Association of Metabolomics With Incidence of Age-Related Macular Degeneration: The UK Biobank Study

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Purpose. The purpose of this study was to identify serum metabolites associated with age-related macular degeneration (AMD) incidence and investigate whether metabolite profiles enhance AMD risk prediction.

Methods. In a prospective cohort study involving 240,317 UK Biobank participants, we assessed the associations of 168 metabolites with AMD incidence using Cox hazards models. Principal component analysis (PCA) captured 90% of the variance in metabolites. These principal components (PCs) were added to the Cox models, with the first PC selected to evaluate model performance using receiver operating characteristic (ROC) curves.

Results. During a median follow-up of 13.69 years, 5199 (2.16%) participants developed AMD. After accounting for demographic, lifestyle, multimorbidity, socioeconomic factors, and genetic predispositions to AMD, 42 metabolites were associated with AMD incidence. Very-low-density lipoprotein (VLDL)-related particles, low-density lipoprotein (LDL)-related particles, three additional lipids particles, and albumin were associated with decreased AMD incidence, whereas glucose increased the risk of AMD incidence. Compared to those in the lowest quartile, individuals in the highest quartile of protective metabolite scores exhibited lower risk of AMD incidence (hazard ratio [HR] = 0.869, 95% confidence interval [CI] = 0.803–0.940, false discovery rate [FDR]-adjusted $P = 1.44 \times 10^{-3}$). However, the AMD-associated metabolites did not enhance predictive performance (both areas under the curve [AUC] = 0.776).

Conclusions. Our findings reveal significant associations between specific metabolites and AMD incidence, highlighting the roles of lipoprotein subclasses, cholesterol subtypes, apolipoproteins, glucose, and albumin. Although metabolomics did not improve risk prediction, certain biomarkers may serve as promising therapeutic targets.

Keywords: age-related macular degeneration (AMD), metabolomics, prediction model, disease biomarkers, UK Biobank

Age-related macular degeneration (AMD) is a leading cause of central visual impairment and blindness globally, particularly among the elderly.[1] With increasing life expectancy and a growing elderly population, the public health and economic burden of AMD has been rising.[2] The pathogenesis of AMD is multifaceted, encompassing environmental, lifestyle, and genetic components.[1] Major risk factors for AMD have been identified, including smoking, body mass index (BMI), chronic comorbidities, and genetic factors, thereby affecting targeted or precision medicine for high-risk individuals.[1,3] Prediction models are increasingly utilized by clinicians to stratify patients based on their risk exposures, thereby enabling the planning of therapies, recommendations, and follow-up frequencies.[4] Over the past 2 decades, genome-wide association studies (GWAS) have identified variants in over 50 genes/loci associated with AMD, indicating significant roles of lipid dysregulation, chronic inflammation, and oxidative stress in AMD development.[5–7] AMD may represent a localized manifestation of broader systemic processes.[8]

Metabolomics, with good potential to understand disease mechanisms following genomics and proteomics, analyzes low molecular weight metabolites in body fluids or within an organism or cell during specific physiological periods.[9] Metabolites reflect the biomedical conditions and cumulative effects of the genome and environmental interactions and are promising for identifying disease biomarkers.[6,10,11] Among the advanced technologies applied to metabolomic studies, nuclear magnetic resonance (NMR)-based metabolic profiling captures the majority of metabolic

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information available through 1H NMR from native serum.[12] It has been used in multiple large-scale epidemiologic and genetic studies.[13,14] Advanced metabolomics should be capable of providing comprehensive insights into the biological processes underlying AMD. Research in this area is still expanding. Currently, there are more reported studies with small sample sizes than with large cohorts, and most are cross-sectional in nature.[15,16]

Here, we utilized data from the UK Biobank cohort, which includes over 500,000 participants and provides extensive phenotypic and genomic details, along with scalar metabolomic states.[17] The current assay quantifies 168 biomarkers, including amino acids, lipid profiles, fatty acids, and metabolites related to carbohydrate metabolism and fluid balance. We aim to identify the metabolites associated with AMD incidence and investigate whether metabolite profiles could enhance the prediction of AMD incidence in a prospective cohort of 240,317 participants from the UK Biobank.

Methods

Study Population

The UK Biobank is a large-scale biomedical database and research resource containing genetic and health information from half a million individuals aged 37 to 73 years in the United Kingdom. Participants from the UK Biobank were enrolled between 2006 and 2010 at 22 recruitment centers across the United Kingdom, with follow-up ongoing. During the baseline assessment, comprehensive baseline data were collected at these centers, including sociodemographics, physical metrics, lifestyle factors, health conditions, and medical and family histories, using standardized surveys.[18] The overall study protocol (http://www.ukbiobank.ac.uk/resources/) is available online. All participants provided written informed consent for the study. The UK Biobank has been approved by the National Information Governance Board for Health and Social Care and the National Health Service (NHS) Northwest Multicenter Research Ethics Committee. This current study was conducted using the UK Biobank Resource (application number: 91320). All research procedures followed the tenets of the Declaration of Helsinki.

Metabolic Biomarker Quantification

The UK Biobank collected blood samples from a subset of consenting participants during the baseline assessment, and the NMR metabolomics measurements for these samples were completed in 2022, covering a total of 275,240 participants. The NMR spectroscopy assay provided an extensive dataset comprising 249 metabolite-related metrics (available at: https://biobank.ctsu.ox.ac.uk/crystal/ukb/docs/Nightingale_biomarker_groups.txt). Of these, the assay directly measures 168 primary metabolites, including amino acids, fatty acids, lipoproteins, various cholesterol subtypes, and markers indicative of inflammation, which partly overlap with conventional clinical predictors, such as glucose and albumin. To maximize unique measurements, we excluded 81 out of the 249 markers that were listed as ratios or percentages, as they were derived from combinations of these primary metabolites. Hence, our study focused on the 168 primary metabolites.[14,17] The abbreviations of the metabolites are shown in Supplementary Table S1.

