and TG, second column in Figure 3), and the results were similar to the trio HDL-C, LDL-C and TG. The MVMR results for Lp(a), CHOL and non-HDL-C were similar to univariable MR results (columns 3, 4 and 5 in Figure 3). The multivariable MR-Egger intercepts showed no evidence of directional pleiotropy effects (Supplementary Table S5, available as Supplementary data at *IJE* online).

We conducted multivariable MR-BMA analyses to select causal serum lipid biomarkers. When the prior probability was set at 0.125 or 0.25 (corresponding to *a priori* of one or two expected causal biomarkers), we found ApoA1 has relatively higher probabilities and causal effects for all AMD subtypes, and TG has the highest probability to be the causal risk factor for intermediate AMD (Supplementary Figure S8 and Supplementary Material, available as Supplementary data at *IJE* online).

Discussion

We systematically evaluated the effects of eight serum lipid biomarkers on the risk of different AMD subtypes. We found that higher genetically predicted HDL-C and ApoA1 levels increased the risk of all AMD subtypes, whereas

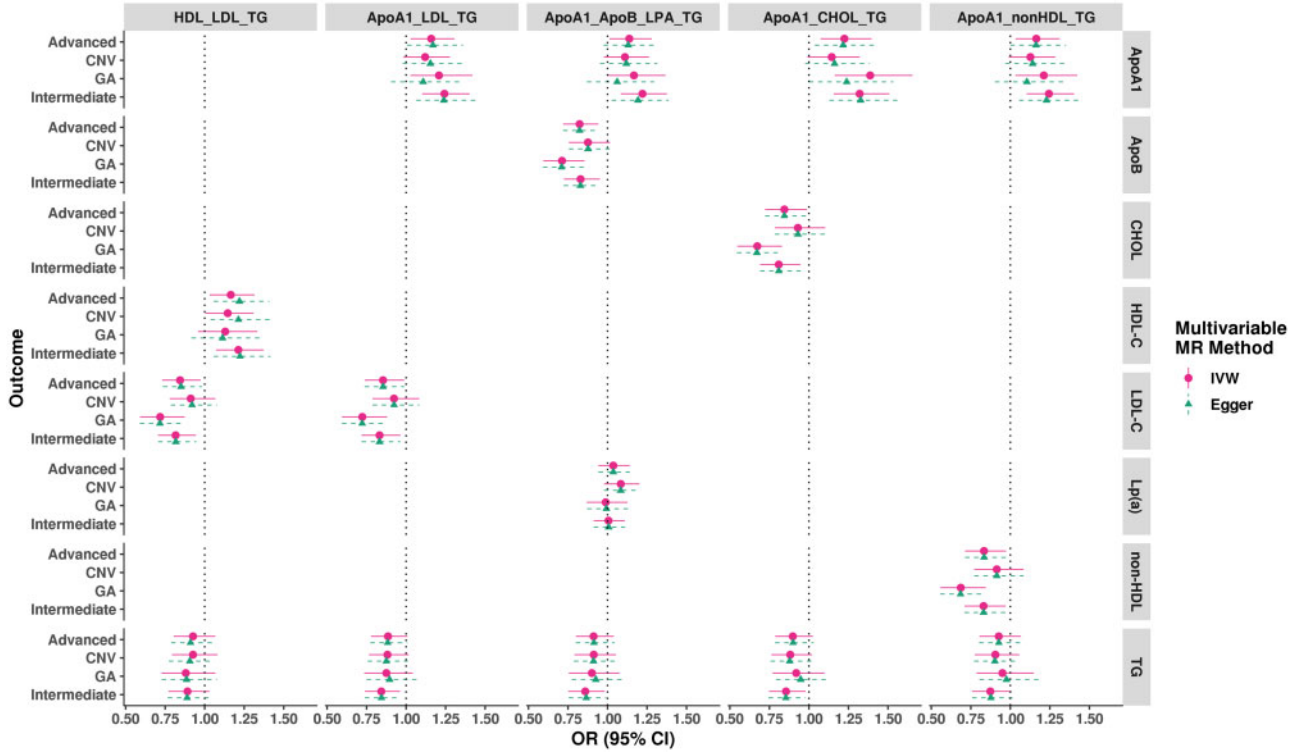

Figure 3 Multivariable MR estimates of the associations between eight serum lipid biomarkers and different AMD subtypes. The *x*-axis is the estimated OR for AMD subtypes per SD increase in genetically predicted lipid concentration levels for each lipid biomarker evaluated. The vertical dashed line is the reference at OR = 1. The *y*-axis lists the different AMD subtypes. The multivariable IVW estimates are shown with a solid line, whereas the multivariable estimates adjusted for the MR-Egger intercept are shown with a dashed line. Each column facet indicates the selected group of exposures in multivariable MR analysis, when all independent SNPs associated with any of the included exposures were fitted

