Stroke

CLINICAL AND POPULATION SCIENCES

Causal Effect of the 25-Hydroxyvitamin D Concentration on Cerebral Small Vessel Disease: A Mendelian Randomization Study

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BACKGROUND: Previous observational studies reported that a lower serum 25-hydroxyvitamin D [25(OH)D] concentration is associated with a higher burden of cerebral small vessel disease (cSVD). The causality of this association is uncertain, but it would be clinically important, given that 25(OH)D can be a target for intervention. We tried to examine the causal effect of 25(OH)D concentration on cSVD-related phenotypes using a Mendelian randomization approach.

METHODS: Genetic instruments for each serum 25(OH)D concentration and cSVD-related phenotypes (lacunar stroke, white matter hyperintensity, cerebral microbleeds, and perivascular spaces) were derived from large-scale genome-wide association studies. We performed 2-sample Mendelian randomization analyses with multiple post hoc sensitivity analyses. A bidirectional Mendelian randomization approach was also used to explore the possibility of reverse causation.

RESULTS: We failed to find any significant causal effect of 25(OH)D concentration on cSVD-related phenotypes (odds ratio [95% CI], 1.00 [0.87–1.16], 1.01 [0.96–1.07], 1.06 [0.85–1.33], 1.00 [0.97–1.03], 1.02 [0.99–1.04], 1.01 [0.99–1.04] for lacunar stroke, white matter hyperintensity, cerebral microbleeds, and white matter, basal ganglia, hippocampal perivascular spaces, respectively). These results were reproduced in the sensitivity analyses accounting for genetic pleiotropy. Conversely, when we examined the effects of cSVD phenotypes on 25(OH)D concentration, cerebral microbleeds were negatively associated with 25(OH)D concentration (0.94 [0.92–0.96]).

CONCLUSIONS: Given the adequate statistical power (>0.8) of the analyses, our findings suggest that the previously reported association between 25(OH)D concentration and cSVD phenotypes might not be causal and partly attributed to reverse causation.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: brain ■ cerebral small vessel disease ■ Mendelian randomization ■ polymorphism, single nucleotide ■ vitamin D deficiency

Cerebral small vessel disease (cSVD) is a common disease entity, a well-known indicator of vascular brain health, and results in neurological disorders such as stroke or dementia.[1–3] There is some evidence supporting that optimal blood pressure control, adequate diet, and physical exercise might prevent the progression of cSVD; yet, these interventions have been not adequately proven to be effective in preventing cSVD in large randomized clinical trials, although some trial evidence supports a benefit of antihypertensive treatment to slow down the progression of cSVD.[3–5]

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Nonstandard Abbreviations and Acronyms

25(OH)D	25-hydroxyvitamin D
BG	basal ganglia
CMB	cerebral microbleed
cSVD	cerebral small vessel disease
GWAS	genome-wide association study
ICH	intracerebral hemorrhage
LD	linkage disequilibrium
PVS	perivascular space
SNP	single-nucleotide polymorphism
WM	white matter
WMH	white matter hyperintensity

Several observational studies have reported a negative association between serum 25-hydroxyvitamin D [25(OH)D] concentration and cSVD.[6–8] Lowering blood pressure through the renin-angiotensin-aldosterone system, attenuating inflammation, and having a neuroprotective effect have been suggested as potential mechanisms.[9,10] However, this association was not reproduced in other studies,[11,12] which led to doubt as to whether it is truly causal.

Mendelian randomization is a method that uses single-nucleotide polymorphisms (SNPs) as a genetic instrumental variable for a given exposure to seek evidence for a causal relation with a given outcome. This method is based on the fact that genetic alleles are assorted randomly during meiosis, making the analysis less prone to confounding and reverse causation than observational studies.[13] In other words, by using MR, one can simulate a randomized controlled trial. Two-sample MR studies derive their estimates from summary statistics of genome-wide association studies (GWAS) for the exposure and the outcome, respectively.[14] Using recent large GWAS of 25(OH)D concentration and cSVD phenotypes, the present study aimed to elucidate whether 25(OH)D concentration is causally associated with 4 cSVD phenotypes, which the GWAS summary statistics were available, lacunar stroke, white matter hyperintensity (WMH) volume, cerebral microbleeds (CMBs), and perivascular spaces (PVSs).