UK Biobank Array Genotyping and AMD Polygenic Risk Score Development

We developed an AMD polygenic risk score (PRS) using data from a recent large-scale GWAS.[6] The PRS was calculated as the weighted sum of the alleles for AMD-associated variants ($P < 5 \times 10^{-8}$), with each allele weighted by its corresponding effect size, $\sum_{i=1}^{n} \hat{\beta}_i * SNP_{(i)}$, where $\hat{\beta}_i$ represents the effect size of $SNP_{(i)}$ on AMD. We standardized the PRS to a mean of 0 and standard deviation (SD) of 1 for analyses. We then validated its predictive ability using Kaplan-Meier survival analysis, distribution comparison, and Harrell's C-index.[19] Detailed methods for genotyping and PRS development are provided in the Supplementary Methods. Supplementary Table S2 provides the list of SNPs used in this study.

Definition of AMD Incidence

Diagnostic information of the UK Biobank cohort was linked with the national Hospital Episode Statistics, cancer, and death registry data.[17] The AMD phenotype was determined based on a combination of primary and secondary International Classification of Diseases Ninth Revision (ICD-9) (data fields 41203 and 41205; code 3625) and ICD Tenth Revision (ICD-10; data fields 41202 and 41204; code H35.3) codes.[17] The ICD date of the first diagnosis was identified by linking health episode statistics (ICD-9: data filed 41281 and ICD-10: data filed 41280). Prevalent AMD cases were defined as individuals diagnosed with AMD at or before baseline enrollment. These individuals were excluded from this analysis. Incident AMD was defined as the first diagnosis of AMD during follow-up. Hospital attendance data were available until October 31, 2022. Due to variations in follow-up frequency and medical visit intervals, the first occurrence of AMD during follow-up visits was considered the end point for those who developed AMD. Follow-up time was calculated from baseline until the date of AMD diagnosis, loss to follow-up, death, or October 31, 2022, whichever came first.

Data Collection

In the UK Biobank study, the majority of participants are of European ancestry (approximately 94%); therefore, the analyses in the present study were limited to individuals of European ancestry. Socioeconomic status was assessed using the Townsend Deprivation Index (TDI).[20] Smoking status was categorized as ever smokers (current/former) and never smokers. Educational attainment was grouped into four levels, from no formal education to a university degree.[20] We used the ICD codes to classify baseline hypertension (ICD-9: 401–405, ICD-10: I10–I13, I15, and O10) and diabetes (ICD-9: 250, and ICD-10: E10–E14). Additional covariates included age at recruitment, sex, BMI, the constructed PRS, and the first 10 principal components of ancestry (to adjust for population substructure). These factors were chosen because of their potential influence on AMD.

Statistical Analysis

The baseline characteristics of participants based on AMD status were summarized using descriptive statistics. Continuous variables were reported as means (SDs), whereas categorical variables were reported as frequencies and percent-

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ages. For NMR biomarkers, we used scaled data that were log-transformed and standardized to have a mean of 0 and SD of 1. Pearson correlation coefficients were calculated to assess correlations of each metabolite (Supplementary Fig. S1). Cox proportional hazards regression models were used to test the association between metabolites and AMD incidence, with follow-up starting at recruitment. To mitigate potential collinearity and high correlation, each of the 168 metabolites was analyzed individually in a multivariable model, adjusted for age at recruitment, sex, the first 10 genetic components, hypertension, diabetes, smoking, BMI, TDI, education level, assessment center, and standardized PRS for AMD. Adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated to express the change in AMD risk per 1 SD increase in each log-transformed metabolite level. In addition, we used a weighted sum method to create protective metabolite scores, as most identified metabolites were protective, to evaluate their cumulative impact on AMD incidence. The multi-metabolite score was defined as the sum of selected metabolites weighted by its β-coefficient from the respective Cox model.[21] Stratification analyses by sex were also performed. For multivariable analyses, P values were corrected using the false discovery rate (FDR) via the Benjamini-Hochberg method, with FDR-adjusted P values < 0.01 being considered statistically significant and FDR-adjusted P values < 0.05 as nominally significant.[22] This threshold was chosen to reduce the likelihood of false positives given the large number of comparisons.

To assess whether circulating NMR biomarkers could enhance AMD risk prediction, principal component analysis (PCA) was used to reduce the dimensionality of correlated NMR biomarkers, yielding fewer uncorrelated principal components (PCs) that retained over 90% of the variance in the original biomarkers. Significant PCs and all PCs accounting for 90% of the variance were incorporated into Cox models separately. The likelihood ratio test compared the fits of nested models with and without the selected PCs. Cox model performance was assessed by generating receiver operating characteristic (ROC) curves and calculating the area under the curve (AUC) to evaluate whether incorporating PCs improved prediction accuracy.[23] All statistical analyses were performed using the R software (version 4.3.3).

Results

Figure 1 shows the flowchart of participant selection. In this prospective cohort study, 5199 (2.16%) participants developed AMD over a median follow-up period of 13.69 years. Baseline characteristics of the 240,317 participants, with or without incident AMD, involved in Cox proportional hazards regression models are shown in Table 1. The mean (SD) age of the total participants was 56.75 (8.02) years, and 54% (n = 129,784) were female participants. Compared with the

