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Original Research

No bidirectional association between serum 25-hydroxyvitamin D and erectile dysfunction: Mendelian randomization and genetic association studies

Xiang Liu[1], Longhua Luo[2], Cong Peng[1], Zixin Wang[1], Jiaming Zhou[2], Xiang Sun[1,*]

[1]Department of Urology, The First Affiliated Hospital of Nanchang University, Nanchang 330019, China
[2]Department of Urology, Gaoxin Branch of the First Affiliated Hospital of Nanchang University, Nanchang 330072, China
*Corresponding author: Department of Urology, The First Affiliated Hospital of Nanchang University, Donghu District, Nanchang 330019, China.
Email: ndyfy01344@ncu.edu.cn

Abstract

Background: The causal relationship between the level of serum 25-hydroxyvitamin D [25(OH)D] and the risk of erectile dysfunction (ED) is still unclear.
Aim: We tried to determine the causal relationship between the level of serum 25(OH)D and ED risk.
Methods: In this study, we used genome-wide association study data from the UK Biobank to analyse the relationship between serum 25(OH)D (as the exposure) and ED (as the outcome). Linkage disequilibrium score regression (LDSC) was used to assess the genetic correlation between 2 traits. The CAUSE (Causal Analysis using Summary Effect estimates) method and Mendelian randomization (MR) were employed to evaluate the bidirectional causal relationship. The MRlap method was utilized to assess the impact of sample overlap on the results. To assess potential heterogeneity and horizontal pleiotropy, we utilized methods such as MR-Egger, MR-PRESSO (Mendelian Randomization Pleiotropy Residual Sum and Outlier), weighted median, and others.
Outcomes: The primary outcome was defined as self or physician-reported ED, or using oral ED medication, or a history of surgery related to ED.
Results: The LDSC analysis did not reveal a significant genetic correlation between serum 25(OH)D and ED ($r_g = 0.2787$, $P = .3536$). Additionally, the CAUSE (P value testing that the causal model is a better fit $>.05$) and MR analyses (odds ratio, 0.8951; 95% confidence interval, 0.7480-1.0710; $P = .2260$) did not support a causal relationship between 25(OH)D and ED, and our study did not detect any heterogeneity and pleiotropy.
Clinical implications: This study provides evidence on whether vitamin D needs to be ingested to prevent or treat ED.
Strengths and limitations: We used LDSC and MR to avoid bias. However, the population in this study was limited to European ancestry.
Conclusion: No causal relationship was found between 25(OH)D and ED.

Keywords: serum 25-hydroxyvitamin D; erectile dysfunction; Mendelian randomization; genome-wide association study; linkage disequilibrium score regression.

Introduction

Erectile dysfunction (ED) is the inability of men to maintain enough erections to have a satisfying sex life.[1] ED is a common sexual dysfunction in men, with an incidence of up to 52% among men 40 to 70 years of age.[2] As a chronic disease, ED impacts both the physical and mental health of the individual. It affects the quality of life for patients and their spouses[3] and may be an early symptom and risk signal of cardiovascular disease.[4]

The apparent age dependence of hypovitaminosis D and ED suggests a potential relationship between these 2 conditions. A cross-sectional study showed that, after adjusting for various variables, men with serum 25-hydroxyvitamin D [25(OH)D] levels below 30 ng/mL exhibited a higher prevalence of ED (prevalence ratio, 1.30; 95% confidence interval, 1.08-1.57).[5] Oxidative stress and inflammation are considered key pathological mechanisms in ED.[6] A series of studies have shown that vitamin D (VD) exerts its antioxidant effects by upregulating cellular glutathione and superoxide dismutase.[7] Additionally, it has the potential to inhibit the production of proinflammatory factors and increase the concentration of anti-inflammatory markers.[8] Various biases in observational studies cannot be avoided, and large-scale randomized controlled trials cannot be conducted due to cost. The causal relationship between the level of serum 25(OH)D and the risk of ED is still unclear. New methods must be used to explore this relationship, which will better inform clinical prevention and treatment decisions for ED.