METHODS

Data Availability and Ethics Statement

All data used for this study is publicly available from the respective GWAS. Ethical approval was not required for this study as we used anonymized summary-level data that were available to the public.

Data

We used summary statistics of the most recent GWASs of both 25(OH)D concentration and cSVD phenotypes: lacunar stroke, WMH volume, CMBs, and PVSs. The exposure dataset was composed of the summary statistics of the most recent and largest GWAS of the 25(OH)D concentration that was derived from an analysis of the UK Biobank population (N=417 580).[15] The outcome dataset was composed of the most recent GWASs of each cSVD phenotype: lacunar stroke (6030 cases and 248 929 controls), WMH volume (N=48 454), CMBs (N=25 862), and PVSs (white matter [WM] PVSs, N=38 598; basal ganglia (BG) PVSs, N=38 903; and hippocampal PVSs, N=38 871).[16–19] 25(OH)D concentration was mostly measured at the initial assessment visit of the UK Biobank, and rank-based inverse-normal transformation was applied. Information on the month of assessment was included as a covariate, taking into account the well-known seasonal variation associated with 25(OH)D concentration,[20] in addition to information on supplement intake. Lacunar stroke was defined according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria[21] or as a subcortical infarct with a diameter ≤15 mm on diffusion-weighted imaging for acute infarcts and on fluid-attenuated inversion recovery or T1 sequences for non-acute infarcts.[16] WMH volume was measured from T1, T2, and fluid-attenuated inversion recovery sequences (with proton density in some cases) usually using a fully automated software, and was transformed with rank-based inverse-normal transformation.[17] CMBs were defined as small, hypointense lesions on susceptibility-weighted imaging or T2*-weighted gradient echo sequences.[18,22] Among participants who had CMBs, 61.3% were classified as having lobar CMB, when they did not have any CMBs in the deep or infratentorial region, while the remaining were classified as mixed CMBs, defined as deep or infratentorial CMBs with a possible combination of CMBs in the lobar region.[18] PVSs were quantified in the cerebral WM, BG, and hippocampus using visual semi-quantitative rating scales or automated computational methods. These were identified as round, ovoid, or linear shape fluid-filled spaces, mimicking the signal of cerebrospinal fluid, usually with a maximum diameter of <3 mm. They exhibit no hyperintense rim on T2-weighted or fluid-attenuated inversion recovery sequences, and are located in areas supplied by perforating arteries.[19,23] Due to the use of different scales across studies, the PVS burden was dichotomized using a cutoff point closest to the top quartile of the semiquantitative scale distribution within each cohort. The selected GWASs included participants of predominantly European ancestry. We used the summary statistics of GWAS conducted in exclusively European ancestry participants except for CMBs, for which available GWAS summary statistics included a small proportion of participants of other ancestries (5.8%).

Bidirectional Mendelian Randomization Analysis

SNPs that met the genome-wide significance level ($P<5\times10^{-8}$) were initially selected as genetic instruments. If no SNPs met the genome-wide significance level, a lower threshold of $P<1\times10^{-5}$ was used. Among the selected SNPs, linkage disequilibrium (LD) clumping with the threshold of $R^2<0.01$ was performed to exclude the correlated SNPs. Heterogeneity among the SNPs was examined using the I^2 statistic of the inverse-variance weighted method.

The primary 2-sample MR analysis was performed using the inverse-variance weighted method along with multiple sensitivity analyses with the weighted median, mode-based, MR-Egger, MR-Lasso, and MR-PRESSO (Mendelian Randomization Pleiotropy Residual Sum and Outlier) methods to rule out

pleiotropy.[24–29] Genetic pleiotropy was assessed using the intercept of the MR-Egger analysis. As the exposure GWAS provided the 25(OH)D concentration unit as rank-based inverse-normal transformed values, the effect size of this study was presented as a change in odds of each cSVD phenotype, the presence versus absence of lacunar stroke, CMBs, and PVSs, and the 1 SD of WMH volume, per 1 SD of the 25(OH)D concentration.

