Genetically decreased vitamin D and risk of Alzheimer disease

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ABSTRACT

Objective: To test whether genetically decreased vitamin D levels are associated with Alzheimer disease (AD) using mendelian randomization (MR), a method that minimizes bias due to confounding or reverse causation.

Methods: We selected single nucleotide polymorphisms (SNPs) that are strongly associated with 25-hydroxyvitamin D (25OHD) levels ($p < 5 \times 10^{-8}$) from the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT) Consortium (N = 33,996) to act as instrumental variables for the MR study. We measured the effect of each of these SNPs on 25OHD levels in the Canadian Multicentre Osteoporosis Study (CaMos; N = 2,347) and obtained the corresponding effect estimates for each SNP on AD risk from the International Genomics of Alzheimer's Project (N = 17,008 AD cases and 37,154 controls). To produce MR estimates, we weighted the effect of each SNP on AD by its effect on 25OHD and meta-analyzed these estimates using a fixed-effects model to provide a summary effect estimate.

Results: The SUNLIGHT Consortium identified 4 SNPs to be genome-wide significant for 25OHD, which described 2.44% of the variance in 25OHD in CaMos. All 4 SNPs map to genes within the vitamin D metabolic pathway. MR analyses demonstrated that a 1-SD decrease in natural log-transformed 25OHD increased AD risk by 25% (odds ratio 1.25, 95% confidence interval 1.03–1.51, $p = 0.021$). After sensitivity analysis in which we removed SNPs possibly influenced by pleiotropy and population stratification, the results were largely unchanged.

Conclusions: Our results provide evidence supporting 25OHD as a causal risk factor for AD. These findings provide further rationale to understand the effect of vitamin D supplementation on cognition and AD risk in randomized controlled trials. *Neurology*® 2016;87:2567–2574

GLOSSARY

AD = Alzheimer disease; **CAD** = coronary artery disease; **CaMos** = Canadian Multicentre Osteoporosis Study; **CI** = confidence interval; **DBP** = vitamin D–binding protein; ***DSM-IV*** = *Diagnostic and Statistical Manual of Mental Disorders, 4th edition*; **GWAS** = genome-wide association study; **IGAP** = International Genomics of Alzheimer's Project; **LD** = linkage disequilibrium; **LDL-C** = low-density lipoprotein cholesterol; **MR** = mendelian randomization; **MS** = multiple sclerosis; **OR** = odds ratio; **RCT** = randomized controlled trial; **SBP** = systolic blood pressure; **SNP** = single nucleotide polymorphism; **25OHD** = 25-hydroxyvitamin D.

Alzheimer disease (AD) is a devastating disease that will continue to exert a significant social and economic burden unless effective forms of prevention are identified. While large-scale observational studies and meta-analyses have suggested that a decreased 25-hydroxyvitamin D (25OHD) level is associated with an increased risk of AD,[1–5] it is difficult to fully protect observational studies from bias due to confounding or reverse causation. These limitations add uncertainty with regard to the causal role of vitamin D in AD etiology. However, it is important to clarify the causal relationship between vitamin D and AD because vitamin D insufficiency is becoming increasingly common[6,7] and repletion can be achieved safely through supplementation.

In the absence of evidence from randomized controlled trials (RCTs), mendelian randomization (MR) can be used to support or contradict the role of a risk factor in disease etiology.[8]

Supplemental data at Neurology.org

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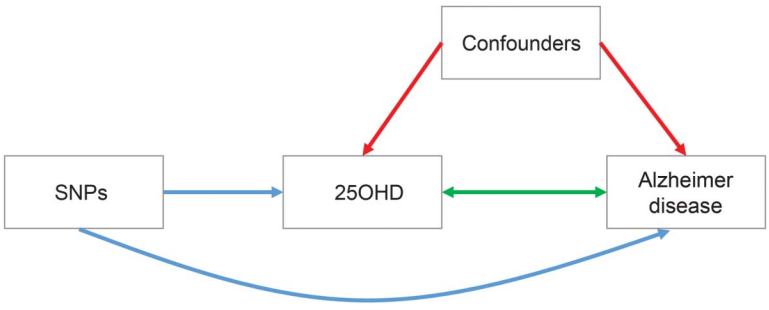
Because single nucleotide polymorphisms (SNPs) are randomly assigned at conception, they are not associated with the behaviors and physiologic processes that may confound observational studies (figure 1).[8] Furthermore, because SNPs are inherited at conception, they are not influenced by reverse causation and can represent lifetime risk due to increased or decreased levels of a risk factor. Given these important advantages, we elected to perform an MR study using SNPs from the largest genome-wide association study (GWAS) for vitamin D (the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits [SUNLIGHT] Consortium[9]; N = 33,996) and summary statistics from the largest GWAS to date for AD (the International Genomics of Alzheimer's Project [IGAP]; N = 17,008 AD cases and 37,154 controls).[10]