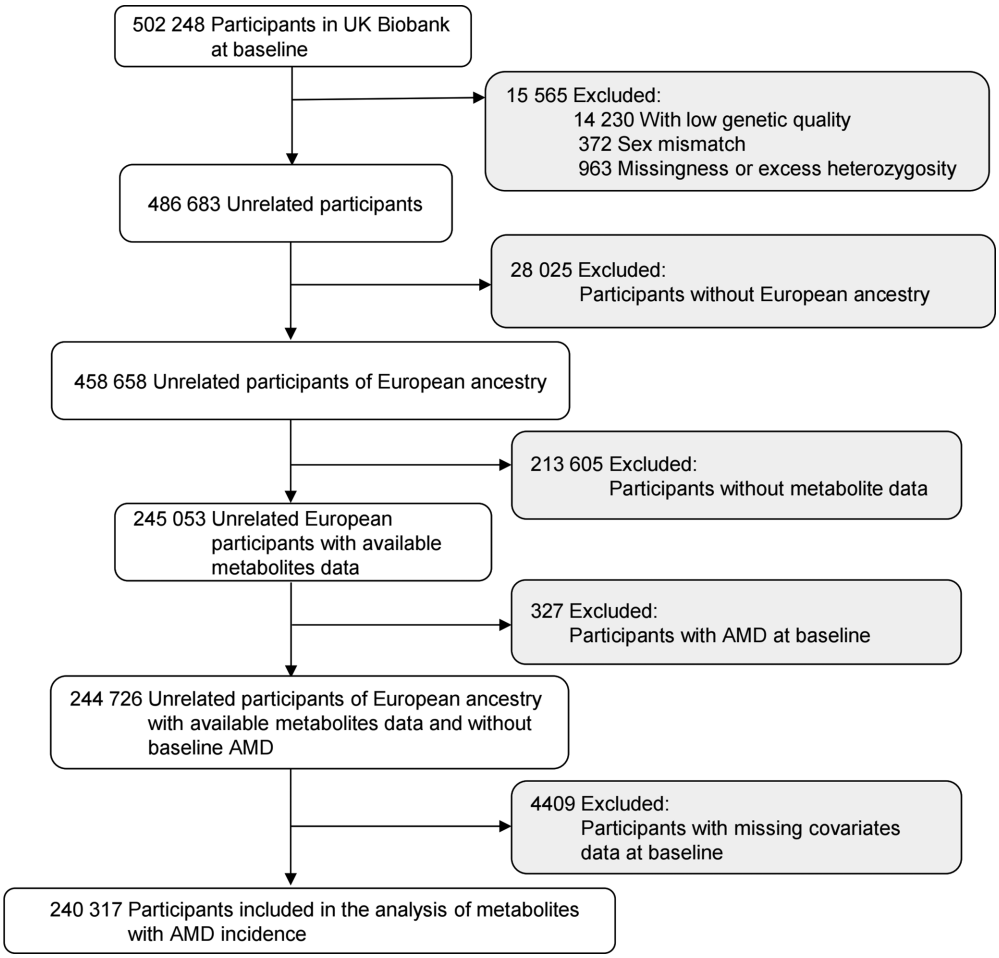

Figure 1. The flowchart of participants selection. AMD, age-related macular degeneration.

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Table 1. Baseline Characteristics of the Study Participants

	Overall (n = 240,317)	New-Onset AMD (n = 5199)	Control (n = 235,118)
Age, mean (SD), y	56.75 (8.02)	62.98 (5.27)	56.61 (8.02)
Sex, n (%)			
F	129,784 (54.0)	3,189 (61.3)	126,595 (53.8)
M	110,533 (46.0)	2,010 (38.7)	108,523 (46.2)
Standardized PRS, mean (SD)	0.00 (1.00)	0.26 (1.03)	−0.01 (1.00)
Smoking, n (%)			
No	130,007 (54.1)	2,535 (48.8)	127,472 (54.2)
Yes	110,310 (45.9)	2,664 (51.2)	107,646 (45.8)
Education level, n (%)			
Level 1	42,526 (17.7)	1,356 (26.1)	41,170 (17.5)
Level 2	65,687 (27.3)	1,117 (21.5)	64,570 (27.5)
Level 3	56,605 (23.6)	1,944 (37.4)	54,661 (23.2)
Level 4	75,499 (31.4)	782 (15.0)	74,717 (31.8)
BMI, mean (SD), kg/m^2	27.41 (4.76)	27.88 (4.88)	27.40 (4.76)
TDI, mean (SD)	−1.52 (2.97)	−1.54 (2.94)	−1.52 (2.97)
Hypertension, n (%)			
No	176,259 (73.3)	3,233 (62.2)	173,026 (73.6)
Yes	64,058 (26.7)	1,966 (37.8)	62,092 (26.4)
Diabetes, n (%)			
No	228,756 (95.2)	4,758 (91.5)	223,998 (95.3)
Yes	11,561 (4.8)	441 (8.5)	11,120 (4.7)

AMD, age-related macular degeneration; BMI, body mass index; PRS, polygenic risk score; SD, standard deviations; TDI, Townsend Deprivation Index.

participants without incident AMD, those who developed AMD were more advanced in age, more likely to be women, smokers, had lower education levels, higher BMI, and were more likely to have hypertension and diabetes (see Table 1).

Validation of Standardized Polygenic Risk Scores

The standardized PRS showed a significant association with AMD incidence in the Cox regression model ($P < 2.0 \times 10^{-16}$). Supplementary Figures S2 to S4 demonstrate a lower AMD-free survival rate in the high PRS group (tertiles) compared to those with low and intermediate genetic risk (all $P_{\text{log-rank}}$ values < 0.001), the effective differentiation between cases and control, and a modest improvement in predictive accuracy. These findings collectively validated the robustness of the constructed PRS.

Associations of Metabolites With AMD Incidence

Figure 2 shows the associations between selected metabolites (those with FDR-adjusted $P < 0.01$) and AMD incidence after adjusting multiple tests. We identified 42 metabolites, out of the total of 168 metabolite measurements, to be associated significantly with AMD incidence (Supplementary Table S3). Significant associations were particularly observed between AMD incidence and the very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) among metabolite particles. Notably, associated metabolite measurements also encompassed one glycolysis-related metabolite (glucose), one related to fluid balance (albumin), and three additional lipids, namely apolipoprotein B (ApoB), total cholesterol minus low-density lipoprotein-C (non-HDL-C), and remnant cholesterol non-HDL-non-LDL-cholesterol (remnant-C).