Linkage disequilibrium score regression (LDSC) is a statistical method used in genomic research. It is primarily employed to analyze and interpret the genetic architecture of complex traits, particularly to estimate the heritability of polygenic

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traits and their shared genetic basis through genome-wide association study (GWAS) data.[9] Mendelian randomization (MR) studies use genetic variants strongly associated with the exposure factor as instrumental variables (IVs) to infer the causal relationship between the exposure and the outcome. Due to the Mendelian law of inheritance, which states that "parental alleles are randomly assigned to offspring during gamete formation," these genetic variants are not influenced by traditional confounding factors such as environmental exposures, socioeconomic status, or behavioral factors. Additionally, because genetic variants are inherited from parents and remain unchanged after birth, the relationship between these variants and the outcome follows a logical temporal sequence. Therefore, MR can overcome the issues of confounding and reverse causality that often plague traditional observational epidemiological studies, thus strengthening the causal inference between exposure and outcome.[10] Our study utilized LDSC and a 2-sample MR approach to address the question, is there a genetic association between serum 25(OH)D levels and the risk of ED?

Methods

Study design

After obtaining GWAS data on the exposure and outcome, we used LDSC to analyze the genetic correlation between the 2 traits. We conducted causal analysis using the CAUSE (Causal Analysis using Summary Effect estimates) method and bidirectional MR to evaluate the bidirectional causal relationship. The MRlap method was employed to assess the impact of sample overlap on the results. The study followed the STROBE-MR (Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization) statement for reporting MR studies.[11]

Exposure data

The GWAS data for serum 25(OH)D comes from the UK Biobank (UKB),[12] encompassing a total of 417 580 European UKB participants who had their 25(OH)D levels measured, and was meta-analyzed with the GWAS data from the SUNLIGHT consortium,[13] which included 79 366 individuals of European ancestry. Identified 143 independent loci associated with 25(OH)D levels. The concentrations of 25(OH)D were quantified using the chemiluminescence immunoassay method, which measures total 25(OH)D concentrations. Measurements of 25(OH)D concentrations below or above the validated assay range (10-375 nmol/L) were excluded.

Outcome data

As the outcome of this study, the GWAS data of ED were downloaded from the IEU Open GWAS Project (https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST006956/), 223 805 participants (6175 cases, 217 630 controls) from the UKB, Partners HealthCare Biobank, and Estonia Genome Center of Tartu University. The primary outcome was defined as self or physician-reported ED, or using oral ED medication, or a history of surgery related to ED (see Table S1).

Genetic association analysis

We used the LDSC method to evaluate the liability-scale heritability (h^2) and genetic correlation (r_g) of the relevant exposures and outcome.[9] We downloaded European linkage disequilibrium (LD) scores from the 1000 Genomes Project (AlkesGroup[broadinstitute.org]), which were used for baseline LDSC intercept, heritability, and genetic correlation analyses. The essence of LDSC is a linear regression, with input data being the results of GWAS analyses. The independent variable in the regression is the LD score value of single nucleotide polymorphism (SNP) loci, in which an LD score for a SNP was defined as the sum of the LD (R^2) with its neighboring loci. The dependent variable, which is central to the algorithm, is a custom-defined statistic that follows a chi-square distribution. By analyzing the intercept from the LDSC regression, we can determine whether there are confounding factors in the GWAS results. If the intercept is close to 1, it indicates no significant confounding factors. However, if the intercept exceeds this range (1 ± 0.05), it suggests the presence of confounding factors. By calculating the LD scores for each SNP in the exposure and outcome GWAS data and performing a collinear regression analysis with the chi-square statistics for each SNP in both the exposure and outcome, we can obtain the heritability of the respective traits as well as the genetic correlation between the exposure and outcome.

