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Causal effect of the 25-Hydroxyvitamin D concentration on ocular diseases: A Mendelian randomization study

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Observational studies report controversial associations between vitamin D levels and ocular diseases. We investigated the potential causal effect of 25-hydroxyvitamin D [25(OH)D] on ocular diseases using two-sample Mendelian randomization (MR). We examined associations between 25(OH)D and various ocular diseases including age-related macular degeneration, cataracts, diabetic retinopathy, myopia, glaucoma, conjunctivitis, keratitis and optic neuritis. Data were from genome-wide association studies of 25(OH)D and ocular diseases. We performed MR analyses using inverse-variance weighted methods with sensitivity analyses. We found no significant causal relationships between 25(OH)D and ocular diseases (all P > 0.05). Tests for heterogeneity (P > 0.05) and pleiotropy (P > 0.05) supported the MR validity. Our MR analysis does not provide evidence supporting a causal relationship between 25(OH)D and ocular disease risk in Europeans. This suggests that previous epidemiological associations may stem from shared biological factors or confounders rather than direct causality. However, further research is needed to fully elucidate the complex relationships between vitamin D and ocular health.

Keywords 25(OH)D concentration, Ocular diseases, Mendelian randomization

Abbreviations

GWAS	Genome-wide association study
IV	Instrumental variants
IVW	Inverse variance weighted
MR	Mendelian randomization
SNP	Single-nucleotide polymorphism
OR	Odds ratio
LD	Linkage disequilibrium
AMD	Age-related macular degeneration
DR	Diabetic retinopathy

Vitamin D, a fat-soluble nutrient, plays a pivotal role in ensuring bone health by enhancing calcium absorption and fostering bone mineralization. While its skeletal benefits are well-acknowledged, the non-skeletal impacts of vitamin D have recently come under the spotlight[1,2]. Deficiency in this vitamin has been linked to a heightened risk of several chronic ailments, including cardiovascular diseases, immune system anomalies, diabetes, and even cancer[3,4]. The nexus between vitamin D and eye health is an emerging area of interest. Although observational studies have hinted at potential associations between vitamin D levels and a range of eye conditions like myopia, age-related macular degeneration (AMD), diabetic retinopathy (DR), and dry eye, the exact causal relationship remains elusive[5]. For instance, when it comes to myopia, vitamin D concentrations might merely reflect the amount of time one spends outdoors[6–8]. In the case of AMD, while some cross-sectional studies advocate the protective role of vitamin D[9,10], extensive prospective research hasn't found any notable influence of vitamin D supplementation on AMD susceptibility[11]. Furthermore, for prevalent eye disorders such as glaucoma[12,13], DR[14,15], and cataracts[16,17], the findings from observational studies are mixed. This inconsistency might stem from the inherent limitations of such studies, which can be influenced by confounding variables, making it challenging to pinpoint causality.

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To overcome the inherent challenges of observational studies, we embarked on a two-sample Mendelian Randomization (MR) study to delve into the potential causal links between vitamin D and prevalent eye conditions. MR is a method that employs single nucleotide polymorphisms (SNPs) as genetic markers for a specific exposure to ascertain causal connections with a particular outcome. In the MR framework, genetic variations that have a strong association with exposure and meet certain criteria are harnessed as instrumental variables (IVs) to probe causal associations with outcomes[18,19]. Given that these genetic markers are randomly determined at conception, they effectively sidestep biases arising from environmental confounders. This positions MR studies in a realm akin to randomized controlled trials. Recent MR explorations have shed light on the fact that 25(OH)D concentrations might not have a causal link with refractive errors[20], but its influence on other eye-related outcomes remains ambiguous. In our research, we employed a dual-sample MR approach to discern the possible causal ties between serum 25(OH)D levels and a spectrum of ocular diseases. These include AMD, senile cataract, DR, myopia, glaucoma, conjunctivitis, allergic conjunctivitis, keratitis, and optic neuritis. Gaining clarity on vitamin D's causal role in ocular health could pave the way for novel preventive and therapeutic strategies for widespread eye ailments that culminate in vision impairment and global blindness.