Among lipid-related metabolites, 26 VLDL-related compositions and 11 LDL-related metabolites were associated with a decreased risk of AMD incidence, nearly across all subtypes (see Fig. 2, Supplementary Table S3). The strongest protective association with AMD incidence was observed with cholesteryl esters in very large VLDL (XL-VLDL-CE; HR = 0.947, 95% CI = 0.920–0.975, FDR-adjusted P = 0.007) among VLDL-related lipids compositions. Regarding LDL-related particles, cholesteryl esters in small LDL (S-LDL-CE) showed the most protective association with AMD incidence (HR = 0.953, 95% CI = 0.926–0.980, FDR-adjusted P = 0.007). Moreover, three additional lipids showed a protective effect for AMD incidence: ApoB (HR = 0.954, 95% CI = 0.927–0.981), remnant-C (HR = 0.956, 95% CI = 0.930–0.984), and non-HDL-C (HR = 0.957, 95% CI = 0.930–0.985), with all FDR-adjusted $P < 0.01$ (see Supplementary Table S3).

Glucose was associated with an increased risk of AMD incidence (HR = 1.058, 95% CI = 1.031–1.086, FDR-adjusted $P = 1.63 \times 10^{-3}$). In contrast, albumin in fluid balance showed the most significant protective effect for AMD incidence (HR = 0.926, 95% CI = 0.900–0.953, FDR-adjusted $P = 2.08 \times 10^{-5}$; see Fig. 2, Supplementary Table S3). Nominal associations with AMD incidence were observed for 53 lipid-related metabolite measurements, including 9 HDL-related components, primarily derived from large HDL particles, in addition to those related to VLDL and LDL. Moreover, one inflammation marker (glycoprotein acetyls), one amino acid (histidine), and four fatty acids showed nominal associations with AMD incidence (see Supplementary Table S3). Notably, none of the small or medium HDL measurements was associated with AMD incidence, except that triglyceride in small HDL showed a borderline association with AMD incidence (see Supplementary Table S3). The protective metabolite scores were defined by 41 metabolites from albumin, VLDL-related measurements, LDL-related measurements, and additional lipids subclasses. Individuals in the highest quartile of protective scores demonstrated the strongest protective effect against AMD incidence (HR = 0.869, 95% CI = 0.803–0.940, FDR-adjusted $P = 1.44 \times 10^{-3}$; Table 2) compared with those in the lowest quartile.

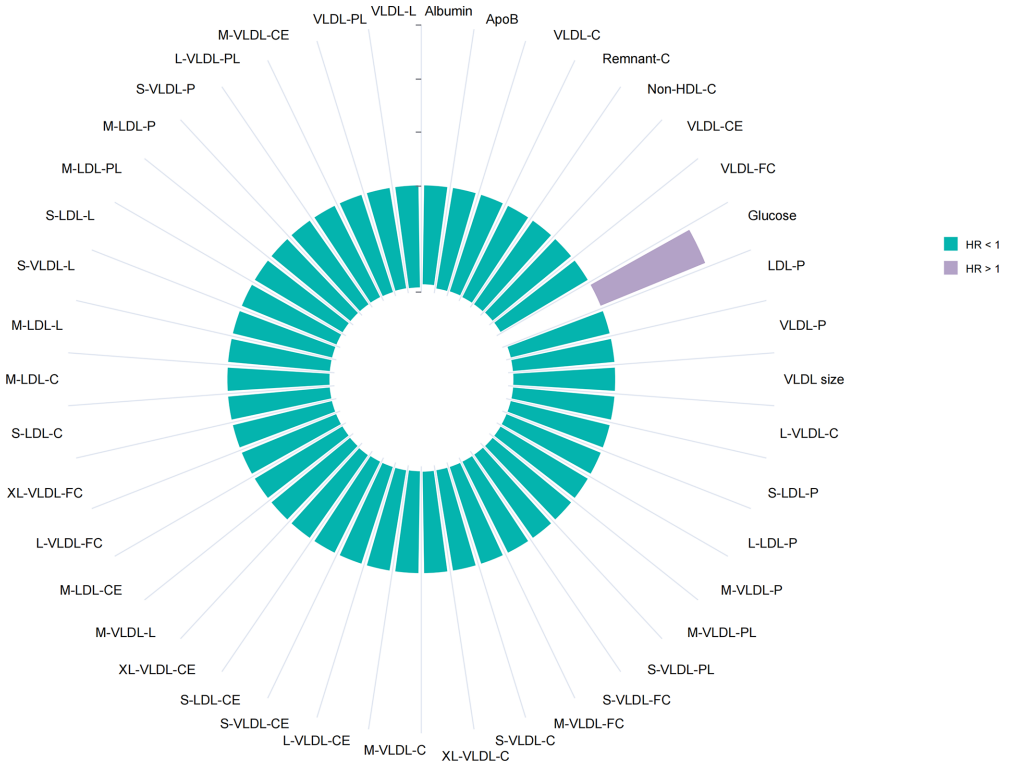

Figure 2. Associations of selected metabolites with AMD incidence in Cox proportion hazards regression model. All were adjusted for age, sex, the first 10 principal components of ancestry, body mass index (BMI), Townsend deprivation index, smoking status, education level, hypertension, diabetes, assessment center, and polygenic risk score (PRS). Hazard ratios (HRs) indicated in hazard for AMD per 1-standard deviation increase in each log-transformed metabolite level (log-transformed + 1). AMD, age-related macular degeneration; HR, hazard ratio. For abbreviations of the metabolites, see Supplementary Table S1.