METHODS Data sources, SNP selection, and genetic effect sizes on 25OHD. We selected SNPs that achieved genome-wide significance ($p < 5 \times 10^{-8}$) for 25OHD in the SUNLIGHT Consortium,[9] the largest GWAS assessing 25OHD levels (N = 33,996). 25OHD was used as a determinant of vitamin D status because this molecule is more stable than the physiologically active form of vitamin D (1,25-dihydroxyvitamin D) and can thereby be reliably measured.[11] Furthermore, cohorts of the SUNLIGHT study used different assays to measure 25OHD, so a *Z* score meta-analysis was used to combine the results of all cohorts. Consequently, the effect of each SNP, which is the regression β coefficient on 25OHD from the SUNLIGHT meta-analysis, was not available. To obtain genetic effect sizes on the nonnormalized scale, we measured the effect of these SNPs on 25OHD in the Canadian Multicentre Osteoporosis Study (CaMos). CaMos was among the largest replication cohorts included in the SUNLIGHT Consortium, involving 2,347 individuals of European descent. We have previously estimated the effect of the SUNLIGHT SNPs on 25OHD in CaMos in an MR analysis.[12] In brief, we used a natural log transformation of 25OHD because of its skewed distribution. We then regressed natural log 25OHD on each effect allele using an additive genetic model, controlling for sex, age, age squared, body mass index, and season of 25OHD measurement. The corresponding effect estimates of the SUNLIGHT SNPs on AD risk were obtained from IGAP, which consisted of 17,008 AD cases and 37,154 controls.[10] Cases were diagnosed by a neurologist using either internationally accepted clinical criteria (such as the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association, DSM-IV, Clinical Dementia Rating, and the Mini-Mental State Examination) or autopsy results.[10]

Figure 1 Mendelian randomization (MR) design using directed acyclic graphs



Red arrows show the potential sources of bias that may influence estimates derived from observational studies. Because it is difficult to fully protect observational studies from confounding, residual confounding from lifestyle factors that determine both vitamin D status and Alzheimer disease (AD) risk is possible. Green arrow denotes potential reverse causation, which is of concern in the investigation of a disease such as AD with a late-life onset. Thus, in observational epidemiology, it is difficult to assess whether decreased vitamin D preceded disease onset. With the MR design, bias due to reverse causation or confounding is greatly reduced because the single nucleotide polymorphisms are assigned before disease onset and are not associated with confounders as a result of the process of randomization at conception.

SNP validation. We have previously used the SUNLIGHT SNPs as instruments for MR analyses of multiple sclerosis (MS) and coronary artery disease (CAD).[12,13] Thus, we and others[14] have previously evaluated whether the 25OHD-associated SNPs violate any of the MR assumptions such as linkage disequilibrium (LD), population stratification, and pleiotropy. If present, these conditions can bias MR estimates. Bias due to LD occurs when SNPs included in the genetic score are found to be correlated with each other ($r^2 > 0.05$). To ensure that the chosen SNPs did not violate this assumption, we measured LD in European samples from the UK10K whole-genome sequencing program (N = 3,781).[15] Population stratification can similarly bias estimates if an SNP is associated with ancestry, which covaries with disease status. To account for population stratification, we restricted our samples to those of European ancestry in CaMos and the IGAP; however, residual population stratification may still exist within European populations.[16] Previous work investigated this in the United Kingdom using the 1958 British Birth Cohort.[14] Pleiotropy in an MR study occurs when the effect of an SNP on AD (the outcome) is independent of 25OHD (the exposure). Therefore, to further explore the possibility of pleiotropy, we performed a literature search in PubMed to see if any of the 25OHD SNPs associate with relevant AD physiologic pathways (details provided in appendix e-1 at Neurology.org). For risk factors that have previously been implicated with AD through MR, we searched through publicly available GWAS datasets to see if any of the 25OHD SNPs associated with these traits at a nominal level ($p < 0.05$) or after a Bonferroni correction for the number of traits tested. On the basis of the results of these analyses, we performed sensitivity analyses in which SNPs were removed if found to be possibly influenced by these factors.

