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Original Research Article

The impact of 25-hydroxyvitamin D and calcium on risk of age-related macular degeneration: a Mendelian randomization study

Xing-Xuan Dong [1], Dong-Ling Chen [1], Yi-Fan Miao [1], Dan-Lin Li [1], Jia-Yan Kai [1], Dan-Ning Hu [2], Xiao-Feng Zhang [3], Lanca Carla [4,5], Grzybowski Andrzej [6], Chen-Wei Pan [1,*]

[1] *School of Public Health, Suzhou Medical College of Soochow University, Suzhou, China;* [2] *New York Eye and Ear Infirmary of Mount Sinai, Icahn School of Medicine at Mount Sinai, New York City, NY, United States;* [3] *Department of Ophthalmology, The Fourth Affiliated Hospital of Soochow University, Suzhou, China;* [4] *Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Instituto Politécnico de Lisboa, Lisboa, Portugal;* [5] *Comprehensive Health Research Center (CHRC), Escola Nacional de Saúde Pública, Universidade Nova de Lisboa, Lisboa, Portugal;* [6] *Institute for Research in Ophthalmology, Foundation for Ophthalmology Development, Poznan, Poland*

A B S T R A C T

Background: The relationships between 25-hydroxyvitamin D [25(OH)D] and calcium and age-related macular degeneration (AMD) are unclear.
Objectives: This study aimed to investigate the causal role of 25(OH)D concentrations, calcium concentrations, and dietary supplements use of vitamin D and calcium on risk of AMD and its subtypes.
Methods: Independent genetic variants associated with 25(OH)D and calcium concentrations were used as instrumental variables in published genome-wide association studies (GWASs) of European ancestry. The bidirectional 2-sample Mendelian randomization (MR) analyses were performed using summary-level data from the UK Biobank and FinnGen datasets. Sensitivity analyses were conducted to ensure the robustness of the MR results. The meta-analyses were conducted using both fixed-effect and random-effect models to provide comprehensive and reliable estimates.
Results: A standard deviation increase in calcium concentrations was linked to a 14%, 17%, and 13% reduction in the likelihood of developing AMD (95% confidence interval [CI]: 0.77, 0.97), wet AMD (95% CI: 0.73, 0.95), and dry AMD (95% CI: 0.75, 1.00), respectively. No significant causal relationships were detected between genetically predicted 25(OH)D concentrations and AMD and its subtypes (all $P > 0.05$). The combined analyses showed that higher calcium concentrations were associated with a reduced risk of overall AMD, with an odds ratio of 0.89 (95% CI: 0.81, 0.98).
Conclusions: This study provides evidence supporting the causal relationship between calcium concentrations and risk of AMD and its subtypes, which may have important implications for the prevention, monitoring, and treatment of AMD.

Keywords: age-related macular degeneration, vitamin D, calcium, Mendelian randomization, prevention

Introduction

Age-related macular degeneration (AMD) is the predominant cause of irreversible visual impairment and severe vision loss among the elderly population [1] and affects nearly 200 million people worldwide [2]. The late stages of AMD can be categorized into 2 main types: wet AMD (neovascular AMD) and dry AMD (atrophic AMD or geographic atrophy) [2,3]. The prevalence and disease burden of AMD are expected to increase owing to the aging population, which may lead to elevated health care expenditures and an amplified social burden [3,4]. Previous studies have shown that in the early stage of AMD, any treatment or preventive procedure is ineffective or has a limited effect on slowing the progression of the disease [2]. In wet AMD, intraocular injection of antivascular endothelial growth factor therapy can effectively inhibit the progression of wet AMD, control neovascularization, reduce macular edema, and improve visual function [2,5,6]. However, limitations of this approach include adverse reactions and severe complications (such as endophthalmitis), substantial interindividual variability (such as no and poor responders), and high treatment costs [6–8]. Furthermore, this therapy may lead to increased rates of geographic atrophy, which may cause severe loss of vision [6]. According to

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; BBB, blood–brain barrier; CI, confidence interval; GWAS, genome-wide association study; IV, instrumental variable; IVW, inverse-variance weighted; MR, Mendelian randomization; MR-PRESSO, Mendelian randomization Pleiotropy Residual Sum and Outlier; OR, odds ratio; RPE, retinal pigment epithelium; SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.

* Corresponding author.
E-mail address: pcwonly@gmail.com (C.-W. Pan).

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the Age-Related Eye Disease Study (AREDS) formulation, vitamin supplements have been recommended to prevent and slow the progression of dry AMD; however, their efficacy is relatively limited. In addition, Syfovre (pegcetacoplan) was recently approved by the Food and Drug Administration as a treatment to decelerate the rate of geographic atrophy growth [9]. Nonetheless, it has been controversial in practice owing to its association with a heightened risk of conversion to wet AMD [9,10]. Therefore, evaluating potential risk factors for AMD is highly valuable because controlling those factors could reduce the incidence of morbidity and disability.

