Elucidating the Role of Vitamin D in Depression

Min-Jung Wang

A Dissertation Submitted to the Faculty of

The Harvard T.H. Chan School of Public Health

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Science

in the Department of Epidemiology

Harvard University

Boston, Massachusetts.

May, 2019

Dissertation Advisor: Dr. Jordan Smoller Min-Jung Wang

Elucidating the Role of Vitamin D in Depression

Abstract

Depression is a debilitating and prevalent mental disorder that creates huge societal and economic burden. Vitamin D has been implicated in several mental disorders, but existing studies on the role of circulating vitamin D (25-hydroxyvitamin D [25(OH)D]) in depression have yielded inconsistent findings. Given that vitamin D levels are readily modifiable through dietary supplementation and/or lifestyle changes, understanding the relationship between vitamin D and depression could lead to cost-effective public health strategies for preventing depression. In this dissertation, we aimed to elucidate this relationship by leveraging data from large genome-wide association studies (GWAS), longitudinal registry, and epidemiologic cohorts.

In Chapter 1, we explored the association between 25(OH)D levels and the risk of incident major depressive disorder (MDD) in a general healthcare population (n=233,970). Low 25(OH)D levels were associated with an increased risk for 5-year MDD. Our study also revealed that these associations were stronger among older adults and white or Asian adults. In Chapter 2, we performed a two-sample bidirectional Mendelian randomization analysis, using summary-level data from the largest GWAS studies of 25(OH)D and depression to date, to evaluate the causal relationship between 25(OH)D and depression. There was no causal association between average lifelong 25(OH)D levels and depression, although the possibility of weaker causal effects or threshold effects remains. In Chapter 3, we examined the association between maternal vitamin D status during pregnancy and offspring depression during childhood and adolescence, and further assessed whether polygenic risk for depression (PRS) modified the association. There was no evidence for a strong association between maternal vitamin D status and

offspring depression during either childhood or adolescence. Additionally, there were no interactions between PRS and maternal 25(OH)D on offspring depression.

These findings suggest that vitamin D deficiency is associated with an increased risk for depression, although small changes in 25(OH)D are not causally related to depression. There was little evidence for an association between prenatal 25(OH)D exposures and early life depression. Further work is needed to understand threshold effects of 25(OH)D on health outcomes and whether there are critical windows of vulnerability to vitamin D exposure.

Table of Contents

List of Figures with Captions

List of Tables with Captions

Acknowledgments

I am tremendously fortunate to have had the support and help of my mentors, family, colleagues, and friends on the path towards this dissertation. First and foremost, I thank my advisor, Dr. Jordan Smoller, for allowing me the freedom to explore research topics that most interested me and pursue important opportunities for my academic and career development. I am also grateful for Jordan's constant encouragement, advice and guidance, and generosity in sharing his expertise in psychiatric genetics. I would also like to thank my research committee, Drs. Erin Dunn, Peter Kraft, and Olivia Okereke for supporting my dissertation work all along, and providing insightful feedback and input that truly strengthened and shaped my dissertation work.

In addition, I thank members of the Smoller lab, Chia-Yen Chen, Karmel Choi, Anne Feng, and Amanda Zheutlin, who generously shared their extensive experiences in research and insights on doctoral life and were always there to encourage and cheer me on; I also thank Ashley Seiger, Emily Madsen, and Kristin Joyce for their help with scheduling and always ensuring that the regulatory aspects of my projects were satisfied. I would also like to thank current and past members of the Dunn lab, in particular, Janine Cerutti, Katherine Crawford, Katie Davis, Becky Mountain, Kristen Nishimi, Olivia Pickett, Tom Soare, and Yiwen Zhu, who helped me at critical stages of my dissertation work and/or provided advice and support on other research projects. Furthermore, I thank them for making my Thursday afternoons so intellectually stimulating and fun at the same time. I am also grateful for my fellow classmates, faculty, and staff at the Harvard T.H. Chan School of Public Health, who have inspired me and made my doctoral journey so fulfilling.

I would like to acknowledge the generous financial support that I received from Mr. Matthew McLennan and Ms. Monika McLennan through the McLennan Family Fellowship and the Department of Epidemiology through the Department of Epidemiology Award. I am also thankful to Eric DiGiovanni and Ellen Furxhi for their administrative support throughout the duration of my program.

I am extremely blessed to have been surrounded by so many wonderful friends, Yinnan Zheng, Zhihui Wang, Chi-Hsuan Chang, Debby Lin, Yu-Tien Hsu, Han Chiu, who have helped me in so many different ways, and made these past five years all the more enjoyable and less stressful. A special thank you to my boy/best friend, Yu-Wei Chen, for his unwavering support and encouragement – pulling me through difficult times and celebrating every small achievement with me. Finally, none of this would have been possible without my parents, Terry and Lyn, who have unconditionally supported me and sacrificed so much to provide me with the best opportunities possible. I thank them especially for always believing in me no matter what I wanted to pursue both academically and professionally. Last but not least, thank you to my sister, Pam, for keeping me company all along, setting up a successful academic path to emulate, and reminding me to take things easy from time to time.

Chapter 1. Serum 25-hydroxyvitamin D and incident major depression in a general healthcare population

Min-Jung Wang[1], Erin C. Dunn[2, 3, 4, 5], Peter Kraft[1, 6], Olivia I. Okereke[1, 3, 7], Karmel W. Choi[1, 2, 3, 4], Jordan W. Smoller[1, 2, 3, 4]

[1] Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA
[2] Psychiatric and Neurodevelopmental Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA
[3] Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA
[4] Stanley Center for Psychiatric Research, The Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA
[5] Henry and Allison McCance Center for Brain Health, Massachusetts General Hospital, Boston, MA, USA
[6] Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA
[7] Department of Medicine, Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA

Abstract

Objective: Emerging evidence suggests vitamin D may be involved in the pathophysiology of depression, however, longitudinal epidemiological studies are sparse and inconclusive. We aimed to investigate the association between serum 25-hydroxyvitamin D [25(OH)D] levels and incident major depression in a large general healthcare population.

Methods: Using longitudinal electronic health records from the Partners Healthcare Research Patient Data Registry, we identified adults (ages ≥18) with at least one measurement of 25(OH)D and no ICD codes for major depressive disorder (MDD) prior to the first 25(OH)D measurement (n=233,970). Vitamin D status was classified based on conventional clinical cut-offs: deficient (<20 ng/mL), insufficient (20-29.9 ng/mL), and normal (≥30 ng/mL); MDD case status was defined using ICD codes and treatment records. The association between 25(OH)D and 5-year incident MDD was assessed using adjusted Cox regression models.

Results: Over a 5-year study period, 10,152 (4.3%) patients developed MDD. Every 10 ng/mL increase in 25(OH)D was associated, on average, with a decreased risk for MDD (adjusted HR (aHR)=0.89; 95% CI: 0.87, 0.90). Compared to adults with normal 25(OH)D levels, people with insufficient (aHR=1.17; 95% CI: 1.12, 1.23) or deficient (aHR=1.37; 95% CI: 1.30, 1.45) levels of 25(OH)D were at increased risk for MDD. The association was stronger among older age groups, and white and Asian adults.

Conclusions: In a large healthcare system population, low 25(OH)D was associated with higher risk for major depression. This association differed by age and race/ethnicity. Further studies to confirm the causal relationship between vitamin D and depression are needed.

Keywords: Vitamin D, risk factor, major depression, EHR

Introduction

Major depressive disorder (MDD) is a highly prevalent and often debilitating mental disorder, estimated to affect 16.6% of adults in the US (1), and currently ranks as the number one leading cause of disability worldwide (2). Major depression has been linked to higher suicide rates, cardiovascular and metabolic diseases, and impaired functioning (3, 4), which cumulatively lead to enormous economic and healthcare costs (3). Given this public health burden, more research is needed to understand the etiology of MDD, which in turn, can inform prevention strategies.

