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Mendelian Randomization Implicates High-Density Lipoprotein Cholesterol–Associated Mechanisms in Etiology of Age-Related Macular Degeneration

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Purpose: Undertake a systematic investigation into associations between genetic predictors of lipid fractions and age-related macular degeneration (AMD) risk.

Design: Two-sample Mendelian randomization investigation using published data.

Participants: A total of 33 526 individuals (16 144 cases, 17 832 controls) predominantly of European ancestry from the International Age-related Macular Degeneration Genomics Consortium.

Methods: We consider 185 variants previously demonstrated to be associated with at least 1 of low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, or triglycerides at a genome-wide level of significance, and test their associations with AMD. We particularly focus on variants in gene regions that are proxies for specific pharmacologic agents for lipid therapy. We then conduct a 2-sample Mendelian randomization investigation to assess the causal roles of LDL-cholesterol, HDL-cholesterol, and triglycerides on AMD risk. We also conduct parallel investigations for coronary artery disease (CAD) (viewed as a positive control) and Alzheimer's disease (a negative control) for comparison.

Main Outcome Measures: Diagnosis of AMD.

Results: We find evidence that HDL-cholesterol is a causal risk factor for AMD, with an odds ratio (OR) estimate of 1.22 (95% confidence interval [CI], 1.03–1.44) per 1 standard deviation increase in HDL-cholesterol. No causal effect of LDL-cholesterol or triglycerides was found. Variants in the *CETP* gene region associated with increased circulating HDL-cholesterol also associate with increased AMD risk, although variants in the *LIPC* gene region that increase circulating HDL-cholesterol have the opposite direction of association with AMD risk. Parallel analyses suggest that lipids have a greater role for AMD compared with Alzheimer's disease, but a lesser role than for CAD.

Conclusions: Some genetic evidence suggests that HDL-cholesterol is a causal risk factor for AMD risk and that increasing HDL-cholesterol (particularly via *CETP* inhibition) will increase AMD risk. *Ophthalmology 2017;124:1165-1174 © 2017 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

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Age-related macular degeneration (AMD) has one of the longest histories of genetic discovery efforts of any disease in the genome-wide association study era.[1] To date, genetic variants in 34 independent loci have been demonstrated to be associated with AMD risk,[2] highlighting several biological mechanisms that provide insight into etiologic processes and may suggest potential therapeutic targets.[3] Several of the genetic variants associated with AMD risk are located in gene regions that also have associations with lipids or lipid-related biology, in particular the *CETP*, *LIPC,* and *APOE* gene regions. Links between lipid deposition and AMD have been hypothesized for more than 50 years.[4] High-density lipoprotein (HDL) cholesterol concentrations have been shown to be positively associated with AMD risk in observational studies, whereas low-density lipoprotein (LDL) cholesterol and triglycerides generally have been found to be negatively associated with risk.[5–7] Previous investigators have suggested mechanistic links between atherosclerosis and pathologic features of AMD, such as soft drusen and lipid deposition in Bruch's membrane, using information about the function of lipid-related genetic variants associated with AMD risk.[8,9] However, links between genetic variants associated with lipid fractions and AMD risk has not been systematically investigated.

Mendelian randomization is the use of genetic variants as proxies for modifiable risk factors.[10,11] A genetic variant that has a specific association with a risk factor can be used to assess the effect of long-term elevated levels of that risk factor on a disease outcome. The approach exploits the random allocation of genetic variants at meiosis, which

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results in genetic variants being independently distributed in the population from potential confounders, and the fixed nature of genetic variants, which results in genetic associations being immune to the influences of environmental factors and reverse causation. Mendelian randomization investigations address the causal question: Do long-term elevated levels of the risk factor lead to increased (or decreased) risk of the disease outcome? Previous Mendelian randomization analyses have suggested that LDL-cholesterol is a causal risk factor for coronary artery disease (CAD) risk,[12,13] but HDL-cholesterol is not.[14]

In this article, we apply a 2-sample Mendelian randomization approach[15] to consider the effects of lipid fractions on AMD risk using 185 genetic variants previously demonstrated to be associated with at least 1 of LDL-cholesterol, HDL-cholesterol, or triglycerides at a genome-wide level of significance. We consider the associations of these variants with lipid fractions taken from the Global Lipids Genetics Consortium on up to 188 577 individuals of European ancestry[16] and associations with AMD risk from the International Age-related Macular Degeneration Genomics Consortium on up to 33 526 individuals (16 144 cases, 17 832 controls) predominantly of European ancestry.[2]

