Lipids in Health and Disease

RESEARCH

Open Access

Associations of human blood metabolome with optic neurodegenerative diseases: a bi-directionally systematic mendelian randomization study

Bin Tong[1,2†], Chubing Long[3†], Jing Zhang[3†], Xin Zhang[1,4], Zhengyang Li[5], Haodong Qi[5], Kangtai Su[5], Deju Zhang[6], Yixuan Chen[3], Jitao Ling[1], Jianping Liu[1], Yunwei Hu[4*] and Peng Yu[1*]

Abstract

Background Metabolic disruptions were observed in patients with optic neurodegenerative diseases (OND). However, evidence for the causal association between metabolites and OND is limited.

Methods Two-sample Mendelian randomization (MR). Summary data for 128 blood metabolites was selected from three genome-wide association study (GWASs) involving 147,827 participants of European descent. GWASs Data for glaucoma (20906 cases and 391275 controls) and age-related macular degeneration (AMD, 9721 cases and 381339 controls) came from FinnGen consortium. A bi-directional MR was conducted to assess causality, and a Mediation MR was further applied to explore the indirect effect, a phenome-wide MR analysis was then performed to identify possible side-effects of the therapies.

Results All the results underwent correction for multiple testing and rigorous sensitivity analyses. We identified N-acetyl glycine, serine, uridine were linked to an elevated risk of glaucoma. 1-arachidonic-glycerol-phosphate-ethanolamine, 4-acetamido butanoate, o-methylascorbate, saturated fatty acids, monounsaturated fatty acids, VLDL cholesterol, serum total cholesterol, X-11,529 were linked to reduced risk of glaucoma. There were 4 metabolites linked to a reduced risk of AMD, including tryptophan betaine, 4-androsten-3beta-17beta-diol disulfate, apolipoprotein B, VLDL cholesterol. We discovered IOP, AS, T2D as glaucoma risk factors, while BMI, AS, GCIPL as AMD factors. And 6 metabolites showed associations with risk factors in the same direction as their associations with glaucoma/AMD. Phenome-wide MR indicated that selected metabolites had protective/adverse effects on other diseases.

†Bin Tong, Chubing Long, and Jing Zhang contributed equally to this work.

*Correspondence:
Yunwei Hu
ndefy22085@ncu.edu.cn
Peng Yu
yu8220182@163.com

Full list of author information is available at the end of the article

Conclusions By integrating genomics and metabolomics, this study supports new insights into the intricate mechanisms, and helps prevent and screen glaucoma and AMD.

Keywords Metabolites, Optic neurodegenerative diseases, Glaucoma, Age-related macular degeneration (AMD), Mendelian randomization (MR), Mediation MR, Phenome-wide MR

Introduction

Glaucoma and age-related macular degeneration (AMD) are both optic neurodegenerative diseases (OND) and are leading causes of irreversible vision impairment and blindness in seniors [1–3]. An analysis by Tham et al. estimated the worldwide number of people with glaucoma to be 76 million, projected to rise to 112 million in 2040 [4]. Another meta-analysis by Wong et al. estimated the global prevalence of AMD to be 196 million individuals in 2020, with a projected increase to 288 million by 2040 [5]. In recent years, high myopia has become increasingly prevalent worldwide. Evidence suggests a connection between high myopia and the development of glaucomatous optic neuropathy and macular degeneration [6]. Glaucoma is an irreversible optic neuropathy due to impairment of the retinal nerve fiber layer (RNFL) and the optic nerve head (ONH). It causes peripheral and even central vision impairment, finally resulting in irreversible blindness. Elevated IOP is the sole recognized changeable risk factor for glaucoma, and all current therapies focus on reducing it [2]. AMD damages the macular portion of the retina, resulting in a gradual decline in central vision [7]. Antivascular endothelial growth factor therapy in the vitreous body, such as bevacizumab, pegaptanib, or ranibizumab, is extremely helpful for treating neovascular AMD. At present, there is no effective treatment for atrophic AMD, while some drugs are being explored [8]. In short, early-stage glaucoma and AMD often occurs without obvious symptoms, posing difficulties for timely diagnosis [9, 10]. Consequently, when the conditions are identified clinically, they often have advanced to stages where treatment options are limited, and vision loss becomes increasingly irreversible.

The human metabolome includes a person's unique metabolic profile, which includes lipids, amino acids, cofactors and vitamins, carbohydrates, energy, peptides, and nucleotides [11, 12]. Metabolomics research may facilitate a more comprehensive understanding of the fundamental disease mechanisms. As early as 2009, Suzana et al. reported alterations in various serum lipids among glaucoma patients, such as cholesterol, low-density lipoprotein cholesterol, and triglycerides [13]. And some researchers have adopted dietary therapy to prevent AMD and have achieved promising results [14]. At present, there are insufficient large-scale randomized controlled trials (RCTs) to assess the causal association between blood metabolites and glaucoma/AMD. Based upon metabolomics and genomics, our article illustrates metabolic pathways of potential screening, prevention, and treatments for glaucoma and AMD, and demonstrates the importance of metabolomics in discovering pharmacological targets.

However, whether metabolites directly or indirectly affect glaucoma/AMD remains unknown. For example, higher monounsaturated fatty acid intake was associated with lower AMD risk [15, 16]. Yuan et al. demonstrated an inverse correlation between monounsaturated fatty acid (MUFA) intake and overweight/obesity through longitudinal analysis of data from the China Health and Nutrition Survey spanning 1991 to 2018 [17]. Additionally, Zhang et al. demonstrated a linear relationship between increasing BMI and elevated AMD risk [18]. Based on the articles mentioned above, it can be speculated that the protective effect of monounsaturated fatty acids on AMD may be mediated by BMI. Therefore, we conducted Mendelian randomization to elucidate whether blood metabolites may affect AMD and glaucoma via intermediate factors.

Mendelian randomization (MR) has been extensively utilized to deduce causal effects [19]. The MR is a significant substitute for offering trustworthy proof in the causal effect of exposures on diseases, particularly when lacking randomized controlled trials (RCTs). To specific, MR employes single nucleotide polymorphisms (SNPs) as instrumental. MR leverages the distributive randomness and and timing priority of genetic variation, effectively mitigating confounding biases and reverse causalities commonly encountered in traditional observational studies [20, 21].

Methods

Study overview

This study consisted of five key parts, as shown in Fig. 1. First, we conducted primary MR to evaluate the causal effects of 128 metabolites on glaucoma and AMD. Then, we conducted two-step MR (step-1 MR: 10 possible risk factors → glaucoma and AMD; step-2 MR: 128 metabolites → identified risk factors), bi-directional MR (glaucoma/AMD → identified metabolites and risk factors), Mediation MR (metabolites → risk factors → glaucoma/AMD) and phe-MR (identified metabolites→ PheWAS) to further explore the uncovered mechanism. Reverse causality can occur when the association between metabolites and OND arises not because metabolites influence OND, but because OND itself alters metabolite levels. The bi-directional MR approach specifically assesses