Accumulating evidence suggests that vitamin D, which acts as a neurosteroid hormone, may play a role in etiology of depression (5-7). Although specific biological pathways linking low vitamin D levels to depression are understudied, several have been postulated. First, vitamin D receptors (VDR) and hydroxylases are expressed in the same human brain regions (e.g., hippocampus and amygdala) implicated in depression pathophysiology (8-10). Second, vitamin D plays a role in regulating autoimmune and inflammatory pathways associated with psychological stress and depression (11-13). Third, vitamin D may exert neuroprotective effects through antioxidant mechanisms, stimulation of neurotrophin production, or formation of essential neurotransmitters (12, 14, 15). Such mechanisms have been supported by animal studies. One study revealed that vitamin D-deprived rodents show persistent changes in brain functioning, and VDR knockout mice display depression-like behaviors, such as lowered activity levels and heightened anxiety (12, 16). Furthermore, active vitamin D metabolites in mice have been shown to affect cholinergic and dopaminergic pathways, which have been associated with depression and other psychiatric conditions (8).

Despite ample support from animal models, longitudinal epidemiological studies to date have been limited. Some studies have estimated that lower 25-hydroxyvitamin D (25(OH)D), the main circulating and stable form of vitamin D (17), is associated with up to a 2-fold higher risk for adult depression (5, 18). However, other studies have reported non-significant findings (19-21). Different

results across studies may be attributable to the study population examined (e.g., studying only men (22), elderly populations (20), patients with cardiovascular disease (23)), sample size (ranging from n=656-7358), methods used to assess depression, or the failure to consider sub-group differences based on age, sex, and race/ethnicity. As such, there is a need for larger studies in general clinical populations with more objective measures for depression.

The current study used longitudinal registry data, namely the Partners Healthcare Research Patient Data Registry (RPDR), to examine the association between serum 25(OH)D levels and incident MDD in a general adult healthcare population. This study aimed to provide further insights on this association by addressing some of the limitations of previous studies by focusing on a much larger, younger, and more diverse population with MDD case status defined by clinician diagnosis and/or treatment. Elucidating the link between vitamin D and depression has important public health implications. Circulating 25(OH)D can be easily and inexpensively elevated through supplementation and/or lifestyle interventions that can be implemented at a population level to prevent depression and other health outcomes.

Methods

Data Source and Study Design

This study analyzed electronic health records (EHRs) extracted through the RPDR (24). The RPDR is a central data warehouse for all inpatient and outpatient records of >6.5 million patients, from two major teaching hospitals (Brigham and Women's Hospital and Massachusetts General Hospital), and affiliated community and special hospitals in the Greater Boston area. Data were examined between January 2000 and December 2016.

Adults were eligible for study inclusion if they had one or more measurements of serum 25(OH)D, had no ninth or tenth versions of the *International Classification of Diseases* (ICD-9/ICD-10)

codes for MDD (296.2x – 296.3x/F32.x-F33.x) prior to their first 25(OH)D measurement, and were at least 18 years of age at the time of their first 25(OH)D measurement (**Supplemental Figure 1.1**). Baseline date was defined as the date of the patient's first recorded 25(OH)D measurement. Patients with 25(OH)D levels that were three standard deviations (SD) above/below the study population mean and/or had a diagnoses of bipolar disorder (BPD) (ICD-9 codes 296.4x-296.8x/ICD-10 codes F31.0x-F31.9x) or manic disorder (ICD-9 codes 296.0x-296.1x/ICD-10 codes F30.1x-F30.4x, F30.8, F30.9) at any time were also excluded. Follow-up time for each patient began at the first measurement of 25(OH)D until the first occurrence of MDD (defined below) or the last recorded date in the RPDR within five years from baseline. Controls with any ICD codes of 296.9/F39 (i.e., Other and unspecified episodic mood disorder) or 311/F32.9 (i.e., Depressive disorder, not elsewhere classified) were excluded to reduce the likelihood of outcome misclassification. A 5-year study window allowed for sufficient accrual of MDD cases, while maintaining biologically plausible relevance of observed 25(OH)D levels to MDD incidence. Previous studies have reported that the effect of lower vitamin D levels may be diluted with follow-up periods longer than 5 years (25). Institutional Review Board approval was granted by the Partners Human Research Committee.

Serum 25(OH)D Measurements

Within the Partners system, serum 25(OH)D levels were assessed using radioimmunoassays (RIA) before 2008, and high-performance liquid chromatography-tandom mass spectrometry (HPLC-MS) thereafter; prior studies have reported high correlation between the two assays (r=0.95) (26). Assays were performed at Clinical Laboratory Improvement Amendment (CLIA)-certified laboratories, and the dates/times of the 25(OH)D assays were available in the EHRs. For patients with multiple measurements taken during the follow-up period, we analyzed only their first 25(OH)D measurement, consistent with EHR studies on 25(OH)D on other health outcomes (27). Of note, among patients with two or more 25(OH)D measures (n=136,863), we found low to moderate variability over 5 years (coefficient of

variation=20.7%; IQR: 0-33.5%), suggesting that a single measurement is likely an acceptable estimate of mean 25(OH)D levels over the study period.

Serum 25(OH)D was included in models both continuously and categorically to assess whether the influence of 25(OH)D on MDD were due to incremental changes and/or threshold effects. Vitamin D status was defined by clinical cut-offs (28, 29): normal (≥30 ng/mL), insufficient (20-29.9 ng/mL) or deficient (<20 ng/mL).

MDD Case Definition and Validation

Incident MDD cases were identified using ICD-9/ICD-10 diagnostic codes. MDD cases were patients who, after a 25(OH)D measurement, met at least one of the following criteria: (1) received ≥2 ICD codes for MDD (296.2x – 296.3x/F32.x-F33.x) at least one month apart; or (2) ≥1 ICD code(s) for MDD *and* treatment with an antidepressant recorded at ≥2 visits; or (3) ≥1 ICD code(s) for MDD *and* treatment with electroconvulsive therapy (ECT). In cases with multiple ICD codes, the date of MDD diagnosis was defined as the earliest recorded ICD code date. Controls were patients who did not fulfill the MDD case definition and did not have any ICD codes for MDD. To ensure the validity of this MDD case definition, two experienced, trained clinicians (JWS, KWC) performed chart reviews on a random set of 50 MDD cases (**Supplementary Methods**). Positive predictive value (PPV), which is the probability that those identified as MDD cases were true cases, was computed to quantify the classification performance of the EHR rule-based MDD definition. Potential cases adjudicated as having insufficient information were removed from the denominator of the PPV calculation. The criteria used to define MDD had a PPV of 92% (95% CI: 84%, 99%).

Statistical Analysis

Kaplan-Meier survival curves and log-rank tests were used to assess the univariate association between 25(OH)D and incident MDD over the five year study period. Multivariable Cox regression models were fit to determine the association between 25(OH)D and incident MDD. Three models were

used to control for a range of potential confounding factors that were shown previously to associate with depression risk and/or vitamin D levels (30, 31) (**Supplementary Methods**). The minimally adjusted model (Model 1) adjusted for age, sex, race/ethnicity, assay, and season of 25(OH)D measurement. Model 2 included all Model 1 covariates and additionally adjusted for vitamin D supplementation, the Charlson-Deyo comorbidity index, and intensity of healthcare use, which could represent a source of ascertainment bias. As vitamin D testing is not systematically performed in healthcare settings, it is possible that those with indications for vitamin D testing are more likely to have both lower vitamin D levels and generally poorer health as well as higher risk for MDD. Therefore, Model 3 built upon Model 2 by including all medical conditions that are indications for vitamin D testing and treatments that may affect circulating 25(OH)D levels. The proportional hazards assumption, which states that the ratio of hazards for any two individuals is constant across time, was tested by including a cross-product interaction term between 25(OH)D and follow-up time in the model. There was no statistical evidence for the violation of the assumption. Interactions between 25(OH)D and age, sex, and race/ethnicity were tested. Results were presented as adjusted hazard ratios (aHR) and 95% confidence intervals (CIs). The statistical significance threshold was set at a two-tailed p-value<0.05. All analyses were performed in STATA 14.0 (StataCorp, College Station, TX) and SAS 9.4 (SAS Institute, Cary, North Carolina).