Materials and methods

Study design

Initially, we pinpointed sources of genome-wide association study (GWAS) data pertinent to vitamin D. Subsequently, we scoured PubMed for ocular diseases potentially linked to vitamin D. Based on our findings, we sought GWAS data that would enable a two-sample MR approach. To probe the causal nexus between the exposure and the outcome, we adopted a two-sample MR methodology, leveraging genetic variations as IVs[21]. For this endeavor, we harnessed aggregated GWAS data on 25(OH)D concentrations as the IVs for our MR analyses. To bolster the credibility of our results, we conducted an array of sensitivity tests. Additionally, we executed one MR analysis prior to and another post the removal of confounding factors. The schematic of our study design is depicted in Fig. 1. This design relies on three key assumptions for Mendelian randomization: Assumption 1: The instrumental variables (genetic variants) are strongly associated with the exposure (25(OH)D concentration). Assumption 2: The instrumental variables are not associated with any confounders of the exposure-outcome relationship. Assumption 3: The instrumental variables influence the outcomes only through the exposure (25(OH)D concentration), and not through any other causal pathway.

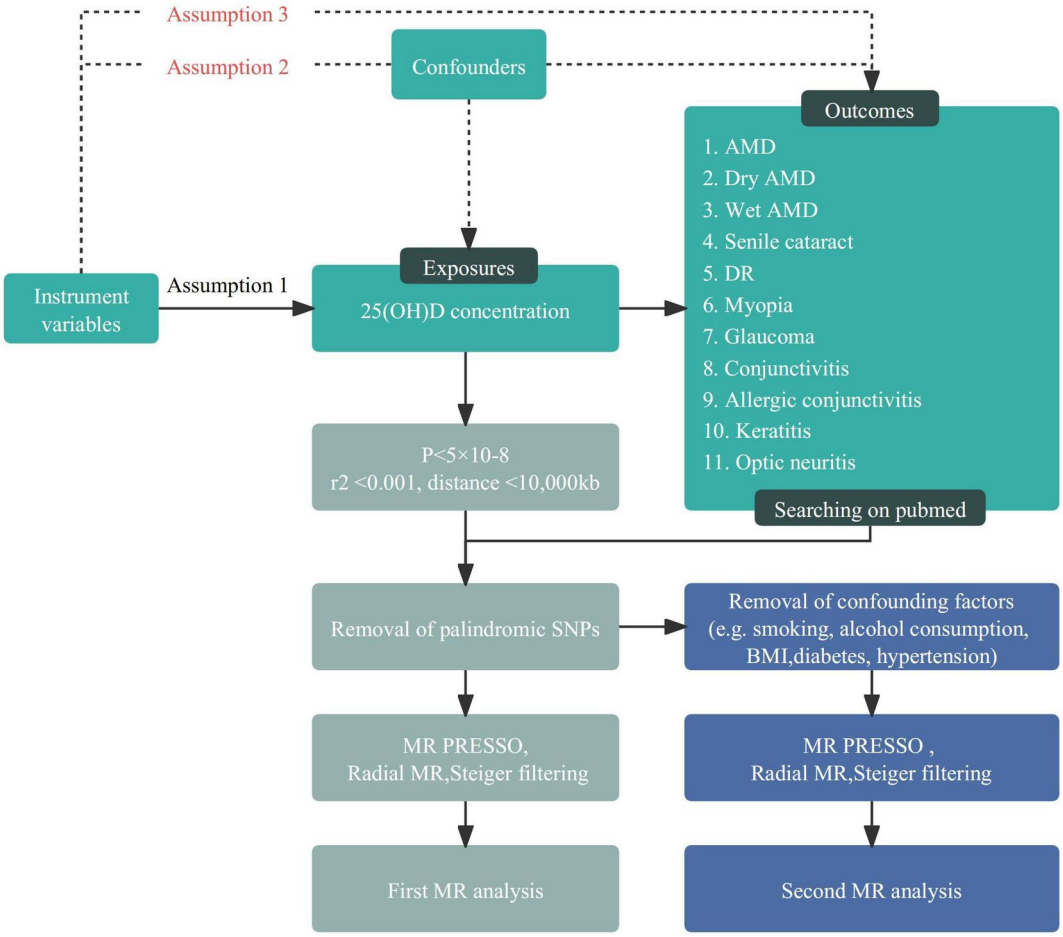

Fig. 1. The study design and workflow of the MR study.