MR estimates. By using the 2-sample MR approach[17] in which genetic effect sizes on AD and 25OHD are obtained from the summary statistics of their respective GWAS (IGAP and CaMos), we were able to test the effect of 25OHD in the largest available genotyped cohort of AD cases. This maximizes statistical power and produces results that are equivalent to 1-sample approaches in which both the exposure and outcome are measured in the same cohort.[17] MR estimates were obtained by weighting the effect of each SNP on AD by its effect on 25OHD. We elected to weight our MR estimates using SD units of natural log 25OHD because SD units are more easily interpreted. To provide a summary measure for the effect of genetically determined 25OHD, including all SNPs genome-wide significant for 25OHD, we combined weighted estimates using a fixed-effects meta-analysis model. In addition, we performed a stratified MR analysis using the same methods in which the

Table 1 Characteristics of vitamin D single nucleotide polymorphisms (SNPs)

					Vitamin D result				AD result	
Locus	SNP	Chr	Vitamin D–decreasing allele	Allele frequency	Effect on vitamin D[a]	*F* statistic[b]	CaMos *p*	SUNLIGHT *p*[c]	OR (95% CI)[d]	AD *p*[d]
GC	rs2282679	4	C	0.30	−0.047	13.38	2.6×10^{-4}	1.9×10^{-109}	1.03 (1.00–1.07)	0.063
CYP2R1	rs10741657	11	C	0.62	−0.052	18.78	1.5×10^{-5}	3.3×10^{-20}	1.01 (0.98–1.04)	0.49
DHCR7	rs12785878	11	G	0.27	−0.056	18.29	2.0×10^{-5}	2.1×10^{-27}	1.02 (0.98–1.06)	0.21
CYP24A1	rs6013897	20	A	0.19	−0.027	3.13	0.077	6.0×10^{-10}	1.02 (0.98–1.06)	0.28

Abbreviations: AD = Alzheimer disease; CaMos = Canadian Multicentre Osteoporosis Study; Chr = chromosome; CI = confidence interval; SNP = single nucleotide polymorphism.
[a] Represents the β coefficient of the vitamin D–decreasing allele on natural log 25OHD in CaMos after adjustment for age, age squared, sex, body mass index, and season of measurement.[12]
[b] Measured in CaMos.
[c] *p* Values extracted from SUNLIGHT.[9]
[d] OR and *p* obtained from the summary statistics of the International Genomics of Alzheimer's Project.

25OHD-associated SNPs located directly in or near loci involved in either vitamin D synthesis or metabolism were analyzed separately to assess the independent effects of these pathways.[14] We next undertook power calculations using an MR power calculator to ensure that our analyses were adequately powered to detect effects.[18,19]

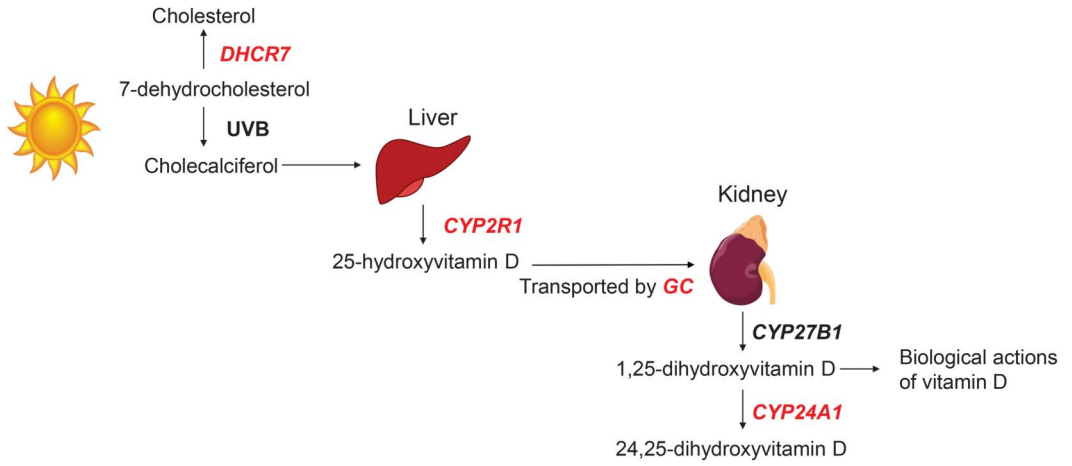
Standard protocol approvals, registrations, and patient consents. All data sources used in this MR study (SUNLIGHT, CaMos, and IGAP) received approval from an ethics standards committee on human experimentation and obtained informed consent from all participants.