Sensitivity Analysis

Given that other methods may better account for observed seasonal variations in 25(OH)D levels (**Supplemental Figure 1.2**), we reran our primary analyses using seasonality-adjusted 25(OH)D, representing mean annual 25(OH)D levels, derived using a cosinor model (32). Details about the derivation of mean annual 25(OH)D are described in the **Supplementary Methods**. Briefly, the cosinor model involved fitting raw 25(OH)D measures against sine and cosine transformations of time (i.e., month of blood sampling) in a linear regression model. Each individual's mean annual 25(OH)D was derived from the intercept and residuals yielded from the model. Published studies have reported the

validity of this method for estimating an individual's mean annual 25(OH)D levels, which could potentially reduce biases in 25(OH)D measurements among adults in North America (33).

Bias Analysis

It is possible that there was residual confounding from variables that were not systematically collected in the EHR, including physical activity, body mass index (BMI), socioeconomic status (SES), smoking, and alcohol consumption (34). We therefore performed a bias analysis (35, 36) to adjust for possible unmeasured confounding by these variables. Briefly, bias factors, representing the potential magnitude of confounding by unmeasured confounders, were derived from a sub-sample of the study cohort who were also part of the Partners Healthcare Biobank cohort (37) and had data on the confounders listed above within one year of their 25(OH)D measurement. These bias factors were used to adjust the effect estimates obtained in the full study cohort, yielding HRs corrected for unmeasured confounding. Bias factors that change the original estimates by >30% indicate large confounding bias, while changes <7% are considered negligible confounding bias (38). Full details of the bias analysis are provided in the **Supplementary Methods**.

Results

Study Cohort Characteristics

Of the 233,970 adult patients included in the study cohort, 10,152 patients (4.3%) developed MDD over the study period (**Figure 1.1**). In the full cohort, the mean 25(OH)D was 28.8 ng/mL (SD=12.2), and 55.3% were classified as vitamin D insufficient or deficient. Baseline characteristics are displayed in **Table 1.1**. The study population had a mean age of 50.6 (SD=15.7) and comprised mainly females (66.3%) and whites (74.9%). Compared to the entire RPDR population (n=~6.5 million), women (RPDR 52.9% vs. study cohort 66.3%) and patients 60 and older (RPDR 53.2% vs. study cohort 68.5%) were overrepresented, but racial/ethnic distributions were similar. Compared to adults with normal

25(OH)D levels, adults who were either vitamin D insufficient or deficient were younger, more likely to be male, have higher Charlson-Deyo comorbidity scores, have higher healthcare use intensity, and were less likely to be on vitamin D supplementation (**Table 1.1**).

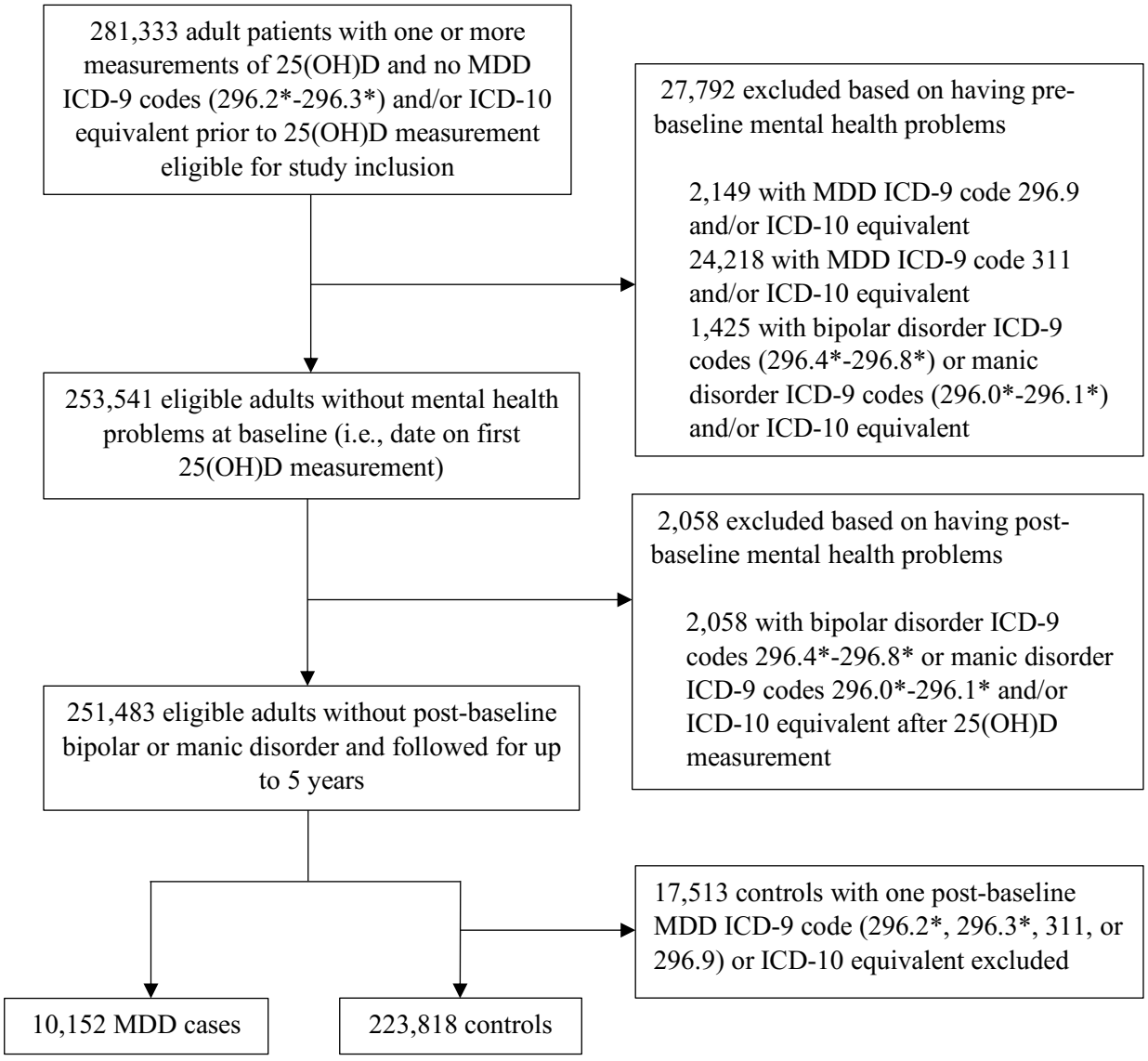


Figure 1.1 Flow chart for study cohort selection

Table 1.1 Study cohort baseline characteristics (n=233,970)

	Full cohort (N=233,970)		Serum 25(OH)D[1]						
			Deficient (N=58,021)		Insufficient (N=71,466)		Normal (N=104,483)		p[2]
	N	%	N	%	N	%	N	%	
Gender									
Male	78,918	33.7	22,206	38.3	26,215	36.7	30,497	29.2	<0.001
Female	155,052	66.3	35,815	61.7	45,251	63.3	73,986	70.8	
Ethnicity									
Asian	11,177	4.8	4,167	7.2	3,826	5.4	3,184	3.0	<0.001
Black	17,362	7.4	8,603	14.8	5,005	7.0	3,754	3.6	
Hispanic	14,693	6.3	5,548	9.6	5,383	7.5	3,762	3.6	
Other	5,109	2.2	2,061	3.6	1,692	2.4	1,356	1.3	
Unknown	10,375	4.4	2,824	4.9	3,269	4.6	4,282	4.1	
White	175,254	74.9	34,818	60.0	52,291	73.2	88,145	84.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p[2]
Age	50.62	15.74	48.85	16.15	49.54	15.56	52.34	15.45	<0.001
	N	%	N	%	N	%	N	%	p[2]
Season of blood draw									
Spring	60,018	25.7	13,408	23.1	18,594	26.0	28,016	26.8	<0.001
Summer	62,938	26.9	17,502	30.2	19,245	26.9	26,191	25.1	
Fall	54,451	23.3	8,797	15.2	15,935	22.3	29,719	28.4	
Winter	56,563	24.2	18,314	31.6	17,692	24.8	20,557	19.7	
Assay									
RIA	40,937	17.5	14,513	25.0	12,006	16.8	14,418	13.8	<0.001
HPLC-MS	193,033	82.5	43,508	75.0	59,460	83.2	90,065	86.2	
Charlson-Deyo index									
0-1	150,485	64.3	34,869	60.1	47,156	66.0	68,460	65.5	<0.001
2-3	49,866	21.3	12,129	20.9	14,849	20.8	22,888	21.9	
4-6	21,298	9.1	6,364	11.0	6,079	8.5	8,855	8.5	
≥7	12,321	5.3	4,659	8.0	3,382	4.7	4,280	4.1	
Past-year vitamin D supplementation use	4,138	1.8	735	1.3	1001	1.4	2402	2.3	<0.001