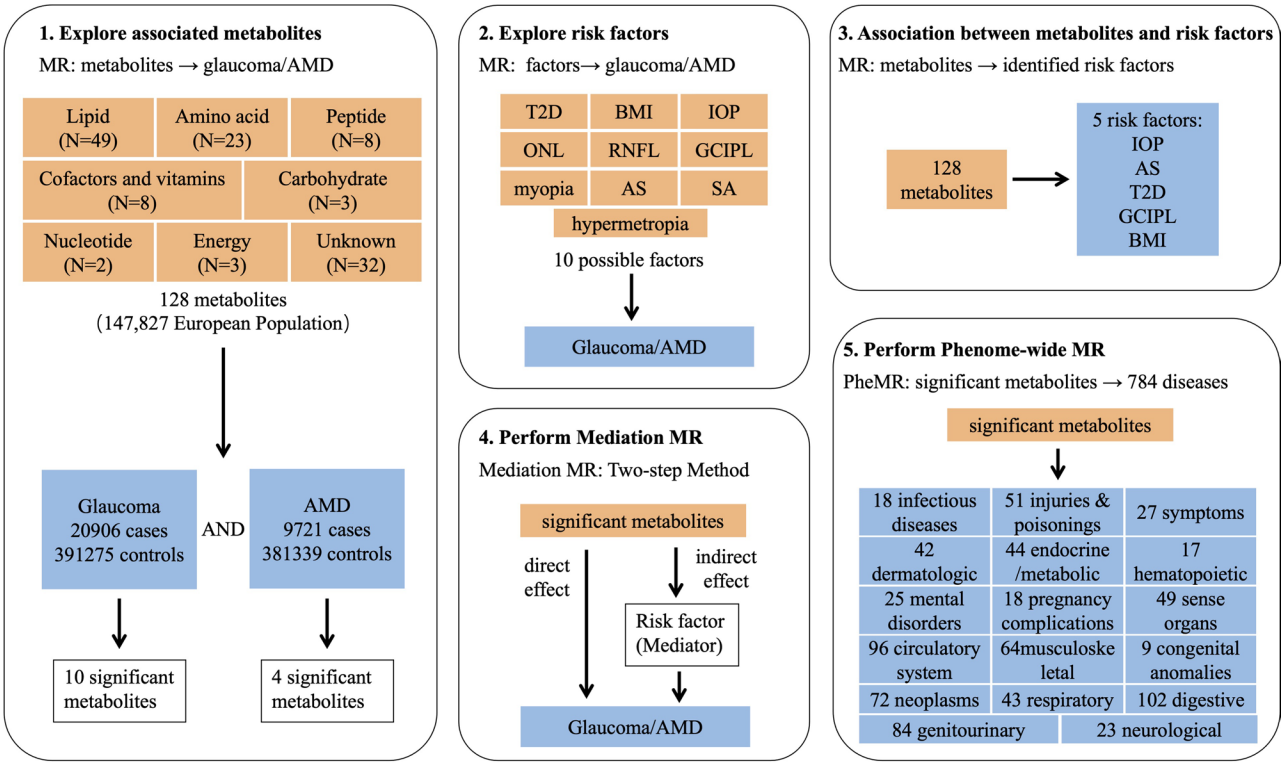

Fig. 1 Overview of this MR study. This study consisted of five key parts. First, we conducted primary MR to evaluate the causal effects of 128 metabolites on glaucoma and AMD. Then, we conducted two-step MR (step-1 MR: 10 possible risk factors → glaucoma and AMD; step-2 MR: 128 metabolites → identified risk factors), bi-directional MR (glaucoma/AMD → identified metabolites and risk factors), Mediation MR (metabolites → risk factors → glaucoma/AMD) and phe-MR (identified metabolites→ PheWAS. The brown box represents exposure in MR, and the blue box represents outcome in MR

whether reverse causality exists between metabolites and OND. The mediation MR approach aims to investigate whether a mediator can mediate the effect of the exposure (metabolites) on the outcome (OND). Generally, MR employs single-nucleotide polymorphisms (SNPs) as instrumental variables (IVs) should follow: IVs (1) are closely linked to exposure, (2) are not linked to confounders, (3) affect outcome only via exposure [22]. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) guideline [23].

Data source

The genetic data for human metabolome were obtained from three huge-scale GWASs summary data involving 147,827 European participants. In short, Shin et al. [24] analyzed 453 metabolic characteristics of 7,824 individuals across two cohorts, including nearly 3 million SNPs; Kettunen et al. [25] explored 453 metabolic characteristics of 24,925 individuals across 14 cohorts, including nearly 12 million SNPs; while Borges et al. scrutinized 249 metabolic characteristics of 115,078 individuals from UK Biobank, including nearly 12 million SNPs. Finally, a total of 469 metabolites are retained after removing redundant metabolites in three GWASs (Table 1, S1-S4).

The primary outcomes were the risk of glaucoma and AMD. The European GWAS summary data for glaucoma (20906 cases and 391275 controls) and AMD (9721 cases and 381339 controls) were obtained from the tenth version of the FinnGen consortium [26]. This is a prospective cohort study in which patients were evaluated using International Classification of Diseases (ICD) diagnosis codes for glaucoma and AMD (Table S3,4,6). Population overlap rate in our study is below 10%, indicating that the causal relationships we derived are reliable [27].

The secondary outcomes that we analyzed were risk factors for glaucoma/AMD. After retrieving relevant literature [2, 3, 28, 29] through PubMed, we identified ten medications that may be the factors for glaucoma and AMD. We also downloaded GWAS summary data for these Mediations, including type 2 diabetes (61,714 European and 1,178 South Asian cases, 593,952 European and 2,472 South Asian controls) [30], body mass index (694,649 European) [31], intraocular pressure (31269 European) [32], outer nuclear layer thickness (31,135 European) [33], retinal nerve fiber layer thickness (31,434 European) [34], ganglion cell inner plexiform layer thickness (31,434 European) [34], myopia (32,650 European cases and 3,973 European controls) [35], hypermetropia (16,035 European cases and 2,695 European controls)

Table 1 Detailed description of human blood metabolites GWASs

Reference	phenotype	Study Cohort	Cohort design	GWAS Sample Size	Population	Female%	Mean Age	Sample material
Shin et al. [24]	453 Metabolites	TwinsUK	An adult twin British registry cohort study	6056	German	93	53.4	1052 Serum, 5004 Plasma
		KORA	Population-based cohort study	1768	British	52	60.8	Serum
Kettunen et al. [25]	123 Metabolites	EGCUT	Population-based cohort study	3,287	Estonian	58	46.3	Plasma
		COROGENE	Case-control study, only controls uesd	828	Finnish	54	53.2	Serum
		FR97	Population-based cohort study	3,661	Finish	45.3	55	Serum
		ERF	Family-based study	2,118	Dutch	58	48.2	Serum
		HBCS	British cohort study	708	Finnish	60	61.3	Serum
		GenMets	Case-control study	572	Finnish	57	55.8	Serum
		LLS	Family-based study	2,227	Dutch	54	59.2	Plasma
		KORA	Population based cohort study	1,745	German	52	60.9	Serum
		NTR	Population based twin study	1,192	Dutch	64	38.8	Plasma
		NFBC 1966	Brith cohort study	4,709	Finnish	51	31.2	Serum
		PROTE	Population based study	597	Estonian	51	38.3	Plasma
		Predict CVD	Cohort study	374	Finnish	37	47.5	Serum
		YFS	Follow up study in children	2,390	Finnish	54	37.7	Serum
		FTC	Population based twin study	664	Finnish	50	23.9	Serum
Borges et al.	249 Metabolites	UK Biobank	Population-based study	115,078	European	NA	NA	Blood
Total	**825 Metabolites**	**/**	**/**	**147,827**	**European**	**/**	/	Blood

[35], ankylosing spondylitis (456,348 European cases and 456,162 European controls) [35], sleep apnea (2,827 European cases and 453,521 European controls) [35] (Table S8).

The summary-level data on human blood Metabolites, glaucoma, AMD, and all the risk factors utilized in our current study were mainly gathered from publically European-descent GWASs. To see more details in Table S16. The ethics committee of the original GWASs approved the protocol and data collection, and each participant provided written informed consent prior to data collection. In addition, this study did not use any individual-level data. As a result, no new approval from the ethical review board was needed.

IVs selection for metabolites and risk factors

For MR, we selected the SNPs with significant associations for human blood metabolites at the genome-wide significance level ($p<5\times10^{-8}$). Then, independent SNPs were selected due to their low linkage disequilibrium (LD) with other SNPs ($r^2<0.001$, clump window 10 kb), indicating their independence as IVs for the specific metabolites. The F-statistic was applied to assess the strength of the IVs. We measured F-statistics for each SNP of exposure ($F=beta^2/se^2$) and removed the weak SNP determined by F-statistics ($F<10$). An essential procedure in MR is ensuring that SNP effects on outcome match the similar allele as effects on exposure. After combining exposure and outcome datasets, we removed incompatible alleles and palindromic SNPs. After that, the remaining SNPs were the real IVs we employed in MR. Furthermore, some sensitivity analyses in MR design required at least three SNPs as IVs [36]. When deleting metabolites with fewer than three strongly linked SNPs after being merged by glaucoma and AMD, 341 of the original 469 metabolites were eliminated. Only 128 blood metabolites passed the selection criteria for the MR study (see Table S1-S4). For IVs of 10 risk factors, glaucoma, and AMD, we set LD to $r^2<0.001$ or clump window 10,000 kb and kept other parameters unchanged.