Table 2. Associations of Protective Metabolite Scores With AMD Incidence

Cumulative Effect	HR (95% CI)	FDR-Adjusted *P* Value
Protective metabolite scores		
Q1	Ref	Ref
Q2	0.940 (0.872–1.014)	0.086
Q3	0.869 (0.804–0.939)	1.40×10^{-3}
Q4	0.869 (0.803–0.940)	1.44×10^{-3}

The analyses were adjusted for age, sex, the first 10 principal components of ancestry, body mass index (BMI), Townsend deprivation index, smoking status, education level, hypertension, diabetes, assessment center, and polygenic risk score (PRS).

AMD, age-related macular degeneration; CI, confidence interval; FDR, false discovery rate; HR, hazard ratio.

Hazard ratios (HRs) indicate the change in hazard for AMD per 1-standard deviation increase in each log-transformed metabolite level (log-transformed + 1).

Subgroup Analyses by Sex

After multiple testing corrections, only albumin reached statistical significance in females (HR = 0.910, 95% CI = 0.877–0.944, FDR-adjusted $P = 8.12 \times 10^{-5}$; Supplementary Table S4). Additionally, 47 lipid-related metabolites, histidine, and 3 fatty acids showed nominally significant associations (all FDR-adjusted $P < 0.05$; see Supplementary Table S4). In contrast, in male participants, only glucose showed a nominal significance (HR = 1.076, 95% CI = 1.037–1.116, FDR-adjusted $P = 0.017$), whereas no other associations were observed (all FDR-adjusted $P > 0.05$; Supplementary Table S5).

Principal Component Analysis for Metabolites in the Prediction Model

The first eight PCs of the NMR biomarkers explained 90% of the total variance present in the 168 metabolites (Supplementary Fig. S5). The associations of these PCs with AMD are shown in Supplementary Table S6. Only PC1 showed statistical significance and was thus included in the Cox regression model for AMD prediction. The PC1 loadings were predominantly derived from VLDL-related metabolites illustrated in Supplementary Figure S6. Additionally, we also evaluated the overall contribution of metabolites in predicting AMD by including the first eight PCs in the model. The likelihood ratio test indicated a significant improvement in model fit when PC1 or the first eight PCs were included ($\chi^2 = 10.656$, $P = 0.001$ and $\chi^2 = 18.479$, $P = 0.018$, respectively; Supplementary Table S7). Figure 3 shows that our Cox regression model had an AUC of 0.776 (95% CI = 0.770–0.781), and the AUC remained the same after adding PC1 (AUC = 0.776, 95% CI = 0.770–0.782). Similarly, adding the first 8 PCs maintained the AUC at 0.776 without significantly enhancing the model (Supplementary Fig. S7).

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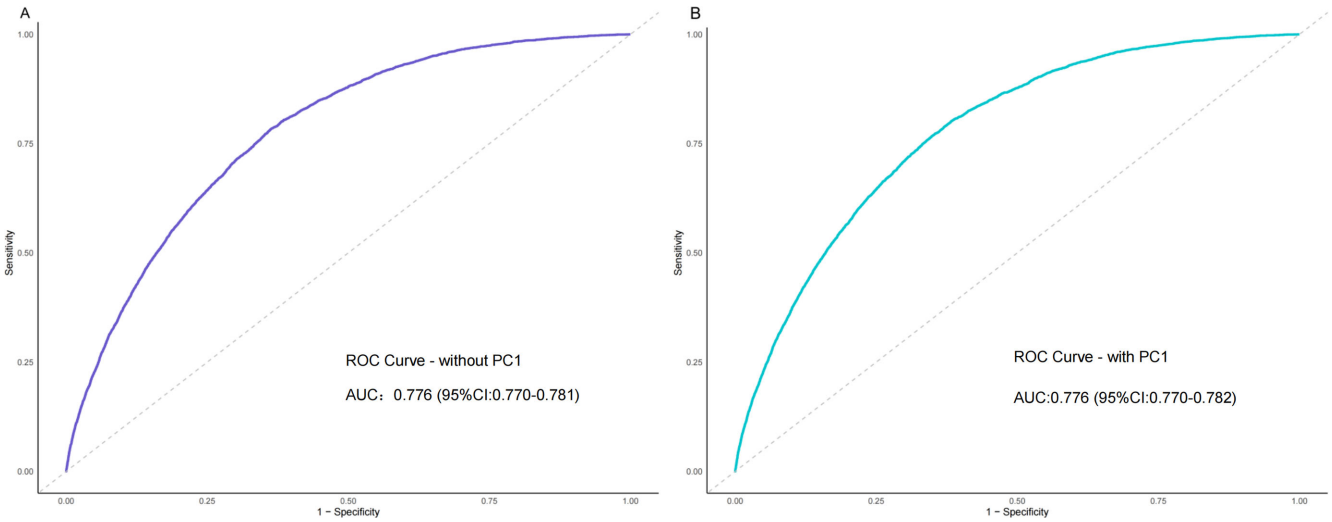

Figure 3. Receiver operating characteristic (ROC) curves of Cox regression model with and without the first principal component. (**A**) Indicates the model was adjusted for age, sex, the first 10 principal components of ancestry, body mass index (BMI), Townsend deprivation index, smoking status, education level, hypertension, diabetes, and polygenic risk score (PRS). (**B**) Additionally adjusted the PC1. Hazard ratios (HRs) indicated in hazard for AMD per 1-standard deviation increase in each log-transformed metabolite level (log-transformed + 1). AUC, the area under the curve; CI, confidence interval; FDR, false discovery rate; HR, hazard ratio; PC, principal component; ROC, receiver operating characteristic.

Discussion

Here, we presented a large longitudinal cohort study utilizing an NMR-based platform to quantify 168 metabolite measurements among 240,317 participants from the UK Biobank. Overall, we identified 42 metabolite measurements that were significantly associated with AMD incidence. Among these significant associations, VLDL-related metabolite particles, LDL-related metabolite particles, albumin, and three other lipid metabolites (ApoB, remnant-C, and non-HDL-C), were associated with a decreased risk of AMD incidence. In contrast, glucose increased the risk of AMD incidence. Additionally, participants in the highest quartile of protective metabolite scores exhibited the strongest protective effect against AMD incidence (HR = 0.869). However, the metabolomics' data from the UK Biobank showed limited predictive value for AMD incidence.