Calcium homeostasis, an essential process for various cellular and biological functions, is regulated primarily by vitamin D [11]. It has been reported that higher concentrations of serum 25-hydroxyvitamin D [25(OH)D] are associated with an higher odds of early AMD and a lower odds of late AMD [12]. Previous studies have suggested that increased vitamin D concentrations and dietary intake are associated with a decreased odds of AMD [13,14]. A recent systematic review and meta-analysis of 16 studies indicated that there is no clear evidence of a definitive association between serum 25(OH)D concentrations and risk of AMD [15]. Blood 25(OH)D concentrations was found to be inversely associated with late AMD in Korean men but not in Korean women, according to a population-based, cross-sectional study [16]. Deficiency in 25(OH)D was associated with wet AMD, but the influence was small and may be affected by residual confounding factors [17]. In addition, Christen et al. [18] found that neither vitamin D3 nor marine ω-3 (n–3) fatty acid supplementation had a significant overall effect on AMD incidence or progression in a nationwide, placebo-controlled, randomized clinical trial.

Calcium pathways have been reported to be closely related to risk factors for AMD, such as aging and drusen accumulation [19,20]. A case–control study revealed dysregulated autophagy and inflammasome activation in the retinal pigment epithelium (RPE) of individuals with advanced AMD, accompanied by reduced intracellular free Ca^{2+} concentrations, indicating an important role for Ca^{2+} signaling in AMD pathogenesis [21]. Furthermore, a clinical study suggested that hydroxyapatite nodules, which are visible within AMD drusen, may serve as indicators of progression to advanced AMD [22]. A secondary analysis revealed that higher levels of dietary and supplementary calcium was associated with a decreased risk of AMD [23]. However, contradictory findings have also been reported. A study revealed that self-reported supplementary calcium consumption was associated with an increased prevalence of AMD [24]. Hence, the available evidence is insufficient to establish whether 25(OH)D and calcium are implicated in the onset and progression of AMD and its subtypes. In addition, in clinical practice, 25(OH)D and calcium have not garnered enough attention as primary prevention and treatment interventions for AMD.

Instrumental variables (IVs) address scenarios where ordinary least squares are not suitable, featuring the following 2 key characteristics: *1*) exogeneity (not influenced by other variables in the model) and *2*) correlation with the endogenous explanatory variable [25]. Mendelian randomization (MR) is a genetic epidemiological approach that uses single nucleotide polymorphisms (SNPs) as IVs, which addresses potential methodologic limitations encountered in traditional studies, such as reverse causation bias and confounding [26]. The objective of this study was to investigate the impact of serum 25(OH)D concentrations, serum calcium concentrations, and dietary supplements use of vitamin D and calcium on risk of AMD.

Methods

Study design

This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian randomization (STROBE-MR) guideline [27]. Figure 1 provides an overview flowchart of the hypothesis and schematic design. As depicted in Figure 1A, 3 fundamental assumptions were made: *1*) genetic variants have a direct impact on exposure, *2*) genetic variants are solely associated with the outcome through exposure, and *3*) the association is independent of any potential confounders [28]. Initially, 2-sample MR analyses were conducted to examine the causal effects of both 25(OH)D and calcium concentrations, as well as their dietary supplements use, on AMD and its subtypes (Figure 1B). Subsequently, a meta-analysis approach was used to consolidate the MR results, enhancing the reliability of the findings (Figure 1C). All data used in this study are publicly available genome-wide association study (GWAS) summary statistics; therefore, no additional ethical approval or informed consent was necessary for this study. To further clarify the practical significance of this phenomenon, we conducted additional analyses to explore the use of dietary supplements of vitamin D and calcium on AMD and its subtypes.

Data sources and genetic instruments selection

The detailed information used in this study is presented in Table 1. Summary statistics for 25(OH)D concentrations were obtained from a GWAS that combined the results of the 2 GWASs (UK Biobank: n = 401,460; author's previous research: n = 42,274) using an inverse-variance weighted fixed-effects meta-analysis [29]. In the UK Biobank, data on 25(OH)D concentrations measured by the diasorin assay (in nmol/L) were available from 465,415 samples, representing 449,978 participants [29]. The GWAS included individuals whose 25(OH)D concentrations were measured at baseline between 2006 and 2010. In the authors' previous GWAS, the authors conducted a meta-analysis, pooling statistics from 19 cohorts, using whole-genome sequencing data from 2619 individuals via the UK10K program, and incorporating deep imputation data from 39,655 individuals who were genotyped genome-wide [29,30]. The methods used to measure 25(OH)D concentrations have been described in detail previously [29,30]. Summary data associated with calcium concentrations as genetic instruments were taken from a GWAS conducted on a cohort of 400,792 European individuals in the UK Biobank [31]. The serum calcium concentrations were adjusted to account for extreme outliers (>1000 times the interquartile range), stratified by sex and menopause status, subjected to an inverse-normal transformation, covariates (ethnic group, alcohol use, smoking status, age, height, and BMI [kg/m^2]) were regressed out, and then the inverse-normal transformation was reapplied. Therefore, the unit of serum calcium concentrations was expressed in SDs. Summary statistics for dietary supplement use of vitamin D and calcium, collected using a binary classification system (yes: 1251; no: 461,682 for vitamin D; yes: 31,393; no: 429,991 for calcium), were obtained from the GWAS pipeline using Phesant-derived variables from the UK Biobank dataset (https://www.ukbiobank.ac.uk/). These binary variables, indicating whether individuals reported using dietary supplements for each respective nutrient, were gathered through an online diet questionnaire.