Statistical analyses

Systematic MR and sensitivity analysis

We applied a two-sample MR system involving sensitivity analyses for primary MR, two-step MR, bi-directional MR, mediation MR, and Phe-MR. In this study, we applied the inverse-variance weighted (IVW) method as a major method to evaluate the associations between exposure and outcome [37]. Due to differences in analysis platforms, experimental conditions, participant populations, and SNPs, heterogeneity may exist in MR analysis, potentially biasing the estimation of causal effects. To assess this heterogeneity, we used Cochran's Q test [38]. We employed a fixed-effect IVW model if no significant heterogeneity was identified, and a random-effect IVW model otherwise. We employed the MR-Egger method [39] and the weighted median method [40] as sensitivity analysis in order to confirm the strength of the causal

connections reported in the IVW method. In our study, only metabolites with P_{FDR} less than 0.05 from the IVW method, and consistent directions between the IVW method and other sensitivity analyses were considered potentially eligible. We additionally utilized a leave-one-out method for sensitivity analysis, in which we removed each SNPs one at a time and estimated the remaining SNP effects. Furthermore, we specifically evaluated possible pleiotropy via the MR-Egger method's intercept and MR-PRESSO [39]. Last, the effect of continuous outcome was reported as beta (β) value with standard error (SE) and the effect of binary outcome was reported as odds ratio (OR) with a 95% confidence interval (CI). An OR greater than 1 indicates that the exposure is a risk factor for the outcome, while an OR less than 1 suggests that the exposure has a protective effect. If the confidence interval (CI) does not cross the null line (i.e., both the lower limit is greater than 1 or the upper limit is less than 1), the result is considered statistically significant. The IVW method underwent correction for multiple testing using the false discovery rate (FDR) method, and $P_{FDR} < 0.05$ were considered significant.

Mediation MR

To calculate the direct and indirect effects of the blood metabolites on glaucoma and AMD, We employed two-steps Mendelian randomization [41]. On top of acquiring the total effect of blood metabolites on glaucoma and AMD (β) from the primary MR, we also calculated two additional effect estimates: (1) the effect of the risk factors on glaucoma and AMD (β_2), and (2) the effect of the blood metabolites on the risk factors (β_1). The indirect effect, meaning causative effect of metabolites on glaucoma and AMD through risk factors, could be calculated by the product method (β_1*β_2). Thus, the proportion of mediation effect could be calculated as ([β_1*β_2]/β), and the SE and CI could be estimated by the Delta method [42].

Phenome-wide MR (Phe-MR)

We performed Phe-MR to identify possible side-effects of the 10 glaucoma-associated metabolites and 4 AMD-associated metabolites, respectively. Data for SNP-outcome effects was derived from the UK Biobank cohort ($N \leq 408{,}961$). They conducted GWAS using the SAIGE v0.29 method to address imbalanced case-control ratios and defined disease based on PheCodes [43]. We removed diseases less than 500 cases, leaving 784 diseases for Phe-MR. SNP-outcome associations were retrieved from the SAIGE GWAS (https://www.leelabsg.org/resources). Phe-MR means the danger/protective effect of each standard deviation rise in blood metabolites level. Phe-MR underwent correction for associated metabolites and 784 diseases using FDR method.

Most statistical analyses were performed in R software (v4.3.2) with the packages TwoSampleMR, data.table, dplyr, R.utils, ggplot, CMplot. We also performed Microsoft Office software.

Results

IVs selected

After screening for significant correlation and low LD SNPs within the initial 469 metabolites, we identified that a total of 4527 SNPs of 177 metabolites met the criteria. We then further filtered out metabolites with fewer than 3 SNPs after merging with OND, finally remaining 128 metabolites with SNP counts ranging from 3 to 247 (Table S1-S4). In the end, 128 blood metabolites were reviewed in the current MR study, and the F statistics for the IVs ranged from 22.7 to 5500.4 (median, 47.0), indicating that our IVs are closely linked to metabolites (Table S2).

We also selected the most suitable IVs for all the ten risk factors, including type 2 diabetes (T2D), body mass index (BMI), intraocular pressure (IOP), outer nuclear layer thickness (ONL), retinal nerve fiber layer thickness (RNFL), ganglion cell inner plexiform layer thickness (GCIPL), myopia, hypermetropia, ankylosing spondylitis (AS), sleep apnea (SA). The F statistics for the IVs of these fators ranged from 20.8 to 1765.2 (median, 42.8) (Table S8). To explore the reverse causality, the F statistics for the IVs of glaucoma ranged from 29.8 to 345.0 (median, 40.7) (Table S6). And the F statistics for the IVs of AMD ranged from 30.1 to 1744.9 (median, 48.0) (Table S6).

Exploration of glaucoma/AMD-associated metabolites

While assessing the relationship between 128 blood metabolites and glaucoma/AMD (Fig. 2), we applied the IVW method as primary analysis. After multiple test adjustments for 128 metabolites using the FDR method ($P_{FDR} < 0.05$), 12 metabolites were discovered to be associated with glaucoma, and 11 with AMD (Table S5). Of these, there are 12 lipids, 5 amino acids, 1 nucleotide, 1 cofactor and vitamin, and 2 unknown (Table S5). The MR-Egger intercept did not indicate any signs of horizontal pleiotropy in the associations between these metabolites and the risk of glaucoma/AMD ($P_{Egger\text{-}intercept} > 0.05$) (Table S5). MR-PRESSO indicated that the IVs selected for these metabolites which are associated with glaucoma did not show any outliers and no pleiotropy ($P_{Global\text{-}Test} > 0.05$) (Table S5). However, the MR-PRESSO method identified significant outliers in some metabolites which are associated with AMD (Table S5). We therefore excluded these outliers and reran MR-PRESSO until all outliers were removed. Even after excluding all outliers from the IVs of LDL cholesterol, remnant cholesterol (non-HDL, non-LDL cholesterol), and clinical LDL cholesterol, pleiotropy persisted ($P_{Global\text{-}Test} < 0.05$),

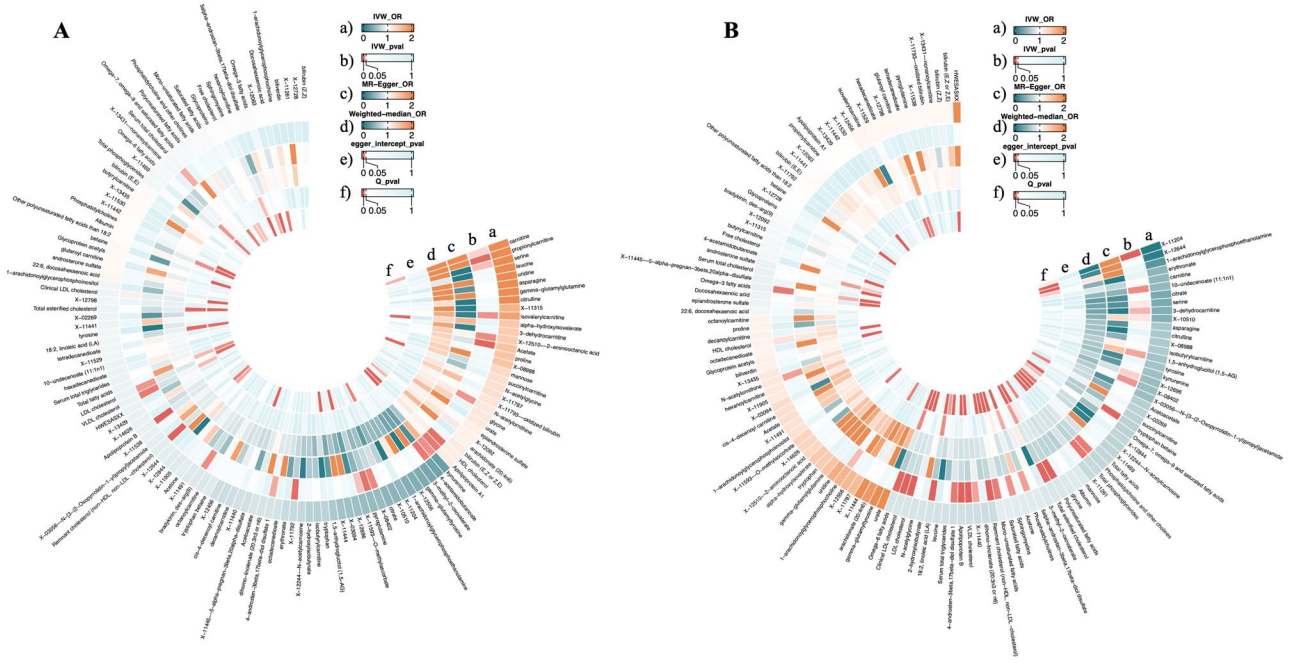

Fig. 2 MR results of all 128 metabolites to glaucoma (**A**) **and AMD** (**B**). (**a**) odds ratio (OR) of IVW method. (**b**) p value of IVW method. (**c**) OR of MR-Egger method. (**d**) OR of Weighted-median method. (**e**) p value of MR-Egger method's intercept to evaluate pleiotropy (**f**) p value of Q test to evaluate heterogeneity. A fixed-effect IVW model was employed if no significant heterogeneity was identified, and a random-effect IVW model otherwise. The effect of binary outcome was reported as OR. P-values uncorrected for multiple testing were presented in this figure, to highlight associations that may be noteworthy when studied alone, and we put the p-values that corrected for multiple testing in the following context

