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Glaucoma

Assessment of Causality Between Diet-Derived Antioxidants and Primary Open-Angle Glaucoma: A Mendelian Randomization Study

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Purpose: This study aimed to investigate the genetic causal relationships among diet-derived circulating antioxidants, primary open-angle glaucoma (POAG), and glaucoma-related traits using two-sample Mendelian randomization (MR).

Methods: Genetic variants associated with diet-derived circulating antioxidants (retinol, ascorbate, β-carotene, lycopene, α-tocopherol, and γ-tocopherol) were assessed as absolute and metabolic instrumental variables. POAG and glaucoma-related traits data were derived from a large, recently published genome-wide association study database; these traits included intraocular pressure (IOP), macular retinal nerve fiber layer (mRNFL) thickness, macular ganglion cell–inner plexiform layer (mGCIPL) thickness, and vertical cup-to-disc ratio (vCDR). MR analyses were performed per outcome for each exposure.

Results: We found no causal association between six diet-derived antioxidants and POAG using the International Glaucoma Genetics Consortium data. For absolute antioxidants, the odds ratios (ORs) ranged from 1.011 (95% confidence interval [CI], 0.854–1.199; $P = 0.895$) per natural log-transformed β-carotene to 1.052 (95% CI, 0.911–1.215; $P = 0.490$) for 1 µmol/L of ascorbate. For antioxidant metabolites, the OR ranged from 0.998 (95% CI, 0.801–1.244; $P = 0.989$) for ascorbate to 1.210 (95% CI, 0.870–1.682; $P = 0.257$) for γ-tocopherol, using log-transformed levels. A similar result was obtained with the FinnGen Biobank. Furthermore, our results showed no significant genetic association between six diet-derived antioxidants and glaucoma-related traits.

Conclusions: Our study did not support a causal association among six diet-derived circulating antioxidants, POAG, and glaucoma-related traits. This suggests that the intake of antioxidants may not have a preventive effect on POAG and offers no protection to retinal nerve cells.

Translational Relevance: This study provides valid evidence regarding the use of diet-derived antioxidants for glaucoma patients.

Introduction

Glaucoma is a group of chronic ophthalmic neurodegenerative diseases and the leading cause of irreversible blindness globally.[1,2] Primary open-angle glaucoma (POAG) is the most common type, affecting approximately 52.7 million people worldwide—a number that is projected to grow to 79.8 million by 2040.[1] Intraocular pressure (IOP) is the only proven modifiable factor for preventing glaucomatous vision loss; however, controlling IOP does not seem to prevent glaucomatous progression in all cases, as the loss of retinal ganglion cells (RGCs) continues regardless of IOP reduction in some patients with glaucoma.[3] Therefore, finding alternative RGC protection treatments is an important research objective.

Oxidative stress plays an essential role in the pathogenesis of glaucoma, potentially due to dysfunction in the trabecular meshwork and RGC damage caused by excessive reactive oxygen species.[4,5] Antioxidants are scavengers of free radicals that diminish oxidative damage and possess the potential to prevent glaucoma and slow its progression. The association between antioxidants and glaucoma has recently attracted interest, due to the safety and accessibility of antioxidants such as vitamins C and E as well as carotenoids. However, the results of previous epidemiologic studies have been inconsistent. Several prospective studies have suggested that dietary intake or supplemental antioxidants are associated with a decreased risk of glaucoma.[6–8] Furthermore, studies have reported that patients with glaucoma exhibited lower serum concentrations of vitamins A, C, and E than the controls.[9,10] Conversely, other cohort studies have not found that diet-derived antioxidants have a protective effect against glaucoma.[11,12] These apparently contradictory results may arise from the confounding factors and reverse causality in observational studies. Moreover, a randomized clinical trial (RCT) has failed to confirm the clinical benefit of a high intake of vegetables and fruits in POAG management.[13] However, this trial had several limitations, including the use of a secondary analysis and uncertain time of glaucoma onset. In sum, the causality between diet-derived antioxidants and glaucoma remains unclear.

Mendelian randomization (MR) is an analytic method for assessing causal relationships between exposures and outcomes based on genetic variation.[14] MR analysis minimizes confounding and avoids reverse causality, as alleles are randomly allocated at conception and are not modified by disease.[15] We estimated the causal association of diet-derived circulating antioxidants and their metabolites with POAG using two-sample MR. Furthermore, we explored genetic associations between diet-derived antioxidants and glaucoma-related traits, including IOP, macular retinal nerve fiber layer (mRNFL) thickness, macular ganglion cell–inner plexiform layer (mGCIPL) thickness, and vertical cup-to-disc ratio (vCDR).