VLDL, which belongs to non-HDL-C levels,[24] was found in the current study to be associated with a reduced risk of AMD incidence for nearly all subparticles. Recent studies suggested that VLDL receptor (VLDLR) deficiency may promote the proliferation and migration of retinal vascular endothelial cells.[25] Evidence from VLDLR knockout mice models has replicated several characteristic phenotypes of retinal angiomatous proliferation (RAP).[26] Additionally, genetic association studies further support the involvement of *VLDLR* in the pathogenesis of AMD, although its functions as a receptor for VLDL remain to be elucidated.[27] Similarly, the LDL receptor (*LRP6*) has also been reported to be involved in vasculature remodeling pathways and implicated in AMD.[27] Recent studies have shown that LDL serves as the primary carrier of cholesterol from systemic circulation to the retinal pigment epithelium and the neurosensory retina.[28]

Interestingly, evaluated VLDL levels in plasma have been identified as a risk factor for cardiovascular disease (CVD), affecting all VLDL subparticles.[24] The association between co-occurrence of AMD and cardiovascular-associated conditions has been previously reported.[29,30] Similar findings were observed in LDL-related compositions, which showed protective effects for AMD incidence in our study. Evidence from observational studies and Mendelian randomization (MR) analyses have identified the association of elevated levels of LDL with a decreased risk of AMD,[31,32] whereas elevated LDL levels are well-established as a risk factor for CVD.[33] Moreover, we identified HDL-related metabolites, primarily in large HDL compositions, suggestively associated with increased AMD risk. The increase of HDL levels in AMD has been reported in a meta-analysis across 14 European cohorts,[34] as well as in earlier results of the Alienor study.[35] Traditionally, HDL cholesterol has been considered the "good cholesterol" due to its protective role in CVD.[36] Thus, the complicated and contrasting effects of VLDL, LDL, and HDL on AMD and CVD underscore the need for further research to elucidate the specific functions of lipoprotein composition in different conditions, suggesting potential underlying biological differences and genetic bases in various disease contexts.[36,37]

Genetic studies on AMD have underscored the roles of lipid-related and complement pathways in its development.[6] Among these, *CETP* and *LIPC* have been genetically linked to lipid and phospholipid contents in HDL particles, with higher concentrations of extra-large HDL particles associated with an increased risk of AMD.[34] Moreover, proteomic studies have shown that HDL particles contain various complement components, which play key roles in AMD pathology.[38] Notably, large HDL subparticles contain the complement factor H (CFH), whereas small and medium HDL subparticles do not.[15] In the present study, no associations were observed for most small or medium HDL particles, largely aligning with previous studies.[15] Hence, particle sizes of HDL cholesterol and its various subclasses, along with genetic factors, may collectively play a complex role in AMD development, although the specific mechanisms have yet to be further investigated.

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Previous observational studies have reported diabetes as a risk factor for AMD,[39] likely due to diabetes-related changes in retinal and choroidal circulation.[40] Accumulating evidence suggested that high-energy-consuming photoreceptors rely on glucose, indicating that dysregulated glucose metabolism may contribute to the pathogenesis of AMD.[28] Our study identified that evaluated glucose level was associated with an increased risk of AMD incidence. Conversely, ApoB had a protective effect on AMD incidence, consistent with previous findings.[31] Albumin as a carrier holds considerable promise for treating various ocular diseases.[41] However, research on its association with AMD incidence has been limited. Based on our current findings, albumin, as both a nutrition-related and antioxidant biomarker in the elderly,[42,43] may reduce the risk of AMD incidence. In addition, histidine, an essential amino acid obtained through diet, showed a suggestive association with AMD incidence in our study. It was also reported to be decreased in patients with late-stage AMD in both the Coimbra and Boston cohorts, correlating with disease severity.[44] These findings highlight the potential role of a balanced diet and nutrition in delaying AMD development and/or progression.

The associations of AMD incidence between male and female participants showed distinct results. In female participants, only albumin was statistically significant, while some lipid metabolites were also suggestively associated with AMD incidence (FDR-adjusted $P < 0.05$), which were not observed in male participants. One explanation for this difference could be the influence of sex hormones, which play a significant role in regulating lipid metabolism.[45] The Study of Women's Health Across the Nation (SWAN) has reported changes in lipid profiles during the menopausal transition in women.[46] Estrogens are believed to have protective influences on various organ systems,[45,47] and also exhibit antioxidant and anti-inflammatory properties that protect against retinal degeneration.[48] This may explain the numerous potentially protective lipid metabolites found in women. The lack of significant associations in the sex-stratified analysis may also be due to the reduced sample size and thus insufficient statistical power. The observed potential sex differences in our study indicate the necessity of considering sex-specific factors in AMD management.

The associations of metabolomics with AMD incidence in the current study did not translate into improved predictive performance, as evidenced by our ROC results. This may be due to the minimal actual contribution of metabolites to AMD prediction. Cumulative analyses showed that even in the highest quartiles of all protective metabolite scores, the cumulative effect yielded an HR of 0.869. Additionally, metabolite levels are significantly influenced by the interaction of genetic and environmental factors, limiting predictive performance.[11] Several studies have evaluated metabolic biomarker panels for distinguishing AMD, achieving predictive accuracies with AUCs ranging from 0.71 to 0.83.[16,49–52] However, these studies primarily focused on cross-sectional data with relatively small sample sizes and different metabolites compared to the current study. Our prospective cohort study from the UK Biobank indicates that incorporating metabolic biomarkers into a prediction model provides limited improvement in distinguishing AMD risk.