RESULTS SNP selection and genetic effect sizes on 25OHD. The SUNLIGHT Consortium identified 4 SNPs genome-wide significant for 25OHD (table 1).[9] These include rs2282679 in *GC* (association with 25OHD: $p = 1.9 \times 10^{-109}$), rs12785878 near *DHCR7* ($p = 2.1 \times 10^{-27}$), rs10741657 near *CYP2R1* ($p = 3.3 \times 10^{-20}$), and rs6013897 in *CYP24A1* ($p = 6.0 \times 10^{-10}$). Notably, all 4 SNPs were located in or near genes that have clear functions within the vitamin D pathway (figure 2). This reduces the potential for pleiotropic effects of these SNPs on AD (whereby the SNP acts on AD independently of 25OHD).

Table 1 displays the effect of the SUNLIGHT SNPs on natural log–transformed 25OHD after adjustment for covariates. Each SNP was an important predictor of natural log 25OHD level. Combined, they explain 2.44% of the variance in this trait.[12] In addition, we observed a trend in which an increased number of 25OHD-decreasing alleles further associated with decreased 25OHD level (nonparametric trend test, $p = 3.3 \times 10^{-19}$), as we have previously described.[12]

SNP validation. Our LD assessment using 3,781 European UK10K samples[15] provided no evidence of LD ($r^2 > 0.05$) between any of the 4 SNPs. Additionally, previous work using data from the 1958 British Birth

Figure 2 Vitamin D pathway



Here we show the vitamin D pathway. Red indicates the gene products with which our single nucleotide polymorphisms (SNPs) have been associated. Note that all 4 SNPs lie in or near genes involved in vitamin D synthesis, transport, or metabolism. UVB = ultraviolet B. Adapted from Mokry et al.[12]

Cohort demonstrated that the allele frequency of rs12785878 (*DHCR7*) varies by geographic region in the United Kingdom.[14] This uneven ancestral distribution was also confirmed by our principal component analysis in CaMos in which rs12785878 (*DHCR7*) associated with non-European ancestry ($p = 2.7 \times 10^{-13}$).[12] Because the prevalence of AD also varies among European subpopulations,[20] we elected to perform a sensitivity analysis that excluded this SNP because it may be a marker for ancestry and thus influenced by residual population stratification.

Previous work by Berry et al.[14] on the 1958 British Birth Cohort found that none of the SUNLIGHT SNPs (rs2282679 [*GC*], rs12785878 [*DHCR7*], rs10741657 [*CYP2R1*], or rs6013897 [*CYP24A1*]) associated with possible confounding lifestyle factors such as socioeconomic position or physical activity.[14] Their regression models also indicated that none of the SNPs associated with relevant biomarkers such as cholesterol or systolic and diastolic blood pressures.[14] Results of our PubMed literature search suggested possible pleiotropic effects for rs12785878 and rs2282679. *DHCR7* encodes an enzyme responsible for transforming 7-dehydrocholesterol to cholesterol. Loss-of-function mutations within this locus have been associated with Smith-Lemli-Opitz syndrome and a range of physical and behavioral effects attributable to impaired cholesterol synthesis.[21] While the analysis by Berry et al.[14] did not support an effect of the *DHCR7* SNP on cholesterol,[14] a recent MR analysis provided strong evidence indicating total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol levels as causal susceptibility factors for AD (table e-1).[22] A search for this SNP in the Global Lipids Consortium[23] yielded a minimum value of $p = 0.01$ across all lipids traits, suggesting that this SNP is nominally associated with cholesterol (table e-1). Therefore, to be conservative, we again elected to perform a sensitivity analysis removing the SNP at *DHCR7*.[22] Our literature search also uncovered an association between vitamin D–binding protein (DBP) and AD pathology, with increased levels of the protein found in the CSF of patients with AD.[24] Furthermore, in vitro and in vivo assays have demonstrated an interaction between DBP and β-amyloid, resulting in reduced β-amyloid aggregation and neuronal cell death.[25] This presents the possibility that rs2282679, which lies within the intron of *GC*, the gene encoding DBP, may influence AD independently of vitamin D. Thus, we performed additional sensitivity analyses removing this SNP. Lastly, MR analyses have also supported systolic blood pressure (SBP) and smoking quantity as causal risk factors for AD (table e-1). Although a search for rs6013897 in the International Consortium for Blood Pressure dataset found a minimum value of $p = 0.05$ for its association with SBP,[26] MR analyses have suggested that vitamin D status is a determinant of arterial blood pressure[27]; thus, SBP is likely an intermediate rather than a pleiotropic effect. None of these potential SNPs were associated with cholesterol, smoking, or blood pressure traits after a Bonferroni correction for the number of traits tested ($p \leq 0.01$).