Methods

Study Design

We performed a two-sample MR analysis of the summary statistics from genome-wide association studies (GWASs) to determine the genetic associations among POAG, glaucoma-related traits, and diet-derived circulating antioxidants, including vitamin A (retinol), vitamin C (ascorbate), vitamin E (α-tocopherol and γ-tocopherol), β-carotene, and lycopene. These antioxidant exposures were categorized into two phenotypes: (1) absolute circulating antioxidants (retinol, ascorbate, β-carotene, and lycopene), measured as actual absolute levels in the blood; and (2) circulating antioxidant metabolites (retinol, ascorbate, α-tocopherol, and γ-tocopherol), quantified as relative concentrations in plasma and/or serum. We have reported this study according to the reporting checklist for Strengthening the Reporting of Observational Studies Using Mendelian Randomization (STROBE-MR).[16,17] The principles of MR are as follows: (1) instrumental variables are associated with exposure; (2) instrumental variables are not associated with confounders; and (3) instrumental variables affect the outcome only by the exposure,[14] as shown in Figure 1A. The schematic overview and framework of the MR analyses in this study are presented in Figure 1B. The data used in the present study are publicly available, and ethical approval was obtained for the original studies.

Selection of Genetic Instrumental Variables

Genetically determined absolute circulating antioxidants (namely, retinol, ascorbate, β-carotene, and lycopene) were identified in recent large GWASs among persons of European ancestry ($P < 5 \times 10^{-8}$; linkage disequilibrium [LD] < 0.001; clump distance = 10,000 kb). Two single nucleotide polymorphisms (SNPs) associated with retinol were identified from a GWAS of 5066 persons.[18] Ten independent SNPs associated with ascorbate were identified from a GWAS of 52,018 persons.[19] One SNP associated with β-carotene was identified from a GWAS of 2344 persons in the Nurses' Health Study.[20] Furthermore, under a relaxed threshold criterion ($P < 5 \times 10^{-6}$; LD < 0.001; clump distance = 10,000 kb),[21] five independent SNPs associated with lycopene were identified from a GWAS of 441 persons.[22]

Information on circulating antioxidant metabolites, retinol, ascorbate, α-tocopherol, and γ-tocopherol was extracted from a metabolic GWAS analysis with relaxed threshold criteria ($P < 1 \times 10^{-5}$), similar to previous studies.[23–25] Twenty-nine independent SNPs associated with retinol and 28 SNPs associated with α-tocopherol were extracted from a recently published GWAS among persons of European ancestry.[26] Fourteen SNPs associated with ascorbate and 13 SNPs associated with γ-tocopherol were identified from another metabolic GWAS analysis of persons of European ancestry.[27]

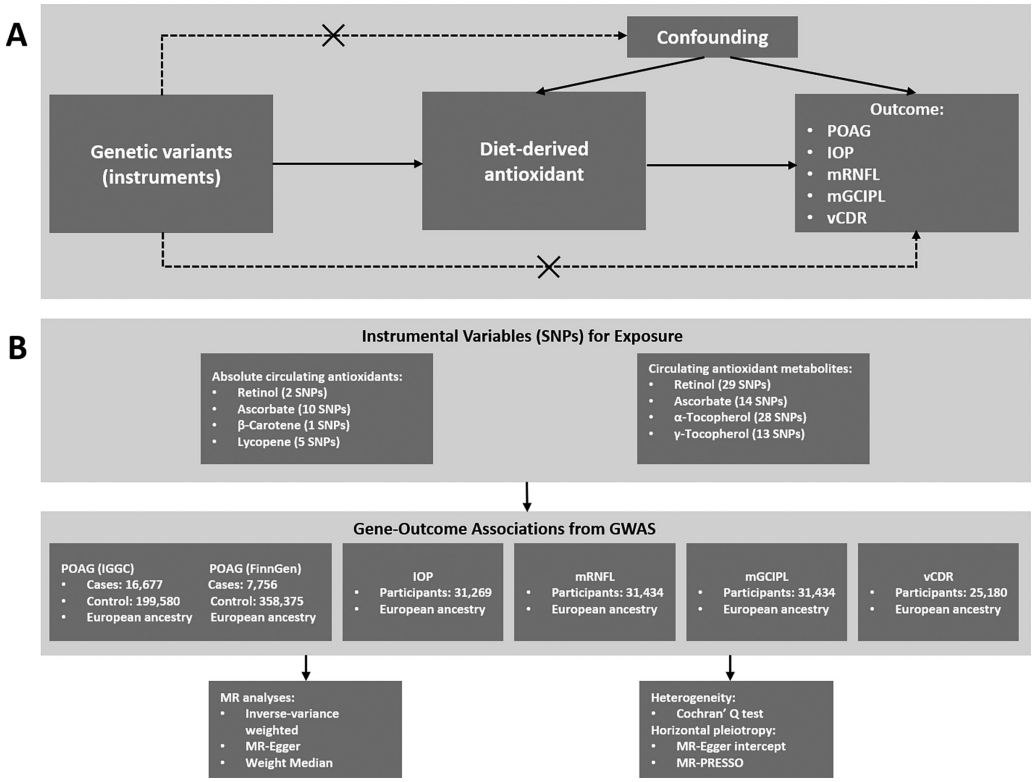

Figure 1. Schematic overview and framework of this MR study design. (**A**) There are three principal assumptions in MR design: (1) instrumental variables are associated with exposure; (2) instrumental variables are not associated with confounders; and (3) instrumental variables affect the outcome only by the exposure. (**B**) Study design and framework of the MR analyses in this study. IGGC, International Glaucoma Genetics Consortium.