Additionally, a reverse MR analysis was performed using each cSVD phenotype as an exposure and the 25(OH)D concentration as an outcome to explore reverse causation between cSVD phenotypes and the 25(OH)D concentration. The selection of the genetic instruments and other analytic methods for this reverse MR analysis were identical to those of the primary analysis described earlier. For CMBs, analysis according to the subtypes of lobar CMBs and mixed CMBs was also performed. The statistical power of each MR analysis was calculated by the method established by Brion et al (https://shiny.cnsgenomics.com/mRnd/).[30]

Cross-Trait LD Score Regression Analysis

Genetic correlations between the 25(OH)D concentration and cSVD phenotypes were examined using cross-trait LD score regression analysis. This analysis determines the estimate of the genetic correlation between 2 phenotypes using genetic covariance and the LD score from the GWAS summary statistics of each.[31,32] The information of the LD structure for the European ancestry was derived from the 1000 genome project.[33]

Statistical significance was considered at a 2-sided $P<0.05$. Analyses were performed using the Mendelian Randomization (version 0.5.0) and MR-PRESSO package for bidirectional MR analysis, and ieugwasr (version 0.1.5) for LD clumping and the LDSC package (version 1.0.1) for cross-trait LD score regression analysis.

RESULTS

GWASs used for this study are summarized in the Table. After data harmonization, the number of independent SNPs selected as instruments for 25(OH)D concentration was 94 for the analysis of association with lacunar stroke, 83 with WMH volume, 95 with CMBs, and 106 for PVSs. The primary 2-sample MR analysis showed no significant association between genetically determined 25(OH)D concentration and cSVD phenotypes; the odds ratios (95% CI) were 1.00 (0.87–1.16) for lacunar stroke, 1.01 (0.96–1.07) for WMH volume, 1.06 (0.85–1.33) for CMBs, 1.00 (0.97–1.03) for WM PVSs, 1.02 (0.99–1.04) for BG PVSs, and 1.01 (0.99–1.04) for hippocampal PVSs. These results were reproduced in the sensitivity analyses (Figures 1A through 1F and 2A through 2F). Moderate heterogeneity was suspected regarding the association between 25(OH)D concentration and WMH volume (I^2=42.7; Table S1). However, the MR-PRESSO analysis, which provides results after excluding outlier SNPs, supported no association between the 25(OH)D concentration and WMH volume (Figure 1). Besides, there was only little heterogeneity regarding the associations between 25(OH)D levels and each of lacunar stroke, CMBs, WM PVSs, BG PVSs, and hippocampal PVSs (I^2=0.0, 11.6, 18.5, 0.0, and 0.0, respectively). The MR-Egger intercept, which is an indicator of genetic pleiotropy, was small and statistically insignificant for all 6 cSVD phenotypes (Table S1).

In reverse MR analyses, 7 SNPs were selected as the genetic instruments for lacunar stroke, 21 SNPs for WMH volume, and 17 for WM PVSs. However, for CMBs, 11 SNPs met the criteria for the *P* value of genome-wide association significance and only 1 SNP remained after LD clumping. Thus, we used the post-clumped single SNP by the Wald method[34] as a primary analysis for CMBs. Also, there was no SNP that met the criteria for the *P* value of genome-wide association

Table. Characteristics of the Genome-Wide Association Studies

Variable	Source GWAS	Ethnicity	N	Adjusted variable
Exposure				
25-hydroxyvitamin D concentration[15]	Nat Commun. 2020. Apr 2;11(1):1647.	European	417 580	Age, sex, assessment month, assessment center, supplement-intake information, genotyping batch, and PCs
Outcome				
Lacunar stroke[16]	Lancet Neurol. 2021 May;20(5):351–361.	European	6030 cases and 248 929 controls	PCs
White matter hyperintensity volume[17]	Nat Commun. 2020. Dec 8;11(1):6285.	European	48 454	Age, sex, PCs, and intracranial volume
Cerebral microbleeds[18]	Neurology. 2020;95:e3331–e3343.	Multiethnic (mainly European, 94.2%)	3556 cases and 22 306 controls	Age, sex, PCs, family, and relations (if applicable)
White matter perivascular spaces[19]	Nat Med. 2023 Apr;29(4):950–962.	European	9324 cases and 29 274 controls	Age, sex, intracranial volume, PCs, and study site
Basal ganglia perivascular spaces[19]	Nat Med. 2023 Apr;29(4):950–962.	European	8913 cases and 29 990 controls	Age, sex, intracranial volume, PCs, and study site
Hippocampal perivascular spaces[19]	Nat Med. 2023 Apr;29(4):950–962.	European	9223 cases and 29 648 controls	Age, sex, intracranial volume, PCs, and study site

GWAS indicates genome-wide association study; and PCs, principal components.