IV selection for MR analyses

MR analysis requires 3 basic assumptions to be satisfied[14]: (1) IVs significantly correlate with the exposure, (2) IVs are not correlated with confounding factors, and (3) IVs affect outcome only through exposure. To fulfill the 3 basic hypotheses, quality control measures were applied to choose IVs that correlate with serum 25(OH)D. First, the chosen IVs must be significantly correlated with the serum 25(OH)D. In this study, the locus range significance threshold was set at a P value below 5×10^{-8} in the analysis of 25(OH)D to ED (in the reverse MR analysis and reverse CAUSE analysis, the P value threshold was set at 5×10^{-6}), and SNPs with minor allele frequencies <0.01 were excluded.[15] Second, because strong linkage disequilibrium may bias the results, we used $r^2 < 0.001$ as a threshold and 10 000 kb as an aggregation window for LD analysis to ensure the independence of IVs.[16] Third, to eliminate confounding factors, we searched every SNPs of IVs in LDtrait (https://ldlink.nih.gov/; population: European; $R^2 = 0.1$; ±500 000 base pair window), excluding SNPs correlated with known confounding factors affecting ED, including hypertension, diabetes, heart disease, smoking, metabolic syndrome, and benign prostatic hyperplasia.[1] Fourth, an essential step in MR is to ensure that the SNP's effect on exposure corresponds to the same allele as its effect on the outcome. To avoid distortions in strand orientation or allelic coding, we removed ambiguous and palindromic SNPs when harmonizing the effects of exposure and outcome.[17] Finally, SNP rs7955128 was excluded for being palindromic with intermediate allele frequencies. The F statistic of SNPs was utilized to assess the robustness and consistency of the association between IVs and serum 25(OH)D. Only SNPs with $F > 10$ were used in our analysis.[18] The calculation of F value refers to equation 1.

$$F = [R^2/(1 - R^2)] \times [(N - K - 1)/K] \quad (1)$$

where N is the sample size of the dataset, K is the number of valid SNPs, and the calculation of the R^2 value refers to

Table 1. The genetic correlations between serum 25(OH)D and ED.

	Serum 25(OH)D	ED
Heritability h^2 (SE)	0.085 (0.015)	0.008 (0.002)
Intercept (single trait)	1.0638	0.979
Genetic correlation r_g (SE)	−0.018 (0.058)	
P value	.758	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ED, erectile dysfunction.

equation 2.[19]

$$R^2 = [2 \times MAF \times (1 - MAF) \times \beta^2]/[(2 \times MAF \times (1 - MAF) \times \beta^2) + (2 \times MAF \times (1 - MAF) \times N \times SE^2)] \quad (2)$$

where MAF represents the minor allele frequency of a common allele, usually a value between 0 and 0.5 and beta represents the regression coefficient. They are usually used to measure the accuracy of the estimation of regression coefficients.

MR analysis

After harmonizing SNPs with the same allele in the exposures and outcome GWAS data, a 2-sample MR analysis was performed. The inverse variance–weighted (IVW) method[20] was used as the primary method to combine the estimates from multiple SNPs in a meta-analysis-like framework, weighting each SNP's effect estimate by its inverse variance, providing a more precise estimation when there is no horizontal pleiotropy in IVs.[21] The heterogeneity of IVs was evaluated using the Cochran Q test and I^2; Q statistics with *P* values <.05 indicated heterogeneity,[20] and I^2 quantifies the heterogeneity, I^2 = (Q-df)/Q∗100%, where df is the degrees of freedom. Negative I^2 values are adjusted to zero, ensuring that the I^2 ranges from 0% to 100%. A value of 0% signifies no detected heterogeneity, while higher values indicate a growing level of heterogeneity.[22] When there was no heterogeneity, the IVW estimate for fixed effects was the primary analysis. Otherwise, the IVW model for random effects was used to generate a more conservative but robust estimate. MR-Egger,[23] weighted median,[21] weighted mode,[24] simple mode,[25] and MR-PRESSO (Mendelian Randomization Pleiotropy Residual Sum and Outlier)[26] comprised the supplementary analysis methods. Even if pleiotropy exists, the MR-Egger and weighted median methods relax this prerequisite (with null IV) to produce unbiased results and are used as sensitivity analyses. MR-PRESSO (NbDistribution = 1000) provides estimates of pleiotropy corrected for levels by removing outliers. When the residual sum of squares observed by the global test corresponds to a *P* value >.05, the estimator considers that no outliers interfere with the results. MR-Egger relaxes the hypothesis that IVs affect outcome only through exposure and estimates whether IVs affect outcome through other pathways by testing the significance of the intercept *P* value in Egger regression; a *P* value <0.05 indicates horizontal pleiotropy. MR Steiger directionality tests were used to ensure that SNPs were more associated with exposure than with outcome to avoid potential reverse causality.[27] As previously described, the intensity of SNPs was quantified by calculating the *F* statistic. SNPs with an *F* statistic ≤10 were not included in the analysis to avoid weak instrument bias. To ensure the MR analysis is based on a reliable foundation, we used the mRnd website (mRnd[cnsgenomics.com]) to calculate the power of the MR analysis of 25(OH)D on ED ($\alpha = 0.05$). Finally, we utilized CAUSE analysis as a supplementary method. By dividing the model into sharing and causal models, CAUSE analysis can distinguish between causal effects and horizontal pleiotropy effects.[28] By comparing the posterior fit of the sharing model and the causal model, we can evaluate whether the data supports a causal effect, thereby minimizing the influence of pleiotropy on the results.