Exposure	Super.pathway	Outcome	IVW.Method	nSNP	FDR_pval	OR(95%CI)
Monounsaturated fatty acids	Lipid	glaucoma	fixed-effect model	169	0.010	0.919(0.878 to 0.962)
4-acetamidobutanoate	Amino acid	glaucoma	fixed-effect model	5	0.015	0.396(0.229 to 0.686)
Saturated fatty acids	Lipid	glaucoma	random-effect model	127	0.023	0.912(0.859 to 0.967)
O-methylascorbate	Cofactors and vitamins	glaucoma	fixed-effect model	11	0.028	0.632(0.470 to 0.852)
Serum total cholesterol	Lipid	glaucoma	fixed-effect model	48	0.028	0.952(0.922 to 0.983)
Serine	Amino acid	glaucoma	fixed-effect model	3	0.032	2.618(1.364 to 5.023)
1-arachidonic-glycerol-phosphate-ethanolamine	Lipid	glaucoma	fixed-effect model	3	0.032	0.384(0.199 to 0.740)
VLDL cholesterol	Lipid	glaucoma	fixed-effect model	144	0.032	0.937(0.897 to 0.979)
Uridine	Nucleotide	glaucoma	fixed-effect model	3	0.037	5.635(1.657 to 19.163)
N-acetylglycine	Amino acid	glaucoma	fixed-effect model	6	0.037	1.313(1.083 to 1.592)
VLDL cholesterol	Lipid	AMD	random-effect model	144	<0.001	0.770(0.699 to 0.848)
Apolipoprotein B	Lipid	AMD	random-effect model	129	<0.001	0.773(0.699 to 0.854)
tryptophan betaine	Amino acid	AMD	fixed-effect model	3	0.007	0.671(0.526 to 0.857)
4-androsten-3beta-17beta-diol disulfate	Lipid	AMD	fixed-effect model	5	0.031	0.773(0.641 to 0.932)

Fig. 3 Summary of significant MR results of blood metabolites to glaucoma and AMD. A fixed-effect IVW model was employed if no significant heterogeneity was identified, and a random-effect IVW model otherwise. The effect of binary outcome was reported as odds ratio (OR) with a 95% confidence interval (CI). The IVW method underwent correction for multiple testing using the false discovery rate (FDR) method, and PFDR < 0.05 were considered significant

so these metabolites were removed from the analysis (Table S5). We then conducted MR and found that the remaining metabolites still showed significant causal relationships with AMD ($P_{IVW} < 0.05$). But after multiple corrections, the causal relationships became less evident ($P_{IVW\text{-}FDR} > 0.05$). And then, we excluded phosphatidylcholine and other cholines, total phosphoglycerides, and monounsaturated fatty acids (Table S5). No indications of reverse causality were found, except for AMD to 4-androsten-3beta-17beta-diol disulfate, and glaucoma to X-11,529 (Table S7). All sensitivity analyses aligned with the IVW method, except for kynurenine to glaucoma, and X-11,204 to AMD (Table S5, Figure S1-S3). Finally, we excluded metabolites with inconsistent sensitivity analyses, retaining 11 metabolites for glaucoma and 4 for AMD (Table S5). N-acetylglycine (OR[95%CI] = 1.313[1.083,1.592], $P_{FDR} = 0.037$), serine (OR[95%CI] = 2.618[1.364,5.023], $P_{FDR} = 0.032$), uridine (OR[95%CI] = 5.635[1.657,19.163], $P_{FDR} = 0.037$) were linked to an elevated risk of glaucoma (Fig. 3, Table S5).

1-arachidonic-glycerol-phosphate-ethanolamine (OR[95%CI]=0.384[0.199,0.740], P_{FDR} = 0.032), 4-acetamidobutanoate (OR[95%CI]=0.396[0.229,0.686], P_{FDR} = 0.015), o-methylascorbate (OR[95%CI]=0.632[0.470,0.852], P_{FDR} = 0.028), X-11,529 (OR[95%CI]=0.878[0.824,0.936], P_{FDR} =0.004), saturated fatty acids (OR[95%CI]=0.912[0.859,0.967], P_{FDR} = 0.023), monounsaturated fatty acids (OR[95%CI]=0.919[0.878,0.962], P_{FDR} = 0.010) VLDL cholesterol (OR[95%CI]=0.937[0.897,0.979], P_{FDR} = 0.032), serum total cholesterol (OR[95%CI]=0.952[0.922,0.983], P_{FDR} = 0.028) were linked to reduced risk of glaucoma (Fig. 3, Table S5). Four metabolites were linked to reduced risk of AMD, including tryptophan betaine (OR[95%CI]=0.671[0.526,0.857], P_{FDR} = 0.007), 4-androsten-3beta-17beta-diol disulfate (OR[95%CI]=0.773[0.641,0.932], P_{FDR} = 0.031),apolipoprotein B (OR[95%CI]=0.773[0.699,0.854], $P_{FDR} = 9.39\times10^{-6}$), VLDL cholesterol (OR[95%CI]=0.770[0.699,0.848], $P_{FDR} = 3.71E^{-06}$), (Fig. 3, Table S5). Specially, VLDL cholesterol concurrently reduces the risk of glaucoma and AMD.

Exploration of potential risk factors for glaucoma/AMD

We employed two-steps MR analyses to explore potential causal mechanisms linking 128 blood metabolites with glaucoma/AMD (step-1 MR: risk factors → glaucoma/AMD; step-2 MR: blood metabolites → risk factors). After multiple test adjustments for 10 risk factors using the FDR method (P_{FDR} < 0.05), we discovered 3 factors that were causally linked to an increased risk of glaucoma and 5 factors for AMD (Table S9). The MR-Egger intercept did not indicate any signs of horizontal pleiotropy in the associations between these risk factors and glaucoma/AMD ($P_{Egger\text{-}intercept}$ > 0.05) (Table S9). No indications of reverse causality were found, except for glaucoma to IOP and AS (Table S10). All sensitivity analyses aligned with the IVW method, except for IOP and ONL to AMD (Table S9). Finally, we excluded metabolites with inconsistent sensitivity analyses, retaining 3 factors for glaucoma and 3 for AMD, including IOP to glaucoma (OR[95%CI]=1.656[1.407,1.949], $P_{FDR} = 1.83\times10^{-8}$), AS to glaucoma (OR[95%CI]=1.015[1.009,1.021], $P_{FDR} = 3.42\times10^{-6}$), T2D to glaucoma (OR[95%CI]=1.055[1.018,1.093], P_{FDR} =0.015), GCIPL to AMD (OR[95%CI]=1.040[1.008,1.074], P_{FDR} =0.040), AS to AMD (OR[95%CI]=1.013[1.003,1.025], P_{FDR} =0.040), BMI to AMD (OR[95%CI]=1.113[1.004,1.235], P_{FDR} = 0.085) (Fig. 4, Table S9). The IVW analysis indicated that BMI's association with AMD did not withstand corrections (P<0.05, P_{FDR}>0.05), while the associations with the other factors remained significant after corrections (P_{FDR}<0.05). Specially, AS increases the risk of glaucoma and AMD, concurrently.

Exploration of causal effects of blood metabolites to glaucoma/AMD risk factors

We further explored the MR analysis of 128 blood metabolites with the five risk factors, namely IOP, AS, T2D, BMI, and GCIPL. After multiple test adjustments for 128 metabolites using the FDR method (P_{FDR}<0.05), 55 blood metabolites showed associations with at least one risk factor, after multiple testing correction adjustments for 128 metabolites (Table S11). Of these, there are 28 lipids, 5 cofactors and vitamins, 5 amino acids, 4 peptides, 2 carbohydrates, 1 energy, and 10 unknown. No horizontal pleiotropy evidence was found, and sensitivity analyses aligned with the IVW method. 6 metabolites showed significant associations with risk factors in the same direction as their significant associations with glaucoma/AMD, suggesting that the associations between 6 metabolites and OND may be mediated by these risk factors (Table S13).