LDL-C, ApoB, CHOL and non-HDL-C levels appeared to be only associated with decreased risk of intermediate and GA AMD. Genetically predicted TG levels were associated with decreased risk of different AMD subtypes. The role of lipids in cardiovascular disease risk is well studied. Compared with cardiovascular disease risk, most of these serum lipid biomarkers showed the opposite direction effects on AMD risk.[48] These findings suggest varying roles of lipids in different AMD stages and subtypes.

Previous observational studies have suggested a potential relationship between lipid biomarkers and AMD risk; however, the results were inconsistent.[13,14,18] We found that genetically elevated HDL-C levels increased the risk of AMD, consistent with findings from previous observational and MR studies.[14–16,18] Typically, HDL-C can mediate reverse cholesterol transport and have atheroprotective functions, such as anti-inflammatory, antioxidant and endothelial cell maintenance.[49] However, dysfunctionally elevated HDL-C could have pro-inflammatory and pro-oxidant roles that impair cholesterol efflux and promote the accumulation of drusen.[50,51] Our results indicate that the effect of HDL-C levels on intermediate AMD (OR 1.34, 95% CI 1.20–1.49) appeared larger than that on advanced AMD, which was also highlighted in a recent observational study,[18] where the effect sizes of estimates were broadly consistent with observational studies. We did, however, find evidence that the effect predicted by these HDL-C genetic instruments are rather heterogeneous. For instance, removing genetic instruments from the gene *CETP* (chromosome 16) attenuated the effect of HDL-C on AMD risk towards the null (e.g. for the CNV subtype); while excluding variants from the gene *LIPC* (chromosome 15) amplified the association (Supplementary Figures S4 and S5, available as Supplementary data at *IJE* online). These results suggest that serum HDL-C risk variants in *CETP* and *LIPC* might have counteracting effects on AMD risk, as discussed in previous literature.[15,18,52] We speculate that HDL-C-related genes may affect AMD risk via different pathways. As the major apolipoproteins in HDL-C particles (genetic correlation 0.96), higher ApoA1 levels also increase the risk of AMD. In our MR-BMA analysis, serum ApoA1 levels have relatively higher probabilities and effects for AMD compared with other lipid biomarkers. A recent study also showed that extra-large and large HDL particles are putative risk factors for AMD.[44]

The relationship between LDL-C and AMD risk has proved controversial in previous observational and genetic studies. For instance, a meta-analysis study showed a

protective tendency between LDL-C levels and AMD risk.[14] Further stratified analysis based on AMD subtypes revealed a protective effect on early stage, but not on late stage. A recent large-scale epidemiologic study also indicated that LDL-C levels were only associated with early AMD.[18] Previous MR studies, by contrast, showed no evidence of association between LDL-C levels and advanced AMD risk.[15,16] In this study, we observed a nominal association between higher LDL-C levels and decreased advanced AMD risk. Importantly, LDL-C levels exhibit a clear protective effect on intermediate and GA AMD subtypes. The nominal association between LDL-C and advanced AMD was likely driven by GA AMD subtype even though only a smaller proportion of advanced AMD cases were GA in the data sets. For ApoB, CHOL and non-HDL-C, all of them were associated with intermediate and GA AMD, but not CNV AMD. Previous observational studies have also shown that drusen are more likely to be involved in the development of GA AMD rather than CNV AMD.[53,54] These results suggest that LDL-C and ApoB may be involved in the formation of drusen in the early and intermediate stages of AMD, and the development of GA AMD;[55] in contrast, their roles in CNV AMD appear limited.