Data Sources of POAG and Glaucoma-Related Traits

The largest GWAS data summary statistics for POAG were extracted from the International Glaucoma Genetics Consortium (IGGC),[28] and 18 studies were included. We used a first-stage meta-analysis comprised of 16,677 cases and 199,580 controls of European descent. The definition of POAG was based on the International Classification of Diseases diagnostic codes (ICD9/ICD10 revisions). Furthermore, we used the FinnGen Biobank (https://r9.finngen.fi/) for sensitivity testing for the association between diet-derived circulating antioxidants and POAG, and there were 7756 cases of POAG and 358,375 controls. We also extracted GWAS summary statistics for glaucoma-related traits among persons of European descent. We obtained the summary statistics for IOP summarizing 12 cohort studies by the IGGC in European-descent populations.[29] We also obtained data on other glaucoma-related traits, including mRNFL thickness,[30] mGCIPL thickness,[30] and vCDR.[29]

Statistical Analysis

We harmonized the exposure and outcome variants and eliminated any possible palindromic SNPs. The harmonization also ensured that the effect alleles belonged to the same allele. Furthermore, we used the PhenoScanner GWAS database (http://www.phenoscanner.medschl.cam.ac.uk/) to screen out SNPs with potential confounders associated with our outcomes ($P < 1 \times 10^{-5}$) to rule out possible pleiotropic effects from the MR analysis.[31] Finally, we calculated the genetic variation (R^2) of the explained phenotype for each instrument variable. Subsequently, we calculated the F-statistics to assess the strength of association for the SNPs,[32] and, to avoid the potential weak bias of instrumental variables, SNPs with F-statistics <10 were excluded.

Mendelian Randomization Analysis

Our MR analysis employed random-effects inverse variance weighting (IVW) regression as the main analysis to assess the causal relationships among diet-derived

circulating antioxidants, POAG, and glaucoma-related traits, as it provides reliable causal estimates in the absence of directional pleiotropy. Furthermore, to assess the robustness of the results, we performed sensitivity analyses using MR–Egger regression and the weighted-median estimator. The weighted-median estimator provides consistent assessment if valid genetic instrumental variables comprise up to 50% of the weights.[33] In the MR–Egger method, the intercept test is used to assess horizontal pleiotropy, which implies that there may be horizontal pleiotropy between genetic instrumental variables if the intercept is not equal to zero.[34] Cochran's Q test was used to assess heterogeneity.[35] In addition, the Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test was used to identify potential outliers with respect to horizontal pleiotropy,[36] and an MR analysis was performed again after removing the outliers to correct for horizontal pleiotropy.

We used a Bonferroni-corrected threshold of $P < 0.00125$ ($P < 0.05/40$, accounting for tests between eight exposures and five outcomes) as evidence of a significant association, and a P value between 0.00125 and 0.05 was considered nominally significant. All statistical analyses in this study were performed using R 4.3.0 (R Foundation for Statistical Computing, Vienna, Austria). MR analyses were performed using the "TwoSampleMR" package.

Results

Characterization of Genetic Instrumental Variables

The Table summarizes the information on the instrumental variables for diet-derived circulating antioxidants and their metabolites. Detailed information on the cohorts contributing to the GWAS for absolute antioxidants is presented in Supplementary Table S1. The summarized information for POAG and glaucoma-related traits is provided in Supplementary Table S2. Details on the SNPs associated with antioxidants, POAG, and glaucoma-related traits are given in Supplementary Tables S3 and S4. F-statistics for all genetic instruments were >10.

Association of Diet-Derived Circulating Antioxidants With POAG

The results of MR analyses are shown in Figure 2 using data from the IGGC study. Diet-derived antioxidant levels showed no significant associations with POAG in the IVW method. For absolute circulating antioxidants, the odds ratio (OR) was 1.027 (95% confidence interval [CI], 0.311–3.395; $P = 0.965$) for natural log-transformed retinol. The OR was 1.052 (95% CI, 0.911–1.215; $P = 0.490$) per 1 µmol/L increase for ascorbate. The OR was 1.011 (95% CI, 0.854–1.199; $P = 0.895$) for natural log-transformed β-carotene. The OR was 1.014 (95% CI, 0.960–1.071; $P = 0.610$) per 1 µg/dL of lycopene. For circulating antioxidant metabolites, the OR was 1.031 (95% CI, 0.950–1.118; $P = 0.466$) for log-transformed retinol per increase. The OR was 0.998 (95% CI, 0.801–1.244; $P = 0.989$) for ascorbate. The OR was 0.998 (95% CI, 0.930–1.072; $P = 0.962$) for α-tocopherol. Similarly, the OR was 1.210 (95% CI, 0.870–1.682; $P = 0.257$) for γ-tocopherol. Furthermore, we performed a sensitivity analysis using data from the FinnGen study and obtained similar results that showed no causal relationship between six diet-derived circulating antioxidants and POAG (Fig. 3).

