



Vision

HDL-cholesterol levels and risk of age-related macular degeneration: a multiethnic genetic study using Mendelian randomization

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Abstract

Background: Dyslipidemia, particularly high-density lipoprotein cholesterol (HDL-C), has recently been implicated in the pathogenesis of age-related macular degeneration (AMD), the leading cause of vision loss. However, epidemiological studies have yielded conflicting results.

Methods: We investigated the causal role of plasma lipid levels in AMD in multiethnic populations comprising 16 144 advanced AMD cases and 17 832 controls of European descent, together with 2219 cases and 5275 controls of Asian descent, using Mendelian

randomization in three models. Model 1 is a conventional meta-analysis which does not account for pleiotropy of instrumental variable (IV) effects. Model 2 is a univariate, inverse variance weighted regression analysis that accounts for potential unbalanced pleiotropy using MR-Egger method. Finally, Model 3 is a multivariate regression analysis that addresses pleiotropy by MR-Egger method and by adjusting for effects on other lipid traits.

Results: A 1 standard deviation (SD) higher HDL-cholesterol level was associated with an odds ratio (OR) for AMD of 1.17 (95% confidence interval: 1.07–1.29) in Europeans ($P=6.88 \times 10^{-4}$) and of 1.58 (1.24–2.00) in Asians ($P=2.92 \times 10^{-4}$) in Model 3. The corresponding OR estimates were 1.30 (1.09–1.55) in Europeans ($P=3.18 \times 10^{-3}$) and 1.42 (1.11–1.80) in Asians ($P=4.42 \times 10^{-3}$) in Model 1, and 1.21 (1.11–1.31) in Europeans ($P=3.12 \times 10^{-5}$) and 1.51 (1.20–1.91) in Asians ($P=7.61 \times 10^{-4}$) in Model 2. Conversely, neither LDL-C (Europeans: OR = 0.96, $P=0.272$; Asians: OR = 1.02, $P=0.874$; Model 3) nor triglyceride levels (Europeans: OR = 0.91, $P=0.102$; Asians: OR = 1.06, $P=0.613$) were associated with AMD. We also assessed the association between lipid levels and polypoidal choroidal vasculopathy (PCV) in Asians, a subtype of AMD, and found a similar trend for association of PCV with HDL-C levels.

Conclusions: Our study shows that high levels of plasma HDL-C are causally associated with an increased risk for advanced AMD in European and Asian populations, implying that strategies reducing HDL-C levels may be useful to prevent and treat AMD.

Key words: HDL-cholesterol, AMD, lipids, Mendelian randomization, genetic association

Key Messages

- In 33 976 European participants, plasma HDL-C levels, but not LDL-C or triglyceride levels, were causally associated with advanced age-macular degeneration (AMD) using a Mendelian randomization approach.
- Similar findings were observed for AMD and its subtype polypoidal choroidal vasculopathy in 7494 Asian samples.
- Our results propose elevated HDL-C as a key risk factor in AMD pathogenesis.

Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among elderly people in the USA and globally.^{1,2} New treatments of AMD such as anti-vascular endothelial growth factor (VEGF) therapy are useful in certain selected cases of neovascular AMD, but expensive and resource-intensive.³ Further understanding the pathogenesis of AMD to discover additional targets and new strategies is a major global research priority. Lipid metabolism has long been hypothesized to play an important role in the development of AMD^{4,5} on the basis of observations that deposits of lipid particles in Bruch's membrane of eyes represent at least 40% of the volume of drusen, an early sign of AMD.^{6,7} Furthermore, genome-wide association studies (GWAS) have identified several high-density lipoprotein cholesterol (HDL-C) genes associated with AMD susceptibility. These genes encode

protein including cholesterol ester transfer protein (CETP),^{8–11} hepatic lipase (LIPC),^{9,10,12,13} Apolipoprotein E (ApoE)^{9,14} and ATP-binding cassette subfamily A member 1 (ABCA1).^{13,15} Finally, small clinical trials suggest that high-dose statins, predominantly used to lower LDL cholesterol (LDL-C), may lead to resolution of AMD signs and vision improvement.¹⁶

However, epidemiological studies have shown conflicting results on the association between plasma lipid levels and AMD. Whereas multiple studies have reported associations between higher HDL-C levels and an increased risk of AMD,^{17–22} others have found no association between HDL-C and AMD risk.^{23–26} The evidence is further complicated by several epidemiological studies that report inverse associations, i.e. that higher HDL-C levels were associated with a lower risk of AMD.^{27–29} In contrast,

there are no evident associations from epidemiological studies between low-density LDL-C or triglycerides and AMD.^{17,19–21,25–27}

Inconsistent associations from epidemiological studies such as between HDL-C and AMD may indicate the presence of confounding, measurement errors, selection bias or reverse causality in the observational studies.^{30,31} Mendelian randomization is an emerging method that takes advantage of the naturally randomized allocation of parental alleles at meiosis to test whether modifiable exposures have a causal role on disease outcomes.^{32,33} Thus, genotypes correlated with the exposure can serve as instrumental variables (IVs) in Mendelian randomization studies.^{34–36} Subsequently, if plasma lipid levels are causally involved in the pathogenesis of AMD, the genetic variants that influence plasma lipid levels should affect the risk of AMD in the predicted direction and magnitude, assuming these genetic variants are conditionally independent of AMD when controlling for lipids and other confounders.

In this study, we examined the causal relationship between plasma lipid levels and advanced AMD using Mendelian randomization based on lipid-associated single-nucleotide polymorphisms (SNPs) as IVs. Our study is the first to evaluate a causal role for HDL-C, LDL-C and triglyceride levels on advanced AMD in a large, multiethnic cohort.