Data sources

The exposure dataset is derived from summary statistics of the most recent and comprehensive GWAS for 25(OH)D concentrations, which analyzed the UK Biobank populations, encompassing 417,580 individuals[22]. The UK Biobank team was responsible for collecting blood samples, measuring 25(OH)D concentrations, and gathering questionnaire data on vitamin D supplement use. Subsequently, in their GWAS analysis, Revez et al. (2020) performed rank-based inverse normal transformation on these 25(OH)D measurements. This transformation ensures effect sizes (β) are expressed in SD units, enabling valid interpretation of variance explained R^2.Their analysis incorporated the month of assessment as a covariate to account for seasonal fluctuations in 25(OH)D concentrations. Regarding supplement use data, Revez et al. employed a multi-step approach: they first conducted a main analysis without considering supplement use, then performed sensitivity analyses with supplement use as a covariate, and finally conducted stratified analyses for supplement users and non-users.

The summary statistics for the GWAS pertaining to ocular diseases are sourced from the R12 GWAS, as released by the FinnGen Consortium. This includes data on AMD (12,495 cases), dry AMD (8,570 cases), wet AMD (6,699 cases), senile cataract (83,886 cases), DR (14,142 cases), myopia (5,406 cases), glaucoma (26,591 cases), conjunctivitis (41,074 cases), allergic conjunctivitis (29,791 cases), keratoconus (16,577 cases), and optic neuritis (1,592 cases)[23].

Table S1 offers a detailed breakdown of the pertinent GWAS data segments. Comprehensive insights into recruitment methodologies and diagnostic criteria can be found in the original publications. It's noteworthy that all datasets employed in our research were exclusively of European descent, ensuring no overlaps or intersections between exposures and outcomes. All the data harnessed for this research is publicly accessible from the respective GWAS. Given that we utilized anonymized, summary-level data available to the public domain, there was no need for ethical approval for this study.

Instrumental variable (IV) selection

We selected multiple distinct SNPs that exhibited a robust association with each exposure trait as IVs to gauge the causal link between 25(OH)D concentration and ocular diseases. The criteria for IV selection were rooted in the foundational hypotheses of MR: (1) The IVs should exhibit a strong correlation with the exposure. (2) The instrumental variants should remain unaffected by any confounders in the relationship between exposure and outcome. (3) The instrumental variants should influence the outcome exclusively via the exposure[24]. To ensure a strong correlation between our IVs and the exposure, we only selected SNPs that reached this genome-wide significance threshold. To pinpoint the IVs, we initially sifted through SNPs linked to the exposure at a genome-wide significance threshold ($P < 5 \times 10 - 8$) and filtered out those exhibiting linkage disequilibrium (LD) ($r2 < 0.001$, distance = 10,000kb)[25]. The curated SNPs were then integrated into the GWAS database for the respective outcomes. In instances where the summary statistics for outcomes lacked SNPs related to the exposure, we refrained from employing proxy SNPs.

To minimize the risk of violating MR Assumptions 2 and 3, each SNP was rigorously assessed for associations with known confounders (e.g., BMI, diabetes, hypertension) using the PhenoScanner database[26,27]. SNPs showing associations with confounders were excluded. Additionally, sensitivity analyses (e.g., MR-Egger regression, MRPRESSO) were employed to detect and account for potential horizontal pleiotropy. While these methods cannot fully 'prove' adherence to MR assumptions, they reduce bias by identifying SNPs with pleiotropic effects or unbalanced associations with confounders. Additionally, outliers were identified and purged using the Radial MR[28]and MRPRESSO tests[29]. SNPs indicating a misaligned causal direction were filtered out via MR Steiger filters[30]. In the final selection, only SNPs boasting instrument strengths (F) exceeding 10 were chosen, adhering to the principles outlined above.