O-methylascorbate was associated with IOP (OR[95%CI]=0.098[0.030,0.322], P_{FDR} = 0.002), and T2D (OR[95%CI]=0.396[0.219,0.713], P_{FDR} = 0.006). VLDL cholesterol was associated with GCIPL (OR[95%CI]=0.713[0.563,0.902], P_{FDR} = 0.031), and BMI (OR[95%CI]=0.946[0.923,0.968], $P_{FDR} = 4.52\times10^{-5}$). Serum total cholesterol was associated with T2D (OR[95%CI]=0.809[0.710,0.922], P_{FDR} = 0.008).Apolipoprotein-B was associated with BMI (OR[

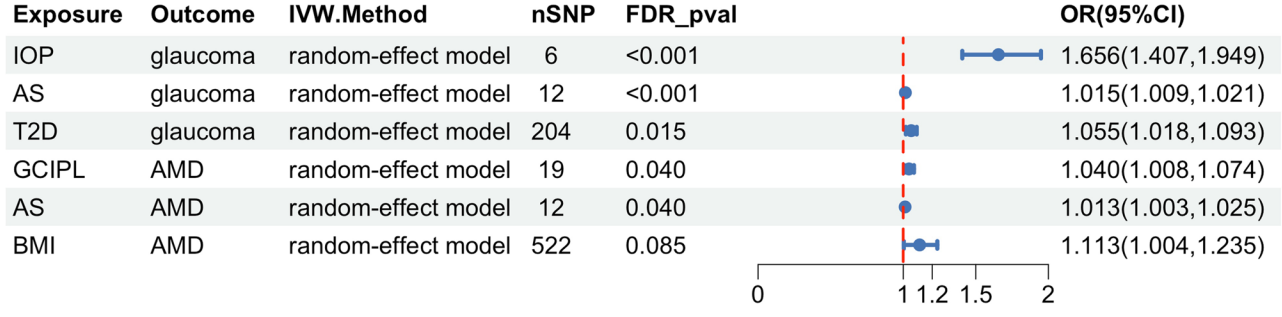

Fig. 4 Summary of significant MR results of risk factors to glaucoma and AMD. A fixed-effect IVW model was employed if no significant heterogeneity was identified, and a random-effect IVW model otherwise. The effect of binary outcome was reported as odds ratio (OR) with a 95% confidence interval (CI). The IVW method underwent correction for multiple testing using the false discovery rate (FDR) method, and PFDR < 0.05 were considered significant.

95%CI] = 0.942[0.920,0.964], $P_{FDR} = 8.64\times10^{-6}$). In particular, o-methyl ascorbate and VLDL cholesterol were associated with more than one risk factor simultaneously. No indications of reverse causality were found, except for BMI to Apolipoprotein B.

Effect of metabolites on glaucoma/AMD through risk factors

To calculate the indirect effect, we utilized the product method ($[\beta_1{}^*\beta_2]/\beta$), and for SE and CI, we used the Delta method. We excluded 2 groups with evidence of reverse causality (Table S13). In general, we found indirect effects of T2D in the relationships between O-methyl ascorbate, Serum total cholesterol and glaucoma, with a mediation proportion of 10.79% and 23.01%, respectively. Additionally, we observed the indirect effects of GCIPL and BMI in the association between VLDL cholesterol and AMD, with a mediation proportion of 4.97% and 2.29%, respectively (Table 2, Table S13). Our results indicate that metabolites primarily impact glaucoma/AMD through direct effect.

.

Phe-MR analysis of identified metabolites

We performed Phe-MR of glaucoma/AMD-associated metabolites to 784 diseases in the UK Biobank, respectively (Table S14). Our Phe-MR indicated that one-SD increase in blood metabolite levels correlates with either an increased or decreased likelihood of a specific disease or trait. We applied the IVW method underwent correction for associated metabolites and 784 diseases using the FDR method, and calculated the pleiotropy and heterogeneity. Only results with significant associations and no pleiotropy were included.

For glaucoma-associated metabolites, we discovered 130 associations with other diseases, of which 85 (65%) were beneficial (to see more details in Table S15). For example, monounsaturated fatty acids were beneficial for 10 diseases of endocrine/metabolic, injuries & poisonings, neoplasms, and circulatory system. Inversely, It was deleterious for digestive, and genitourinary disease. Simple and unspecified goiter was the most prominent association, beta = -0.834, $P_{FDR} = 8.82\times10^{-8}$. O-methylascorbate was beneficial for 28 diseases of circulatory system, musculoskeletal, respiratory, mental disorders, injuries & poisonings, endocrine/metabolic, digestive, genitourinary, respiratory. Inversely, It were deleterious for neoplasms, musculoskeletal. Other mental disorder was the most prominent associations, beta = -0.687, $P_{FDR} = 7.83\times10^{-5}$ (Figure S4, Table S15).

For AMD-associated metabolites, we discovered 162 associations with other diseases, of which 69 (43%) were beneficial (to see more details in Table S15). For example, VLDL cholesterol was beneficial for 11 diseases of genitourinary, mental disorders, digestive, hematopoietic, pregnancy complications, musculoskeletal, endocrine/metabolic, circulatory system, injuries & poisonings. Inversely, It was deleterious for 25 diseases of neoplasms, endocrine/metabolic, endocrine/metabolic, circulatory system, digestive, mental disorders, digestive, musculoskeletal, neurological, and respiratory. Disorders of lipoid metabolism was the most prominent association, beta = 0.866, $P_{FDR} = 1.39\times10^{-63}$(Figure S4, Table S15).

Discussion

The growing prevalence of an aging population has underscored the importance of caring the health of seniors [44]. Visual impairments and blindness have a substantial impact on the quality of life for seniors, presenting a significant public health concern. Optic neurodegenerative diseases (OND), including glaucoma and age-related macular degeneration (AMD), are major causes of irreversible visual impairment and blindness in seniors [1–3]. Furthermore, there were only a few achievable medication options for glaucoma and AMD [45]. In this study, we utilized Mendelian randomization (MR) to identify causal relationships between human blood metabolites and glaucoma or AMD.

To our knowledge, our research is the most comprehensive MR study systematically evaluating the causal role of human blood metabolites on glaucoma and AMD in the European population. Therefore, our MR study enhances the evidence supporting an association between blood metabolites and OND risk. Through GWAS summary data for 128 human blood metabolites involving 147,827 European participants, our study discovered that 10 metabolites were linked to glaucoma, with serine, uridine, and N-acetylglycine exerting deleterious effects,

Table 2 Mediation MR results. β = total effect of blood metabolites on glaucoma and AMD, β_1 = the effect of blood metabolites on the risk factors, β_2 = the effect of the risk factors on glaucoma and AMD. The proportion of the mediation effect could be calculated as ($[\beta_1{}^*\beta_2]/\beta$), and the SE and CI could be estimated by the Delta method

Exposure	Mediator	Outcome	beta			Mediation/Indirect effect			Proportion of mediation effect
			β	β1	β2	beta	se	OR [95%CI]	
O-methylascorbate	T2D	glaucoma	-0.458	-0.927	0.053	-0.049	0.024	0.952(0.902,0.989)	10.79%
Serum total cholesterol	T2D	glaucoma	-0.049	-0.212	0.053	-0.011	0.005	0.989(0.977,0.997)	23.01%
VLDL cholesterol	GCIPL	AMD	-0.261	-0.339	0.039	-0.013	0.008	0.987(0.970,0.999)	4.97%
VLDL cholesterol	BMI	AMD	-0.261	-0.056	0.107	-0.006	0.003	0.994(0.987,1.000)	2.29%

while 1-arachidonic-glycerol-phosphate-ethanolamine, 4-acetamidobutanoate, O-methylascorbate, serum total cholesterol, saturated fatty acids, VLDL cholesterol, and monounsaturated fatty acids exhibiting protective effects. We also identified 3 factors that exhibited deleterious effects, including IOP, T2D, and AS. We further discovered that the effect of O-methyl ascorbate and Serum total cholesterol was possibly mediated by T2D. As for AMD, we identified 4 metabolites that exerted protective effects, including tryptophan betaine, 4-androsten-3beta-17beta-diol disulfate, apolipoprotein B, VLDL cholesterol. We aldo identified 3 factors that exhibited deleterious effects, including GCIPL, BMI, and AS. We further discovered that the effect of VLDL cholesterol was possibly mediated by GCIPL and BMI. In addition, we employed Phe-MR to foresee the side effects of interfering with highlighted metabolites. For glaucoma-associated metabolites, we discovered 130 associations with other diseases, of which 85 (65%) were beneficial. For AMD-associated metabolites, we discovered 162 associations with other diseases, of which 69 (43%) were beneficial.