Due to the use of GWAS data for both exposure and outcome from the UKB in this study, the impact of sample overlap on the results cannot be ignored. Because directly calculating the rate of sample overlap is challenging, we use the MRlap package to correct the IVW results (MR threshold = 5×10^{-8}, MR pruning distribution =10 000, MR pruning LD = 0.001).[29] By observing the differences between the results generated by the IVW method before and after correction, we can determine whether the sample overlap has a significant impact on the results. If the impact is significant, the corrected *P* value should be considered as the primary result.

MR analyses were performed using the 2-sample MR, MRlap, and CAUSE packages in R 4.4.0 (R Foundation for Statistical Computing). LDSC analyses were performed using the LDSC (LDore) v1.0.1 in Python 3.12.1 (Python Software Foundation).

Results

The heritability (h^2) estimated using LDSC for serum 25(OH)D and ED was 8.48% (SE = 1.51%) and 0.76% (SE = 0.19%), respectively. The 2 phenotypes showed a weak genetic correlation (r_g [SE] = −0.0179 [0.058]; *P* = 0.7583) (Table 1, Table S2) illustrates the details of LDSC analysis results. Comprehensive details regarding all the SNPs utilized in MR analyses can be located in Tables S3 and S5. The *F* statistic for all SNPs surpassed 10, suggesting a minimal risk of weak instrument bias in our analysis. Table 2 illustrates the results of MR and reverse MR. Figures 1 and 2 show the effect plots produced by MR and reverse MR. In short, in the MR analysis between serum 25(OH)D and the ED risk, no significant cause association was found. Certain SNPs were discarded from our study due to their close correlation with confounding factors related to outcome (Table S7). Cochran's Q test did not detect heterogeneity between IVs (*P* > .05). We found no evidence of horizontal pleiotropy according to the significance of the intercept *P* value in Egger regression (*P* intercept >.05). The MR-PRESSO analysis results indicate no outliers found among the SNPs. MR Steiger directionality tests further determine the direction of the causal relationship between serum 25(OH)D and ED. In the MR analysis of 25(OH)D on ED, the statistical power reached 86% (R^2 = 0.024, odds ratio = 1.171),[5] which guaranteed

Table 2. MR analysis results.

Exposure	Outcome	Methods	nsnp	OR (95% CI)	*P*	*P*-Cochran's Q	*P*-intercept	Global test *P* value	Correct causal direction
Serum 25(OH)D	ED	Inverse variance weighted	103	0.895 (0.748-1.071)	.226	.816		.833	TURE
		MR-Egger		1.033 (0.772-1.380)	.829	.827	.223		
		Weighted median		0.813 (0.606-1.091)	.168				
		Simple mode		0.861 (0.468-1.584)	.631				
		Weighted mode		0.917 (0.708-1.187)	.511				
ED	Serum 25(OH)D	Inverse variance weighted	6	0.051 (0.975-1.053)	.384	.741		.721	TURE
		MR-Egger		0.051 (0.975-1.052)	.552	.851	.303		
		Weighted median		0.986 (0.965-1.007)	.182				
		Simple mode		0.986 (0.965-1.008)	.388				
		Weighted mode		0.051 (0.975-1.054)	.410				

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; ED, erectile dysfunction; MR, Mendelian randomization; OR, odds ratio; SNP, single nucleotide polymorphism; TURE, SNPs were more associated with exposure than with outcome.

Table 3. Results of MRlap analysis.

Exposure	Outcome	Corrected Effect	SE	*P*	*P* difference
Serum 25(OH)D	ED	0.004	0.013	.750	.777
ED	Serum 25(OH)D	0.137	0.866	.875	.899

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ED, erectile dysfunction.

Table 4. The results of causal analysis using summary effect estimates.