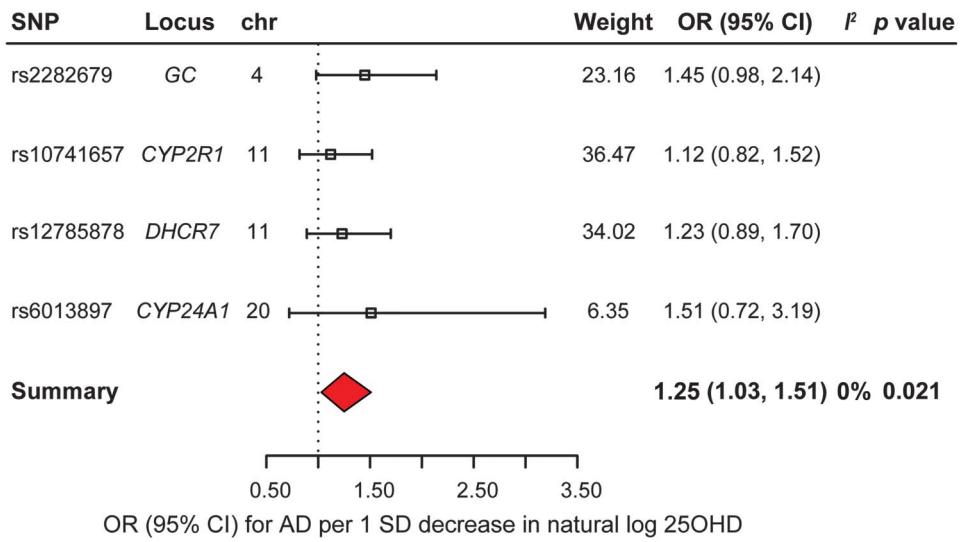
MR estimates. MR analyses demonstrated that a 1-SD decrease in genetically determined natural log–transformed 25OHD increased the odds of AD by 25% (odds ratio [OR] = 1.25, 95% confidence interval [CI] = 1.03–1.51, $p = 0.021$, figure 3). The genetic score including all 4 25OHD SNPs had 100% power to detect an effect of a 25% increase in the odds of AD. We observed similar results after removing the *DHCR7* locus that were suggestive of an effect of 25OHD on the odds of AD (OR = 1.26, 95% CI = 1.00–1.60, $p = 0.052$, figure 4). When the *GC* locus, encoding DBP, was removed because of possible independent effects of DBP, we observed a suggestive effect of 25OHD on AD risk; however the 95% CIs included the null (OR = 1.19, 95% CI = 0.96–1.45, $p = 0.11$, figure 4).

Furthermore, stratified MR analysis to assess to independent effects of vitamin D metabolism (*GC* and *CYP24A1*) and synthesis (*DHCR7* and *CYP2R1*) found SNPs involved in metabolism to be stronger predictors of AD risk by increasing the odds of AD by 46% ($OR_{metabolism} = 1.46$, 95% CI = 1.03–2.07, $p = 0.032$, figure 4). In contrast, we observed a suggestive effect of SNPs involved in the synthesis of vitamin D on AD, yet the 95% CIs included the null ($OR_{synthesis} = 1.17$, 95% CI = 0.93–1.46, $p = 0.17$, figure 4).

DISCUSSION Our results demonstrated that a decreased 25OHD level was associated with risk of AD, in which a 1-SD decrease in the natural log–transformed 25OHD resulted in a 25% increase in the risk of AD ($p = 0.021$). These results are consistent with results from previous observational studies[1–5] but, because of the nature of the MR analysis, are less prone to confounding and reverse causation. This study, along with the results of observational analyses, provides a rationale to investigate whether vitamin D supplementation can reduce AD risk in RCTs.