Sensitivity analysis was performed for instrumental variables with over three SNPs (Figs. 2, 3). The results obtained with the MR–Egger and weighted-

Table. Summary of Instrumental Variables for Diet-Derived Antioxidants

Exposure	Sample Size	P	SNPs, n	Unit	PMID
Absolute circulating antioxidants					
Retinol	5066	5×10^{-8}	2	µg/L in log-transformed scale	21878437
Ascorbate	52,018	5×10^{-8}	10	µmol/L	33203707
β-Carotene	2344	5×10^{-8}	1	µg/L in log-transformed scale	23134893
Lycopene	441	5×10^{-6}	5	µg/dL	26861389
Circulating antioxidant metabolites					
Retinol	8247	1×10^{-5}	29	$\log_{10}$-transformed metabolite concentration	36635386
Ascorbate	2085	1×10^{-5}	14	$\log_{10}$-transformed metabolite concentration	24816252
α-Tocopherol	8192	1×10^{-5}	28	$\log_{10}$-transformed metabolite concentration	36635386
γ-Tocopherol	7725	1×10^{-5}	13	$\log_{10}$-transformed metabolite concentration	24816252

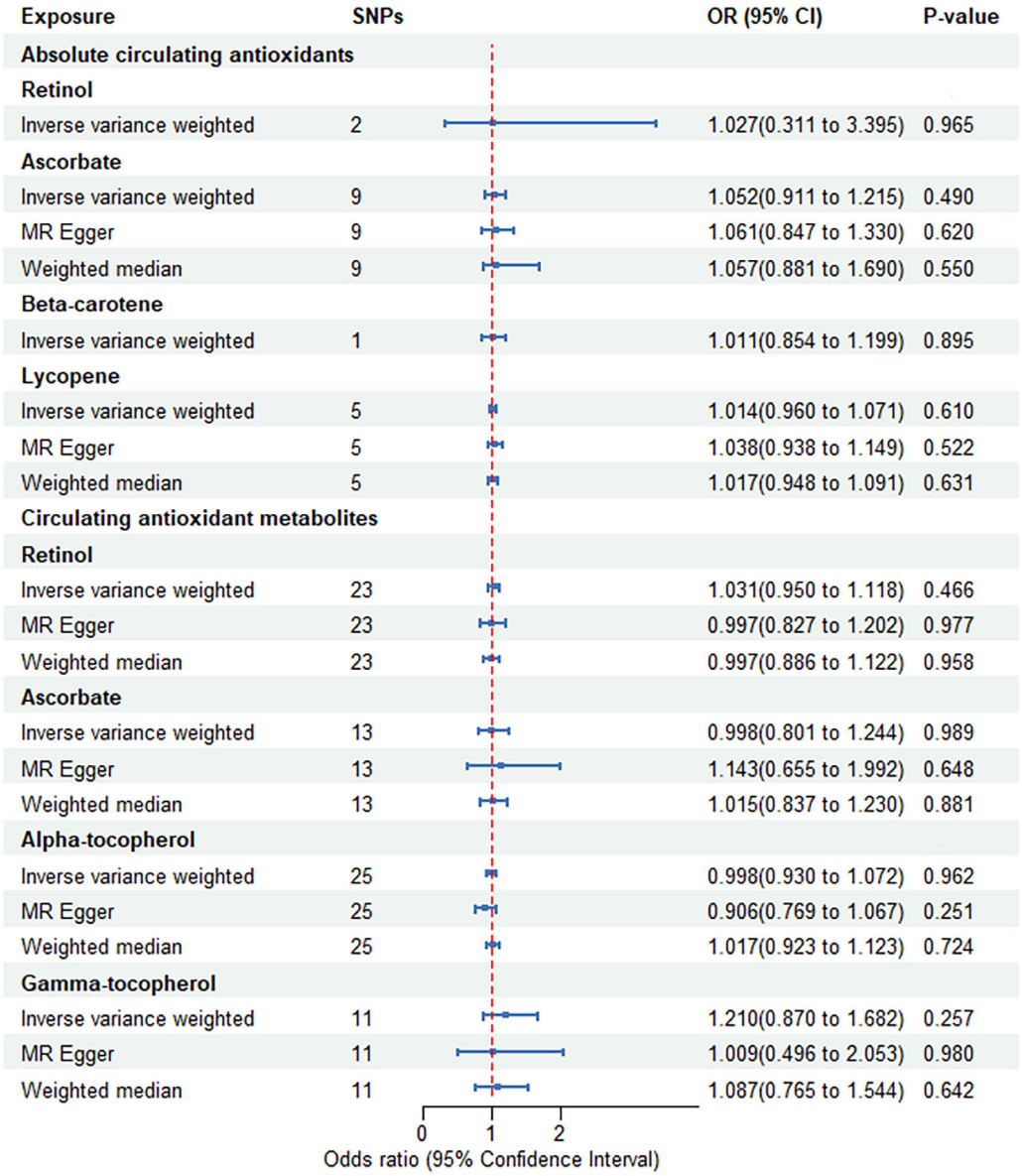

Figure 2. Genetic association between diet-derived circulating antioxidants and POAG using the IGGC. Estimated odds ratios represent the effect per 1 µmol/L of ascorbate, natural loggaucoma; IOP β-carotene, natural log-transformed retinol, and 1 µg/dL of lycopene on POAG.

median methods were consistent with the IVW regression analyses. Cochran's Q test detected no heterogeneity except for ascorbate in the IGGC study ($P = 3.50 \times 10^{-5}$) (Supplementary Tables S5) and ascorbate ($P = 0.044$) and γ-tocopherol ($P = 0.034$) in the FinnGen Biobank. The MR–Egger intercept test showed no significant horizontal pleiotropy for any diet-derived antioxidant. Furthermore, the MR-PRESSO test identified two outlier SNPs for ascorbate of circulating antioxidant metabolites and glaucoma in the IGGC study (rs8057559 and rs6713914; $P < 0.001$) (Supplementary Table S5). MR analyses showed that the risk of POAG did not change substantially after removing the outliers. The MR-PRESSO test did not detect outlier SNPs for the other outcomes.

Association of Diet-Derived Circulating Antioxidants and Glaucoma-Related Traits

Figure 4 shows the results for glaucoma-related traits, reflecting no significant associations with diet-derived antioxidants. For absolute circulating antioxidants, β ranged from −0.455 (95% CI, −2.172 to 1.263; $P = 0.604$) for mRNFL thickness to 0.319 (95% CI, −1.272 to 1.910; $P = 0.694$) for IOP per natural log-