Methods

Study design

We conducted a global Mendelian randomization analysis in 16 144 cases and 17 832 controls of European descent, in 26 studies from North America, Europe, Australia and Israel, as part of the International AMD Genomics Consortium (IAMDGC),¹⁵ as well as 2219 cases and 5275 controls of Asian descent from four studies conducted in Singapore, Japan, Korea and China, as part of the Genetics of AMD in Asian (GAMA) Consortium (Table 1).⁸ Mendelian randomization employs genetic variants as IVs to estimate the causal effect of a risk factor on an outcome, in the presence of unmeasured confounding (Figure 1), given that the genotypes are conditionally independent of the disease status.^{30–33}

Our study consisted of three stages. First, we used lipid-associated independent SNPs previously identified in a large-scale GWAS in Europeans ($P < 5 \times 10^{-8}$) as IVs.^{37,38} We examined the effect of a given SNP on the risk of advanced AMD in European individuals. The causal effects of lipid traits on AMD in Europeans was estimated using three methods of Mendelian randomization analysis of multiple IVs. Second, we used the same set of SNPs as IVs and tested the association of these SNPs with lipid traits in Asian populations. These SNPs were carried forward for

subsequent association analysis of advanced AMD in Asian participants. The causal effects in Asians were estimated in the same approach as in Europeans. Separate analyses in Europeans and Asians ensure the validity of the two-sample Mendelian randomization approach, which assumes that the underlying population is homogeneous.^{35,39,40} Finally, we combined the causal effects of lipids on AMD in both populations using the fixed-effects meta-analysis.

All studies were performed with the approval of local Medical Ethics Committees.^{8,15,37} Signed informed consent form was obtained from all participants in accordance with the Declaration of Helsinki.

Study populations and instrumental variables

To conduct Mendelian randomization analyses in Europeans, we extracted summary association results for HDL-C, LDL-C and triglycerides from the lipid SNPs identified through a meta-analysis of GWAS in 188 578 European-ancestry individuals from the Global Lipids Consortium.^{37,38} These 185 genetic variants collectively accounted for 6.4% of the variance in HDL-C, 6.9% of the variance in LDL-C and 5.2% of variance in triglyceride, as estimated using the R package 'gtx'. We examined the association of SNPs with advanced AMD in 16 144 cases and 17 832 controls in 26 studies as part of the International AMD Genomics Consortium (IAMDGC) (Table 1).¹⁵ Two SNPs (rs1998013, rs16831243) were not available in GWAS data from IAMDGC, thus the analyses were conducted on 183 SNPs in Europeans including 96 SNPs associated with HDL-C, 80 SNPs with LDL-C and 60 SNPs with triglycerides.

For the Asian study, we tested the associations between these 185 SNPs and lipid traits in 25 420 Asians in 13 studies from the Asian Genetic Epidemiology Network (AGEN) Consortium (Supplementary Tables 1 and 2, available as Supplementary Data at *IJE* online). We further examined their associations with advanced AMD in 2219 cases and 5275 controls from four studies from the GAMA Consortium (Table 1).⁸ We excluded 36 SNPs that were unavailable, with imputation quality < 0.9 or that were not in Hardy-Weinberg equilibrium ($P < 1 \times 10^{-5}$) in AGEN or GAMA datasets. The four SNPs most strongly associated with lipids in AGEN samples (*CETP* rs3764261, *APOE* rs4420638, *PTLP* rs6124760 and *LCAT* rs8059305), not in the 185-SNPs list and not in LD ($r^2 < 0.2$) with these SNPs, were also included for the Asian study. This left 153 SNPs in our analysis, including 81 associated with HDL-C, 67 with LDL-C and 49 with triglycerides.

Diagnosis of AMD

The diagnosis of advanced AMD was based on clinical examinations using dilated fundus photography,

Table 1. Baseline characteristics of advanced AMD cases and controls by regions

Population	No. of Studies	Regions	Ethnicity	Advanced AMD	Control	Case recruitment	Control recruitment	AMD phenotyping	
European	26		Caucasian	16 144 (3235) ^b	17 832	Clinic/population-based/spouses/volunt.	Clinic/population-based/spouses/volunt.	FA, OCT, self-report ^d	
	13	USA	Caucasian						
Asian	9	Europe ^a	Caucasian	2219 (1062) ^c	5275	Clinic/population-based/spouses/volunt.	Clinic/population-based/spouses/volunt.	FA, OCT, self-report	
	3	Australia	Caucasian			Clinic	Clinic	FA, OCT	
	1	Israel	Caucasian			Clinic	Clinic	FA, OCT	
	4								
	1	Singapore	Chinese			Clinic	Population-based	FA, ICG, OCT	
	1	Hong Kong	Chinese			Clinic	Population-based	FA, ICG, OCT	
	1	Japan	Japanese			Clinic	Clinic	FA, ICG, OCT	
1	Korea	Korean	Clinic	Clinic	FA, ICG, OCT				

^aSample collections in UK, Germany, Netherlands, etc., as well as from the European Genetic Database. ^bNumber of samples in parentheses to indicate the number of subjects with geographic atrophy (GA) in at least one eye and no evidence of choroidal neovascular (CNV) in either eye. ^cNumber of samples in parentheses to indicate the number of subjects with polypoidal choroidal vasculopathy (PCV) in at least one eye. In Asian samples, ICG was performed to diagnose patients with PCV. ^dPractitioner-confirmed self-report. AMD, age-related macular degeneration; FA, fluorescein angiography; OCT, optical coherence tomography; ICG, indocyanine green angiography; volunt., recruitment of volunteers.

fluorescent angiography and optical coherence tomography. Cases with other macular diseases such as central serous chorioretinopathy or choroidal neovascularization due to high myopia and angioid streaks were excluded.