Statistical analysis

In our MR investigation, we utilized a spectrum of methods to discern the causal link between 25(OH)D concentrations and ocular diseases[29,31]. Our primary analysis hinged on the inverse variance weighting (IVW) method. This approach amalgamates Wald estimates for each SNP via a meta-analysis technique to produce a comprehensive estimate. We favored the IVW method due to its superior accuracy and testing power, especially when the foundational MR assumptions were satisfied[32]. To assess the robustness of our findings and address potential violations of MR assumptions—specifically horizontal pleiotropy, we also incorporated alternative methods like MR-Egger regression, weighted median (WM), Robust Adjusted Profile Score(RAPS), simple mode and weighted mode. Each method operates under distinct statistical assumptions: RAPS adjusts for pleiotropy and weak instrument bias through profile likelihood estimation, providing robust causal inference without requiring specific pleiotropy distribution assumptions. Weighted median delivers consistent causal estimates even if up to 50% of the genetic variants are invalid, assigning weights based on the inverse variance of each variant's effect size. The simple mode method determines causal inference by selecting the modal value derived from Wald ratio estimates across individual genetic instruments. Weighted mode identifies causal effects supported by the largest cluster of SNPs, prioritizing more precise estimates through inverse-variance weighting. MR Egger tests for directional pleiotropy and estimates causal effects under the assumption that pleiotropic effects are independent of the genetic variants' associations with the exposure.

Outliers were identified and pruned using the MRPRESSO and radial MR techniques[28,29]. The MR Steiger filter was deployed to ascertain the causal trajectory of each identified SNP concerning exposure and outcome, discarding those SNPs that indicated reverse causality[30]. The potential pleiotropy of IVs within the outcome's GWAS dataset was scrutinized using the MR Egger intercept, with P values exceeding 0.05 suggesting an absence of significant pleiotropy[29]. The IVs' heterogeneity within the outcome's GWAS dataset was evaluated using both MR Egger and IVW against Cochran's Q statistics. A *P*value surpassing 0.05 indicated homogeneity[33]. To visually assess the causal influence and the robustness of our findings, we employed scatter and funnel plots,

respectively. We also executed "leave-one-out" analyses, systematically excluding each instrumental SNP to gauge its individual impact.

The strength of the IVs was assessed using the F-statistic, calculated as $F = \beta^2/SE^2$, where β represents the effect size of the SNP on 25(OH)D concentration (i.e., the change in 25(OH)D per allele), and SE denotes the standard error of the effect size estimate[34]. An F-statistic exceeding 10 was interpreted as indicating strong instrument strength, minimizing the risk of weak instrument bias.

To account for multiple testing across the various ocular diseases examined, we applied the Benjamini-Hochberg procedure to control the false discovery rate (FDR) within each MR method. We calculated the proportion of variance explained (R^2) by our genetic instruments for each outcome using the formula: R^2 = (2beta^2maf*(1-maf)) / (2beta^2maf*(1-maf) + (se^2)2nmaf(1-maf)), where beta is the effect size, maf is the minor allele frequency, se is the standard error, and n is the sample size.

All our statistical endeavors were executed using R (version 4.4.2). The MR analyses were facilitated by tools such as Two sample MR (version 0.6.9)[35], Radial MR (version 1.1)[28], and MRPRESSO (version 1.0)[29].