Table 1.1 (continued)

	Full cohort (N=233,970)		Serum 25(OH)D						
			Deficient (N=58,021)		Insufficient (N=71,466)		Normal (N=104,483)		p[2]
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
Healthcare use density	0.010	<0.01, 0.02	0.011	<0.01, 0.03	0.010	<0.01, 0.02	0.010	<0.01, 0.02	<0.001
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**p[2]**
Indicators for vitamin D testing									
History of rickets	20	0.0	6	0.0	5	0.0	9	0.0	0.81
History of osteomalacia	68	0.0	17	0.0	15	0.0	36	0.0	0.27
History of osteopatia	14,660	6.3	2,671	4.6	3,749	5.2	8,240	7.9	<0.001
History of chronic kidney disease	7,084	3.0	2,573	4.4	1,904	2.7	2,607	2.5	<0.001
History of liver disease	6,261	2.7	2,297	4.0	1,857	2.6	2,107	2.0	<0.001
History of pancreatic insufficiency	931	0.4	340	0.6	261	0.4	330	0.3	<0.001
History of malabsorption syndromes	7,571	3.2	1,905	3.3	2,286	3.2	3,380	3.2	0.69
History of hyper-/hypo-thyroidism	6,664	2.8	1,646	2.8	1,954	2.7	3,064	2.9	0.05
History of pathological fractures	2,213	0.9	668	1.2	637	0.9	908	0.9	<0.001
History of chronic bronchitis	1,767	0.8	643	1.1	480	0.7	644	0.6	<0.001
History of type 2 Diabetes	23,561	10.1	8,036	13.9	7,001	9.8	8,524	8.2	<0.001
History of hypercalcemia	2,691	1.2	955	1.6	754	1.1	982	0.9	<0.001
History of antiseizure medication	22,745	9.7	5,983	10.3	6,655	9.3	10,107	9.7	<0.001
History of glucocorticoid medication	14,096	6.0	3,547	6.1	4,267	6.0	6,282	6.0	0.55

[1]Vitamin D status defined by the following 25(OH)D cut-offs: Deficient, <20 ng/mL; Insufficient, 20-29.9 ng/mL; Normal, ≥30 ng/mL

[2]Chi-squared test for categorical variables; ANOVA for normally distributed continuous variables; Kruskall-Wallis for skewed continuous variables

Serum 25(OH)D and 5-year Incident MDD

Serum 25(OH)D levels were inversely associated with five-year MDD incidence (log-rank test p<0.0001) (**Figure 1.2**), with the highest and lowest MDD incidence in the deficient and normal categories, respectively. **Table 1.2** displays the HR estimates for incident MDD according to continuous and categorical 25(OH)D. After adjusting for covariates, every 10 ng/mL increase in 25(OH)D was associated, on average, with an 11% reduction in MDD risk (HR=0.89, 95% CI: 0.87, 0.90). This 10 ng/mL difference is equivalent to moving from the deficient to insufficient or insufficient to normal category. In addition, compared to patients with normal 25(OH)D levels, patients with insufficient (HR=1.17; 95% CI: 1.12, 1.23; p<0.0001) or deficient levels (HR=1.37; 95% CI: 1.30, 1.45; p<0.0001) had a 17-37% increased risk for MDD. Deficient 25(OH)D levels also conferred an increased risk for MDD compared to insufficient 25(OH)D levels (HR=1.17; 95% CI: 1.11, 1.23; p<0.0001).

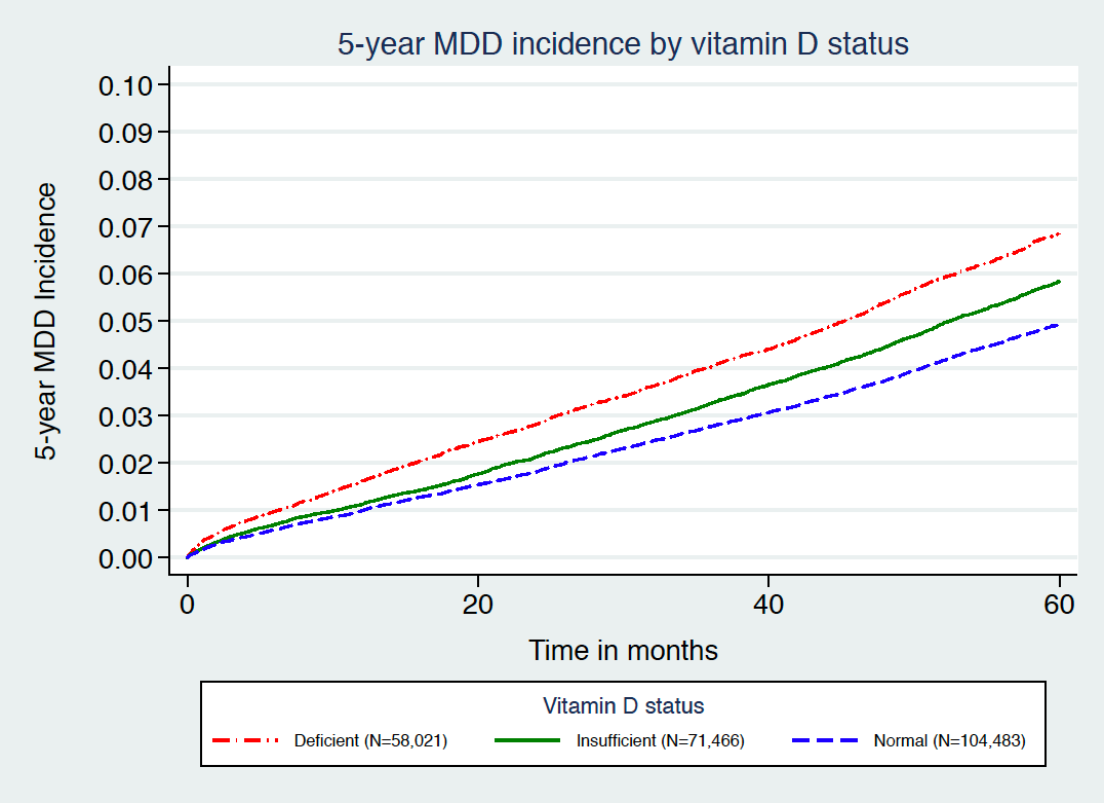


Figure 1.2 Kaplan-Meier 5-year major depressive disorder (MDD) incidence estimates by vitamin D status

Table 1.2 Association between serum 25(OH)D levels and incident MDD using Cox regression analysis

Serum 25(OH)D	MDD cases		Model 1			Model 2			Model 3		
	N	%	HR	95% CI	p	HR	95 % CI	p	HR	95 % CI	p
Continuous (per 10 ng/mL increase)	10,152	4.3	0.87	0.85, 0.88	<0.0001	0.88	0.86, 0.90	<0.0001	0.89	0.87 0.90	<0.0001
Clinical Cut-offs											
Normal (≥30 ng/mL)	4,104	3.9	ref	ref	ref	ref	ref	ref	ref		ref
Insufficient (20-29.9 ng/mL)	3,154	4.4	1.19	1.13, 1.25	<0.0001	1.40	1.33, 1.47	<0.0001	1.17	1.12, 1.23	<0.0001
Deficient (<20 ng/mL)	2,894	5.0	1.46	1.39, 1.54	<0.0001	1.18	1.13, 1.24	<0.0001	1.37	1.30, 1.45	<0.0001
Insufficient vs. Deficient											
Insufficient (20-29.9 ng/mL)	3,154	4.4	ref	ref	ref	ref	ref	ref	ref		ref
Deficient (<20 ng/mL)	2,894	5.0	1.23	1.17, 1.30	<0.0001	1.18	1.12, 1.24	<0.0001	1.17	1.11, 1.23	<0.0001