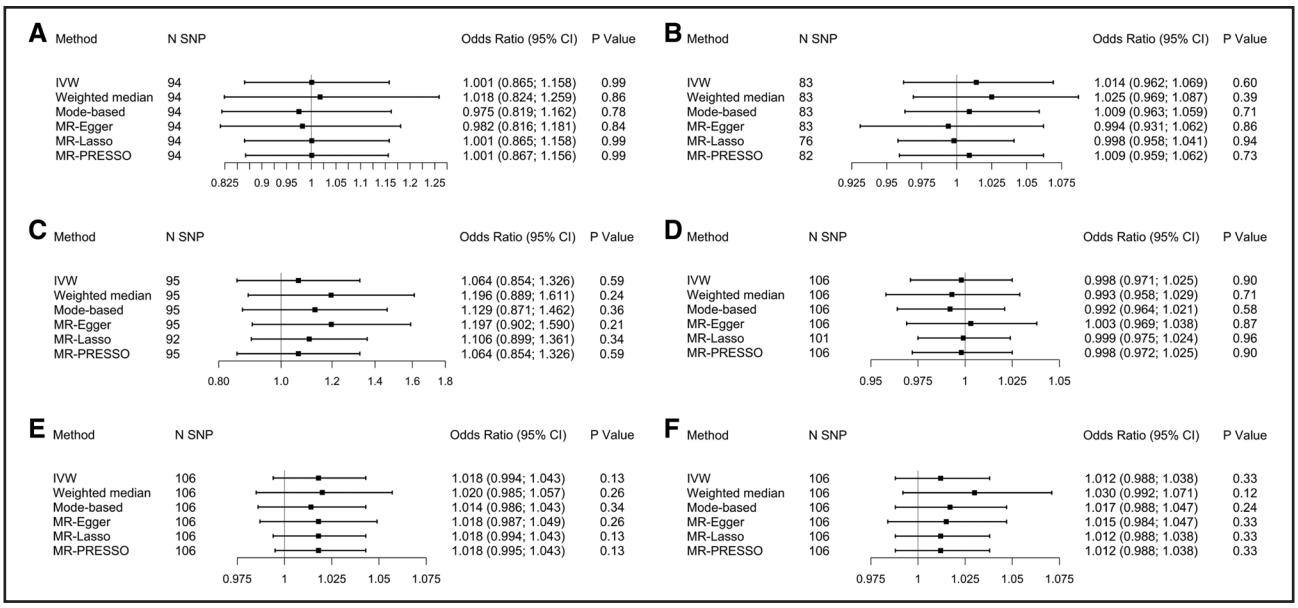

Figure 1. Results of the Mendelian randomization analysis for the effect of the 25(OH)D concentration on cerebral small vessel disease phenotypes.

A, Lacunar stroke; (**B**) white matter hyperintensity volume; (**C**) cerebral microbleeds; (**D**) white matter perivascular spaces; (**E**) basal ganglia perivascular spaces; and (**F**) hippocampal perivascular spaces. 25(OH)D indicates 25-hydroxyvitamin D; IVW, inverse-variance weighted; and SNP, single-nucleotide polymorphism.

significance for BG PVSs and hippocampal PVSs, so we used a lenient threshold of $P<1\times10^{-5}$ for these analyses. The primary analysis using the inverse-variance weighted method showed no significant association of genetic liability to lacunar stroke, PVSs, or genetically determined WMH volume with 25(OH)D concentration