Results

MR analysis of 25(OH)D concentration on ocular diseases (First MR analysis)

Based on the design of the prior study, we obtained the IVs required for conducting the MR analyses (refer to Table S2for specific information). These R^2 values, ranging from 1.8 to 2.3%. All instrumental SNPs demonstrated strong statistical power, with F-statistics exceeding the recommended threshold of 10, confirming minimal risk of weak instrument bias (Table S2).In this Mendelian randomization study, which included 55–87 IVs, we utilized the IVW methodology to investigate the causal associations between 25 hydroxyvitamin D concentrations and the risk of major ocular diseases. Our analysis revealed no statistically significant causal associations between 25 hydroxyvitamin D concentrations and various major ocular diseases, including AMD (OR = 0.937; 95%CI = 0.796–1.103; *P* = 0.437), Dry AMD (OR = 0.933; 95%CI = 0.781–1.115; *P* = 0.446), Wet AMD (OR = 0.843; 95%CI = 0.692–1.028; *P* = 0.091), senile cataract (OR = 0.975; 95%CI = 0.909–1.047; *P* = 0.490), DR (OR = 1.059; 95%CI = 0.923–1.214; *P* = 0.417), myopia (OR = 1.063; 95%CI = 0.872–1.296; *P* = 0.547), conjunctivitis (OR = 1.031; 95%CI = 0.954–1.115; *P* = 0.444), allergic conjunctivitis (OR = 1.060; 95%CI = 0.967–1.163; *P* = 0.213), optic neuritis (OR = 0.856; 95%CI = 0.581–1.261; *P* = 0.432) and keratitis (OR = 1.062; 95%CI = 0.948–1.189; *P* = 0.299). However, the IVW method showed a significant causal relationship between 25(OH)D concentration and glaucoma (OR = 1.124; 95%CI = 1.000–1.262; *P* = 0.050), with the MR-Egger method producing contradictory results (OR = 0.970; 95%CI = 0.796–1.183; *P* = 0.765), suggesting potential invalidity of the causal relationship[36].

The main findings are exhibited in Fig. 2 and Table S3. For a visual representation of the causal effect of each 25(OH)D concentration-associated SNP on ocular diseases, please refer to the forest plot in Appendix A, whereas the scatter plots comparing SNP effects on 25(OH)D concentration with SNP effects on ocular diseases can also be found in Appendix A.

Robustness

The integrity of our findings was bolstered by our meticulous Mendelian randomization analyses, which consistently exhibited no signs of heterogeneity or pleiotropy. The funnel plots in Appendix A further attested to the lack of discernible horizontal pleiotropy across all outcomes. Additionally, the MR-Egger regression method was employed to probe any potential horizontal pleiotropy between SNPs and outcomes, and it consistently indicated an absence of such pleiotropy (as detailed in Table S4). Sensitivity analysis results, presented in Table S4, further confirmed the absence of heterogeneity, as evidenced by Cochran's IVW and the MR Egger Q test. Moreover, the leave-one-out sensitivity analysis plot underscored that no individual SNP exerted undue influence on the causality, further solidifying the reliability of our conclusions (refer to Appendix A).

MR analysis of 25(OH)D concentration on ocular diseases (Second MR analysis)

To more rigorously adhere to the core tenets of MR and to validate the consistency of our findings, we meticulously removed potential confounders as outlined in our initial study design. Specifically, we identified and excluded a total of 19 SNPs that showed associations with these recognized confounders (details provided in Table S5). Leveraging these refined IVs, we conducted a secondary MR analysis. This subsequent analysis mirrored our initial findings, reaffirming that there wasn't a significant causal link between serum 25 hydroxyvitamin D concentrations and the susceptibility to the majority of prevalent eye conditions. Both heterogeneity and pleiotropy tests yielded standard results, further endorsing the solidity of our MR analysis. Comprehensive details are presented in Fig. 3 and Table S6.

Discussion

This research predominantly harnessed MR analysis to probe the relationships between 25(OH)D concentrations and various ocular diseases, drawing from expansive GWAS datasets. Our two-sample MR analysis did not reveal statistically significant causal link between 25(OH)D levels and prevalent ophthalmic conditions such as AMD, senile cataracts, DR, and others. However, it is crucial to note that the absence of statistically significant associations does not definitively prove the lack of a causal relationship. Instead, our results suggest that if causal effects exist, they may be smaller than what our study was powered to detect, or that the relationship may be more complex than our model could capture.