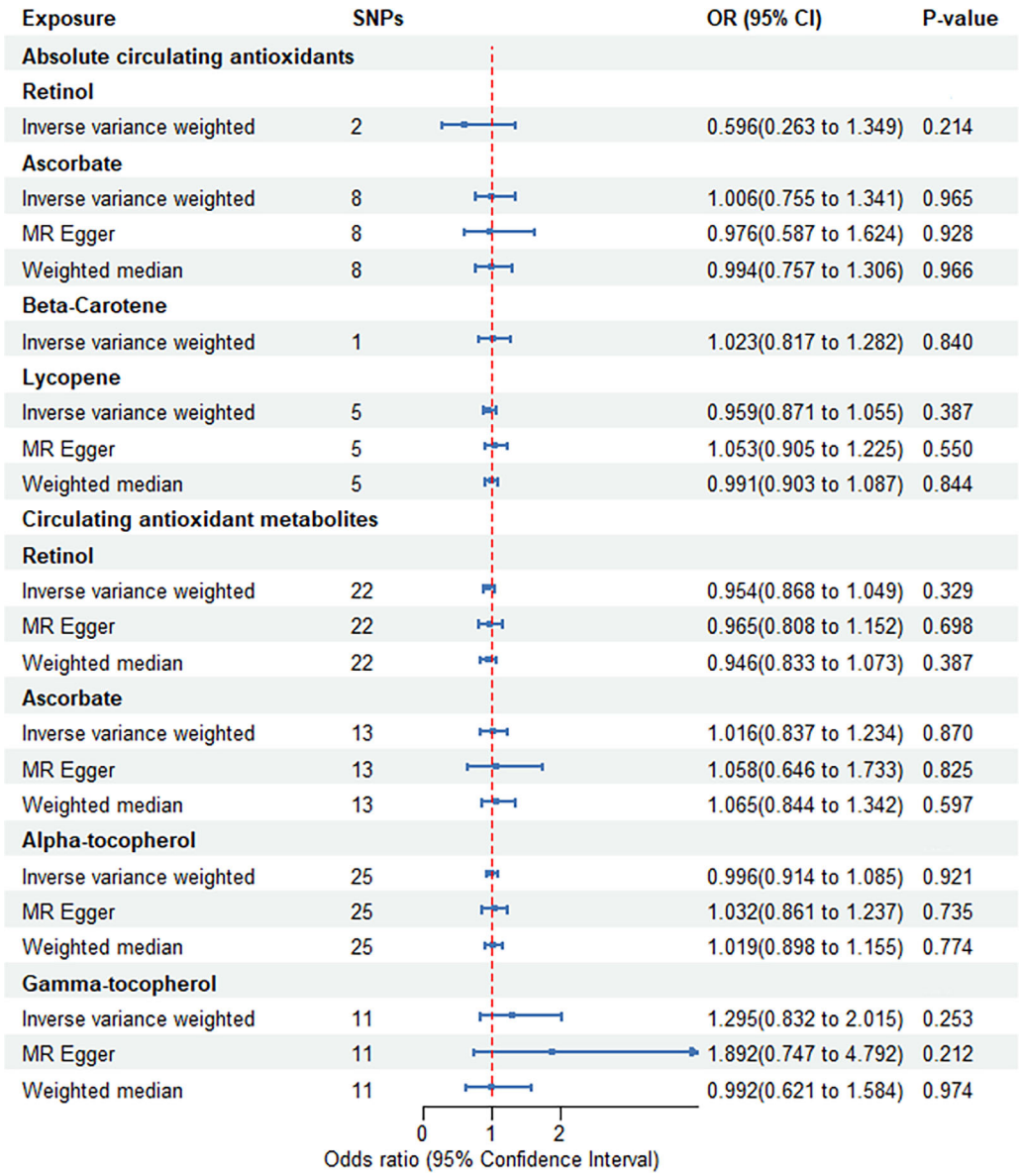

Figure 3. Genetic association between diet-derived circulating antioxidants and POAG using the FinnGen Biobank. Estimated odds ratios represent the effect per 1 µmol/L of ascorbate, natural logate, natural lognterval. natural log-transformed retinol, and 1 µg/dL of lycopene on POAG.

transformed retinol. Per 1-µmol/L increase for ascorbate, β (except for mGCIPL thickness) ranged from −0.182 (95% CI, −0.449 to 0.086; $P = 0.183$) for IOP to 0.114 (95% CI, −0.266 to 0.494; $P = 0.556$) for mRNFL thickness. For natural log-transformed β-carotene, β ranged from −0.203 (95% CI, −0.828 to 0.421; $P = 0.523$) for mGCIPL thickness to 0.084 (95% CI, −0.392 to 0.559; $P = 0.730$) for mRNFL thickness. For log-transformed retinol per increase, β ranged from −0.186 (95% CI, −0.511 to 0.140; $P = 0.264$) for mGCIPL thickness to 0.108 (95% CI, −0.002 to 0.217; $P = 0.054$) for IOP. For circulating antioxidant metabolites, β ranged from −0.074 (95% CI, −0.278 to 0.129; $P = 0.474$) for IOP to 0.021 (95% CI, −0.294 to 0.335; $P = 0.898$) for mGCIPL thickness per log-transformed retinol. Also, β ranged from −0.158 (95% CI, −0.464 to 0.147; $P = 0.310$) for IOP to 0.005 (95% CI, −0.006 to 0.016; $P = 0.363$) for vCDR per log-transformed ascorbate. The β values ranged from −0.047 (95% CI, −0.191 to 0.096; $P = 0.518$) for IOP to 0.054 (95% CI, −0.211 to 0.319; $P = 0.689$) for mGCIPL thickness per log-transformed α-tocopherol. Finally, β ranged from −0.263 (95% CI, −0.917 to 0.391; $P = 0.431$) for mRNFL thickness to 0.407 (95% CI, −0.179 to 0.993; $P = 0.173$) for IOP per log-transformed γ-tocopherol.