The GWAS data associated with AMD and its subtypes were obtained from the UK Biobank (n = 403,837; case = 3810; control = 400,027) and FinnGen (AMD: n = 403,837 and 357,849; wet AMD: n

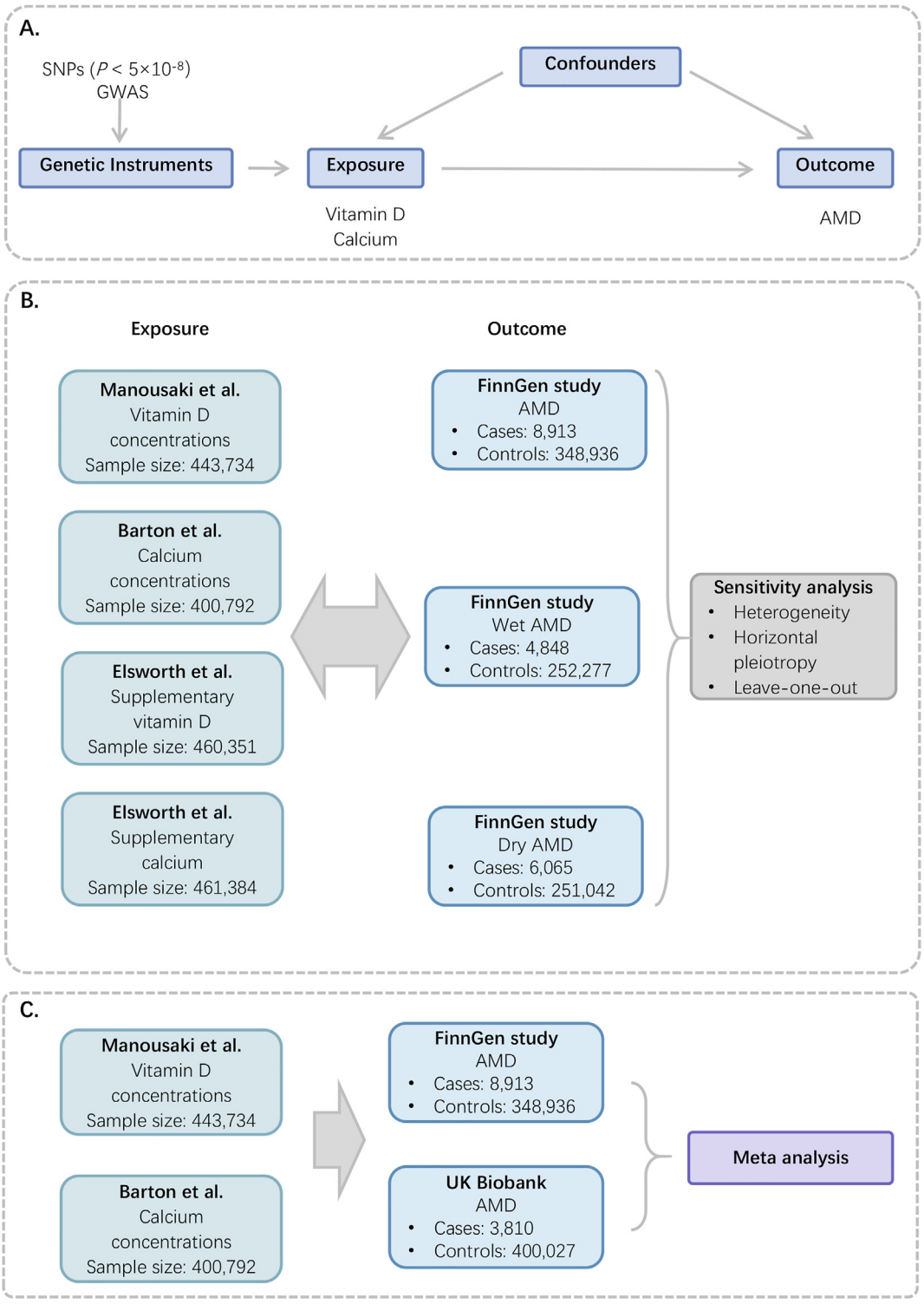

FIGURE 1. Schematic overview of the study design. AMD, age-related macular degeneration; 25(OH)D, 25-hydroxyvitamin D.

= 257,125; dry AMD: n = 257,107) [32]. In the FinnGen and UK Biobank, AMD was defined as the age-related loss of vision in the central portion of the retina (macula) secondary to retinal degeneration, according to the International Statistical Classification of Diseases, 10th Revision (H35.3). All GWAS data were obtained from the IEU GWAS database (https://gwas.mrcieu.ac.uk/), GWAS catalog (https://www.ebi.ac.uk/gwas/), and FinnGen Data Freeze 9 (https://r9.finngen.fi/).

A genome-wide significance threshold of $P < 5 \times 10^{-8}$ was used to select genetic IVs. SNPs exhibiting high linkage disequilibrium were excluded using a strict r^2 cutoff of 0.001, and a clumping window larger than 10,000 kb was applied. F-statistics were calculated as $(\beta/SE)^2$ to evaluate risk of weak instrumental bias, with a minimum value of 10 considered sufficient for MR analysis. The protocol and data collection procedures were approved by the ethics committees of

TABLE 1
Description of genome-wide association studies summary statistics.