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; HR: hazard ratio; MDD: major depressive disorder
Model 1: Adjusted for age, sex, race/ethnicity, assay, season
Model 2: Model 1 covariates + healthcare use intensity + vitamin D supplementation + Charlson-Deyo comorbidity index
Model 3: Model 2 covariates + medical conditions and therapies indicating vitamin D testing

Serum 25(OH)D and 5-year Incident MDD within Subgroups

We found evidence suggesting that the association between 25(OH)D levels and MDD was modified by age (p=0.001) and race/ethnicity (p=0.006), but not sex (p=0.12). Specifically, the association between vitamin D deficiency and MDD risk was stronger among older adults (age≥40; HR=1.37-1.48) compared to younger adults (HR=1.28) (**Table 1.3**). Furthermore, the association between vitamin D deficiency and MDD risk was only statistically significant in Asian (HR=1.38; p=0.04) and white (HR=1.46; p<0.0001) individuals, but not black (HR=1.09; p=0.37) or Hispanic (HR=1.09; p=0.34) individuals.

Sensitivity Analysis

Seasonality-adjusted mean annual 25(OH)D levels were strongly and positively correlated with raw 25(OH)D levels (Pearson's r=0.98); the absolute difference between the two measures was small (mean=-0.05, SD=2.18). 9.7%, 17.3%, and 8.9% of those with deficient, insufficient, and normal 25(OH)D levels, respectively, were reclassified either one category up or down. Those in the insufficient group were slightly more likely to be reclassified to the deficient (9.8%) than normal (7.5%) group. However, models fit with the mean annual 25(OH)D levels yielded almost identical estimates to those in the primary analyses (**Supplemental Table 1.1**), suggesting the robustness of the results to different methods of seasonality adjustments.

Bias Analysis

Additional analyses were performed to assess the robustness of the HR estimates to corrections for potential unmeasured confounding. 1,119 individuals in the study cohort were eligible for inclusion in the Partners Biobank sub-cohort used to calculate bias factors. Compared to the full cohort, the sub-cohort was slightly over-represented in including patients with normal 25(OH)D levels (55.0% in sub-cohort vs. 44.7% in the full cohort), whites (sub-cohort, 87.7% vs. full cohort, 74.9%), and vitamin D supplementation users (sub-cohort, 6.6% vs. full cohort, 1.8%). In the sub-cohort, more than half

exercised for less than 2.5 hours per week, were not obese, never smoked, had at least one alcohol drink per week, were employed in the past 5 years, and completed college or graduate school (**Supplemental Table 1.2**).

Bias factors ranged between 0.87 and 1.17, with physical activity showing the largest confounding effects on the association between 25(OH)D and MDD. After correcting for confounding bias induced by physical activity, the aHR estimates attenuated (Vitamin D insufficiency: HR=1.07, 95% CI: 1.03, 1.13; deficiency: HR=1.17, 95% CI: 1.11, 1.24), but remained statistically significant (**Supplemental Table 1.3**). Estimates were also robust to corrections for all other confounders.

Table 1.3 The association between serum 25(OH)D levels and incident MDD, stratified by age group, sex, and race/ethnicity

	MDD cases		Serum 25(OH)D[1]											
			Continuous			Normal			Insufficient			Deficient		
	N	%	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Age group														
<40	3,296	5.4	0.90	0.86, 0.92	<0.0001	ref	ref	ref	1.14	1.04, 1.24	0.004	1.28	1.17, 1.41	<0.0001
40-59	4,194	4.2	0.86	0.84, 0.89	<0.0001	ref	ref	ref	1.21	1.12, 1.30	<0.0001	1.48	1.36, 1.60	<0.0001
≥60	2,662	3.6	0.90	0.87, 0.92	<0.0001	ref	ref	ref	1.19	1.09, 1.31	<0.0001	1.37	1.23, 1.51	<0.0001
Sex														
Females	7,391	4.8	0.90	0.88, 0.91	<0.0001	ref	ref	ref	1.17	1.11, 1.23	<0.0001	1.32	1.24, 1.40	<0.0001
Males	2,761	3.5	0.85	0.82, 0.89	<0.0001	ref	ref	ref	1.19	1.09, 1.31	<0.001	1.51	1.37, 1.66	<0.0001
Race/ethnicity														
Asian	308	2.8	0.86	0.77, 0.96	0.01	ref	ref	ref	1.21	0.90, 1.64	0.21	1.38	1.02, 1.86	0.04
Black	808	4.7	0.91	0.85, 0.98	0.01	ref	ref	ref	0.93	0.75, 1.14	0.47	1.09	0.90, 1.32	0.37
Hispanic	992	6.8	0.94	0.89, 1.01	0.08	ref	ref	ref	1.08	0.92, 1.27	0.36	1.09	0.92, 1.29	0.34
White	7,480	4.3	0.87	0.86, 0.90	<0.0001	ref	ref	ref	1.18	1.12, 1.25	<0.0001	1.46	1.37, 1.55	<0.0001

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; HR: hazard ratio; MDD: major depressive disorder

All models adjusted for assay, season, healthcare use intensity, vitamin D supplementation, Charlson-deyo comorbidity index, and medical conditions and therapies indicating vitamin D testing, and mutually adjusted for the other two tested variables

[1]Vitamin D status defined by the following 25(OH)D cut-offs: Deficient, <20 ng/mL; Insufficient, 20-29.9 ng/mL; Normal, ≥30 ng/mL. Continuous 25(OH)D was analyzed as per 10 ng/mL increment.

Discussion

In this large, general healthcare cohort, we found that low 25(OH)D levels were associated with incident MDD over a five-year period, independent of potential confounders. Compared to adults with normal 25(OH)D levels, the risk of incident MDD was almost 20-40% higher among adults with insufficient and deficient levels, respectively. The association was robust to corrections for potential confounding bias and was stronger among older age groups (age>40 years), and white and Asian adults.

To our knowledge, this is the largest study to date exploring the association between 25(OH)D and incident MDD (N =233,970), over 30-fold larger than the largest prior study (23). The current study extends existing work on the prospective association between 25(OH)D levels and incident depression (19, 20, 23, 39-41) by focusing on a larger and ethnically/racially diverse cohort with a broader age range. Most existing studies have examined elderly populations (aged ≥55) because of the high prevalence and severe health consequences of vitamin D deficiency in this vulnerable group (42, 43). However, in recent years, vitamin D deficiency has also become a health concern among younger adults, given the increasing proportion of the population with night-shift work and working, living, and exercising indoors, resulting in reduced sunlight exposure (42, 44). Indeed, studies suggest vitamin D deficiency affects 30-50% of children and young adults even in geographical regions that receive ample sunlight (45, 46). There is, however, a lack of studies exploring the association between vitamin D levels on incident depression in younger populations. In this study, we examined the association in a younger cohort comprising mainly individuals aged ≤60. Our findings provide preliminary evidence to support that vitamin D deficiency may also increase MDD risk among younger adults.

Findings from our subgroup analysis in older adults (ages >60) add to the literature on vitamin D deficiency and late-life depression, which has so far yielded mixed conclusions. Our findings, which reveal a 1.37-fold higher risk of incident MDD among older adults with vitamin D deficiency, are consistent with that from the most recent and largest epidemiological study to date. In a community-

dwelling Irish population, adults with deficient vitamin D levels had a 1.49-fold higher risk of incident depression, compared to adults with sufficient levels (18). Relative to previous studies in elderly cohorts, our analysis had higher statistical power due to a substantially larger sample (n=73,815). We also had sufficient power to make comparisons across all three vitamin D categories and observed significantly different risks for incident MDD even between the insufficient and deficient groups. Prior studies that collapsed vitamin D categories may have diluted the observed effects, which could partially explain their non-significant findings.