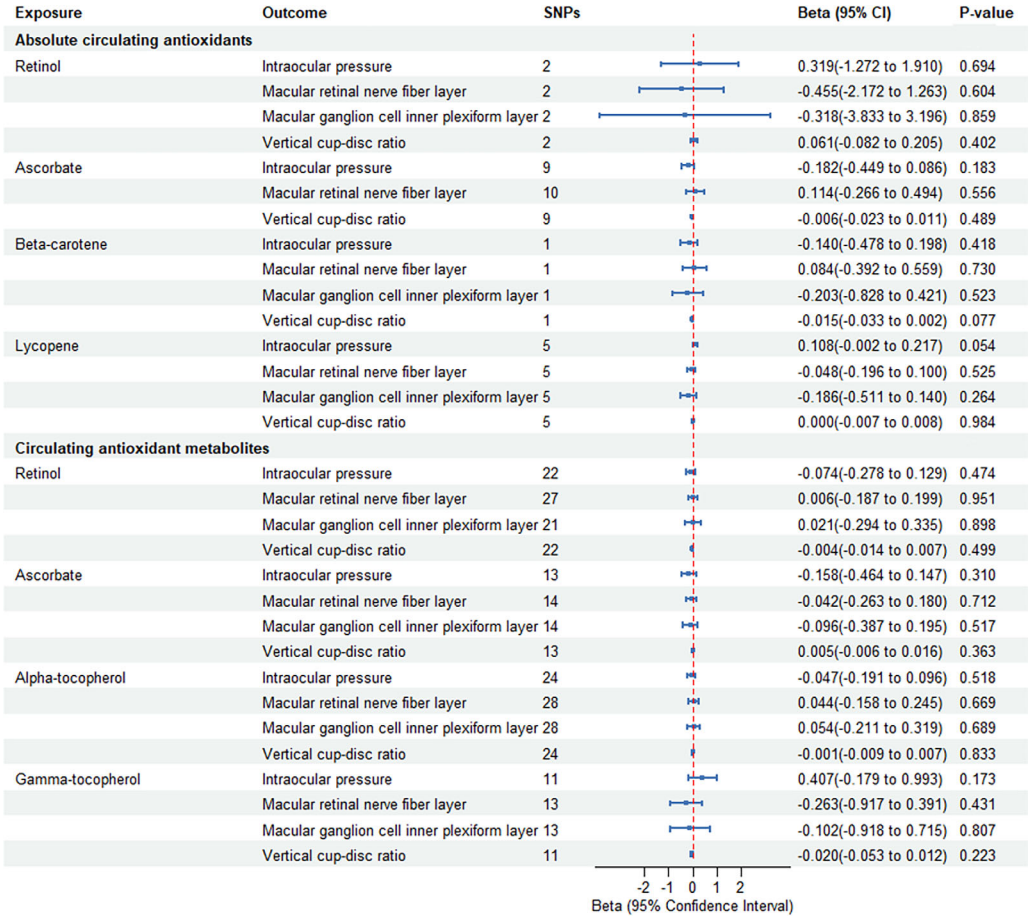

Figure 4. Genetic association between diet-derived circulating antioxidants and glaucoma-related traits. Estimated beta represents the effect per increase in log-transformed antioxidant metabolite concentrations on glaucoma-related traits. The results were obtained using an IVW method.

Sensitivity analyses showed that the MR–Egger and weighted-median methods generated consistent results with the IVW regression (Fig. 4). Cochran's Q test detected no heterogeneity except for lycopene with mGCIPL thickness ($P = 0.014$) (Supplementary Tables S6–S9). There is no evidence of the presence of horizontal pleiotropy for any of the antioxidants according to the MR–Egger intercept and MR-PRESSO tests except for the association between IOP and retinol (rs79548267; $P = 0.036$) and ascorbate (rs9606290; $P = 0.030$) for circulating antioxidant metabolites (Supplementary Table S6). MR analyses showed that the OR estimates did not change significantly for the association between IOP and retinol after removing the outliers. After removing the outliers, there was a nominal correlation between ascorbate and IOP ($\beta = -0.418$; 95% CI, -0.730 to -0.107; $P = 0.023$).

Discussion

Our study used two-sample MR to estimate the genetic relationships among six diet-derived circulating antioxidants, POAG, and glaucoma-related traits. Instrumental variables were used as proxies for absolute antioxidant levels and their metabolites. Our results indicate that diet-derived antioxidants are unlikely to have a protective effect against POAG and are not significantly genetically associated with glaucoma-related traits. These findings suggest that the previously observed associations between antioxidants and glaucoma may not be causal.

Evidence that dietary-derived antioxidants are protective against glaucoma is inconclusive. Most cross-sectional studies have reported that supplementation with vitamins or a high intake of fruits

and vegetables is associated with a decreased risk of glaucoma.[37–39] However, a cross-sectional study analyzing data from the US National Health and Nutrition Examination Survey (NHANES), 2005–2006, reported that supplementary vitamin C, but not vitamin A or E, was associated with decreased risk of glaucoma.[40] Furthermore, cohort data from the Nurses' Health Study indicated that supplementation with vitamins A, C, and E, as well as dietary intake of green leafy vegetables high in vitamins, yielded mixed results.[6,41] The aforementioned observational studies relied on self-reported intake, without objective measurements of antioxidants or nutrients, and the effect of confounding factors on POAG cannot be excluded. Moreover, although a meta-analysis of five primarily cross-sectional observational studies concluded that the dietary intake of vitamins A and C reduced POAG risk by 55% and 61%, respectively, no such effect was observed for vitamins B1 and E[42]; however, the included studies had high heterogeneity and different definitions of POAG, with two studies using self-reported data. Our study provides evidence, using genetic instrumental variables, that diet-derived antioxidants have no causal relationship with glaucoma.

Our study identified no causal relationship between six diet-derived antioxidants and POAG, but other antioxidant relationships still should be clarified. For example, a recent study suggested that diet supplementation of zinc, selenium, and magnesium was associated with a lower risk of glaucoma.[43] Conversely, previous studies have also indicated that dietary intake of magnesium and selenium may increase the risk of glaucoma.[7,44,45] Similarly, one study suggested a lower likelihood of glaucoma with increased dietary consumption of omega-3,[46] whereas other studies found either no correlation or an increased risk of glaucoma.[7,47,48] Additionally, there are contradictory findings regarding the intake of vitamin B and its association with glaucoma occurrence.[7,12,39] Therefore, it is necessary to conduct further studies to confirm the relationship between other antioxidants and glaucoma.