The relationship between vitamin D and ocular diseases has been a focal point of numerous studies, most of which have been observational in nature. Such studies inherently come with a plethora of confounding factors, leading to divergent conclusions. For instance, when examining the link between vitamin D and AMD, an earlier

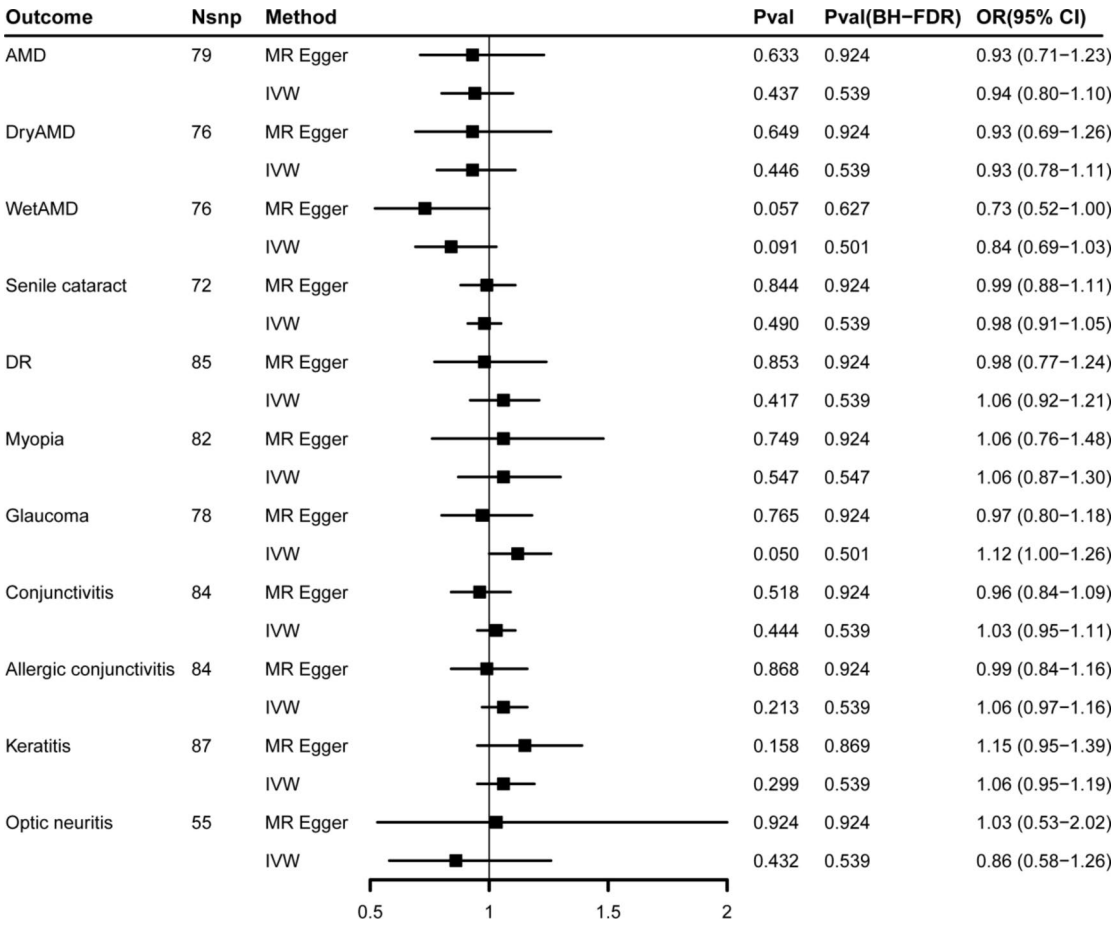

Fig. 2. First MR analysis of 25(OH)D concentration on ocular diseases.