Intriguingly, vitamin D deficiency was associated with higher MDD risk in white and Asian adults, but not in black or Hispanic adults. Similar racial differences have been observed in studies examining the association between serum 25(OH)D levels and other health outcomes including bone mineral density, stroke, and mortality (47-51). For instance, in the Multi-Ethnic Study of Atherosclerosis (MESA), lower 25(OH)D was associated with an increased risk for incident coronary heart disease in white and Chinese participants, but not black or Hispanic participants (50). The reason for racial differences in the association of low 25(OH)D levels with incident depression remains unknown. One explanation is that black individuals have lower levels of vitamin D binding proteins and higher levels of bioavailable 25(OH)D, therefore total 25(OH)D measured using standard clinical assays may not be accurate for defining vitamin D status in these populations (50, 52). Other potential mechanisms such as variation in circulating 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels and genetic differences in VDR affinity may also explain lower susceptibility to the adverse effects of low serum 25(OH)D in black populations (53-55). Future studies evaluating the prospective association between 25(OH)D and MDD in ethnic minority populations are needed.

Vitamin D may directly impact depression risk through plausible biological mechanisms including the regulation of serotonin synthesis, neuritogenesis, neuromodulation, neuroprotection, or downregulation of inflammatory responses (8). On the other hand, the observed associations could be explained by non-causal or indirect vitamin D effects. First, low vitamin D may be a marker of lifestyle risk factors and chronic illnesses that contribute to the development of depression (20, 22, 56). However,

in the current study, the association between vitamin D and MDD incidence persisted after adjustment for baseline medical comorbidity and when restricted to younger populations with less severe chronic conditions and presumably more active lifestyles. Second, the effect of vitamin D on psychiatric traits may in fact be mediated through calcium suppression and increases in parathyroid hormone (PTH) levels (57). Indeed, prior studies have shown associations between hyperparathyroidism and impaired cognition and depression (58, 59). Additional work is needed to unravel the relationship between vitamin D, PTH, and depression.

Key strengths of this study include a large sample size and examination of 25(OH)D levels collected before MDD diagnosis, minimizing the likelihood of reverse causation. Further, by leveraging data from the subset of participants who were further characterized in the Partners Biobank research cohort, we were able to demonstrate the robustness of the estimates to potential biases induced by unmeasured confounders such as physical activity. However, the study is not without limitations. First, diagnoses were limited to those captured by clinical documentation in the EHR. This may have led to outcome misclassification, although such misclassification would likely have biased our estimates towards the null. Second, while we attempted to account for potential selection bias resulting from differential vitamin D testing due to factors related to both 25(OH)D and MDD, the adjustments may have been incomplete. Third, patients in the study population were all adults, predominantly white, and mainly from the Greater Boston Area, which is located at a higher latitude with sunlight attenuation during winter months. Therefore, these results may not be generalizable to children or adolescents and populations in different geographic locations or with different demographic distributions. Fourth, some covariates were poorly captured in the EHRs, which could have led to residual confounding. For example, only 2% of the study population had recorded past-year vitamin D supplementation use, compared to the 28% past-month vitamin D supplementation use reported in the National Health and Nutrition Examination Survey (NHANES) (60), indicating possible underestimation of dietary supplementation use. It was also not possible to account for changes in vitamin D supplementation use after vitamin D testing, such that patients who were tested to be vitamin D deficient started to take vitamin D

supplements. Nonetheless, in these cases, the results would likely have been biased towards the null, and the true effect sizes would have been larger. Lastly, the Partners Biobank sub-cohort used to derive bias factors to correct for unmeasured confounding had slightly different demographic distributions to the full study population, thus corrections for confounding bias may be incomplete. Nonetheless, comparable associations between these confounders and depression have been reported in other populations (61-63), suggesting the validity of the bias corrections.

Our findings support an association between 25(OH)D levels and MDD, which persisted after adjustments for a range of confounders and correction for confounding bias. However, as with all observational studies, causality cannot be inferred. Trials such as the Vitamin D and Omega-3 Trial-Depression Endpoint Prevention (VITAL-DEP) are underway to evaluate the effect of vitamin D supplementation on depression (64), which could provide firmer conclusions on the causal relationship. If truly causal, the normalization of 25(OH)D in the population may be a cost-effective and safe public health strategy for preventing depression.

Acknowledgements

We would like to thank the Partners Biobank for providing health information data. The Partners Biobank is supported by the National Human Genome Research Institute of the National Institute of Health (Grant NHGRI U01HG008685).

References

1. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005;62:593-602.
2. Depression and Other Common Mental Disorders Global Health Estimates. Geneva, World Health Organization; 2017.
3. Lepine JP, Briley M. The increasing burden of depression. Neuropsychiatr Dis Treat. 2011;7:3-7.
4. Thase ME. Managing medical comorbidities in patients with depression to improve prognosis. J Clin Psychiatry. 2016;77 Suppl 1:22-27.
5. Anglin RE, Samaan Z, Walter SD, McDonald SD. Vitamin D deficiency and depression in adults: systematic review and meta-analysis. Br J Psychiatry. 2013;202:100-107.
6. Ju SY, Lee YJ, Jeong SN. Serum 25-hydroxyvitamin D levels and the risk of depression: a systematic review and meta-analysis. J Nutr Health Aging. 2013;17:447-455.
7. Okereke OI, Singh A. The role of vitamin D in the prevention of late-life depression. J Affect Disord. 2016;198:1-14.
8. Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front Neuroendocrinol. 2013;34:47-64.
9. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat. 2005;29:21-30.
10. Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, Hewison M. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab. 2001;86:888-894.
11. Mathieu C, van Etten E, Decallonne B, Guilietti A, Gysemans C, Bouillon R, Overbergh L. Vitamin D and 1,25-dihydroxyvitamin D3 as modulators in the immune system. J Steroid Biochem Mol Biol. 2004;89-90:449-452.
12. McCann JC, Ames BN. Is there convincing biological or behavioral evidence linking vitamin D deficiency to brain dysfunction? FASEB J. 2008;22:982-1001.
13. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009;65:732-741.
14. Wang Y, Chiang YH, Su TP, Hayashi T, Morales M, Hoffer BJ, Lin SZ. Vitamin D(3) attenuates cortical infarction induced by middle cerebral arterial ligation in rats. Neuropharmacology. 2000;39:873-880.

15. Buell JS, Dawson-Hughes B. Vitamin D and neurocognitive dysfunction: preventing "D"ecline? Mol Aspects Med. 2008;29:415-422.
16. Burne TH, Johnston AN, McGrath JJ, Mackay-Sim A. Swimming behaviour and post-swimming activity in Vitamin D receptor knockout mice. Brain Res Bull. 2006;69:74-78.
17. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? J Am Coll Cardiol. 2008;52:1949-1956.
18. Briggs R, McCarroll K, O'Halloran A, Healy M, Kenny RA, Laird E. Vitamin D Deficiency Is Associated With an Increased Likelihood of Incident Depression in Community-Dwelling Older Adults. J Am Med Dir Assoc. 2018.
19. Chan R, Chan D, Woo J, Ohlsson C, Mellstrom D, Kwok T, Leung P. Association between serum 25-hydroxyvitamin D and psychological health in older Chinese men in a cohort study. J Affect Disord. 2011;130:251-259.
20. Jovanova O, Aarts N, Noordam R, Carola-Zillikens M, Hofman A, Tiemeier H. Vitamin D serum levels are cross-sectionally but not prospectively associated with late-life depression. Acta Psychiatr Scand. 2017;135:185-194.
21. Zhao G, Ford ES, Li C, Balluz LS. No associations between serum concentrations of 25-hydroxyvitamin D and parathyroid hormone and depression among US adults. Br J Nutr. 2010;104:1696-1702.
22. Lee DM, Tajar A, O'Neill TW, O'Connor DB, Bartfai G, Boonen S, Bouillon R, Casanueva FF, Finn JD, Forti G, Giwercman A, Han TS, Huhtaniemi IT, Kula K, Lean ME, Punab M, Silman AJ, Vanderschueren D, Wu FC, Pendleton N, group Es. Lower vitamin D levels are associated with depression among community-dwelling European men. J Psychopharmacol. 2011;25:1320-1328.
23. May HT, Bair TL, Lappe DL, Anderson JL, Horne BD, Carlquist JF, Muhlestein JB. Association of vitamin D levels with incident depression among a general cardiovascular population. Am Heart J. 2010;159:1037-1043.
24. Nalichowski R, Keogh D, Chueh HC, Murphy SN. Calculating the benefits of a Research Patient Data Repository. AMIA Annu Symp Proc. 2006:1044.
25. Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, Peto R. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. Am J Epidemiol. 1999;150:341-353.
26. Jafri L, Khan AH, Siddiqui AA, Mushtaq S, Iqbal R, Ghani F, Siddiqui I. Comparison of high performance liquid chromatography, radio immunoassay and electrochemiluminescence immunoassay for quantification of serum 25 hydroxy vitamin D. Clin Biochem. 2011;44:864-868.