In European samples, advanced AMD cases were defined as choroidal neovascularization (CNV) and/or geographic atrophy (GA) in at least one eye and age at first diagnosis ≥ 50 .¹⁵ Among the 16 144 AMD cases, 10 749 (67%) had CNV, 3235 (20%) had GA and 2160 (13%) had a mixture of both phenotypes. A detailed description of phenotyping is provided in the [Supplementary Note](#) (available as [Supplementary Data](#) at *IJE* online).

In Asian samples, Indocyanine green angiography was performed to diagnose advanced AMD patients with polypoidal choroidal vasculopathy (PCV)⁴¹; PCV is common in Asians with distinct clinical characteristics compared with typical choroidal neovascular AMD (tAMD).^{42,43} Of the 2119 AMD cases, 1062 (50.1%) were classified as tAMD and 1157 (49.9%) as PCV.

Statistical analysis

The effect sizes of genetic variants associated with lipid traits were estimated in the standard deviation (SD) of the normally transformed trait values using a linear regression model.³⁷ We estimated the relationship between the given SNP and AMD using logistic regression analysis with AMD as the outcome, as described in previous publications.^{8,15}

Models for Mendelian randomization analyses

We conducted three models to estimate the causal association between lipids and AMD with multiple SNPs as IVs in each population.

Model 1 is a conventional meta-analysis, where we combined individual Mendelian randomization odds ratio ($OR_{i, lipid-AMD}$) estimate for each SNP i using a fixed-effects model.^{35,40} The association between lipids and AMD at SNP i was calculated as $\beta_{i, lipid-AMD} = \frac{\beta_{i, snp-AMD}}{\beta_{i, snp-lipid}}$. $\beta_{i, snp-lipid}$ is the estimate of SNP i effect on normalized lipid trait values. $\beta_{i, snp-AMD}$ is the natural logarithm of $OR_{i, snp-AMD}$ estimate of SNP i on the AMD risk. The variance of $\beta_{i, lipid-AMD}$ at SNP i was calculated using the delta method⁴⁴:

$$var(\beta_{i, lipid-AMD}) = \left(\frac{\beta_{i, snp-AMD}}{\beta_{i, snp-lipid}} \right)^2 \left(\left(\frac{se(\beta_{i, snp-lipid})}{\beta_{i, snp-lipid}} \right)^2 + \left(\frac{se(\beta_{i, snp-AMD})}{\beta_{i, snp-AMD}} \right)^2 \right).$$

A Cochran's Q test was applied to estimate heterogeneity I^2 of the IV estimates across SNPs. We performed a

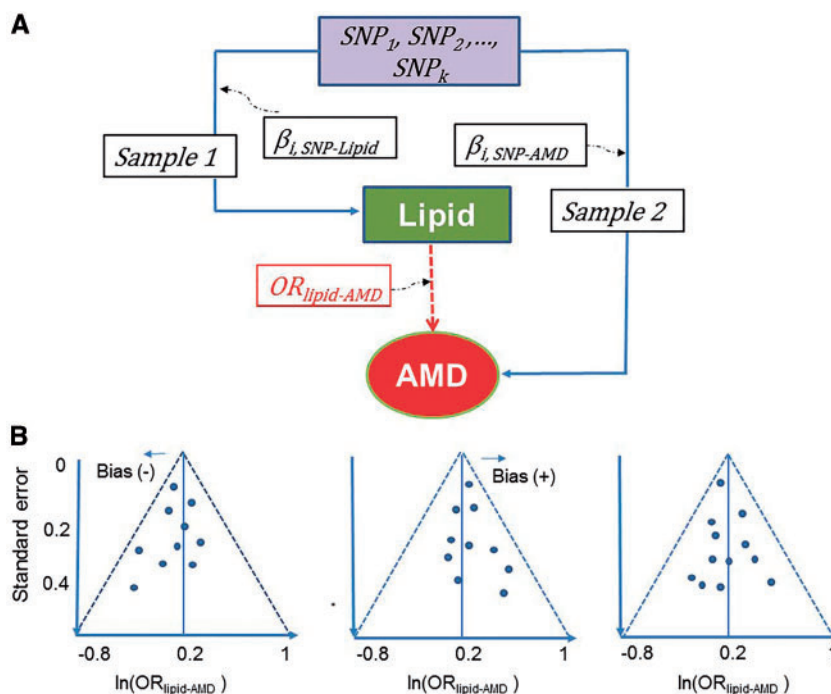


Figure 1. Diagram for Mendelian randomization and an illustration of unbalanced pleiotropy. (A) A set of lipid-associated independent Single-nucleotide polymorphisms ($SNP_1, SNP_2, \dots, SNP_k$) are used as instrument variables (IVs) to infer the causal association between the exposure (lipid) and the disease outcome (AMD). The effects of each SNP i (i up to 183) on lipid level ($\beta_{i, SNP-Lipid}$) and AMD ($\beta_{i, SNP-AMD}$) are assessed separately in sample 1 and sample 2 as shown in graph. The dashed arrow from lipid trait to AMD indicates the hypothesized causal association. Three models are used in the Mendelian randomization analysis: Model 1—conventional meta-analysis of individual IV estimates, $\log(OR_{i, lipid-AMD})$, at each SNP i where $\log(OR_{i, lipid-AMD}) = \frac{\beta_{i, SNP-AMD}}{\beta_{i, SNP-Lipid}}$; Model 2—inverse variance weighted (IVW) univariate regression: $\beta_{i, SNP-AMD} \sim \beta_{i, SNP-Lipid}$; Model 3—IVW multivariate regression: $\beta_{i, SNP-AMD} \sim \beta_{i, SNP-HDL} + \beta_{i, SNP-LDL} + \beta_{i, SNP-Triglycerides}$. AMD, age-related macular degeneration; OR, odds ratio. (B) An illustration of unbalanced pleiotropy. We assume that the true OR between lipid trait and AMD is 0.2 at the log scale (straight vertical line). In the funnel plots, each dot represents an individual IV SNP, with magnitude of the IV estimation on x-axis and standard error on y-axis. Unbalanced/directional pleiotropy refers to the presence of negative (–) bias where the IV estimates have mean shift to the left of the true value (left panel) and positive (+) bias where the IV estimates have mean shift to the right of the true value (middle panel). MR-Egger method is preferred to account for unbalanced pleiotropy due to unknown pathways. Systematic bias is not present in the balanced pleiotropy (right panel).