meta-analysis encompassing 11 observational studies posited that elevated vitamin D levels conferred a protective effect against AMD[37]. Yet, a subsequent, more extensive meta-analysis incorporating 18 observational studies found no discernible association between the two[38]. A recent MR study suggests that calcium concentrations may have a protective effect against AMD, rather than vitamin D concentrations. The narrative around vitamin D and senile cataracts is similarly nuanced. While certain studies have touted the protective benefits of vitamin D supplementation against senile cataracts[39], a recent trial has countered this claim, suggesting that routine high-dose vitamin D supplementation might not significantly reduce the necessity for cataract surgery, especially among older adults residing in regions with low vitamin D deficiency prevalence[40]. The discourse on vitamin D and DR is equally multifaceted. A myriad of studies hint at an inverse relationship between vitamin D levels and DR[41–43], yet comprehensive systematic reviews have found no concrete association between them[44]. We acknowledge that the genetic architecture of DR is complex, which presents certain challenges for MR analyses using GWAS summary statistics. However, numerous recent studies have successfully applied MR methods to investigate DR, contributing valuable insights into its etiology[45]. Our study continues this important trend while recognizing the need for cautious interpretation of results. The multifactorial nature of DR suggests that genetic variants may explain only a portion of the phenotypic variance, highlighting the importance of integrating both genetic and environmental data in future research. As for the connection between vitamin D and myopia, the prevailing consensus is that there isn't a direct link. Instead, vitamin D levels are more likely indicative of time spent outdoors, serving as a biomarker rather than a causative factor[46,47]. The findings for other eye-related conditions are similarly varied, underscoring the complexity of the relationship between vitamin D and ocular health. In essence, while a multitude of studies have delved into the interplay between vitamin D concentrations and eye diseases, the results remain inconclusive. The intricate mechanisms and relationships between vitamin D levels and ophthalmological conditions warrant further exploration and understanding[5,15,48].

Our study boasts multiple strengths. Foremost, we utilized MR analysis to discern the causal nexus between 25(OH)D concentrations and common eye ailments, effectively sidelining potential confounders. Additionally, our two-sample MR methodology, which leveraged distinct exposure and outcome summary datasets, was designed to mitigate biases.

However, the depth of our study also brings to light certain limitations that warrant discussion. The use of summary statistics, while efficient, inherently limits the granularity of insights. Individual-level data could have allowed for more detailed subgroup analyses, potentially revealing patterns or relationships that summary statistics might miss. While we took steps to mitigate this risk, including the exclusion of SNPs associated with