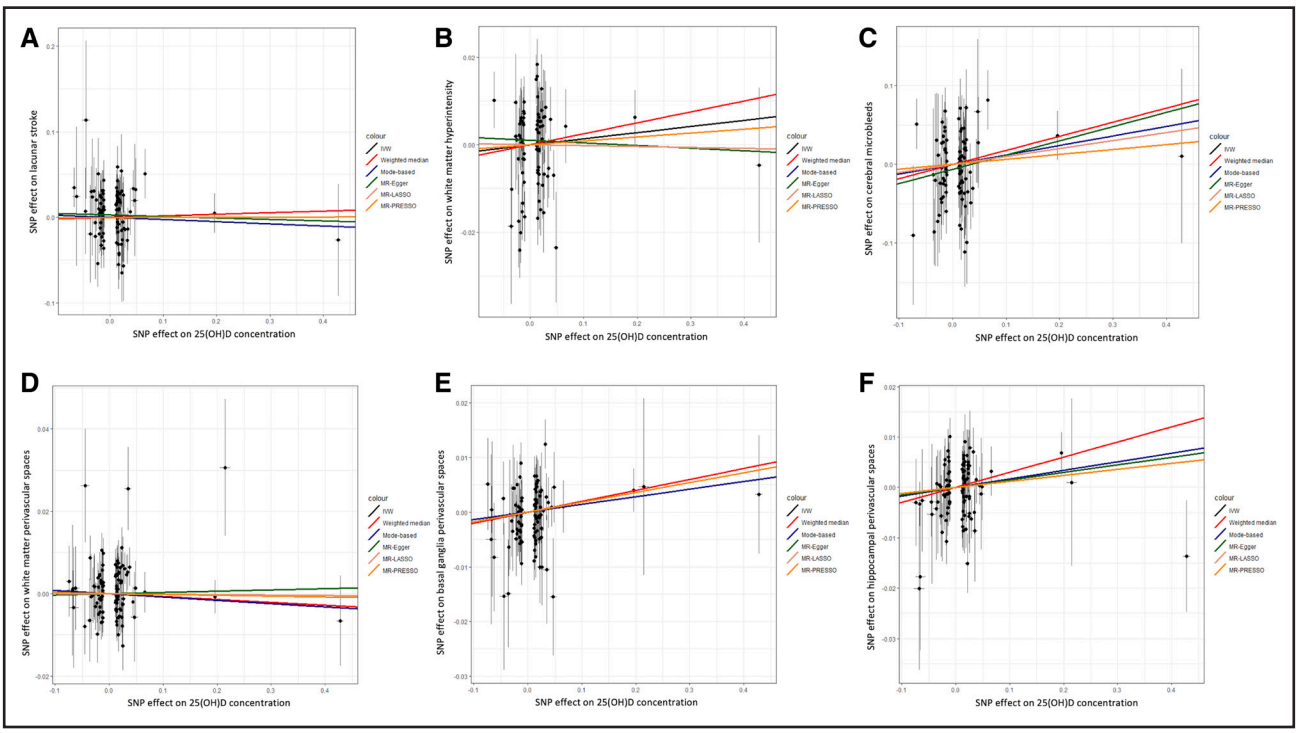

Figure 2. Scatter plot of the Mendelian randomization analysis results for the effect of the 25(OH)D concentration on cerebral small vessel disease phenotypes.

A, Lacunar stroke; (**B**) white matter hyperintensity volume; (**C**) cerebral microbleeds; (**D**) white matter perivascular spaces; (**E**) basal ganglia perivascular spaces; and (**F**) hippocampal perivascular spaces. 25(OH)D indicates 25-hydroxyvitamin D; IVW, inverse-variance weighted; and SNP, single-nucleotide polymorphism.