random-effect meta-analysis to combine $\beta_{i, lipid-AMD}$ estimates if $I^2 > 40\%$.

Model 2 is an inverse variance weighted (IVW) univariate regression-based analyses. We regressed the effect of SNP i and AMD associations ($\beta_{i, SNP-AMD}$) against the effect of SNP i and lipids associations ($\beta_{i, SNP-Lipid}$), with the regression line forced through the origin, as previously described.³⁹ The regression was weighted on the inverse variance of $\beta_{i, SNP-AMD}$.

Model 3 is an IVW multivariable regression-based analysis accounting for pleiotropic effects of other lipid traits. The analytic framework is the same as Model 2 except accounting for other lipid levels.³⁹ For example, to estimate $\beta_{HDL-AMD}$, we regressed the effects of SNP and AMD associations ($\beta_{i, SNP-AMD}$) against the effect sizes of SNP and HDL-C associations ($\beta_{i, SNP-HDL}$) by adjusting for the effect of SNP and LDL-C associations ($\beta_{i, SNP-LDL}$), as well as SNP and triglycerides associations ($\beta_{i, SNP-triglycerides}$).

For Models 2 and 3, we conducted 10 000 bootstraps, and our effect estimate is the mean of the bootstraps

with the 95% confidence interval (CI) determined from the empirical distribution. We further combined the $OR_{lipid-AMD}$ estimates from Europeans and Asians using the fixed-effects meta-analysis. The same analytic approach was repeated in Asians for AMD subtypes: PCV and tAMD. The R statistical software v3.2.5 was used in the analyses.

Unbalanced pleiotropy

Horizontal pleiotropy occurs where the genetic variant influences the disease outcome through a different pathway from the exposure under investigation.^{45,46} If there is no systematic bias and the pleiotropic effects happen to cancel out across a set of independent variants (instruments), the Mendelian randomization estimate remains valid. If the horizontal pleiotropy is unbalanced (or directional), it might result in a biased estimate as the mean bias term across variants is not equal to zero. Without a full knowledge of underlying pathways for each genetic variant, Mendelian randomization Egger regression (MR-Egger)

provides a statistical method to account for unbalanced pleiotropy of genetic IVs.⁴⁵

In the analyses, conventional Model 1 does not account for pleiotropy. Model 3 adjusts for other lipid traits that may mediate unbalanced pleiotropy. We applied the MR-Egger method^{45–47} as an additional sensitivity analysis in both Models 2 and 3, accounting for unbalanced pleiotropy due to unknown pathways. The presence of unbalanced pleiotropy was inferred if the intercept term was not zero using Egger's test.⁴⁵ If there was evidence of unbalanced pleiotropy (P -pleiotro < 0.05), we used the estimate from the MR-Egger test rather than a traditional Mendelian randomization estimate (i.e. intercept forced to 0).

Sensitivity analysis

We performed sensitivity analyses to test the robustness of the results. First, we assessed the impacts of SNPs that were genome-wide significantly associated with AMD on Mendelian randomization estimation. Two loci showed strongest genome-wide significance for AMD: *CETP* (rs9989419, rs5880) and *LIPC* (rs261342, rs1532085) in Europeans and *CETP* (rs3764261) in Asians. We therefore evaluated the $OR_{lipid-AMD}$ estimations by removing the SNPs at these two loci in analyses and SNPs at four loci previously showing association with AMD, including *CETP*, *LIPC*, *APOE* and *ABCA1*.^{13–15} We further assessed $OR_{lipid-AMD}$ estimations by omitting all SNPs showing associations with AMD ($P < 1 \times 10^{-4}$). Second, we performed an additional analysis restricting on a subset of SNPs showing associations with lipids at p -value < 0.05 (referred as restricted SNPs; no. of SNPs = 82) and at p -value $< 5 \times 10^{-8}$ in Asians (referred as strong IVs; no. of SNPs = 20).

Statistical power

Our power calculation suggested the Asian study has approximately 89% power to detect a true OR as large as 1.4 for AMD per SD of lipid level, under the assumption that the proportion of lipid variance explained by SNP IVs at $R^2 \sim 5\%$ and type-1 error of 0.05 (Supplementary Table 3, available as Supplementary Data at *IJE* online).⁴⁸ The statistical power becomes 69% and 39% if the true ORs for AMD decrease to 1.3 and 1.2, respectively. In contrast, the European study has more than 97% power to detect the association in all the scenarios above.

Results

We first evaluated the effects on AMD risk for lipid-associated SNPs in 16 144 cases and 17 832 controls of European descent, and 2219 cases and 5275 controls of

Asian descent, respectively. The study characteristics and sample sizes for advanced AMD cases and controls are shown in Table 1. Among all loci related to plasma lipids in European populations, *CETP* and *LIPC* loci showed associations with AMD at P -value $< 5 \times 10^{-8}$ (Supplementary Table 4, available as Supplementary Data at *IJE* online).