Variables	PMID/GWAS ID	Year	Sample size	Case	Control	Traits	Population	Source
Exposure								
Serum 25-hydroxyvitamin D	32059762	2020	443,734	—	—	Serum 25-hydroxyvitamin D concentrations	European ancestry individuals	UK Biobank and a meta-analysis
Calcium	34226706	2021	400,792	—	—	Calcium concentrations	European ancestry individuals	UK Biobank
Supplementary vitamin D	ukb-b-12648	2018	460,351	17,879	442,472	Vitamin and mineral supplements: vitamin D	European ancestry individuals	UK Biobank
Supplementary calcium	ukb-b-7043	2018	461,384	31,393	429,991	Mineral and other dietary supplements: calcium	European ancestry individuals	UK Biobank
Outcome								
Age-related macular degeneration	34017140	2021	403,837	3,810	400,027	Macular degeneration (SPA correction)	European ancestry individuals	UK Biobank
Age-related macular degeneration	—	2022	357,849	8,913	357,849	Age-related macular degeneration (whether dry or wet)	European ancestry individuals	FinnGen
Wet age-related macular degeneration	—	2022	257,125	4,848	252,277	Wet age-related macular degeneration	European ancestry individuals	FinnGen
Dry age-related macular degeneration	—	2022	257,107	6,065	251,042	Dry age-related macular degeneration (includes geographic atrophy)	European ancestry individuals	FinnGen

Abbreviation: SPA, saddle-point approximation.

the original articles and GWAS, and written informed consent was obtained from each participant before data collection.

Statistical analysis

The main analysis applied both the fixed and random-effects inverse-variance weighted (IVW) approach to obtain causal estimates. Simultaneous use of both fixed-effect and random-effect models of IVW allows for the consideration of heterogeneity among IVs, enhancing the reliability and robustness of MR analysis. In addition, supplementary approaches such as MR-Egger, weighted median, and weighted mode were using to investigate causal relationships. MR-Egger can detect certain violations of standard IV assumptions and provide an effect estimate that is immune to these breaches [33]. The weighted median estimator maintains consistency even when $\leq$50% of the data originate from invalid IVs, demonstrating superior finite-sample type 1 error rates, compared with IVW [34]. Although the power of the weighted mode in detecting a causal effect was lower than that of IVW and the weighted median methods, it surpassed that of MR-Egger regression [35]. These methods can serve as sensitivity analyses for MR analysis, verifying the robustness of the results. The Cochran Q test was conducted to assess heterogeneity among different IVs [36]. Leave-1-out analysis was performed to measure the potential influence of each IV on the overall results by sequentially excluding each SNP at a time. To evaluate horizontal pleiotropy, the MR-Egger regression test was used, where a nonzero intercept indicated the presence of pleiotropy [33]. The Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) method was also used to further control potential pleiotropy [37]. To assess statistical power, the online tool for binary outcomes in MR studies (https://github.com/kn3in/mRnd) was used, with an α level set at 0.05. A meta-analysis approach using both fixed and random effects was used to enhance the reliability of the MR results. In meta-analysis, using both fixed-effect and random-effect models concurrently is advantageous for evaluating the impact of various models on study outcomes, as well as for assessing the extent of heterogeneity and testing its significance. With a limited number of original articles, the fixed-effect model may lack sufficient power, whereas the random-effect model provides more accurate estimates of population effects and heterogeneity variance [38]. Thus, these 2 models offer researchers essential insights to comprehend the results and select the appropriate statistical model. All statistical analyses were conducted using Stata software (version 12.0; StataCorp) and R software (version 4.3.1; R Core Team). A significance level of $<$0.05 (2-sided) was used to indicate statistical significance.

Results

None of the SNPs used as IVs for genetically predicted 25(OH)D and calcium concentrations were in linkage disequilibrium with the known AMD risk loci ($r^2 < 0.1$). The F-statistics for the genetic instruments were all above 10, suggesting that our MR study did not experience weak instrument bias [39].

25(OH)D concentrations and AMD

According to the IVW results, no significant causal effect of 25(OH)D on AMD or its subtypes was found (Figure 2). Specifically, genetically predicted 25(OH)D concentrations were linked to a 5% increased risk of AMD (fixed effects—95% confidence interval [CI]: 0.82, 1.36; random effects—95% CI: 0.77, 1.43), an 8% reduced risk of wet AMD (fixed effects—95% CI: 0.65, 1.28; random effects—95% CI: 0.61, 1.39), and a 14% higher risk of dry AMD, although these associations