27. Maguire LH, Song M, Strate LE, Giovannucci EL, Chan AT. Higher serum levels of vitamin D are associated with a reduced risk of diverticulitis. Clin Gastroenterol Hepatol. 2013;11:1631-1635.
28. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine S. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-1930.
29. Rosen CJ. Clinical practice. Vitamin D insufficiency. N Engl J Med. 2011;364:248-254.
30. Cuomo AG, N.; Goracci, A.; Fagiolini, A. Depression and Vitamin D Deficiency: Causality, Assessment, and Clinical Practice Implications. Neuropsychiatry. 2017;7.
31. Fiske A, Wetherell JL, Gatz M. Depression in older adults. Annu Rev Clin Psychol. 2009;5:363-389.
32. Barnett AG, Dobson AJ: Analysing seasonal health data. Berlin ; London, Springer; 2010.
33. Sachs MC, Shoben A, Levin GP, Robinson-Cohen C, Hoofnagle AN, Swords-Jenny N, Ix JH, Budoff M, Lutsey PL, Siscovick DS, Kestenbaum B, de Boer IH. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2013;97:1243-1251.
34. Bertone-Johnson ER. Vitamin D and the occurrence of depression: causal association or circumstantial evidence? Nutr Rev. 2009;67:481-492.
35. Arah OA, Chiba Y, Greenland S. Bias formulas for external adjustment and sensitivity analysis of unmeasured confounders. Ann Epidemiol. 2008;18:637-646.
36. Vanderweele TJ, Arah OA. Bias formulas for sensitivity analysis of unmeasured confounding for general outcomes, treatments, and confounders. Epidemiology. 2011;22:42-52.
37. Karlson EW, Boutin NT, Hoffnagle AG, Allen NL. Building the Partners HealthCare Biobank at Partners Personalized Medicine: Informed Consent, Return of Research Results, Recruitment Lessons and Operational Considerations. J Pers Med. 2016;6.
38. Janes H, Dominici F, Zeger S. On quantifying the magnitude of confounding. Biostatistics. 2010;11:572-582.
39. Almeida OP, Hankey GJ, Yeap BB, Golledge J, Flicker L. Vitamin D concentration and its association with past, current and future depression in older men: The Health In Men Study. Maturitas. 2015;81:36-41.
40. Milaneschi Y, Shardell M, Corsi AM, Vazzana R, Bandinelli S, Guralnik JM, Ferrucci L. Serum 25-hydroxyvitamin D and depressive symptoms in older women and men. J Clin Endocrinol Metab. 2010;95:3225-3233.
41. Toffanello ED, Sergi G, Veronese N, Perissinotto E, Zambon S, Coin A, Sartori L, Musacchio E, Corti MC, Baggio G, Crepaldi G, Manzato E. Serum 25-hydroxyvitamin d and the onset of late-life

depressive mood in older men and women: the Pro.V.A. study. J Gerontol A Biol Sci Med Sci. 2014;69:1554-1561.
42. Boucher BJ. The problems of vitamin d insufficiency in older people. Aging Dis. 2012;3:313-329.
43. Cherniack EP, Florez H, Roos BA, Troen BR, Levis S. Hypovitaminosis D in the elderly: from bone to brain. J Nutr Health Aging. 2008;12:366-373.
44. Tonnesen R, Hovind PH, Jensen LT, Schwarz P. Determinants of vitamin D status in young adults: influence of lifestyle, sociodemographic and anthropometric factors. BMC Public Health. 2016;16:385.
45. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney RC, Saluja B, Ganie MA, Singh S. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. Am J Clin Nutr. 2005;82:477-482.
46. McGrath JJ, Kimlin MG, Saha S, Eyles DW, Parisi AV. Vitamin D insufficiency in south-east Queensland. Med J Aust. 2001;174:150-151.
47. De Silva DA TM, Allen JC Jr, Ikram MK, Wong TY, Tan EK, Lee WL. Ethnic Differences in Association of Vitamin D Levels with Incident Stroke Cases between Ethnic Chinese and South Asians. Austin Journal of Clinical Neurology. 2017;4:1102-1104.
48. Gutierrez OM, Farwell WR, Kermah D, Taylor EN. Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporos Int. 2011;22:1745-1753.
49. Michos ED, Reis JP, Post WS, Lutsey PL, Gottesman RF, Mosley TH, Sharrett AR, Melamed ML. 25-Hydroxyvitamin D deficiency is associated with fatal stroke among whites but not blacks: The NHANES-III linked mortality files. Nutrition. 2012;28:367-371.
50. Robinson-Cohen C, Hoofnagle AN, Ix JH, Sachs MC, Tracy RP, Siscovick DS, Kestenbaum BR, de Boer IH. Racial differences in the association of serum 25-hydroxyvitamin D concentration with coronary heart disease events. JAMA. 2013;310:179-188.
51. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med. 2008;168:1629-1637.
52. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369:1991-2000.
53. Bell NH, Greene A, Epstein S, Oexmann MJ, Shaw S, Shary J. Evidence for alteration of the vitamin D-endocrine system in blacks. J Clin Invest. 1985;76:470-473.
54. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res. 1998;13:325-349.

55. Levin GP, Robinson-Cohen C, de Boer IH, Houston DK, Lohman K, Liu Y, Kritchevsky SB, Cauley JA, Tanaka T, Ferrucci L, Bandinelli S, Patel KV, Hagstrom E, Michaelsson K, Melhus H, Wang T, Wolf M, Psaty BM, Siscovick D, Kestenbaum B. Genetic variants and associations of 25-hydroxyvitamin D concentrations with major clinical outcomes. JAMA. 2012;308:1898-1905.
56. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014;2:76-89.
57. Gronli O, Kvamme JM, Jorde R, Wynn R. Vitamin D deficiency is common in psychogeriatric patients, independent of diagnosis. BMC Psychiatry. 2014;14:134.
58. Hoogendijk WJ, Lips P, Dik MG, Deeg DJ, Beekman AT, Penninx BW. Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. Arch Gen Psychiatry. 2008;65:508-512.
59. Jorde R, Waterloo K, Saleh F, Haug E, Svartberg J. Neuropsychological function in relation to serum parathyroid hormone and serum 25-hydroxyvitamin D levels. The Tromso study. J Neurol. 2006;253:464-470.
60. Ganji V, Milone C, Cody MM, McCarty F, Wang YT. Serum vitamin D concentrations are related to depression in young adult US population: the Third National Health and Nutrition Examination Survey. Int Arch Med. 2010;3:29.
61. Fluharty M, Taylor AE, Grabski M, Munafo MR. The Association of Cigarette Smoking With Depression and Anxiety: A Systematic Review. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco. 2017;19:3-13.
62. Haynes JC, Farrell M, Singleton N, Meltzer H, Araya R, Lewis G, Wiles NJ. Alcohol consumption as a risk factor for anxiety and depression: results from the longitudinal follow-up of the National Psychiatric Morbidity Survey. Br J Psychiatry. 2005;187:544-551.
63. Lucas M, Mekary R, Pan A, Mirzaei F, O'Reilly EJ, Willett WC, Koenen K, Okereke OI, Ascherio A. Relation between clinical depression risk and physical activity and time spent watching television in older women: a 10-year prospective follow-up study. Am J Epidemiol. 2011;174:1017-1027.
64. Okereke OI, Reynolds CF, 3rd, Mischoulon D, Chang G, Cook NR, Copeland T, Friedenberg G, Buring JE, Manson JE. The VITamin D and OmegA-3 TriaL-Depression Endpoint Prevention (VITAL-DEP): Rationale and design of a large-scale ancillary study evaluating vitamin D and marine omega-3 fatty acid supplements for prevention of late-life depression. Contemporary clinical trials. 2018;68:133-145.