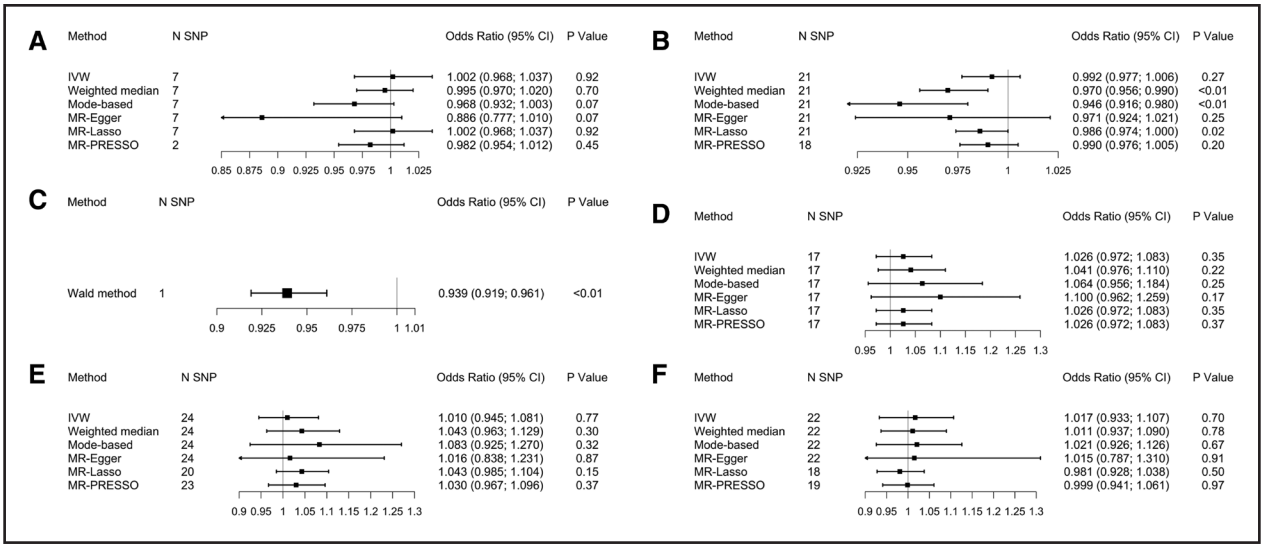

Figure 3. Results of reverse Mendelian randomization analysis for the effect of cerebral small vessel disease phenotypes on the 25(OH)D concentration.

A, Lacunar stroke; (**B**) white matter hyperintensity volume; (**C**) cerebral microbleeds; (**D**) white matter perivascular spaces; (**E**) basal ganglia perivascular spaces; and (**F**) hippocampal perivascular spaces. 25(OH)D indicates 25-hydroxyvitamin D; IVW, inverse-variance weighted; and SNP, single-nucleotide polymorphism.

(Figures 3A, 3B, and 3D through 3F). In the sensitivity analysis, a significant negative association between WMH volume and 25(OH)D concentration was indicated by the weighted median, mode-based analysis, and MR-Lasso analysis, while a negative association between lacunar stroke and the 25(OH)D concentration was only suggestive based on the mode-based and MR-Egger analyses (Figures 3A, 3B, 4A, and 4B). However,

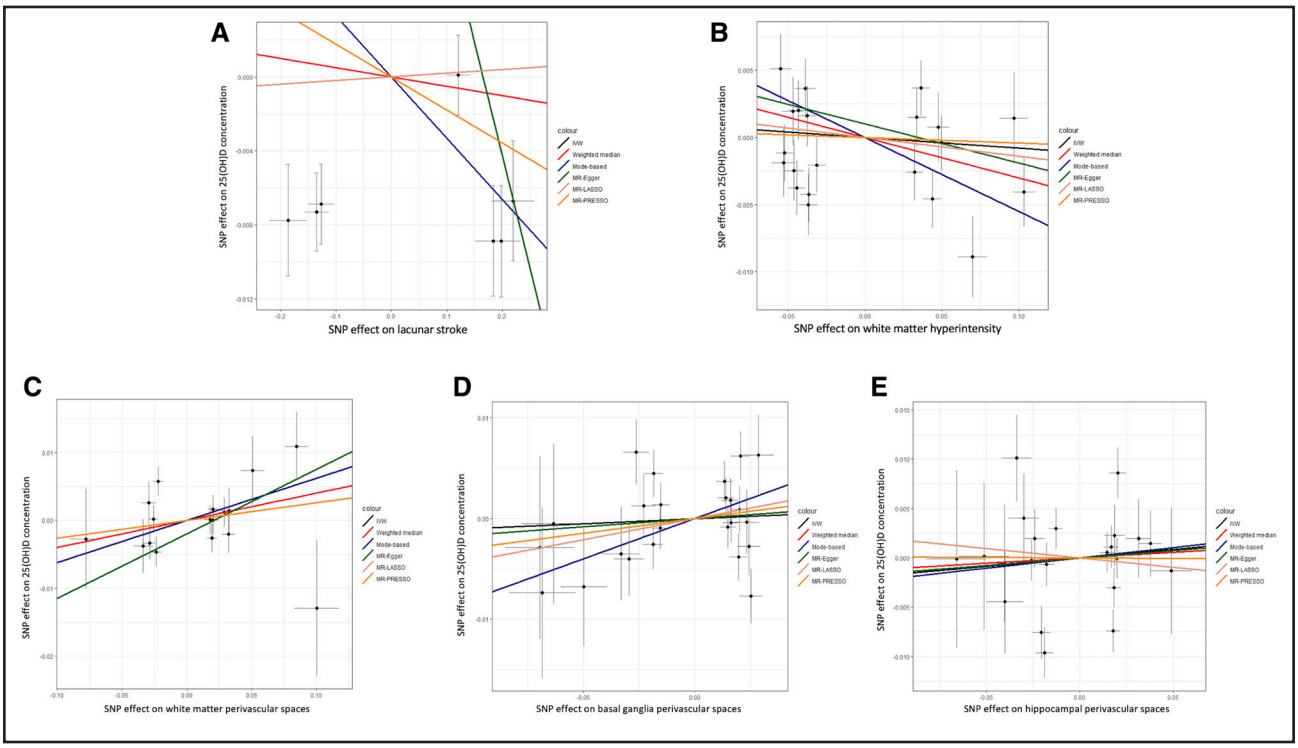

Figure 4. Scatter plot of the reverse Mendelian randomization analysis results for the effect of cerebral small vessel disease phenotypes on the 25(OH)D concentration.