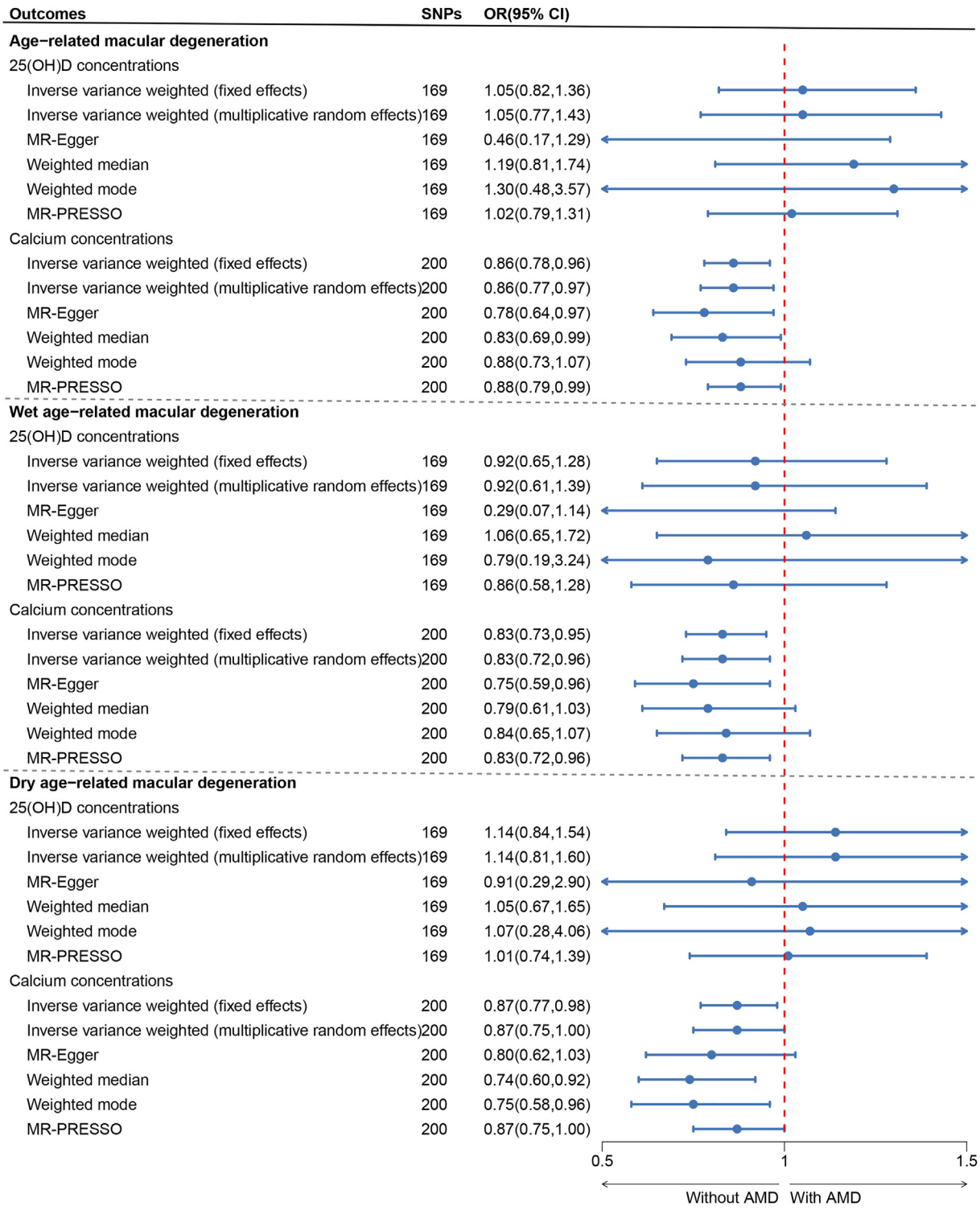

FIGURE 2. Mendelian randomization estimations inferring causality of 25-hydroxyvitamin D ($n = 443,734$) and calcium concentrations ($n = 400,792$) on age-related macular degeneration ($n = 357,849$) and its subtypes (wet: $n = 257,125$; dry: $n = 257,107$). MR analysis results estimates from the application of inverse-variance weighted, MR-Egger, weighted median, weighted mode, and MR-PRESSO methodologies. The main results (ORs and 95% CIs) of 25(OH)D and calcium concentrations for age-related macular degeneration and its subtypes were calculated using both fixed-effects and random-effects inverse-variance weighted regression. An OR >1 indicates an increased risk of age-related macular degeneration, while an OR <1 indicates a reduced risk. Specifically, calcium concentrations were found to significantly reduce risk of age-related macular degeneration by 14% (95% CI: 0.77, 0.97), wet age-related macular degeneration by 17% (95% CI: 0.73, 0.95), and dry age-related macular degeneration by 13% (95% CI: 0.75, 1.00). The full results are shown in Supplemental Figures 1–3 and Supplemental Figures 10–12. CI, confidence interval; MR, Mendelian randomization; OR, odds ratio; PRESSO, Pleiotropy Residual Sum and Outlier; SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D.