Exposure	Model	Gamma (95% CI)	Eta (95% CI)	Q (95% CI)	*P* value
Serum 25(OH)D	Sharing	NA	−0.18 (−2.76 to 2.77)	0.03 (0 to 0.22)	.989
	Causal	−0.03 (−0.21, 0.14)	−0.11 (−2.68 to 2.68)	0.04 (0 to 0.24)	
ED	Sharing	NA	0 (−0.03 to 0.03)	0.07 (0 to 0.31)	.971
	Causal	0 (−0.02, 0.01)	0 (−0.03 to 0.03)	0.08 (0 to 0.34)	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; ED, erectile dysfunction.

the reliability of the result. For detailed results, please refer to Tables S4 and S6. The MRlap approach enhanced the reliability of the IVW method results (corrected effect P = 0.750) and demonstrated that sample overlap in the dataset does not significantly impact the results (P difference = 0.777) (Table 3). In the bidirectional CAUSE analysis, no significant horizontal or vertical pleiotropy was detected (gamma and eta values in all models did not significantly deviate from 0). Additionally, there was no evidence to suggest a significant causal relationship between the 25(OH)D and the ED (P value testing that the causal model is a better fit >.05) (Table 4). Figure 3 shows the results of shared and causal models in CAUSE analysis.

Discussion

We used LDSC for the first time to explore whether serum 25(OH)D is causally correlated with ED risk. In our study, strongly associated genetic instrument variants from GWAS data in European populations was identified to conduct MR studies on causal relationship between the level of serum 25(OH)D and the ED risk at the gene prediction level. Finally, no significant genetic causal association was found in our analyses.

Penile erection is a complex physiological phenomenon, precisely regulated and coordinated by the vascular, nervous, endocrine systems, and penile erectile tissue. This process involves the engorgement of penile arteries, relaxation of trabecular smooth muscle, and occlusion of cavernous veins.[30] Vascular ED is one of the most common types of organic ED, with endothelial dysfunction playing a crucial role in its development.[31] As the protective effects of VD on endothelial function become increasingly recognized,[7] and given the widespread prevalence of both ED and VD deficiency in elderly men, the association between VD deficiency and ED has been frequently explored. However, the causal relationship between the two remains unclear. A study involving 150 ED patients found that serum 25(OH)D levels in patients with organic ED were significantly lower than those in patients with psychogenic ED. Moreover, serum 25(OH)D levels in patients with arteriogenic ED were significantly lower than in those with nonarteriogenic ED,[32] indicating the importance of VD in maintaining vascular function. Research by Zhang et al[33] also demonstrated that the 5-item International Index of Erectile Function scores of ED patients positively correlated with serum 25(OH)D levels and negatively correlated with carotid intima-media thickness. Furthermore, an increase in carotid intima-media thickness and a decrease in VD levels were shown to be related.[34]