Metabolite levels can potentially be modified through dietary interventions, pharmacological agents, or supplements. Implementing such treatments requires further research to establish optimal dosing, duration, and delivery methods. However, metabolites within the human body are highly interconnected, and changes in one metabolite can affect entire metabolic pathways. Therefore, we prefer dietary interventions rather than direct injections of these metabolites. Of course, further experiments are needed to prove either approach. Due to the various lipids that are found to have a risk-reducing effect on glaucoma and AMD in our results, we strongly recommend patients relieve their symptoms by increasing nut intake, or individuals eat nuts for disease prevention. This opinion is consistent with a study conducted by Amirul Islam et al. [46]. Similarly, Sala-Vila et al. found nuts could reduce cognitive decline in elders through brain fMRI [47]. Furthermore, additional clinical trials are required to evaluate the potential of discovered metabolites for therapy.

In previous similar MR study, Hysi et al. [48] found that O-methyl ascorbate significantly reduces IOP, which aligns with prior research on the antihypertensive and antioxidative characteristics of ascorbate chemicals. Hu et al. [49] discovered that T2D increased the risk of primary open-angle glaucoma (POAG) in European population while not in Asian population, and no evidence indicating that higher levels of HbA1c and fasting glucose are associated with an increased risk of POAG. However, Wang et al. [50] found associations between T2D, SBP, fasting glucose, and HbA1c with the risk of glaucoma, and no evidence supporting a causal association of BMI and blood lipids with glaucoma. Han et al. [51] conducted a MR study between 8 lipids and AMD, revealing that LDL cholesterol, ApoB, total cholesterol, triglycerides, and non-HDL cholesterol were linked to a reduced risk of AMD, whereas HDL cholesterol and ApoA1 were associated with an elevated risk of AMD. Wang et al. [52] performed a MR study between n-6 polyunsaturated fatty acids and AMD, discovering that circulating linoleic acid was a preventive factor while circulating arachidonic acid was a risk factor.

Overall, our study's findings partially align with previous research. As for glaucoma, decreased d-serine levels significantly enhance the survival rate of retinal ganglion cells (RGCs) and reduce apoptosis through treated by d-amino acid oxidase in an experimental rat model of glaucoma [53]. Qin et al. compared saturated fatty acids in plasma of primary angle-closure glaucoma patients ($n=181$) with non-glaucoma individuals ($n=340$), and demonstrated that higher plasma levels of total saturated fatty acids were associated with a decreased risk of PACG ($p=0.008$) [54]. Hysi et al. [48] found that O-methyl ascorbate significantly reduces IOP through MR, which aligns with prior research on the antihypertensive and antioxidative characteristics of ascorbate chemicals. MUFAs, particularly oleic acid, have been shown to reduce inflammation and oxidative stress, which is a significant contributing factor to both glaucoma and AMD [55]. Furthermore, These fatty acids are known to reduce inflammation and oxidative stress, thereby mitigating the progression of glaucoma by preserving retinal ganglion cells and preventing damage to the retinal nerve fiber layer [56]. A study by Haydon et al. found that uridine diphosphate (UDP) derivatives may have therapeutic potential for glaucoma by activating the P2Y6 receptor on microglia, thereby promoting their phagocytic clearance function and reducing intraocular pressure [57]. There is no direct evidence to reveal the relationship between VLDL and glaucoma. However, some indications may suggest their connection. Loewen et al. found VLDL receptor in müller cells was increased 3-fold ($p=0.001$) when rats were anoxic [58]. Jiang et al. discovered the absence of VLDL receptors can trigger vascular endothelial cells and greatly enhance angiogenesis in the retina [59]. Previous studies of total cholesterol showed inconsistent results, possibly due to methodological problems in traditional observational methods. The study by Rajesh et al. found that primary open-angle glaucoma patients ($n=50$) had significantly ($P<0.001$) higher mean total cholesterol levels (205 ± 37 mg/dl) compared to controls (178 ± 23 mg/dl), suggesting a strong positive association between elevated serum total cholesterol and an increased risk of developing POAG [60]. Research on the relationship between 4-acetamidobutanoate, N-acetylglycine, 1-arachidonic-glycerol-phosphate-ethanolamine

and glaucoma is very limited, which deserved further exploration.

As for AMD, previous research is also consistent with our findings. The abnormal vascular invasion observed in the retinas of mice with VLDL receptor defects, specifically into normal avascular photoreceptor cells, may suggest neovascular age-related macular degeneration (AMD) and potentially indicate a protective role of VLDL against AMD [61]. ApoB levels were associated with decreased risk of intermediate AMD, but not with choroidal neovascularization [51]. However, further studies are needed to verify its specific mechanism. Betaine was shown to inhibit the activation of the Akt pathway, thereby attenuating the increased expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1α. These findings suggest that betaine may potentially delay the progress of retinal vascular diseases [62]. 4-androsten-3beta-17beta-diol disulfate, a steroid sulfate derived from androstenediol, it functions through both traditional androgen receptor (AR) signaling and alternative pathways within the retina, impacting neural differentiation, survival, and development [63]. By activating AR pathways, it reduces oxidative stress, inflammation, and cell apoptosis, contributing to its neuroprotective effect. Additionally, its androgenic properties allow it to interact with GABAA receptors in the central nervous system, further supporting its protective role in AMD [64].

Based on our Mediation MR results, we attempted to discuss the mechanisms by which these metabolites influence OND. A cross-sectional study found that 16 out of 50 patients (28%) in the diabetes group had POAG, while in control group was 6%, which is statistically significant ($p<0.05$) [65]. Furthermore, in a population-based study by Gavin et al., higher serum total cholesterol levels were associated with increased intraocular pressure in individuals with diabetes mellitus ($p=0.001$) [66]. Additionally, the association between T2D and glaucoma is well-established, as diabetes can lead to vascular changes affecting the eye [67]. Research shows that higher total cholesterol levels, including VLDL, correlate with increased BMI, a known risk factor for various diseases [68]. Additionally, increased BMI has been associated with a higher risk of advanced AMD, highlighting the importance of weight management in ocular health [69]. The presence of atherogenic lipoproteins, including VLDL, further relates to cardiovascular risks, which may also impact ocular health [70]. All of the above suggests that while the mediating effect may be small, its clinical significance should not be overlooked, making it worthy of further exploration.

Based on our Phe-MR results, we further discuss the role of these metabolites in other neurodegenerative diseases. Research suggests that reducing the release of neurotoxic d-serine from A1 inflammatory astrocytes may have therapeutic benefits for mild to late-stage Alzheimer's disease [71]. Genetically predicted elevated levels of O-methylascorbic acid may be associated with a reduced risk of developing delirium [72]. Increased levels of apolipoprotein B in the cerebrospinal fluid of Alzheimer's disease patients have been linked to t-tau, p-tau, and four synaptic markers in pre-symptomatic individuals [73]. Furthermore, in our previously published review, our team comprehensively summarized the role of lipid metabolism dysregulation in the pathogenesis of Alzheimer's and Parkinson's diseases, the application of lipid monitoring, and emerging lipid-targeting therapeutic strategies [74].

There are several noteworthy benefits in our study. First, it was the first comprehensive MR investigation that integrates genomics and metabolomics data to uncover novel causal associations of metabolites and glaucoma, AMD. Second, we were the first to explore whether some risk factors mediate the association between metabolites and glaucoma, AMD. Then, we used Phe-MR to thoroughly evaluate potential on-target side effects of medicative metabolites. Additionally, our research utilized carefully designed GWASs with large sample sizes, allowing for the creation of strong causal inferences with significant statistical power. At last, our study utilized an MR design, largely avoiding residual confounding and reverse causation. To specific, we employed multiple sensitivity analyses to ensure the robustness of our MR estimates.

Still, there are a few intrinsic limitations in this article. First, given the limited number of SNPs that reached significance, we lowered our selection criteria. Each SNP had the F statistics above 10, indicating that no weak instruments had been selected. Second, all participants in our study were of European ancestry, reducing the risk of misleading associations from population stratification bias. However, this limits the generalizability of our findings to non-European populations. And then, the PheCode system was based on hospital diagnoses, which may have underrepresented illness with mild symptoms. Last, metabolites within the human body are highly interconnected, and changes in one metabolite can affect entire metabolic pathways. We hope that our study can inspire future research exploring non-linear interactions between metabolites and OND.

Conclusion

In short, our MR study indicates a potential causal relationship of blood metabolites on glaucoma and AMD. These findings could serve as valuable circulating metabolical indicators for preventing and screening glaucoma and AMD. Thus, our study further proves the association between metabolism and optic neurodegenerative diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02337-0.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements
We thank the authors and participants in all of the GWASs we used for sharing their findings publically available.