At present, there are no epidemiologic studies on the association between diet-derived antioxidants and IOP. Previous studies have confirmed increased oxidative stress levels in the aqueous humor of patients with glaucoma.[49,50] Oxidative stress contributes to trabecular meshwork dysfunction,[51,52] obstructing aqueous humor outflow and leading to elevated IOP. However, in our study, diet-derived antioxidants were not found to have a significant IOP-lowering effect.

Preventing or delaying glaucoma progression remains a challenge worldwide. In two RCTs, supplementation with antioxidants improved inner retinal function and vision-related quality of life in patients with glaucoma,[53,54] suggesting that antioxidants may slow glaucoma progression. However, these studies had short follow-up periods (8–12 months) and relatively small samples (43–109 participants) and did not assess structural changes in RGCs. In contrast, Hui et al.[55] reported that the intake of vitamin B3 improved inner retinal function but did not slow circumpapillary RNFL thinning in patients with glaucoma. Additionally, in another RCT with a 2-year follow-up, supplementation with oral antioxidants did not slow the progression of peripapillary RNFL and macular ganglion cell complex in patients with POAG.[56] Combined with our findings, this suggests that diet-derived antioxidants may not protect RGCs.

Our study determined that diet-derived antioxidants had no causal relationship with POAG and no protective effect on glaucoma-related traits. A cross-sectional study reported that the serum levels of vitamins A, C, and E were not associated with glaucoma.[40] Another study showed that patients with normal-tension glaucoma had lower serum levels of vitamin C, but not vitamins A or E, compared to the controls.[57] Furthermore, a meta-analysis found no evidence of a relationship between plasma or serum vitamin levels and POAG.[42] These study findings suggest that antioxidants in the blood may not be associated with glaucoma risk. Furthermore, previous studies have shown inconsistent antioxidant activity in serum and aqueous humor in glaucoma patients.[49,58] Therefore, we hypothesize that circulating antioxidant levels may not accurately represent antioxidant capacity and that enhancing antioxidant levels in the blood does not necessarily produce additional antioxidant effects in ocular structures. However, this hypothesis requires further verification. Patients with obstructive sleep apnea (OSA) have a higher risk of POAG compared to those without OSA,[59,60] and it is believed that oxidative stress plays a significant role.[61] Therefore, it is equally unlikely that diet-derived antioxidants can be protective against POAG related to OSA.

This study has several strengths. First, to the best of our knowledge, we are the first to investigate a causal relationship among diet-derived antioxidants, POAG, and glaucoma-related traits using genetic instrumental variables, excluding the residual confounding and reverse causality bias in observational studies and avoiding the exposure of participants to unnecessary risks and hazards in clinical trials. Second, we used two independent datasets of instrumental variables for both absolute circulating antioxidants and their metabolites. Similar results were obtained in MR analyses with both absolute blood and metabolite levels,

especially for retinol and ascorbate, thus demonstrating the robustness of our findings. Third, we also used the FinnGen Biobank in our analysis, in addition to using the largest recent summary statistics POAG data, and obtained consistent results. Finally, in addition to analyzing the causal relationship between antioxidants and glaucoma, we included glaucoma-related traits to clarify the association between diet-derived antioxidants and glaucoma progression.

However, our study has several limitations, as well. First, GWAS data were extracted from populations of European descent, but glaucoma exhibits significant race-dependent differences.[62] Therefore, our findings cannot be directly generalized to other racial populations. Second, as there were only one and two SNPs for the absolute circulating antioxidants β-carotene and retinol, we could not perform MR–Egger, weighted-median, or MR-PRESSO analyses. However, these instrumental variables are not associated with risk factors for POAG or glaucoma-related traits in the PhenoScanner database, indicating that horizontal pleiotropy is unlikely. Future research should find more antioxidant-related sites and improve the strength of the instrumental variables. Third, a nonlinear model may be more suitable for exploring the causal associations among diet-derived antioxidants, POAG, and glaucoma-related outcomes. However, as published reports include only summary statistics and no individual-level data, we could not perform nonlinear analyses. Finally, we could not completely exclude the possibility that other untested antioxidants may have protective effects on glaucoma and its associated traits.

Conclusions

Our findings did not support a causal association between genetically determined diet-derived circulating antioxidants of vitamins A, C, and E; β-carotene; or lycopene with POAG risk. Furthermore, our analyses observed no significant genetic association between six diet-derived antioxidants and glaucoma-related traits. Therefore, supplemental antioxidants may not offer clinical benefits to patients with POAG or elevated IOP and are not protective of RGCs in the general population.

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