Using a Mendelian randomization approach, we found an association between genetically raised plasma HDL-C and the risk for advanced AMD in both European and Asian populations in all three models. A small unbalanced pleiotropy was noticed in the Asian population only (Table 2 and Supplementary Table 5, available as Supplementary Data at *IJE* online) with the funnel plots of individual IV estimates for SNPs in Supplementary Figure 1 (available as Supplementary Data at *IJE* online). Using conventional meta-analysis, we found that a 1-SD higher plasma HDL-cholesterol level was associated with an OR for AMD of 1.30 (95% CI 1.09–1.55) in Europeans ($P = 0.003$) and of 1.42 (1.11–1.80) in Asians ($P = 0.004$; Model 1; Table 2). IVW regression-based analysis showed a consistent trend of magnitude and direction of the estimates (Models 2 and 3). After accounting for pleiotropic effects of the SNPs on LDL-C and triglyceride levels in Model 3, there was an increase of 17% (1–29%) for the risk of AMD per 1-SD increase in HDL-C in Europeans ($P < 0.001$) and 58% (24–100%) in Asians ($P < 0.001$).

No evidence of associations was observed between LDL-C and AMD risk in either Europeans ($P \geq 0.150$) or Asians ($P \geq 0.498$) for all three models (Table 2). An association was noted in the meta-analysis (Model 1) and weighted univariate regression (Model 2) in the European dataset for triglycerides. However, after accounting for pleiotropic effects, no associations were found (Model 3). Similarly, no associations between AMD and LDL-C or AMD and triglycerides were observed in Asians (Figure 2).

We subsequently performed sensitivity analyses with a subset of SNPs to assess the robustness of the findings. We first evaluated the impact of the two most influential genes, *CETP* and *LIPC*, which showed genome-wide associations for AMD. Of note, *CETP* and *LIPC* HDL-C increasing alleles had opposite effects on AMD (Figure 2). In both European and Asian studies, the effects of HDL-C on the risk of AMD was greatly reduced after *CETP* variants were removed, but strengthened after *LIPC* variants were removed (Supplementary Table 6, available as Supplementary Data at *IJE* online). The association between HDL-C and AMD risk remained when both *CETP* and *LIPC* variants were omitted (Europeans: OR = 1.36; 1.20–1.53; Asians: OR = 1.48, 1.05–2.07; Table 3). Further removing the SNPs at four loci previously suggesting genome-wide significance with AMD and those

Table 2. Causal association of lipid levels and risk of advanced AMD

Lipid	Population	# Case/control	P_{pleiotro}	I^2 (%)	Model 1			Model 2			Model 3		
					OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
HDL-C	Europeans	16 144/17 832	0.794	69.9	1.30	1.09–1.55	3.18×10^{-3}	1.21	1.11–1.31	3.12×10^{-5}	1.17	1.07–1.29	6.88×10^{-4}
	Asians	2219/5275	0.046	17.0	1.42	1.11–1.80	4.24×10^{-3}	1.51	1.20–1.91	7.61×10^{-4}	1.58	1.24–2.00	2.92×10^{-4}
	All	18 363/23 107			1.34	1.16–1.54	4.70×10^{-5}	1.24	1.15–1.34	7.05×10^{-8}	1.22	1.12–1.33	5.63×10^{-6}
LDL-C	Europeans	16 144/17 832	0.346	63.2	1.03	0.88–1.20	0.715	0.95	0.88–1.02	0.150	0.96	0.89–1.03	0.272
	Asians	2219/5275	0.050	0	1.12	0.81–1.53	0.498	1.08	0.77–1.50	0.665	1.02	0.77–1.36	0.874
	All	18 363/23 107			1.04	0.91–1.20	0.533	0.95	0.88–1.02	0.186	0.96	0.89–1.04	0.306
Triglycerides	Europeans	16 144/17 832	0.255	72.3	0.67	0.53–0.84	6.04×10^{-4}	0.86	0.77–0.95	3.74×10^{-3}	0.91	0.82–1.02	0.102
	Asians	2219/5275	0.023	0	1.02	0.81–1.29	0.870	0.95	0.74–1.20	0.649	1.06	0.84–1.34	0.613
	All	18 363/23 107			0.82	0.70–0.97	0.0186	0.87	0.79–0.95	3.07×10^{-3}	0.95	0.83–1.08	0.440

Model 1: Conventional meta-analysis of the estimates across multiple SNPs; Model 2: inverse variance weighted (IVW) univariate regression analysis; Model 3: IVW multivariable regression analysis, accounting for pleiotropic effects from other lipid traits. In both Models 2 and 3, the Egger regression method was used if there was evidence showing the unbalanced pleiotropy ($P < 0.05$). I^2 , heterogeneity statistics of inter-SNP IV estimates; P_{pleiotro} , P -value for the presence of unbalanced pleiotropy. All independent SNPs associated with lipid levels available in European study (no. of SNPs = 183) and Asian study (no. of SNPs = 153). In Model 1 and Model 2, only SNPs showing association with the respective lipid level were included in the analysis (see 'Methods' section). In Model 3, all SNPs available were included for multivariable analysis. OR, odds ratio for AMD per 1-SD unit increase of lipid levels; $OR_{\text{lipid-AMD}}$ in European and Asian data was estimated separately by three models; $OR_{\text{lipid-AMD}}$ in combined data was calculated using fixed-effects meta-analysis of combining estimates from European and Asian studies. HDL-C, high-density lipoprotein cholesterol; LDL-C, LDL cholesterol.

showing suggestive evidence of association ($P < 1 \times 10^{-4}$) for AMD resulted in similar conclusions in the magnitude and direction of the IV estimators. Meanwhile, the sensitivity analysis did not change the non-significant results for LDL-C and triglycerides (Supplementary Table 7, available as Supplementary Data at IJE online).