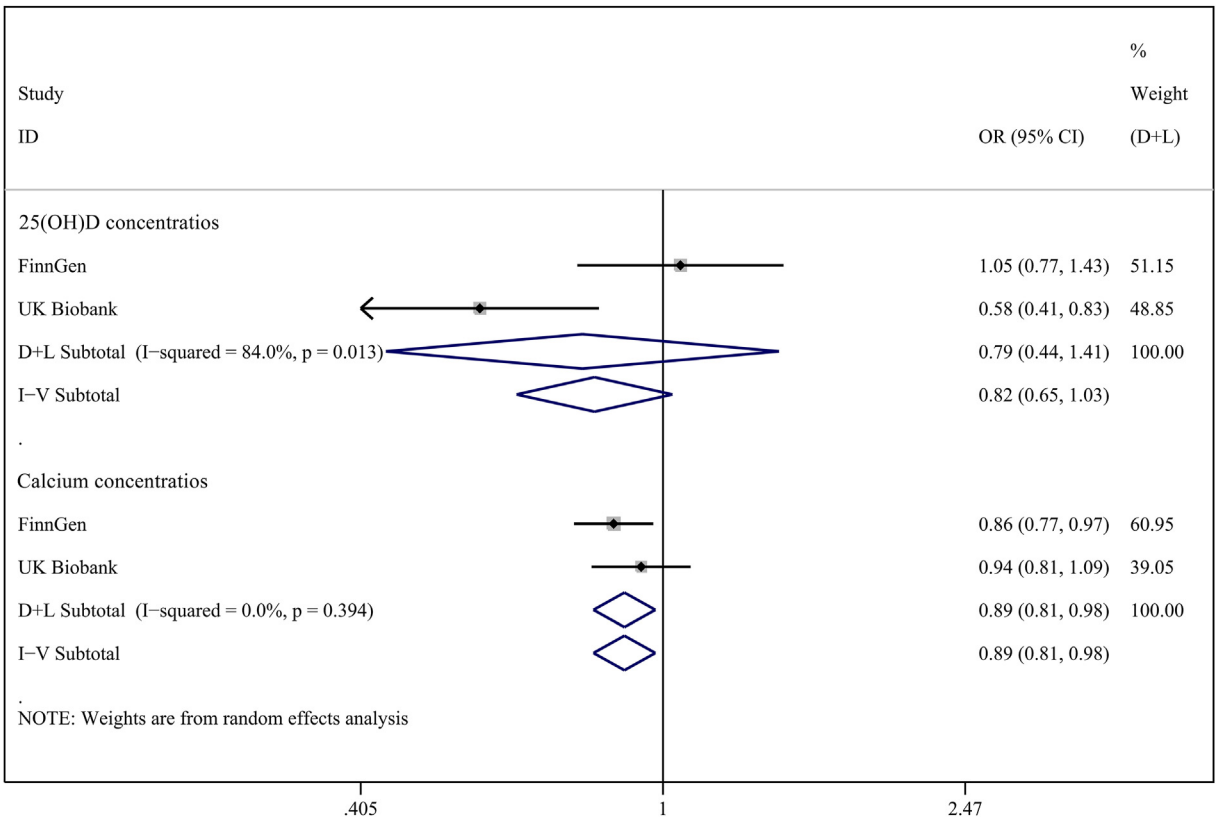

FIGURE 3. Associations between genetically predicted 25-hydroxyvitamin D (n = 443,734) and calcium concentrations (n = 400,792) and age-related macular degeneration in the UK Biobank (n = 403,837) and FinnGen (n = 357,849) and combined analyses of both samples. The size of the boxes represents the relative weights of the MR results. The diamond represents the overall effect estimate of the meta-analysis, where its center denotes the point estimate, and its width signifies the 95% CI around the point estimate of the pooled effect. Serum calcium concentrations were associated with an 11% reduced risk of age-related macular degeneration (95% CI: 0.81, 0.98). The effect estimates (ORs) and 95% CIs were combined using both fixed-effects and random-effects models. CI, confidence interval; OR, odds ratio; 25(OH)D, 25-hydroxyvitamin D.

did not reach statistical significance (fixed effects—95% CI: 0.84, 1.54; random effects—95% CI: 0.81, 1.60) (Figure 2; Supplemental Figures 1–3). Similar results were also found for the MR-Egger, weighted median or weighted mode estimates (all $P > 0.05$). Cochran' Q test revealed significant heterogeneity between 25(OH)D and AMD and wet AMD (AMD—Cochran Q: 245.42; $P < 0.001$; wet AMD—Cochran Q: 253.45; $P < 0.001$). No evidence of horizontal pleiotropy was estimated by MR-Egger regression (all P for intercept > 0.05). The MR-PRESSO test identified 4, 1, and 2 outliers for AMD, wet AMD, and dry AMD, respectively. There was no evidence of reverse causality between AMD and its subtypes and 25(OH)D concentrations (all $P > 0.05$) (Supplemental Figures 4–6). No significant associations were observed between dietary supplements of 25(OH)D and AMD and its subtypes (all $P > 0.05$) (Supplemental Figures 7–9).

Calcium concentrations and AMD

This MR study suggested that genetically predicted calcium concentrations were causally associated with AMD based on the IVW method (Figure 2). In detail, each SD increase in calcium concentrations was associated with a 14%, 17%, and 13% reduction in risk of AMD (fixed effects—odds ratio [OR]: 0.86; 95% CI: 0.78, 0.96; random effects—OR = 0.86; 95% CI: 0.77, 0.97), wet AMD (fixed effects—OR: 0.83; 95% CI: 0.73, 0.95; random effects—OR: 0.86; 95% CI: 0.72, 0.96), and dry AMD (fixed effects—OR: 0.87; 95% CI: 0.77, 0.98; random effects—OR: 0.87; 95% CI: 0.75, 1.00) (Figure 2; Supplemental Figures 10–12), respectively. Two outliers were identified by MR-PRESSO in the association between calcium concentrations and AMD. No significant potential outliers were found in the associations between calcium concentrations and AMD subtypes. Cochran Q test revealed significant heterogeneity between calcium concentrations and AMD and dry AMD (P for Cochran Q < 0.05). MR-Egger regression showed no significant horizontal pleiotropy (AMD: P for intercept = 0.27; wet AMD: P for intercept = 0.33; dry AMD: P for intercept = 0.45). Reverse MR revealed no significant effects of AMD or its subtypes on calcium concentrations (all $P > 0.05$) (Supplemental Figures 13–15). In addition, MR analysis revealed no significant causal effects of dietary calcium supplements on AMD or its subtypes (all $P > 0.05$) (Supplemental Figures 16–18).