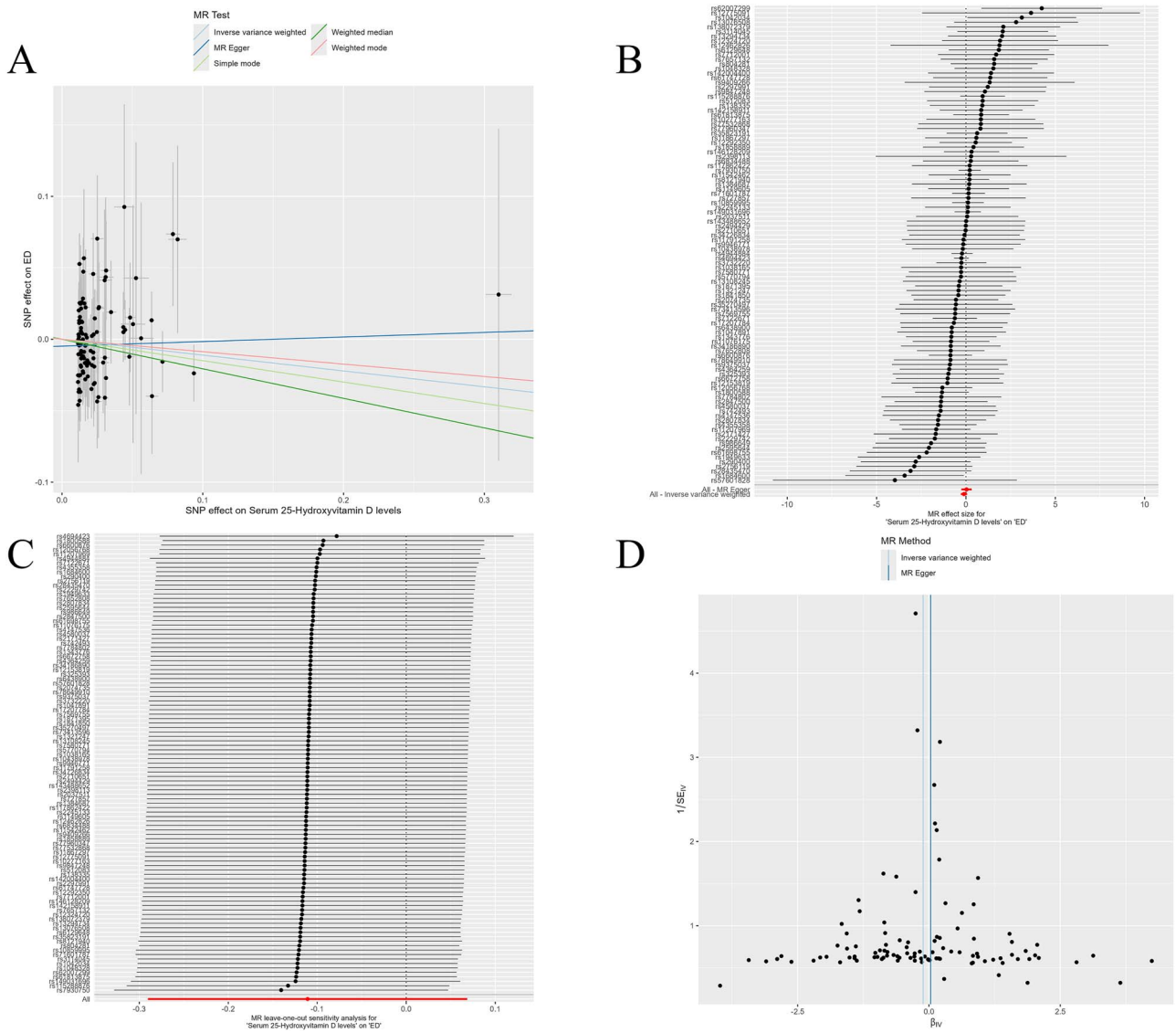

Figure 1. A: Scatter plot of the causal effect of serum 25-hydroxyvitamin D levels on erectile dysfunction. B: MR effect size for serum 25-hydroxyvitamin D levels on erectile dysfunction. C: Leave-one-out analysis of the effect of serum 25-hydroxyvitamin D levels on erectile dysfunction. D: Funnel plot of the causal effect of serum 25-hydroxyvitamin D levels on erectile dysfunction.

VD may influence nitric oxide (NO) levels in blood vessels by regulating the expression and activity of NO synthase in endothelial cells,[7] and VD deficiency may impair NO-dependent arterial dilation in the penis.

Observational studies often suffer from the influence of various confounding factors, making it difficult to determine the sequence of changes in serum 25(OH)D levels and the onset of ED and thus challenging to establish a causal relationship between the two. However, in a randomized controlled trial lasting 3 years, subjects who regularly took VD capsules showed a significant increase in serum 25(OH)D levels compared with the placebo group but did not experience the expected benefits, as the incidence of ED was not significantly lower than in the placebo group.[35] A meta-analysis including 7 studies involving a total of 4132 subjects also failed to demonstrate a significant association between VD levels and the risk of ED.[36] Additionally, an observational study assessing the correlation between VD levels and ED in patients with lower urinary tract symptoms found that the association between VD deficiency and moderate-to-severe ED was only significant in the subgroup of patients over 60 years of age. In the overall cohort, no significant association was observed between moderate-to-severe ED and serum 25(OH)D levels,[37] suggesting that VD deficiency and ED might be parallel outcomes of aging rather than directly related conditions.

Our study aimed to explore the potential causal relationship between circulating serum 25(OH)D levels and the risk of ED from a genetic perspective. The analysis was based on large publicly available GWAS datasets, with ample sample sizes ensuring statistical power while minimizing the likelihood of false positives and negatives.[38] We estimated the heritability and genetic correlation of the relevant traits using LDSC to better understand the genetic basis of serum 25(OH)D and ED as well as their genetic overlap. To reduce the impact of confounding factors and reverse causation, we employed MR and CAUSE analyses. We selected IVs that are significantly associated with the target traits but not with confounding factors, and used various methods to detect pleiotropy and heterogeneity to ensure the validity of the 3 main MR assumptions.