Previous observational studies have shown that higher TG levels reduce the risk of early stage AMD but not late stage.[14,18] In our univariable MR analysis, raised TG levels were associated with decreased risk of different AMD subtypes; however, the effect size on CNV AMD subtype was smaller and was not that robust based on MR-PRESSO outlier-corrected tests. In this study, we find no evidence of the association between Lp(a) and AMD risk. Serum Lp(a) levels are mainly genetically determined by genetic variations in the *LPA* gene region,[56,57] none of which showed association with AMD risk. We found a SNP rs7412 in *APOE* that is both associated with Lp(a) concentration and AMD risk. However, apoE proteins are thought to influence Lp(a) catabolism through lipoprotein receptor clearance pathways such as LDL receptor (maintains the plasma levels of LDL) rather than directly affecting Lp(a) assembly or secretion.[58]

These findings aid us in the understanding of lipid metabolism in drusen formation and AMD development, as well as the clinical implications of modifying blood lipid concentrations in preventing AMD. The clinical hallmark of early stage AMD is the presence of drusen, with ~40% of drusen content comprised of lipids. Lipids may be involved in the initiation and formation of drusen in the early and intermediate stages. This is supported by the associations between HDL-C/LDL-C/TG and intermediate AMD. Both CNV and GA AMD are subtypes of advanced AMD, the late stage of AMD that can cause vision loss. This study shows that LDL-C and TG are associated with GA AMD, and their roles in CNV AMD appear limited, suggesting different pathogenesis pathways for GA and CNV AMD subtypes. Currently, there are no effective medications for GA subtype, and the anti-vascular endothelial growth factor therapies for CNV are also not curative.[12] These MR findings suggest the potential utility of lipid modifying therapies in AMD treatment, and shed light on the different roles of lipid subfractions on different AMD subtypes (Figure 2). A recent study also showed that high-dose statins may have a particular role in large drusenoid deposits in AMD patients, and result in regression of large drusen and improvement of visual acuity.[59] Further clinical trials are warranted to investigate different lipid-modifying drugs in specific AMD subtypes rather than in a broad range of AMD subtypes.

A strength of this study is that we used large-scale data sets with standard protocols to measure lipid biomarkers; this allowed us to systematically evaluate the effects of lipids on AMD risk. Compared with traditional observational studies, MR findings are less likely to be affected by confounding or bias from reverse causation. To the best of our knowledge to date, this is the first study to have comprehensively evaluated the relationships between lipid/lipoprotein biomarkers and different AMD stages and subtypes through a MR framework. In particular, unlike some previous studies we have considered a wide range of lipids and lipoproteins. Dyslipidaemia has been involved in the formation of drusen, which are characterized in the early stage of AMD. This study based on different AMD stages and subtypes provides new insights into the role of lipids in AMD risk and development. At the same time, our results should be interpreted in light of the study's limitations. Firstly, as this study is based on European ancestry participants, the generalizability of our findings to other ethnic groups needs further investigation. Moreover, in a MR framework, the genetically predisposed biomarker changes are assumed to have a linear and lifetime effect on AMD risk. The potentially non-linear relationships and short-term effects of these biomarkers are unclear. This study indicates the role of circulating lipids on AMD risk; further studies are needed to investigate the effects of retina-specific lipid metabolism on AMD risk. Finally, in this study we used publicly available AMD samples and were unable to assess the potential selection bias due to competing risk, such as coronary artery disease (CAD). We performed an exploratory analysis computing the genetic correlation between CAD and AMD and found the correlations were close to zero (data not shown). We also conducted a MVMR analysis of CAD, LDL-C and TG (as three exposures) on AMD risk, and found no evidence of association between CAD and AMD risk (Supplementary

Figure S9, available as Supplementary data at *IJE* online). The MVMR results for LDL-C were essentially unchanged relative to the previous MVMR results in Figure 3, suggesting that broadly speaking our typically elderly AMD samples were not enriched for cardioprotective genetic factors; these results suggest our MR findings are unlikely to be driven by competing risk of conditions with shared aetiology.[60]

Conclusion

This study provides genetic evidence that elevated circulating HDL-C and ApoA1 levels increase the risk of all AMD subtypes, whereas LDL-C, ApoB, CHOL and non-HDL-C levels are particularly associated with decreased risk of intermediate and GA AMD. The inconsistent results from previous studies can be partly explained by the large heterogeneity of AMD disease (different stages and subtypes) in these studies. This study provides new insights into the pathogenesis of AMD. Further studies are warranted to investigate the role of lipid metabolism in drusen formation and AMD development in the early and intermediate stages, and the utility of lipid pathways for therapeutic treatment in preventing AMD.

Data Availability

UK Biobank data are available through the UK Biobank Access Management System https://www.ukbiobank.ac.uk/. The International Age-related Macular Degeneration Genomics Consortium data are available from the database of Genotypes and Phenotypes (dbGaP, study accession: phs001039.v1.p1). Applications are assessed for meeting the required criteria for access.

Supplementary Data

Supplementary data are available at *IJE* online.

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Conflict of Interest

None declared.

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