Author contributions
B.T, C.B.L, and J.Z conceptualized and designed the study. B.T and C.B.L performed the statistical analyses. B.T. and J.Z. were responsible for data visualization. B.T. and C.B.L wrote the initial draft of the manuscript. All authors critically reviewed and approved the final version of the manuscript. X.Z, Z.Y.L, H.D.Q, K.T.S, D.J.Z, Y.X.C, J.L, and J.P.L contributed to data acquisition and analysis. Y.W.H and P.Y contributed to the conception and study design, drafting the text, and preparing the figures as the corresponding authors.

Funding
This research was supported by the National Natural Science Foundation of China (Grant Nos. 81760150 and 82160162 to J.P.L; No. 82160371 to J.Z.; No. 82360204 to YW.H; Grant Nos. 82100869 and 82360162 to P.Y); Natural Science Foundation in Jiangxi Province grant [20224ACB216009 to J.Z]; the Jiangxi Province Thousands of Plans (Grant No. jxsq2023201105 to P.Y); Young Elite Scientists Sponsorship Program by JXAST (No. 2023QT05 to J.Z.) and the Hengrui Diabetes Metabolism Research Fund (No. Z-2017-26-2202-4 to P.Y). Natural Science Foundation of Jiangxi Province (No. 20224BAB216049 to YW.H), and Science and technology project of Jiangxi Administration of Traditional Chinese Medicine (No. 2023B0368 to YW.H).

Data availability
The original contributions presented in the study are included in the article/Table S16. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics approval and consent to participate
Specific ethical approval was not required for this study because all data were obtained from sources available to the public.

Consent for publication
Not appliable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Endocrinology and Metabolism, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi 330006, China
[2]School of Ophthalmology and Optometry, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi 330006, China
[3]Department of Anesthesiology, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi Province 330006, China
[4]Ophthalmic Center, The Second Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang, China
[5]The First Clinical Medical College, Jiangxi Medical College, Nanchang University, Nanchang, Jiangxi 330006, China
[6]Food and Nutritional Sciences, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China

Received: 1 August 2024 / Accepted: 21 October 2024
Published online: 04 November 2024

References

1. Wareham LK, Liddelow SA, Temple S, Benowitz LI, Di Polo A, Wellington C, Goldberg JL, He Z, Duan X, Bu G, et al. Solving neurodegeneration: common mechanisms and strategies for new treatments. Mol Neurodegener. 2022;17:23.
2. Stein JD, Khawaja AP, Weizer JS. Glaucoma in Adults-Screening, diagnosis, and management: a review. JAMA. 2021;325:164–74.
3. Guymer RH, Campbell TG. Age-related macular degeneration. Lancet. 2023;401:1459–72.
4. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology. 2014;121:2081–90.
5. Wong WL, Su X, Li X, Cheung CM, Klein R, Cheng CY, Wong TY. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2:e106–116.
6. Haarman AEG, Enthoven CA, Tideman JWL, Tedja MS, Verhoeven VJM, Klaver CCW. The complications of myopia: a review and Meta-analysis. Invest Ophthalmol Vis Sci. 2020;61:49.
7. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. Lancet. 2012;379:1728–38.
8. Gragoudas ES, Adamis AP, Cunningham ET Jr., Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med. 2004;351:2805–16.
9. Zang P, Hormel TT, Hwang TS, Bailey ST, Huang D, Jia Y. Deep-learning–aided diagnosis of diabetic retinopathy, age-related macular degeneration, and glaucoma based on structural and angiographic OCT. Ophthalmol Sci. 2023;3:100245.
10. Kastner A, King AJ. Advanced glaucoma at diagnosis: current perspectives. Eye. 2020;34:116–28.
11. Jacob M, Lopata AL, Dasouki M, Abdel Rahman AM. Metabolomics toward personalized medicine. Mass Spectrom Rev. 2019;38:221–38.
12. Yu B, Li AH, Metcalf GA, Muzny DM, Morrison AC, White S, Mosley TH, Gibbs RA, Boerwinkle E. Loss-of-function variants influence the human serum metabolome. Sci Adv. 2016;2:e1600800.
13. Pavljasević S, Asćerić M. Primary open-angle glaucoma and serum lipids. Bosn J Basic Med Sci. 2009;9:85–8.
14. Souied EH, Delcourt C, Querques G, Bassols A, Merle B, Zourdani A, Smith T, Benlian P. Oral docosahexaenoic acid in the prevention of exudative age-related macular degeneration: the Nutritional AMD Treatment 2 study. Ophthalmology. 2013;120:1619–31.
15. Yasukawa T, Sasaki M, Motomura K, Yuki K, Kurihara T, Tomita Y, Mori K, Ozawa N, Ozawa Y, Yamagishi K, et al. Association between fatty acid intakes and age-related Macular Degeneration in a Japanese Population: JPHC-NEXT Eye Study. Transl Vis Sci Technol. 2023;12:3.
16. Montserrat-de la Paz S, Naranjo MC, Bermudez B, Lopez S, Moreda W, Abia R, Muriana FJ. Postprandial dietary fatty acids exert divergent inflammatory responses in retinal-pigmented epithelium cells. Food Funct. 2016;7:1345–53.
17. Yuan X, Wei Y, Jiang H, Wang H, Wang Z, Dong M, Dong X, Zhang J. Longitudinal relationship between the Percentage of Energy Intake from macronutrients and Overweight/Obesity among Chinese adults from 1991 to 2018. Nutrients 2024;16.
18. Zhang QY, Tie LJ, Wu SS, Lv PL, Huang HW, Wang WQ, Wang H, Ma L. Overweight, obesity, and risk of age-related Macular Degeneration. Invest Ophthalmol Vis Sci. 2016;57:1276–83.
19. Smith GD, Ebrahim S. Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003;32:1–22.
20. Gupta V, Walia GK, Sachdeva MP. Mendelian randomization': an approach for exploring causal relations in epidemiology. Public Health. 2017;145:113–9.
21. Tudball MJ, Smith GD, Zhao Q. Almost exact mendelian randomization. arXiv Preprint arXiv:220814035. 2022.
22. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA. 2017;318:1925–6.
23. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, VanderWeele TJ, Higgins JPT, Timpson NJ, Dimou N, et al. Strengthening the reporting of Observational studies in Epidemiology using mendelian randomization: the STROBE-MR Statement. JAMA. 2021;326:1614–21.
24. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, Arnold M, Erte I, Forgetta V, Yang TP, et al. An atlas of genetic influences on human blood metabolites. Nat Genet. 2014;46:543–50.