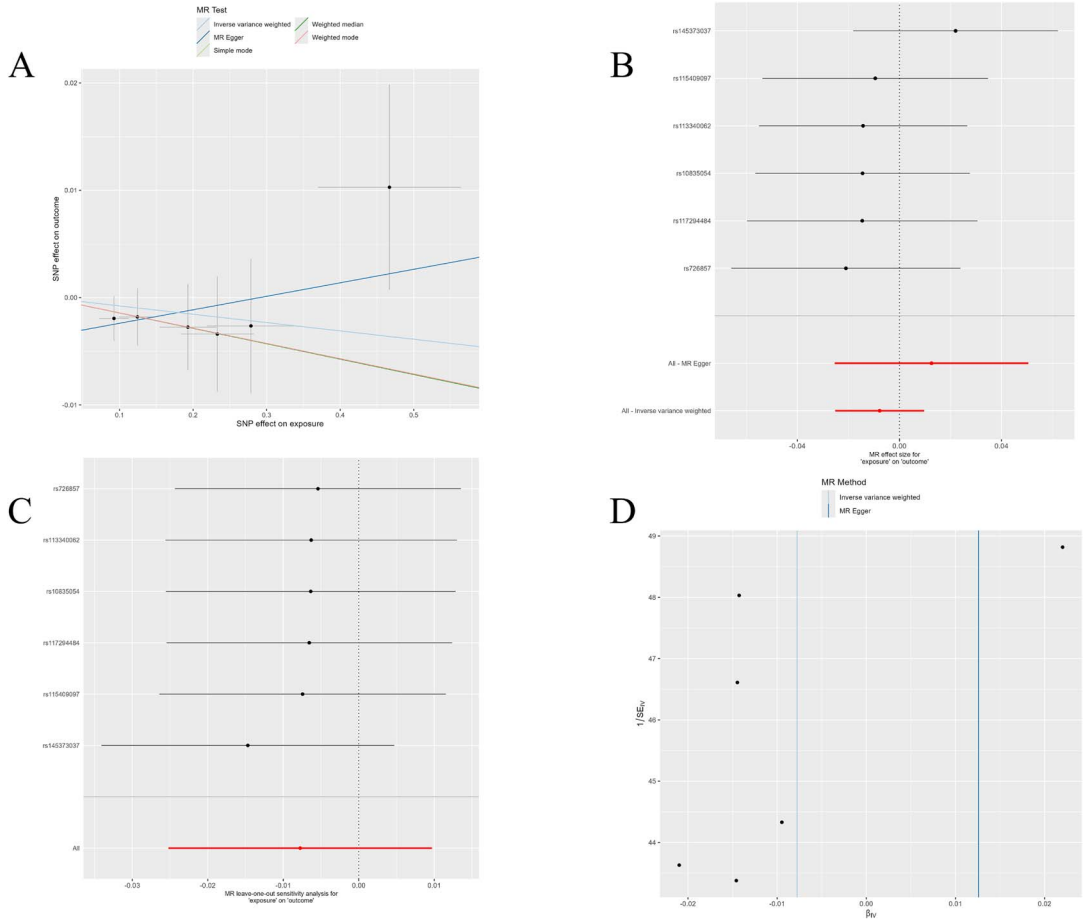

Figure 2. Scatter plot of the causal effect of erectile dysfunction on serum 25-hydroxyvitamin D levels. B: MR effect size for erectile dysfunction on serum 25-hydroxyvitamin D levels. C: Leave-one-out analysis of the effect of erectile dysfunction on serum 25-hydroxyvitamin D levels. D: Funnel plot of the causal effect of erectile dysfunction on serum 25-hydroxyvitamin D levels.