A, Lacunar stroke; (**B**) white matter hyperintensity volume; (**C**) white matter perivascular spaces; (**D**) basal ganglia perivascular spaces; and (**E**) hippocampal perivascular spaces. 25(OH)D indicates 25-hydroxyvitamin D; IVW, inverse-variance weighted; and SNP, single-nucleotide polymorphism.

there was no association between PVS phenotypes and 25(OH)D concentration (Figures 3D through 3F and 4D through 4F). Furthermore, substantial heterogeneity was suspected regarding these phenotypes (I^2=72.4, 88.1, 37.4, 43.2, and 64.4 for the WMH volume, lacunar stroke, WM PVSs, BG PVSs, and hippocampal PVSs, respectively). The MR-PRESSO analysis showed no significant association after excluding the outlier SNPs, and the MR-Egger intercepts were not statistically significant (Figures 3A, 3B, and 3D through 3F; Table S1).

In contrast, the reverse MR analysis for CMBs based on the analysis using a single, post LD-clumped SNP showed a significant negative association between CMBs and the 25(OH)D concentration (odds ratio [95% CI], 0.94 [0.92–0.96]; Figure 3C).

We found no association between the 25(OH)D concentration and CMB subtypes, with the exception of a positive association identified in the MR-Lasso analysis for the reverse MR analysis between lobar CMBs and 25(OH)D concentration (odds ratio [95% CI], 1.01 [1.00–1.02]). It should, however, be noted that a lenient threshold of $P<1\times10^{-5}$ was applied for the reverse MR analysis, given that no SNP met the initial *P* value threshold, resulting in decreased statistical power (as detailed in Table S2).

The statistical power was ≥80% in most of the bidirectional MR analysis regarding associations between 25(OH)D concentration and WMH volume or CMBs, mode-based, and MR-Egger analysis regarding the bidirectional association between the 25(OH)D concentration and lacunar stroke. For PVSs, some of the sensitivity analysis such as weighted median method regarding the association between 25(OH)D concentration and hippocampal PVS and both mode-based and MR-Egger methods for the association between WM PVS and 25(OH)D concentration in the reverse MR analysis held ≥80% statistical power, while only mode-based analysis achieved this for BG PVS (Table S3).

The cross-trait LD score regression analysis did not show any genetic correlation between 25(OH)D concentration and lacunar stroke, WMH volume, or PVSs (Table S4). The genetic correlation between 25(OH)D concentration and CMBs could not be estimated, probably because of the lack of statistical power due to the small sample size of the source GWAS for CMBs.

DISCUSSION

Our results suggest that a low serum 25(OH)D concentration is not a direct cause of cSVD, which contradicts findings of previous observational studies.[6–8] This result was supported by the absence of genetic correlation between 25(OH)D concentration and cSVD phenotypes in our study. However, the results of the reverse MR analysis suggested that cSVD phenotypes, especially CMBs, were negatively associated with serum 25(OH)D concentration; thus, cSVD burden reduced the serum 25(OH)D concentration.

There were a series of observational studies about the associations between serum 25(OH)D concentration and cSVD phenotypes. Two small studies of single hospital-based adult outpatients undergoing magnetic resonance imaging (N=56) and community-dwelling elderly people receiving home care (N=318) showed that a lower 25(OH)D concentration was associated with a higher burden of cSVD phenotypes, such as a larger WMH volume or lacunar stroke.[7,8] This negative association was reproduced by another study of hospitalized patients with stroke (N=759): a 25(OH)D deficiency was associated with severe WMH, lacunes, and deep CMBs in a dose-dependent manner.[6] However, the weakness of the aforementioned studies, including the small sample size and cross-sectional study design, might have limited generalizability of the causality of relationship. Furthermore, a well-designed, prospective, population-based cohort (N=888) could not find an association between the baseline 25(OH)D concentration and WMH grade cross-sectionally or WMH progression during an ≈10-year follow-up.[11]