25. Kettunen J, Demirkan A, Würtz P, Draisma HH, Haller T, Rawal R, Vaarhorst A, Kangas AJ, Lyytikäinen LP, Pirinen M, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat Commun. 2016;7:11122.
26. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner KM, Reeve MP, Laivuori H, Aavikko M, Kaunisto MA, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. 2023;613:508–18.
27. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample mendelian randomization. Genet Epidemiol. 2016;40:597–608.
28. Jayaram H, Kolko M, Friedman DS, Gazzard G. Glaucoma: now and beyond. Lancet. 2023;402:1788–801.
29. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. Lancet. 2018;392:1147–59.
30. Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, Yengo L, Lloyd-Jones LR, Sidorenko J, Wu Y, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. Nat Commun. 2018;9:2941.
31. Koskeridis F, Evangelou E, Said S, Boyle JJ, Elliott P, Dehghan A, Tzoulaki I. Pleiotropic genetic architecture and novel loci for C-reactive protein levels. Nat Commun. 2022;13:6939.
32. Bonnemaijer PWM, Leeuwen EMV, Iglesias AI, Gharahkhani P, Vitart V, Khawaja AP, Simcoe M, Höhn R, Cree AJ, Igo RP, et al. Multi-trait genome-wide association study identifies new loci associated with optic disc parameters. Commun Biol. 2019;2:435.
33. Currant H, Fitzgerald TW, Patel PJ, Khawaja AP, Webster AR, Mahroo OA, Birney E. Sub-cellular level resolution of common genetic variation in the photoreceptor layer identifies continuum between rare disease and common variation. PLoS Genet. 2023;19:e1010587.
34. Currant H, Hysi P, Fitzgerald TW, Gharahkhani P, Bonnemaijer PWM, Senabouth A, Hewitt AW, Atan D, Aung T, Charng J, et al. Genetic variation affects morphological retinal phenotypes extracted from UK Biobank optical coherence tomography images. PLoS Genet. 2021;17:e1009497.
35. Jiang L, Zheng Z, Fang H, Yang J. A generalized linear mixed model association tool for biobank-scale data. Nat Genet. 2021;53:1616–21.
36. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife. 2018;7.
37. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013;37:658–65.
38. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327:557–60.
39. Burgess S, Thompson SG. Erratum to: interpreting findings from mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32:391–2.
40. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some Invalid instruments using a weighted median estimator. Genet Epidemiol. 2016;40:304–14.
41. Burgess S, Daniel RM, Butterworth AS, Thompson SG. Network mendelian randomization: using genetic variants as instrumental variables to investigate mediation in causal pathways. Int J Epidemiol. 2015;44:484–95.
42. Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron J, Taylor AE, Davies NM, Howe LD. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. Eur J Epidemiol. 2021;36:465–78.
43. Zhou W, Nielsen JB, Fritsche LG, Dey R, Gabrielsen ME, Wolford BN, LeFaive J, VandeHaar P, Gagliano SA, Gifford A, et al. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. Nat Genet. 2018;50:1335–41.
44. Cheng X, Yang Y, Schwebel DC, Liu Z, Li L, Cheng P, Ning P, Hu G. Population ageing and mortality during 1990–2017: a global decomposition analysis. PLoS Med. 2020;17:e1003138.
45. Hirooka K. Advanced Research in Glaucoma: treatment and postoperative approaches. J Pers Med 2023;13.
46. Amirul Islam FM, Chong EW, Hodge AM, Guymer RH, Aung KZ, Makeyeva GA, Baird PN, Hopper JL, English DR, Giles GG, Robman LD. Dietary patterns and their associations with age-related macular degeneration: the Melbourne collaborative cohort study. Ophthalmology. 2014;121:1428–e14341422.
47. Sala-Vila A, Valls-Pedret C, Rajaram S, Coll-Padrós N, Cofán M, Serra-Mir M, Pérez-Heras AM, Roth I, Freitas-Simoes TM, Doménech M, et al. Effect of a 2-year diet intervention with walnuts on cognitive decline. The Walnuts and healthy aging (WAHA) study: a randomized controlled trial. Am J Clin Nutr. 2020;111:590–600.
48. Hysi PG, Khawaja AP, Menni C, Tamraz B, Wareham N, Khaw KT, Foster PJ, Benet LZ, Spector TD, Hammond CJ. Ascorbic acid metabolites are involved in intraocular pressure control in the general population. Redox Biol. 2019;20:349–53.
49. Hu Z, Zhou F, Kaminga AC, Xu H. Type 2 diabetes, fasting glucose, Hemoglobin A1c levels and risk of primary Open-Angle Glaucoma: a mendelian randomization study. Invest Ophthalmol Vis Sci. 2022;63:37.
50. Wang K, Yang F, Liu X, Lin X, Yin H, Tang Q, Jiang L, Yao K. Appraising the effects of metabolic traits on the risk of Glaucoma: a mendelian randomization study. Metabolites 2023;13.
51. 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;50:325–36.
52. Wang K, Zhong Y, Yang F, Hu C, Liu X, Zhu Y, Yao K. Causal effects of N-6 polyunsaturated fatty acids on age-related Macular Degeneration: a mendelian randomization study. J Clin Endocrinol Metab. 2021;106:e3565–72.
53. Zhang X, Zhang R, Zhou X, Wu J. Decreased d-Serine levels prevent retinal ganglion cell apoptosis in a Glaucomatous Animal Model. Invest Ophthalmol Vis Sci. 2018;59:5045–52.
54. Qin Y, Feng X, Luo H, Liu S, Wang X, Wang X, Yang Q, Zhao Q, Cao Y, Li J, et al. Association between plasma free fatty acid levels and primary angle-closure glaucoma based on a mass spectrometry metabolomics analysis. Acta Ophthalmol. 2022;100:e204–12.
55. Galbis-Estrada C, Pinazo-Durán MD, Cantú-Dibildox J, Marco-Ramírez C, Díaz-Llópis M, Benítez-del-Castillo J. Patients undergoing long-term treatment with antihypertensive eye drops responded positively with respect to their ocular surface disorder to oral supplementation with antioxidants and essential fatty acids. Clin Interv Aging. 2013;8:711–9.
56. Reyes-Goya C, Santana-Garrido Á, Espinosa-Martín P, Vázquez CM, Mate A. Wild and cultivated olive trees: nutraceutical insights of extra virgin olive oils in cardiovascular and ocular diseases. Biochim Biophys Acta Mol Basis Dis. 2024;1870:166904.
57. Haydon PG, Lee J. Methods for treating glaucoma using uridine diphosphate derivatives. Google Patents; 2015.
58. Loewen N, Chen J, Dudley VJ, Sarthy VP, Mathura JR Jr. Genomic response of hypoxic Müller cells involves the very low density lipoprotein receptor as part of an angiogenic network. Exp Eye Res. 2009;88:928–37.
59. Jiang A, Hu W, Meng H, Gao H, Qiao X. Loss of VLDL receptor activates retinal vascular endothelial cells and promotes angiogenesis. Invest Ophthalmol Vis Sci. 2009;50:844–50.
60. Joshi RS, Adatiya VH. Study of the relationship between serum lipid levels and primary open-angle glaucoma. Indian J Ophthalmol. 2023;71:1948–52.
61. Joyal JS, Sun Y, Gantner ML, Shao Z, Evans LP, Saba N, Fredrick T, Burnim S, Kim JS, Patel G, et al. Retinal lipid and glucose metabolism dictates angiogenesis through the lipid sensor Ffar1. Nat Med. 2016;22:439–45.
62. Kim YG, Lim HH, Lee SH, Shin MS, Kim CJ, Yang HJ. Betaine inhibits vascularization via suppression of akt in the retinas of streptozotocin-induced hyperglycemic rats. Mol Med Rep. 2015;12:1639–44.
63. Wang T, Huang C, Li J, Wu X, Fu X, Hu Y, Wu G, Yang C, Chen S. Causal influence of plasma metabolites on age-related macular degeneration: a mendelian randomization study. Med (Baltim). 2024;103:e39400.
64. Galindez SM, Keightley A, Koulen P. Differential distribution of steroid hormone signaling networks in the human choroid-retinal pigment epithelial complex. BMC Ophthalmol. 2022;22:406.
65. Desai A, Patel D, Sapovadia A, Mehta P, Brahmbhatt J. A study of relation between primary open angle glaucoma and type II diabetes mellitus. 2018.
66. Tan GS, Wong TY, Fong CW, Aung T. Diabetes, metabolic abnormalities, and glaucoma. Arch Ophthalmol. 2009;127:1354–61.
67. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med. 2001;345:790–7.
68. van Leiden HA, Dekker JM, Moll AC, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD, Polak BC. Blood pressure, lipids, and obesity are associated with retinopathy: the hoorn study. Diabetes Care. 2002;25:1320–5.
69. Seddon JM, Reynolds R, Rosner B. Associations of smoking, body mass index, dietary lutein, and the LIPC gene variant rs10468017 with advanced age-related macular degeneration. Mol Vis. 2010;16:2412–24.
70. Marston NA, Giugliano RP, Melloni GEM, Park JG, Morrill V, Blazing MA, Ference B, Stein E, Stroes ES, Braunwald E, et al. Association of Apolipoprotein B-Containing Lipoproteins and risk of myocardial infarction in individuals with and without atherosclerosis: distinguishing between particle concentration, type, and content. JAMA Cardiol. 2022;7:250–6.

71. Balu DT, Pantazopoulos H, Huang CCY, Muszynski K, Harvey TL, Uno Y, Rorabaugh JM, Galloway CR, Botz-Zapp C, Berretta S, et al. Neurotoxic astrocytes express the d-serine synthesizing enzyme, serine racemase, in Alzheimer's disease. Neurobiol Dis. 2019;130:104511.
72. Long C, Lin D, Zhang L, Lin Y, Yao Q, Zhang G, Li L, Liu H, Ying J, Wang X, Hua F. Association between human blood metabolome and the risk of delirium: a mendelian randomization study. Front Endocrinol (Lausanne). 2023;14:1332712.
73. Picard C, Nilsson N, Labonté A, Auld D, Rosa-Neto P, Ashton NJ, Zetterberg H, Blennow K, Breitner JCB, Villeneuve S, Poirier J. Apolipoprotein B is a novel marker for early tau pathology in Alzheimer's disease. Alzheimers Dement. 2022;18:875–87.
74. Tong B, Ba Y, Li Z, Yang C, Su K, Qi H, Zhang D, Liu X, Wu Y, Chen Y, et al. Targeting dysregulated lipid metabolism for the treatment of Alzheimer's disease and Parkinson's disease: current advancements and future prospects. Neurobiol Dis. 2024;196:106505.

Publisher's note