Replication and meta-analyses

According to the replication analyses of summary statistics from the UK Biobank, the direction of the effects estimated between genetically predicted calcium concentrations and AMD was consistent but did not reach statistical significance (Figure 3). A statistically significant combined association of genetically predicted calcium concentrations with AMD was observed (OR: 0.89; 95% CI: 0.81, 0.98) in meta-analyses of data from 2 consortia (Figure 3). The combined analysis of genetically predicted 25(OH)D concentrations did not support a causal relationship with AMD (OR: 0.82; 95% CI: 0.65, 1.03).

Discussion

This study used a bidirectional 2-sample MR and meta-analysis approach to investigate the causal associations between genetically predicted 25(OH)D and calcium concentrations and risk of AMD and its subtypes. The results obtained from the IVW approach and combined analyses suggested an association between genetically determined calcium concentrations and AMD, as well as wet AMD. However, no significant causal effects of 25(OH)D concentrations, vitamin D supplement use, or calcium supplement use on risk of AMD, wet AMD, or dry AMD were observed.

Vitamin D is a fat-soluble vitamin that plays a crucial role in promoting overall well-being and maintaining proper functioning of the human body, including the regulation of gene expression, modulation of the immune system, and promotion of angiogenesis [40,41]. Dietary sources of vitamin D typically account for no >5% to 10% of the total intake; the remainder may come from the skin or other sources. Each additional 100 IU of daily oral vitamin D intake leads to an elevation in serum 25(OH)D of ~1 ng/mL (2.5 nmol/L) [42]. According to a single-center cross-sectional study, the occurrence of subretinal fibrosis in patients classified under Clinical Age-Related Maculopathy Staging 5 may correlate with lower plasma 25(OH)D concentrations [43]. Itty et al. [44] conducted a case–control study revealing lower mean 25(OH)D concentrations and a higher prevalence of vitamin D deficiency among older adults diagnosed with wet AMD. A prospective study indicated that high 25(OH)D concentrations, approximately >70 nM, are associated with a reduced likelihood of developing incident early AMD, though not late AMD, suggesting a potential protective effect against AMD onset [45]. However, a previous case–control study found no evidence supporting the association between AMD and serum vitamin D concentrations [46], which is consistent with our own findings. A prespecified ancillary study of the vitamin D and ω-3 (n–3) trial indicated that the vitamin D3 and marine ω-3 (n–3) fatty acid may have no influence on the incidence and progression of AMD [18]. A meta-analysis further revealed that there is currently no objective association between vitamin D and AMD concentrations, and longitudinal studies investigating this relationship are currently limited [15]. It is important to consider that certain significant associations may be influenced by factors such as sample size and reverse causation, which can affect the reliability and generalizability of the results. Therefore, further well-designed longitudinal studies with larger sample sizes are necessary.

Calcium is an essential element for the human body and plays a pivotal role in various vital physiological functions, such as the maintenance of calcium homeostasis, growth and development, and nerve conduction [47]. The expert from the National Osteoporosis Foundation recommended a total daily intake of 1200 mg calcium for women aged older than 51 y and men aged older than 71 y, and 1000 mg/d for men aged older than 50 y [48]. In a secondary analysis of participants in randomized clinical trials, dietary calcium intake exhibited a significant association with late-stage AMD development, while no significant association was observed with intermediate-stage AMD [23]. As proposed by Gopinath et al. [49], the pathophysiologic processes underlying the development of early AMD (drusen and abnormalities in the RPE) are distinct from those involved in the transition from early to late AMD (neovascularization and geographic atrophy). Additionally, in a distinct cross-sectional study, participants reporting daily calcium intake exceeding 800 mg demonstrated higher odds of AMD diagnosis than those who did not report calcium supplementation [24]. Previous cross-sectional studies have demonstrated that reduced consumption of both supplementary and dietary calcium was significantly associated with an increased risk of AMD [23,49,50], aligning with our findings. A previous study proposed that calcium may delay the progression of AMD by enhancing the utilization and metabolism of copper [51]. Therefore, we hypothesized that the relationship between calcium concentrations and AMD does not adhere to a linear pattern but rather demonstrates an inverted U-shaped or inverted L-shaped distribution. Further empirical research is necessary to validate the accuracy of this hypothesis.