Additional sub-study analyses performed in Asians using the restricted SNPs and strong IVs showing association for HDL-C ($P < 0.05$ and $P < 5 \times 10^{-8}$, respectively) did not substantially change the estimated effect size (Supplementary Table 8, available as Supplementary Data at IJE online). Using restricted SNPs and strong IVs also yielded similarly non-significant results for LDL-C and triglycerides in the Asians.

Finally, we performed subgroup analyses for two clinical AMD subtypes in Asian participants: PCV and typical AMD (tAMD). We found that HDL-C was associated with both PCV (OR = 1.62; 1.18–2.22) and tAMD (OR = 1.56, 1.16–2.09; Table 4). Using the restricted SNPs and strong IVs, the association between HDL-C and AMD subtypes was largely unchanged. Likewise, there was no evidence of an association between LDL-C and triglycerides with the risk of AMD subtypes. After *CETP* and *LIPC* SNPs were excluded, the magnitudes of IV effects of HDL-C on the risk of PCV and tAMD were reduced (Supplementary Table 9, available as Supplementary Data at IJE online).

Discussion

In this study, we performed a global meta-analysis from European and Asian populations to determine the causal relationship between plasma lipids and advanced AMD using a Mendelian randomization approach. We found that higher plasma HDL-C was causally associated with an increased risk of AMD in both Europeans and Asians. In contrast, neither LDL-C nor triglycerides were associated with the risk of AMD. Our study, to the best of our knowledge, is the first report providing genetic evidence for a causal link between HDL-C levels and AMD in a multiethnic cohort. Mendelian randomization reduces the influence of unknown or unmeasured confounders that may give rise to spurious associations between exposures and disease outcomes. Thus, our study helps clarify conflicting outcomes from previous epidemiological studies on plasma lipids and AMD risk. It provides strong support to the hypothesis that elevated levels of plasma HDL-C are a causal risk factor during the development of AMD—one that is easy to monitor in at-risk individuals.

Several lines of evidence support a role for dyslipidemia in AMD pathogenesis. First, there is histological evidence that drusen, the earliest sign of AMD, contain lipid material.^{6,7} Second, biochemical evidence suggests that intra-

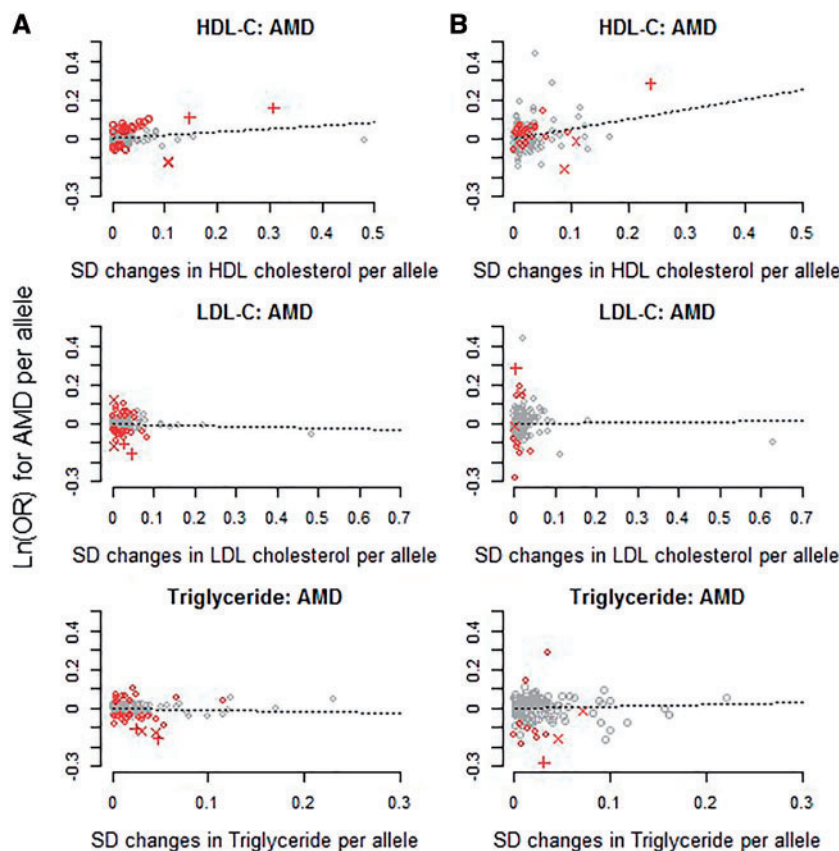


Figure 2. Scatter plots of association of lipids and AMD estimated from European and Asian populations. For each scatter plot, changes in lipid level (SD changes per lipid-level-increasing allele) are plotted against $\ln(\text{OR})$ of AMD. Dashed line is the fitted line from Model 3. +, *CETP* rs9989419 and rs5880 for Europeans and *CETP* rs3764261 for Asians; x, *LIPC* rs261342 and rs1532085 for all populations; o, SNPs in light gray not associated with AMD ($P \geq 0.05$); others marginally associated with AMD ($P < 0.05$).