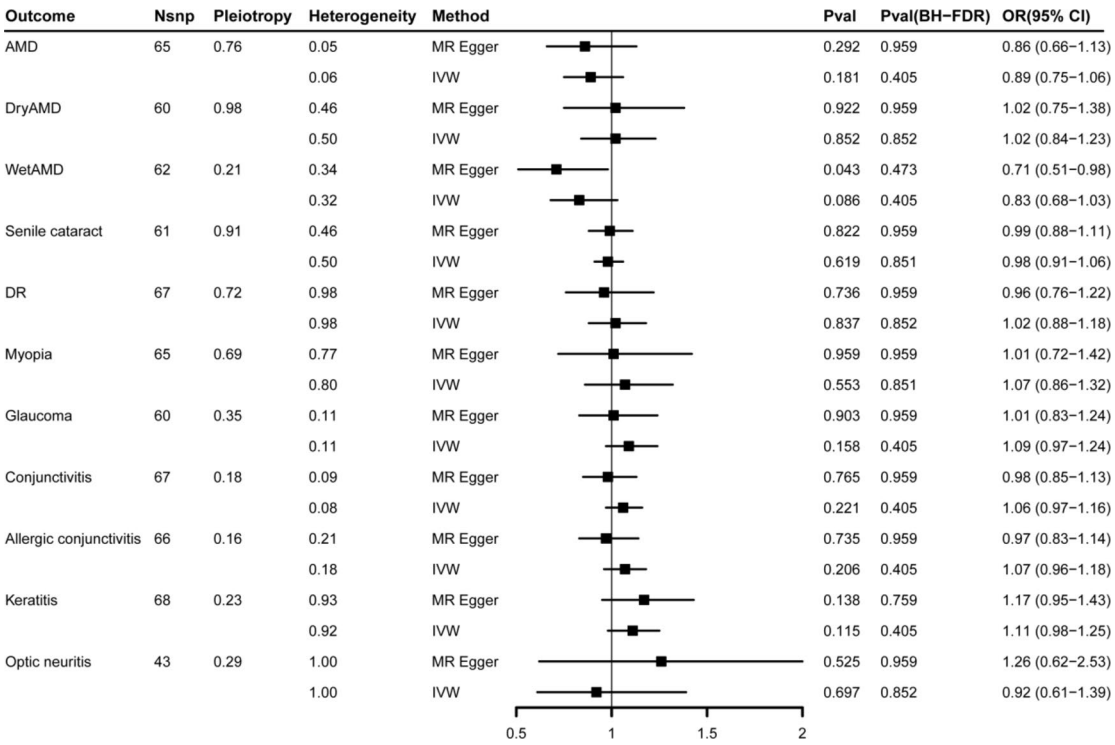

Fig. 3. Second MR analysis of 25(OH)D concentration on ocular diseases.

known confounders and the use of multiple MR methods, we cannot rule out the possibility of pleiotropic effects acting through unknown or unmeasured confounders. If several variants have pleiotropic effects that act via the same confounder, the pleiotropic effects and instrument strengths could be correlated, potentially biasing our results. This limitation is common to many MR studies and underscores the need for cautious interpretation of our findings. The demographic focus on European individuals raises questions about the broader applicability of our findings. Different genetic backgrounds and environmental exposures in non-European populations might lead to different relationships between 25(OH)D concentrations and ocular diseases. Furthermore, the use of sensitivity analyses in the context of non-associations warrants discussion. Most MR sensitivity tests, such as those for pleiotropy and heterogeneity, are designed to assess the robustness of positive findings rather than confirm null results. In our study, while these tests did not indicate significant pleiotropy or heterogeneity, their interpretation in the context of non-significant primary results should be cautious. The absence of evidence for these potential biases does not necessarily strengthen the case for a true null effect, but rather suggests that if biases exist, they were not detected by our methods.

It is important to note that while our MR analysis did not find evidence for causal associations between 25(OH)D concentrations and various ocular diseases, this does not definitively prove the absence of any causal relationship. MR methods are designed to test for causal associations rather than conclusively demonstrate non-causation. Our findings suggest that if causal relationships exist, they may be weaker than previously thought or potentially masked by complex biological interactions not captured in our analysis. Additionally, the relatively low statistical power in our study, primarily driven by imbalanced case-control ratios in the outcome datasets, suggests that subtle causal effects might exist below our detection threshold.Therefore, our results should be interpreted as a lack of evidence for causality rather than proof of no causal relationship.

Conclusion

Through a rigorous MR analysis based on extensive GWAS datasets, we found no significant evidence for causal links between 25(OH)D concentrations and common ocular diseases. This suggests that previous observational studies may have been influenced by confounders or shared biological factors rather than direct causal relationships. Future research should aim to incorporate more diverse study populations, utilize individual-level data where possible, and explore potential gene-environment interactions that our current approach may not have captured. Additionally, alternative methodologies and longitudinal studies could provide complementary insights to our MR findings.

Data availability

Summary statistics for ocular diseases were downloaded from FinnGen Consortium at https://www.finngen.fi/en/access_results.Summary statistics for 25(OH)D concentrations were downloaded from: https://cnsgenomics.com/content/data.

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Author contributions
Ling Liu and Zhichao Ruan conceived, initiated, and supervised the project. Xinxin Luo collected and analyzed the data, and wrote a draft of the manuscript. The authors read and approved the final manuscript.

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