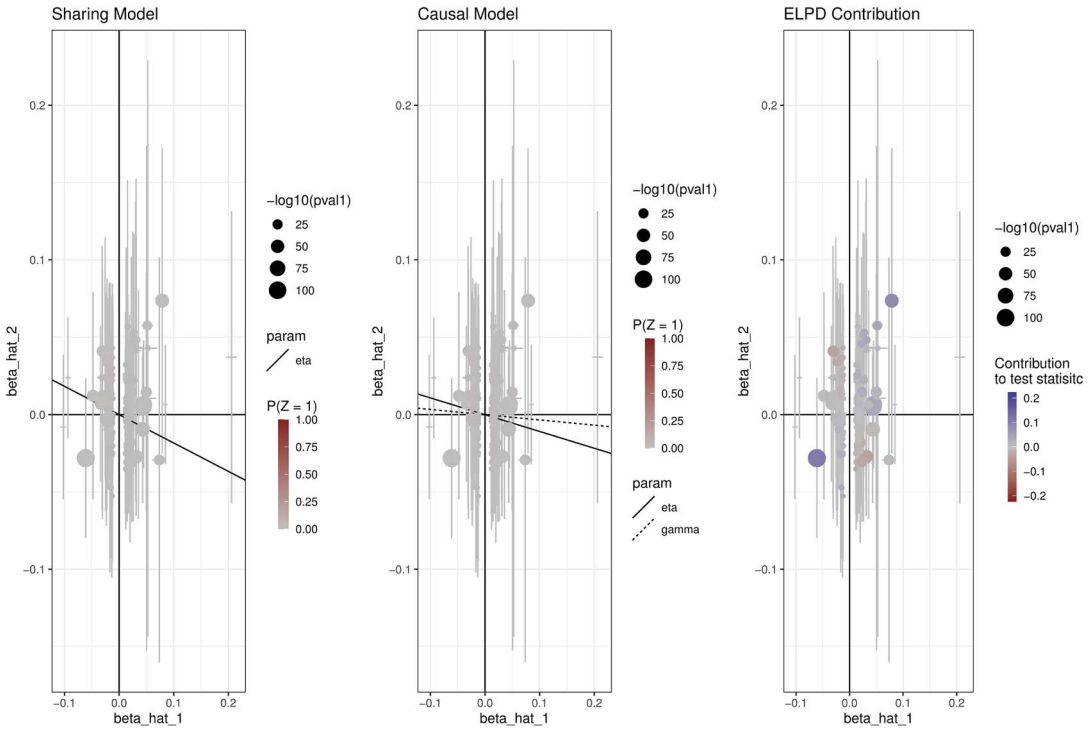

Figure 3. Effect estimates for serum 25-hydroxyvitamin D (horizontal axis) are plotted against estimates for erectile dysfunction (vertical axis). Error bars have length 1.96 times the standard error of the estimate. gamma: causal effect; eta: sharing effect.

However, the limitations of this study should not be overlooked. First, because ED only occurs in men, the use of abstract-level GWAS data prevents us from analyzing the nonlinear relationship between serum 25(OH)D levels and ED risk, as well as from conducting sex-stratified analyses. Second, in the LDSC analysis, the heritability of ED is relatively low, at just 0.76%. Additionally, the intercept for the analysis of 25(OH)D reached 1.0631, indicating the presence of a small amount of bias due to population stratification or other confounding factors. These biases could potentially affect the genetic correlation analysis. Third, while LDSC and MR results suggest that genetic correlation and causal association might not exist at the genetic level, they do not rule out the biological plausibility of such a relationship, which requires further mechanistic studies for confirmation. Finally, as the GWAS data for both traits are derived from European populations, caution should be exercised when generalizing the findings to other populations.

Conclusion

In our LDSC and MR study, ED risk was not significantly associated with serum 25(OH)D levels, which implies that supplementing VD may not be beneficial for the prevention of ED. We expect future GWASs with larger sample sizes to arrive at more credible conclusions.

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Author contributions

Xiang Liu and Longhua Luo contributed equally to this work. Conceptualization: Z.W. and C.P.; methodology: J.Z.; formal analysis: X.L.; writing—original draft preparation: L.L.; writing—review and editing: S.X. All authors have read and agreed to the published version of the manuscript.

Supplementary material

Supplementary material is available at *Sexual Medicine* online.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability statement

The datasets presented in this study are publicly available. Details are provided in the main text and Table S1. No additional ethical approval was required because only publicly available abstract-level data were used. The R packages used in the analysis are also publicly available and can be obtained on the R website (https://cran.r-project.org/web/packages/).

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