As a result of the aging demographics of Western countries, the prevalence of AD is expected to rise.[28] In addition, the prevalence of vitamin D insufficiency is increasing,[29] and these trends may have important public health implications. One such implication is the economic burden and long-term care costs associated with AD.[30] For instance, the direct medical costs are estimated at $19,177 annually in the United States.[31] In contrast, an annual supply of 1000 IU

Figure 3 Forest plot of main results

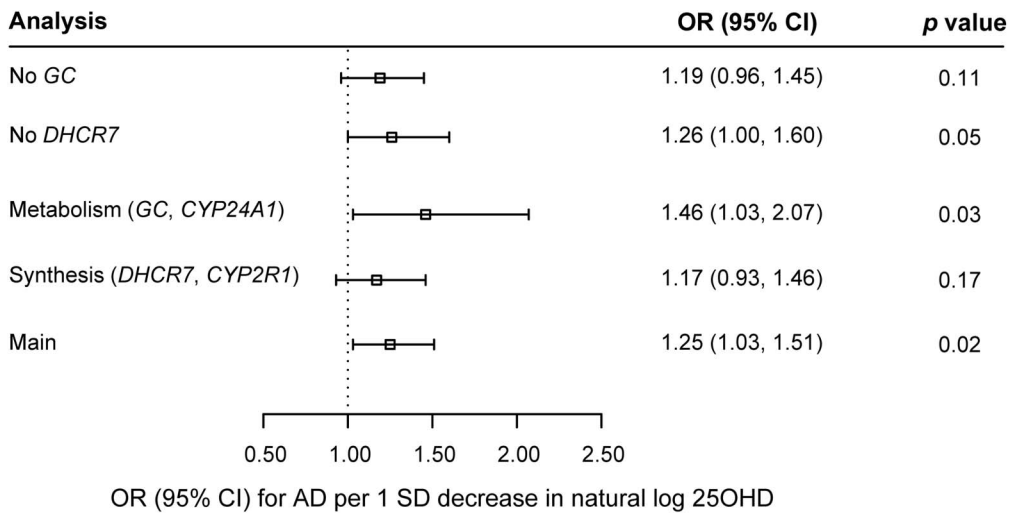


Forest plot of our main mendelian randomization analysis. Boxes and error bars represent the effect of an individual single nucleotide polymorphism (SNP) on Alzheimer disease (AD) weighted by its effect on natural log 25-hydroxyvitamin D (25OHD) in SD units. Red diamond represents the summary estimate for the effect of a 1-SD decrease in genetically determined natural log 25OHD on the odds of AD. Chr = chromosome; CI = confidence interval; OR = odds ratio.

vitamin D supplements satisfying the Institute of Medicine's intake guidelines for sufficiency[32] costs approximately $30 to $40. Therefore, ensuring vitamin D sufficiency among individuals at high risk for AD may be explored as a cost-effective approach to reduce risk if clinical trial evidence supports a role for vitamin D administration in the prevention of AD.

Our MR approach has several important advantages. First, the potential bias due to confounding is greatly reduced because genetic variation is not associated with the self-selected lifestyle factors that may influence observational analyses.[14] In addition, because genetic variation is allocated at conception, our analysis is protected from reverse causation. This is important because AD is characterized by a long preclinical phrase, which renders it difficult to determine whether an exposure precedes the pathologic changes in the brain. Our analysis also captures the lifetime risk of AD due to genetically decreased vitamin D, which is important because a single vitamin D measurement is unlikely to be an accurate predictor of a disease that manifests in the seventh or eighth decade of life. Lastly, by using the 2-sample MR approach, we were able to test the effect of vitamin

Figure 4 Forest plot of sensitivity analyses



Forest plot of our sensitivity and stratified mendelian randomization analyses plotted against our main analysis for comparison. AD = Alzheimer disease; CI = confidence interval; OR = odds ratio; 25OHD = 25-hydroxyvitamin D.

D in a large cohort of patients with AD (N = 17,008 AD cases and 37,154 controls). Such 2-sample approaches have statistical power equivalent to an approach using individual-level data[17]; however, few cohorts have accrued as many cases and controls for AD.