This study has several limitations. First, our sample was limited to participants of European ancestry from the UK Biobank, which may limit the generalizability of our findings to other populations. Future research should investigate the impacts of metabolites on AMD risk in populations of different ethnic backgrounds across multiple cohorts. Second, although we adjusted for potential confounders, the observational nature of our study did not eliminate the risk of residual or unknown confounders. Third, reliance on clinical coding for disease diagnosis might have resulted in under-reporting, potentially affecting the observed associations. Future studies should consider using multiple data sources, such as age care databases, pharmaceutical records, and primary care logs, to improve case detection and reduce bias. Fourth, opposite roles of HDL, VLDL, and LDL were found between AMD and CVD. Despite adjusting for hypertension in Cox models, defining the earliest loss to follow-up, death, or AMD diagnosis as the end point to account for competing risks, and assuming random censoring in the statistical models, there can still be potential influence of severe CVD on reduced life expectancy, which may affect AMD risk. Fifth, given the chronic nature of AMD progression, the age of onset is difficult to ascertain and can only be inferred from medical records (date of diagnosis), which is an inherent limitation when dealing with large datasets. Additionally, we lacked data to classify AMD according to the International Age-Related Maculopathy (ARM) Epidemiological Study Group[53] or the Beckman Initiative's clinical classification.[54] This limitation restricts our ability to evaluate the relationship between metabolites and different stages of AMD. Future research in cohorts with well-defined AMD stages would be beneficial.

Conclusions

This study provides new evidence supporting the association between metabolomic profiles and AMD incidence. We identified 42 metabolites, including some lipoprotein subclasses, cholesterol subtypes, albumin, and glucose, that were associated with AMD incidence. Further research into the specific functions of lipid compositions will improve the understanding of AMD pathophysiology. Sex-stratified analyses indicated potential differences in the association of lipid metabolites with AMD incidence between male and female participants, emphasizing the necessity for considering sex-specific factors in AMD management. Moreover, although metabolomics did not significantly improve AMD risk prediction, our findings indicate that the metabolomics of AMD could potentially aid in the development of new therapeutic targets and strategies.

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References

1. Fleckenstein M, Keenan TDL, Guymer RH, et al. Age-related macular degeneration. *Nat Rev Dis Primers*. 2021;7:31.
2. Soubrane G, Cruess A, Lotery A, et al. Burden and health care resource utilization in neovascular age-related macular degeneration: findings of a multicountry study. *Arch Ophthalmol*. 2007;125:1249–1254.