Table 3. Causal association of HDL-cholesterol and risk of advanced AMD in a subset of data

Study (no. of cases/controls)	SNPs	OR	95% CI	<i>P</i>	Unbalanced pleiotropy
Europeans	All SNPs	1.17	1.07–1.29	6.88×10^{-4}	Absent
	Exclude <i>CETP</i> and <i>LIPC</i> SNPs	1.36	1.20–1.53	1.85×10^{-6}	Absent
	Exclude <i>CETP</i> , <i>LIPC</i> , <i>APOE</i> and <i>ABCA1</i> SNPs	1.26	1.11–1.43	3.72×10^{-4}	Present
	Exclude SNPs ($P < 1 \times 10^{-4}$)	1.18	1.04–1.34	0.0132	Absent
Asians	All SNPs	1.58	1.24–2.00	2.92×10^{-4}	Present
	Exclude <i>CETP</i> and <i>LIPC</i> SNPs	1.48	1.05–2.07	0.0243	Present
	Exclude <i>CETP</i> , <i>LIPC</i> , <i>APOE</i> and <i>ABCA1</i> SNPs	1.34	0.94–1.94	0.116	Present
	Exclude SNPs ($P < 1 \times 10^{-4}$)	1.23	0.93–1.62	0.157	Present

SNPs showing evidence of the association for AMD ($P < 1 \times 10^{-4}$) were removed. IVW multivariable regression analysis (Model 3) was used in the Mendelian randomization analysis. Egger regression method was applied in the presence of unbalanced pleiotropy. OR, odds ratio for AMD per 1-SD unit increase of HDL-cholesterol level; All SNPs, all independent SNPs associated with lipid levels and are available in Europeans (no. of SNPs = 183) and Asians (no. of SNPs = 153); *CETP* SNPs (rs9989419, rs5880, rs3764261), *LIPC* SNPs (rs261342, rs1532085), *APOE* SNPs (rs6859, rs7254892, rs4420638) and *ABCA1* (rs1883025, rs2472509) were excluded, respectively.

retinal lipid transport is facilitated by proteins that also regulate systemic lipid metabolism.^{49,50} Third, genetic studies have reported variants in several cholesterol-related genes that increase the risk of AMD.^{8–13} Fourth, animal experiments have demonstrated that impaired macrophage cholesterol efflux through HDL-mediated reverse

cholesterol transport may lead to a pro-angiogenic status such as that in AMD.^{51,52} However, despite the strong biological rationale, the relationship between plasma lipid levels and AMD from epidemiological studies has been inconsistent. Recently, a prospective study in Europeans showed that plasma HDL-C was associated with an

Table 4. HDL-cholesterol and risk of AMD subtypes in Asian population

		Unbalanced pleiotropy	HDL-C			LDL-C			Triglycerides		
			OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
PCV	All SNPs	Absent	1.62	1.18–2.22	3.22×10^{-3}	0.95	0.64–1.38	0.783	1.11	0.82–1.50	0.511
	Restricted SNPs	Absent	1.69	1.21–2.36	2.98×10^{-3}	0.97	0.64–1.48	0.905	1.17	0.84–1.63	0.364
	Strong IVs	Absent	1.80	1.25–2.65	6.66×10^{-3}	1.34	0.75–2.41	0.334	1.26	0.86–1.80	0.245
tAMD	All SNPs	Present	1.56	1.16–2.09	3.80×10^{-3}	1.13	0.79–1.60	0.500	1.06	0.79–1.43	0.678
	Restricted SNPs	Present	1.69	1.24–2.30	1.36×10^{-3}	1.14	0.79–1.66	0.481	1.07	0.79–1.46	0.649
	Strong IVs	Present	1.45	1.01–2.06	5.69×10^{-2}	0.99	0.60–1.62	0.959	1.00	0.71–1.43	0.999

Restricted SNPs associated with lipids from AGEN consortium ($P < 0.05$, no. of SNPs = 82). Strong IVs of SNPs associated with lipids from AGEN consortium ($P < 5 \times 10^{-8}$, no. of SNPs = 20). IVW multivariable regression analysis (Model 3) was used in the Mendelian randomization analysis. Egger regression method was applied in the presence of unbalanced pleiotropy. PCV, polypoidal choroidal vasculopathy (1062 cases vs 5275 controls); tAMD, typical neovascular AMD (1157 cases vs 5275 controls); OR, odds ratio for disease per 1-SD unit increase of lipid levels; IV, instrumental variable used in Mendelian randomization analysis; All SNPs, all independent SNPs associated with lipid levels and are available in Europeans ($N = 183$) and Asians ($N = 153$).

increased risk of early AMD at an OR of 1.62 per mmol/L (95% CI, 1.19–2.22) and with advanced AMD at an OR of 2.03 per mmol/L (95% CI, 1.02–4.05),²¹ but causality for this relationship has remained unclear.

One important assumption for Mendelian randomization is that the effect of a genetic variant on disease outcomes is mediated through its influence on the intermediate trait (here, the respective lipid fraction). In reality, genetic variants associated with HDL-C are likely to associate with other lipid traits and unknown pathways. We have attempted to address this pleiotropy through the IVW regression-based multivariable analysis, which uses multiple genetic variants associated with other lipid levels to simultaneously estimate the causal effect, conditioning upon other lipid traits.³⁹ We also employed Egger methods to deal with the presence of unbalanced pleiotropy,^{45,46} which might occur with other connected pathways between SNPs and AMD. Our data suggest that the findings of there being of a casual association between HDL-C and AMD remains robust despite adjusting for pleiotropic effects. In addition, there was no evidence that the estimates differed greatly from each other beyond *CETP* and *LIPC* variants, providing reassurance that bias from pleiotropy is very limited for the majority of SNPs.