In this study, calcium concentrations demonstrated a more pronounced impact on wet AMD than that on dry AMD. Wet AMD is characterized by neovascularization leading to leakage, subsequent intraretinal/subretinal fluid, hemorrhages, and fibrosis [2]. A previous study indicated that an allosteric inhibitor of end-binding protein 3 prevents neovascularization and choroidal neovascularization by modulating pathologic calcium signaling [52]. Although a case–control study proposed the potential utility of serum trace elements as predictive biomarkers for wet AMD, it did not establish a significant correlation between serum calcium concentrations and this condition [53]. A retrospective analysis involving 259,562 subjects revealed a potential association between the use of second-generation calcium channel blockers and an elevated risk of developing wet AMD [54], which aligns with our observations. We postulate that the protective effect of calcium against wet AMD may exhibit a specific threshold, which warrants further investigation in subsequent studies. Furthermore, it is essential to consider factors that influence calcium concentrations in the future, including diet, lifestyle, and genetics, which may provide additional insights for clinicians to assess and address individuals with wet AMD.

We also found that calcium has an impact on dry AMD. Dry or atrophic AMD is characterized by RPE dysfunction, photoreceptor loss, retinal degeneration, and macular drusen [55]. A community-based cross-sectional survey conducted in Japan revealed a significant correlation between serum calcium concentrations and the presence of large drusen [56]. This study further indicated a notably higher prevalence of hypocalcemia in individuals with large drusen than in individuals in the control group [56]. Increased intracellular Ca^{2+} influx has been proposed to contribute to lipofuscin accumulation in retinal pigment epithelial cells, subsequently leading to retinal degeneration [57]. However, the specific role of calcium in the formation of RPE and drusen has not been determined. Furthermore, calcium plays an important role in protecting the blood–brain barrier (BBB), which is formed by the brain microvascular endothelial cells lining the wall of brain capillaries. Alterations in BBB integrity play a key role in the development of multiple brain disorders [58]. The adhesion molecule and thrombospondin, supports the attachment of human endothelial and smooth muscle cells to culture dishes and is similar to the basement membrane in vivo. The attachment of cells to thrombospondin is mediated by calcium-dependent mechanisms [59]. Therefore, calcium plays a role in cell adhesion and maintains the integrity of the BBB. It is interesting to explore the role of calcium in maintaining ocular blood vessel integrity in the blood-retina barrier, which may play an important role in the pathogenesis of AMD.

Our study contributes novel insights into the clinical management of AMD, particularly in light of the limited availability of effective preventative medications for dry AMD [60]. Vitamin D serves as a precursor in calcium and phosphorus metabolism, thereby regulating the concentrations of these elements in human body [61]. Notably, our study did not establish a direct causal association between 25(OH)D and AMD. Therefore, it is plausible that the association between 25(OH)D and AMD may be influenced by calcium. However, a complete dismissal of the link between 25(OH)D and AMD is not

warranted. In clinical practice, physicians may consider integrating calcium therapy into the treatment regimen for AMD, alongside 25(OH)D as an adjunct. Simultaneously, it is imperative to carefully monitor the effectiveness and safety of calcium therapy in individuals with AMD to generate robust evidence for clinical treatment. Future studies should further investigate the role of calcium in the pathogenesis and treatment of AMD, thereby providing additional evidence to guide clinical practice.

To our knowledge, this is the first MR study investigating the genetic associations between 25(OH)D and calcium concentrations and risk of AMD. The main strengths of this study include the use of a 2-sample MR design and the utilization of outcome datasets from 2 different sources, which enhance the robustness of our findings. However, several limitations should be acknowledged. First, the data used in the analyses primarily included individuals of European descent, owing to the lack of GWAS summary statistics for other ancestries. Although this design helps minimize bias from population stratification, it may limit the generalizability of our findings to other populations. Second, our analysis considered only the dichotomous diagnosis of AMD rather than the disease course or progression, which may overlook potential associations that could be specific to certain stages of AMD. Third, it is important to note that the genetic instruments used in MR studies approximate average effects over the life course. Therefore, the biological association between calcium concentrations and risk of AMD could be more complex than what was presented in our study. Finally, the lack of GWAS data on dietary and supplementary vitamin D and calcium intake precludes the quantification of the relationship between the different sources (such as diet and supplements) of these nutrients and AMD. However, we conducted further MR analyses to examine the impact of 25(OH)D and calcium supplements on AMD, which did not reveal significant associations. Future randomized controlled trials are warranted to provide a more comprehensive understanding of the relationship between calcium concentrations and the onset and development of AMD.

In conclusion, our study provides evidence of a causal relationship between higher calcium concentrations and a reduced risk of AMD, particularly wet AMD. These findings underscore the clinical importance of calcium in the context of AMD and indicate its potential utility in monitoring, early detection, and targeted interventions for both prevention and treatment. Further research is warranted to validate these findings and elucidate the underlying mechanisms through which calcium influences risk of AMD.

Author contributions

The authors' responsibilities were as follows – X-XD, C-WP: designed research; X-XD, D-LC, Y-FM: conducted research, provided essential reagents, or provided essential materials; D-LL, J-YK: provided essential reagents or provided essential materials; X-XD, D-LC: wrote the article; C-WP: had primary responsibility for final content; D-NH, X-FZ, CL, AG: provided critical revision of the manuscript for important intellectual content; and all authors: read and approved the final manuscript.

Conflicts of interest

There authors report no conflicts of interests.

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Data availability

All summary statistics used in this 2-sample MR are available online from each genome-wide association study. Statistical code is available on the request by directly contacting the corresponding author (e-mail: pcwonly@gmail.com).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.06.018.

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