Investigative Ophthalmology & Visual Science

3. Lambert NG, ElShelmani H, Singh MK, et al. Risk factors and biomarkers of age-related macular degeneration. *Prog Retin Eye Res*. 2016;54:64–102.
4. Sobrin L, Seddon JM. Nature and nurture - genes and environment - predict onset and progression of macular degeneration. *Prog Retin Eye Res*. 2014;40:1–15.
5. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385–389.
6. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*. 2016;48:134–143.
7. Chen LJ. Genetic association of age-related macular degeneration and polypoidal choroidal vasculopathy. *Asia Pac J Ophthalmol (Phila)*. 2020;9:104–109.
8. Cheung CM, Wong TY. Is age-related macular degeneration a manifestation of systemic disease? New prospects for early intervention and treatment. *J Intern Med*. 2014;276:140–153.
9. Deng Y, Shuai P, Wang H, et al. Untargeted metabolomics for uncovering plasma biological markers of wet age-related macular degeneration. *Aging (Albany NY)*. 2021;13:13968–14000.
10. Fiehn O. Metabolomics–the link between genotypes and phenotypes. *Plant Mol Biol*. 2002;48:155–171.
11. Lauwen S, de Jong EK, Lefeber DJ, den Hollander A. Omics biomarkers in ophthalmology. *Invest Ophthalmol Vis Sci*. 2017;58:Bio88–Bio98.
12. Soininen P, Kangas AJ, Würtz P, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst*. 2009;134:1781–1785.
13. Wu Q, Li J, Sun X, et al. Multi-stage metabolomics and genetic analyses identified metabolite biomarkers of metabolic syndrome and their genetic determinants. *EBioMedicine*. 2021;74:103707.
14. Buergel T, Steinfeldt J, Ruyoga G, et al. Metabolomic profiles predict individual multidisease outcomes. *Nat Med*. 2022;28:2309–2320.
15. Acar İ E, Lores-Motta L, Colijn JM, et al. Integrating metabolomics, genomics, and disease pathways in age-related macular degeneration: the EYE-RISK Consortium. *Ophthalmology*. 2020;127:1693–1709.
16. Laíns I, Kelly RS, Miller JB, et al. Human plasma metabolomics study across all stages of age-related macular degeneration identifies potential lipid biomarkers. *Ophthalmology*. 2018;125:245–254.
17. Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203–209.
18. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol*. 2017;186:1026–1034.
19. Harrell FE, Jr, Lee KL, Califf RM, Pryor DB, Rosati RA. Regression modelling strategies for improved prognostic prediction. *Stat Med*. 1984;3:143–152.
20. Mutz J, Roscoe CJ, Lewis CM. Exploring health in the UK Biobank: associations with sociodemographic characteristics, psychosocial factors, lifestyle and environmental exposures. *BMC Med*. 2021;19:240.
21. Ahola-Olli AV, Mustelin L, Kalimeri M, et al. Circulating metabolites and the risk of type 2 diabetes: a prospective study of 11,896 young adults from four Finnish cohorts. *Diabetologia*. 2019;62:2298–2309.
22. Kim KI, van de Wiel MA. Effects of dependence in high-dimensional multiple testing problems. *BMC Bioinformatics*. 2008;9:114.
23. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*. 2011;12:77.
24. Heidemann BE, Koopal C, Bots ML, Asselbergs FW, Westerink J, Visseren FLJ. The relation between VLDL-cholesterol and risk of cardiovascular events in patients with manifest cardiovascular disease. *Int J Cardiol*. 2021;322:251–257.
25. Xia CH, Lu E, Liu H, Du X, Beutler B, Gong X. The role of Vldlr in intraretinal angiogenesis in mice. *Invest Ophthalmol Vis Sci*. 2011;52:6572–6579.
26. Hu W, Jiang A, Liang J, et al. Expression of VLDLR in the retina and evolution of subretinal neovascularization in the knockout mouse model's retinal angiomatous proliferation. *Invest Ophthalmol Vis Sci*. 2008;49:407–415.
27. Haines JL, Schnetz-Boutaud N, Schmidt S, et al. Functional candidate genes in age-related macular degeneration: significant association with VEGF, VLDLR, and LRP6. *Invest Ophthalmol Vis Sci*. 2006;47:329–335.
28. Joyal JS, Sun Y, Gantner ML, et al. Retinal lipid and glucose metabolism dictates angiogenesis through the lipid sensor Ffar1. *Nat Med*. 2016;22:439–445.
29. Duan Y, Mo J, Klein R, et al. Age-related macular degeneration is associated with incident myocardial infarction among elderly Americans. *Ophthalmology*. 2007;114:732–737.
30. Wong TY, Tikellis G, Sun C, Klein R, Couper DJ, Sharrett AR. Age-related macular degeneration and risk of coronary heart disease: the Atherosclerosis Risk in Communities Study. *Ophthalmology*. 2007;114:86–91.
31. Han X, Ong JS, Hewitt AW, Gharahkhani P, MacGregor S. The effects of eight serum lipid biomarkers on age-related macular degeneration risk: a Mendelian randomization study. *Int J Epidemiol*. 2021;50:325–336.
32. Wang Y, Wang M, Zhang X, et al. The association between the lipids levels in blood and risk of age-related macular degeneration. *Nutrients*. 2016;8:663.
33. Silverman MG, Ference BA, Im K, et al. Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: a systematic review and meta-analysis. *JAMA*. 2016;316:1289–1297.
34. Colijn JM, den Hollander AI, Demirkan A, et al. Increased high-density lipoprotein levels associated with age-related macular degeneration: evidence from the EYE-RISK and European Eye Epidemiology Consortia. *Ophthalmology*. 2019;126:393–406.
35. Cougnard-Grégoire A, Delyfer M-N, Korobelnik J-F, et al. Elevated high-density lipoprotein cholesterol and age-related macular degeneration: the Alienor study. *PLoS One*. 2014;9:e90973.
36. Sacks FM, Jensen MK. From high-density lipoprotein cholesterol to measurements of function: prospects for the development of tests for high-density lipoprotein functionality in cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2018;38:487–499.
37. van Leeuwen EM, Emri E, Merle BMJ, et al. A new perspective on lipid research in age-related macular degeneration. *Prog Retin Eye Res*. 2018;67:56–86.
38. Zhang Y, Gordon SM, Xi H, et al. HDL subclass proteomic analysis and functional implication of protein dynamic change during HDL maturation. *Redox Biol*. 2019;24:101222.
39. Chen X, Rong SS, Xu Q, et al. Diabetes mellitus and risk of age-related macular degeneration: a systematic review and meta-analysis. *PLoS One*. 2014;9:e108196.
40. Choi JK, Lym YL, Moon JW, Shin HJ, Cho B. Diabetes mellitus and early age-related macular degeneration. *Arch Ophthalmol*. 2011;129:196–199.
41. Tiwari R, Sethiya NK, Gulbake AS, Mehra NK, Murty USN, Gulbake A. A review on albumin as a biomaterial for ocular drug delivery. *Int J Biol Macromol*. 2021;191:591–599.

Investigative Ophthalmology & Visual Science

42. Arques S. Human serum albumin in cardiovascular diseases. *Eur J Intern Med*. 2018;52:8–12.
43. De Simone G, di Masi A, Ascenzi P. Serum albumin: a multifaced enzyme. *Int J Mol Sci*. 2021;22:10086.
44. Laíns I, Duarte D, Barros AS, et al. Human plasma metabolomics in age-related macular degeneration (AMD) using nuclear magnetic resonance spectroscopy. *PLoS One*. 2017;12:e0177749.
45. Palmisano BT, Zhu L, Eckel RH, Stafford JM. Sex differences in lipid and lipoprotein metabolism. *Mol Metab*. 2018;15:45–55.
46. El Khoudary SR, Shields KJ, Janssen I, et al. Cardiovascular fat, menopause, and sex hormones in women: the SWAN Cardiovascular Fat Ancillary Study. *J Clin Endocrinol Metab*. 2015;100:3304–3312.
47. Inaraja V, Thuissard I, Andreu-Vazquez C, Jodar E. Lipid profile changes during the menopausal transition. *Menopause*. 2020;27:780–787.
48. Cascio C, Deidda I, Russo D, Guarneri P. The estrogenic retina: the potential contribution to healthy aging and age-related neurodegenerative diseases of the retina. *Steroids*. 2015;103:31–41.
49. Li M, Zhang X, Liao N, et al. Analysis of the serum lipid profile in polypoidal choroidal vasculopathy. *Sci Rep*. 2016;6:38342.
50. Kersten E, Dammeier S, Ajana S, et al. Metabolomics in serum of patients with non-advanced age-related macular degeneration reveals aberrations in the glutamine pathway. *PLoS One*. 2019;14:e0218457.
51. Laíns I, Chung W, Kelly RS, et al. Human plasma metabolomics in age-related macular degeneration: meta-analysis of two cohorts. *Metabolites*. 2019;9:127.
52. Mitchell SL, Uppal K, Williamson SM, et al. The carnitine shuttle pathway is altered in patients with neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2018;59:4978–4985.
53. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol*. 1995;39:367–374.
54. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology*. 2013;120:844–851.

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