We observed considerable heterogeneity between the effects of *CETP* and *LIPC* SNPs, relative to lipid-associated SNPs, on AMD risk. Intriguingly, *CETP* HDL-raising alleles were associated with an increased risk of AMD, whereas *LIPC* HDL-raising alleles with a reduced risk of AMD—a directionality that is consistent with what has been observed previously.¹² The mechanisms behind this remain unclear and could be either related to lipid-regulatory functions of these genes in the bloodstream, or as yet undescribed functions specific to the eye. For instance, lipids play a central role in the build-up of

sub-retinal deposits of drusen that are considered as early hallmarks of AMD.^{53,54} *LIPC* rs10468017-T allele, which positively correlated with HDL-increasing *LIPC* rs1532085-A allele in our study ($r^2 = 0.64$), has been shown to be associated with the decreased risk of drusen progression,⁵⁵ which might well explain a protective effect of this gene. Likewise, *CETP* might have specific functions in the eye and modify AMD risk by modulating levels or composition of retinal lipoproteins that facilitate removal of harmful insoluble cholesteryl esters.²⁵ Future studies will need to address the exact mechanisms that causally link *CETP* and *LIPC* with AMD, for instance by studying the impact of *CETP* and *LIPC* variants on the full retinal lipoprotein profile and drusen formation during AMD pathogenesis.

The IV estimation between HDL-C and AMD suggests a larger effect in Asians than in Europeans. This could be partially related to heterogeneity of AMD subtypes in different ethnic populations. In the European samples, although the majority of AMD patients present with CNV, 20% is GA.¹⁵ Conversely, the percentage of GA is very low (less than 7%) in Asian AMD patients⁵⁶ and thus this phenotype was not included in our Asian sub-study. On the other hand, PCV is more common in Asians, accounting for almost half of advanced AMD Asian samples. PCV shares some common features with typical neovascular AMD, but also demonstrates distinct clinical characteristics.⁵⁷ Our subgroup analysis for PCV suggests a similar causal association between HDL-C levels and PCV, but slightly larger effect sizes compared with typical AMD, which warrants further investigation.

Burgess and Davey-Smith recently also proposed a genetic link between HDL-C and AMD in Europeans.⁵⁸ They approximated beta and standard errors for SNP-AMD association from publicly released *P*-values and allele

frequencies in the Mendelian randomization analysis. Our study now validates their findings with the original datasets and further tightens the link between HDL-C and AMD by narrowing variation of the Mendelian randomization estimators. The OR and 95% CI estimation in our study using the univariate regression (Model 2) is 1.21 (95% CI, 1.11–1.31, $P = 3.12 \times 10^{-5}$) vs 1.22 (1.03–1.44; $P = 0.02$) in their study and 1.17 (1.07–1.29, $P = 3.12 \times 10^{-4}$) vs 1.18 (1.01–1.38; $P = 0.03$), respectively, in the multivariable regression (Model 3).

There are limitations inherent in the genetic approach used in our study. To infer causalities, we have used all lipid-associated SNPs from the European population. Some of these SNPs are not associated with lipids in Asians, and therefore may not be appropriate instruments for Mendelian randomization. We have attempted to address this ‘weak’ IV problem through our sensitivity analysis by using a set of associated SNPs. Second, it cannot be excluded that some genetic variants have direct, lipid-independent effects on AMD and other unknown risk factors, which could artificially inflate Mendelian randomization estimates in our study. Third, SNPs conferring small genetic effects might not be detected in Asians due to the still relatively modest sample sizes.

Nevertheless, our study has significant research and clinical implications. Our data provide evidence that plasma HDL-C levels are causally involved in the pathogenesis of AMD, and therefore modulation of HDL-C metabolism might represent a novel means of preventing or retarding AMD. Drugs that elevate HDL-C are used for the treatment of cardiovascular diseases. For instance, the most widely used class of lipid-modifying drugs (i.e. statins) has weak effects on HDL-C (generally < 10% increase).⁵⁹ Some studies have suggested a reduction in AMD risk following statin therapy,^{16,60} whereas the findings have not been consistently reproduced.⁶¹ Although less commonly prescribed following HPS2-THRIVE clinic trial results,⁶² Niacin (vitamin B3) is the most potent HDL-raising drug, with an average 21% increase in HDL-C.⁶³ Following epidemiological associations between HDL-C and risk of cardiovascular disease, the last few decades have seen the development of a new generation of drugs that target CETP in order to raise HDL-C levels and reduce cardiovascular outcomes.^{64,65} Phase III trials of three such drugs—dalcetrapib, torcetrapib and evacetrapib—were terminated prematurely due either to cardiovascular safety concerns (torcetrapib) or to a lack of efficacy at pre-specified interim analyses (dalcetrapib and evacetrapib).⁶⁶ Anacetrapib is a CETP inhibitor which raised HDL-C levels by 138% in addition to lowered LDL-C levels by 40% in a phase II trial.⁶⁷ A large Phase III cardiovascular outcome trial in ~30 000 patients has recently

completed (Clinical trial id: NCT01252953); results are expected in mid-2017. The impact of lipid-modifying therapies on AMD risk is currently unknown. Our results emphasize that the potential effects of HDL-raising drugs on AMD and related phenotypes should be further investigated.

In summary, we performed a global meta-analysis using lipid-associated genetic variants to investigate the influence of plasma lipid levels on the risk of AMD. Our study suggests a causal relationship between elevated HDL-C levels and an increased risk of advanced AMD, whereas levels of LDL-C and triglycerides were not found to be associated with AMD. Our results support further investigation into whether strategies to reduce HDL-C levels could be used to prevent or treat AMD.

Supplementary Data

Supplementary data are available at *IJE* online.

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