MR analysis is an analogue of a randomized controlled trial, and it can control for unmeasured confounding and reverse causation, which is commonly found in observational studies.[13,34] It would be infeasible and somewhat unethical to randomize vitamin D deficiency, but MR analysis made it possible to seek evidence for a causal effect of a low 25(OH)D concentration on cSVD phenotypes.[34] Additionally, MR analyses estimate a lifelong effect of an exposure, which would be impossible in a randomized clinical trial. Thus, MR analysis would be a good choice for answering such a question of whether a vitamin D supplement has a beneficial effect on preventing cSVD. Moreover, we were able to secure a statistical power >90% in most of the analyses for the WMH volume and CMBs, and a power >80% in some analyses for lacunar stroke and PVSs. We were confident that we could confirm the associations between the 25(OH)D concentration and cSVD phenotypes in this study (Table S3).

Our results contradict those of a recent MR study based on the patients with ischemic stroke or myocardial infarction (N=441) and controls (N=5331) from Hong Kong.[35] The study showed that vitamin D is causally protective against recurrent ischemic vascular events. However, stroke is a heterogeneous disease entity, and no protection against lacunar stroke does not mean no protection against large artery atherosclerosis or cardioembolism.[21,36] Recently, a 2-sample MR study using publicly available summary statistics from published GWASs reported that lower 25(OH)D concentrations were associated with an increased risk of intracerebral hemorrhage (ICH), which is another phenotype of cSVD.[37] The difference in results between this study and ours could be

attributed to the difference in pathogenesis between ICH and CMB. In this recent ICH study, 25(OH)D concentration was negatively associated with nonlobar (deep) ICH, but not with lobar ICH. However, in our study, most patients had lobar CMBs, and the data for those with strictly nonlobar CMBs were unavailable.

It is quite interesting that reverse causation between cSVD phenotypes, especially CMBs, and the 25(OH)D concentration was observed in our study. people with a higher cSVD burden, who are prone to stroke or vascular cognitive impairment, are susceptible to poor nutritional status and frailty, which leads to lack of outdoor activity and a lower 25(OH)D concentration eventually.[38,39] This association may be prominent with CMBs, as CMBs in our study were mainly lobar or mixed types suggestive of cerebral amyloid angiopathy, and Alzheimer disease shares a pathophysiology with cerebral amyloid angiopathy.[40,41] The Rotterdam study, a prospective population-based cohort study, found that a lower 25(OH)D concentration was associated with prevalent stroke, not with incident stroke, which implies that a lower 25(OH)D is a consequence rather than a cause of stroke.[42] Reverse causation is an important bias that should be considered in observational studies to investigate causal inference.[43] Our results suggest that the negative association between the 25(OH)D concentration and cSVD burden might have been driven by reverse causation. Yet, people with CMBs may be at risk of 25(OH)D deficiency and might be suggested as a target population for screening serum 25(OH)D levels and providing supplementation if needed. This finding might add value to this field, as there is not enough evidence for screening for vitamin D deficiency in asymptomatic adults.[44]

Our study has several limitations. First, because the GWASs used in our study were mostly based on the patients with European ancestry, generalizability of our findings to other ethnicities might be limited. Second, we were unable to account for the nonlinear association in the analysis, which needs a single dataset with all the exposure and outcome variables along with information of the genetic instruments[45,46] However, a recent study that performed an nonlinear MR analysis using the UK Biobank database showed there was no association between the 25(OH)D concentration and WMH volume.[47] Third, the subjects of each GWAS study were derived from either population-based cohorts or hospital-based cohorts and this difference in the source population may be a potential source of bias. Forth, it should be noticed that there are other cSVD phenotypes, such as cortical atrophy, superficial hemosiderosis, or microinfarcts, which were not analyzed in this study as GWAS results are not available yet.[3] Lastly, although we performed various sensitivity analyses, possibilities of uncontrolled pleiotropies or heterogeneities cannot be excluded.

CONCLUSIONS

This 2-sample MR analysis using recent large GWASs indicates that 25(OH)D concentration does not have a causal effect on cSVD phenotypes. The association shown in previous observational studies might have been derived from unmeasured confounding and reverse causation, especially for CMBs.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S4
STROBE-MR checklist

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