The investigation consists of 4 related components. First, we consider whether the 185 variants are more associated with AMD risk than would be expected by chance alone and highlight those variants associated with AMD risk at a Bonferroni-corrected significance threshold. Second, we consider individual genetic variants in gene regions that are proxies for specific pharmacologic agents that have been or are being developed for lipid therapy. Third, we perform univariable Mendelian randomization analyses for the effects of LDL-cholesterol, HDL-cholesterol, and triglycerides on AMD risk, and undertake sensitivity analyses using the MR-Egger[17] and weighted median[18] methods that make weaker assumptions than those in a standard Mendelian randomization analysis. Fourth, we perform a multivariable Mendelian randomization analysis for the effects of the lipid fractions on AMD risk.[19] For part of these analyses, we also test for heterogeneity in the models to see whether genetic associations with AMD risk vary more than would be expected on the basis of the associations of the variants with the lipid fractions alone.

For a positive control, we also consider genetic associations with CAD risk, because lipid fractions are known to influence CAD risk. For a negative control, we consider genetic associations with Alzheimer's disease risk, a disease that has an age profile of cases similar to AMD, but which is not known to be linked to lipid fractions or lipid-related variants[20] (with the exception of variants in the *APOE* gene region that are strong predictors of Alzheimer's disease[21]). In each analysis, we compare the genetic associations and causal estimates obtained for AMD with those for CAD and Alzheimer's disease. Associations with CAD risk are taken from the Coronary Artery Disease Genome wide Replication and Meta-analysis plus Coronary Artery Disease (CARDIoGRAMplusC4D) consortium on up to 171 191 individuals (60 801 cases, 110 390 controls) mostly of European ancestry.[22] Associations with Alzheimer's disease risk are taken from the International Genomics of Alzheimer's Project consortium on up to 54 162 individuals (17 008 cases, 37 154 controls) of European ancestry (discovery phase only).[23]

Methods

All analyses were performed using R (version 3.3.1). All statistical tests are 2 sided. This article used only publically available data and thus did not require specific ethical approval. Ethical approval for the original studies can be found in the original source articles. This research adhered to the Declaration of Helsinki.

Genetic Associations of Variants with Disease Outcomes

Genetic associations with LDL-cholesterol, HDL-cholesterol, and triglycerides were obtained from the Global Lipids Genetics Consortium[16]; associations with AMD risk were obtained from the International Age-related Macular Degeneration Genomics Consortium[2]; associations with CAD risk were obtained from the CARDIoGRAMplusC4D consortium[22]; and associations with Alzheimer's disease were obtained from the International Genomics of Alzheimer's Project.[23] Associations with AMD risk are for advanced AMD cases (defined as "geographic atrophy or choroidal neovascularization in at least 1 eye and age at first diagnosis $\geq$50 years") versus controls (no advanced or intermediate AMD; intermediate AMD is defined as "pigmentary changes in the retinal pigment epithelium [RPE] or more than 5 macular drusen greater than 63 μm in diameter and age at first diagnosis $\geq$50 years").

Beta-coefficients and standard errors for all variants are available for download, except for the associations with AMD risk. For these, we took the *P* values and directions of associations that are published by the International Age-related Macular Degeneration Genomics Consortium (http://csg.sph.umich.edu/abecasis/public/amd2015/), and converted the *P* values to *z* scores. We used published association estimates (beta-coefficients and standard errors) with AMD risk for the 34 genome-wide significant variants (see Table 1 in reference 2), and the assumption that the standard error of the beta-coefficient from a logistic regression analysis is proportional to $1-\sqrt{MAF(1-MAF)}$, where *MAF* is the minor allele frequency (assuming that the sample size was the same for all variants).[24] This means that the standard error multiplied by $\sqrt{MAF(1-MAF)}$ should be constant for all variants. We took the average value of this expression for the 34 genome-wide significant variants and divided by $\sqrt{MAF(1-MAF)}$ to estimate the standard errors for the remaining variants. We then multiplied the estimated standard error by the published *z* score to obtain the beta-coefficient for each variant and used the published direction of association to orientate this coefficient.

To assess the validity of this approach, we repeated it first dividing the 34 variants for which beta-coefficients are provided at random into 2 equal groups of 17. We then found the average value of the constant [standard error multiplied by $\sqrt{MAF(1-MAF)}$] using the first 17 variants and used this to calculate the beta-coefficients for the associations of the remaining 17 variants. We then compared the calculated values of the beta-coefficients for these variants with their values provided by the consortium. The correlation between the calculated and actual values of the beta-coefficients was 0.993. This suggests that the approach was valid and that the beta-coefficients calculated for the 185 lipid-related variants are close to the true values.