Our analysis also has limitations worth consideration. While we undertook multiple steps to examine pleiotropy, residual bias is possible because the exact function of these SNPs is unknown. Because the IGAP used a case-control design, our results represent risk of incident AD. Therefore, we cannot conclude whether vitamin D influences AD progression, nor can we determine the 25OHD level at which AD risk is abated. We also note that when the *GC* locus was removed, the 95% CIs of our estimates included the null. This may suggest that DBP (encoded by *GC*) may have distinct effects on AD risk. This has been supported by functional studies demonstrating a direct action of DBP on β-amyloid– and AD-related pathology.[25] However, DBP is responsible for transporting 80% to 90% of 25OHD in the body,[33–35] and 25OHD and its metabolite, 24,25-dihydroxyvitamin D, have been shown to be important predictors of DBP concentrations.[36] Furthermore, the effect of 25OHD on parathyroid hormone levels, an indicator of 25OHD activity, has been shown to be unchanged after adjustment for DBP.[37] This suggests that the actions of 25OHD are independent of the proportion bound and unbound to DBP.[37] Thus, it is unclear whether DBP operates entirely distinctly from vitamin D or rather acts as an intermediate along this pathway. Nonetheless, we cannot rule out whether the observed association of vitamin D on AD risk is due predominantly to the actions of DBP. The removal of the *DHCR7* locus, because of possible pleiotropic effects with cholesterol, is conservative, as suggested by previous work that found no evidence for an association of *DHCR7* with total cholesterol, high-density lipoprotein cholesterol, or LDL-C. Further MR analyses of 25OHD with *DHCR7* and *CYP2R1* used as instruments did not support an effect of 25OHD on LDL-C or remnant cholesterol.[38] Thus, the loss of statistical power incurred after the removal of these SNPs from the MR analysis likely outweighs the protection from possible pleiotropic effects.

We have previously used this approach to address whether genetically decreased vitamin D influences the risk of MS and CAD.[12,13] Despite using similar methods, we obtained some notable differences in our results for MS, CAD, and AD. First, the effect of a 1-SD decrease in natural log transformed was strongest for MS, with an OR of 2.02 (95% CI = 1.65–2.46, $p = 7.72 \times 10–12$).[12] In contrast, our MR analysis did not support a causal role of vitamin D in CAD (OR = 0.99, 95% CI = 0.84–1.17, $p = 0.93$).[13] Three of the 4 vitamin D–associated SNPs (DHCR7, CYP24A1, and CYP2R1) were nominally associated with MS in the International Multiple Sclerosis Genomics Consortium ($p < 0.05$), with the DHCR7 SNP achieving genome-wide significance for MS ($p < 5 \times 10^{-8}$).[12] Associations of this strength were not observed for any of the 4 vitamin D SNPs with AD ($p > 0.05$ for all) or CAD ($p > 0.6$ for all). Interestingly, *GC*, which was the weakest instrument for MS, was the strongest instrument for AD (MS: $p = 0.062$: AD: $p = 0.063$). This finding could suggest that DBP, which is encoded by *GC*, is driving the observed relationship between vitamin D and AD. Additional GWAS and MR studies of DBP levels are necessary to help clarify the role of this protein.

While previous work has identified possible risk factors for AD such as cholesterol and blood pressure (or an exposure related to high blood pressure) with much larger effect sizes (e.g., $OR_{LDL\text{-}C} = 2.13$, 95% CI = 2.12–2.50, $p = 3.0 \times 10^{-87}$; $OR_{SBP} = 0.75$, 95% CI = 0.62–0.91, $p = 3.4 \times 10^{-3}$),[22] our results identify vitamin D as an additional factor that can provide a smaller yet important reduction in risk. This provides critical insight into a disease that remains poorly understood and furthermore offers a simple mechanism for individuals to decrease their risk of AD by ensuring vitamin D sufficiency. Because MR analyses have demonstrated that cholesterol influences vitamin D levels[38] and vitamin D affects blood pressure,[27] whether the effect of vitamin D on AD is partially mediated by these factors warrants further investigation.

Thus, our MR analysis provides evidence to support a causal role of vitamin D in the risk of AD. However, long-term RCTs are required to test whether supplementation may prevent AD.

AUTHOR CONTRIBUTIONS

Lauren E. Mokry conceived the design of the experiment, drafted the manuscript, and analyzed and interpreted the data. Dr. Stephanie Ross, John A. Morris, Dr. Despoina Manousaki, and Dr. Vincenzo Forgetta revised the manuscript. Dr. J. Brent Richards conceived the design of the experiment, revised the manuscript, and provided interpretation of the data.

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DISCLOSURE

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