Appendix

Supplementary Methods

MDD Chart Reviews

Chart reviews on 50 random identified MDD cases were performed by two trained and experienced clinicians (JWS, KWC), who reviewed all medical notes in the patient's records to determine diagnosis of MDD or "not enough information" to make a decision. Guidelines for assigning diagnosis were based on DSM-V criteria for MDD, and a confidence level of low or intermediate/high was assigned to patients that were adjudicated to be an MDD case. Cases for which there was uncertainty were discussed with the senior psychiatrist (JWS) until consensus was reached. Two cases did not have sufficient information for classification, and of the remaining 48 patients, 4 were classified as having no evidence of MDD, 6 were classified as possible MDD cases (low confidence), and 38 were classified as probable/definite MDD cases (intermediate/high confidence).

Assessment of Covariates

Demographic factors including age, sex, and race/ethnicity were retrieved from the closest record prior to the date of study entry. Season of vitamin D testing was derived from the recorded date of blood draw. To account for the general burden of chronic medical conditions, ICD-9 coding algorithms were used to derive the Deyo modification to the Charlson co-morbidity index (1, 2), which has previously been validated in clinical populations (3, 4). This algorithm was implemented in R (5), using the *medicalrisk* package (6). Prescription of vitamin D supplementation, including cholecalciferol (D_3), calcitriol ($1,25\text{-}(OH)_2D_3$), and ergocalciferol (D_2), was assessed using prescription records within the one-year period prior to the 25(OH)D measurement. To determine if detection of MDD was higher among patients with lower vitamin D levels due to higher healthcare utilization, which could reflect a possible

ascertainment bias, we derived a variable to quantify and control for the intensity of healthcare utilization. Healthcare facility use density was calculated by summing the number of inpatient and outpatient visits and dividing by the amount of time the patient had been in the Partners Healthcare system prior to the 25(OH)D measurement. Similar equations have been used previously to quantify intensity of healthcare utilization in patients in the Partners Healthcare system (7). History of medical conditions that are indications for vitamin D testing (8, 9), including rickets, osteomalacia, osteoporosis, chronic kidney disease, liver disease, pancreatic insufficiency, malabsorption syndromes (e.g., IBD, cystic fibrosis), hyper- or hypo-thyroidism, non-traumatic fractures, chronic bronchitis, type II diabetes, hypercalcemia, were defined by the presence of corresponding ICD codes prior to baseline. Treatments known to affect vitamin D metabolism (9-11), such as anti-seizure and/or glucocorticoid medication, were identified using medication records. Age, Deyo-Charlson comorbidity index, and healthcare facility use were analyzed as continuous variables, and all other covariates were modelled categorically.

Derivation of Mean Annual 25(OH)D

The cosinor model takes the sine and cosine transformations of time and fits them as predictors of 25(OH)D in a linear regression model (12). The coefficients from the model are then transformed to obtain the amplitude and phase shift of the sine curve. In the cosinor model, 25(OH)D is modeled as a sine wave, characterized by phase shift (location of peak and trough on time axis), height (vertical shift of sine wave), and amplitude (distance between mean to peak or trough location of the curve). The mean annual 25(OH)D concentration in the study sample was estimated by the coefficient for the model intercept which, in combination with the residuals of the models, was used to derive the mean annual 25(OH)D levels for each individual.

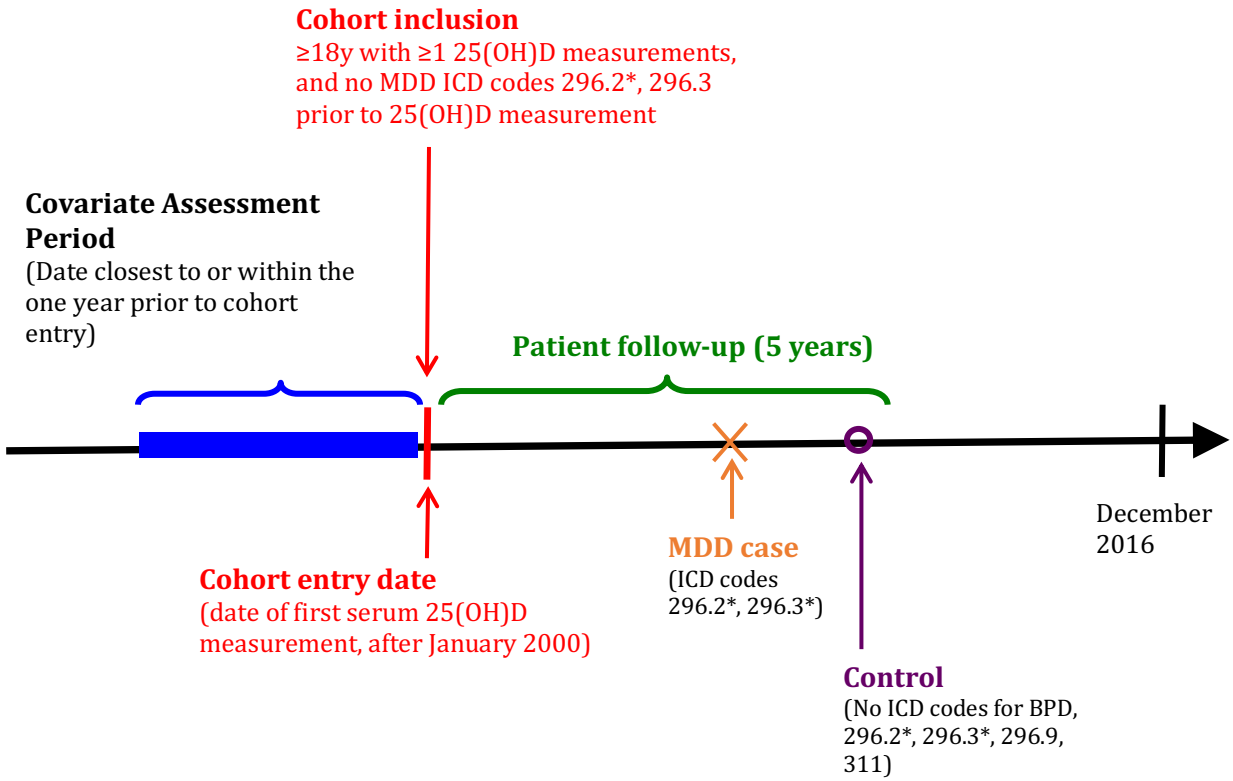
Bias Analysis

Confounders assessed in the bias analysis were defined and coded as follows: physical activity was defined as the total hours of moderate to intense activity per week and categorized into tertiles (<0.4, 0.4-2.49, >2.5 hours/week); body mass index (BMI; kg/m^2) was grouped into two categories based on WHO BMI categories (non-obese: BMI<30; obese BMI≥30) (13); socioeconomic factors included employment in the past 5 years (yes or no) and highest educational attainment (high school or less than high school, some college, or college/graduate); smoking status was classified as current, past, and never smokers; and alcohol consumption was grouped into 3 categories (<1, 1-6, or 7-12 drinks/week). In the Partners Biobank sub-cohort, we obtained estimates for the association between these confounders and MDD among those with normal vitamin D levels (RR_{dz0}) and the prevalence of different levels of the confounders (P_{Z0}, P_{z1}). Using this information, bias factors for each confounder were calculated (Equation (1)) and used to correct the HR estimates and confidence intervals from the main analysis. Analyses were performed in SAS 9.4.

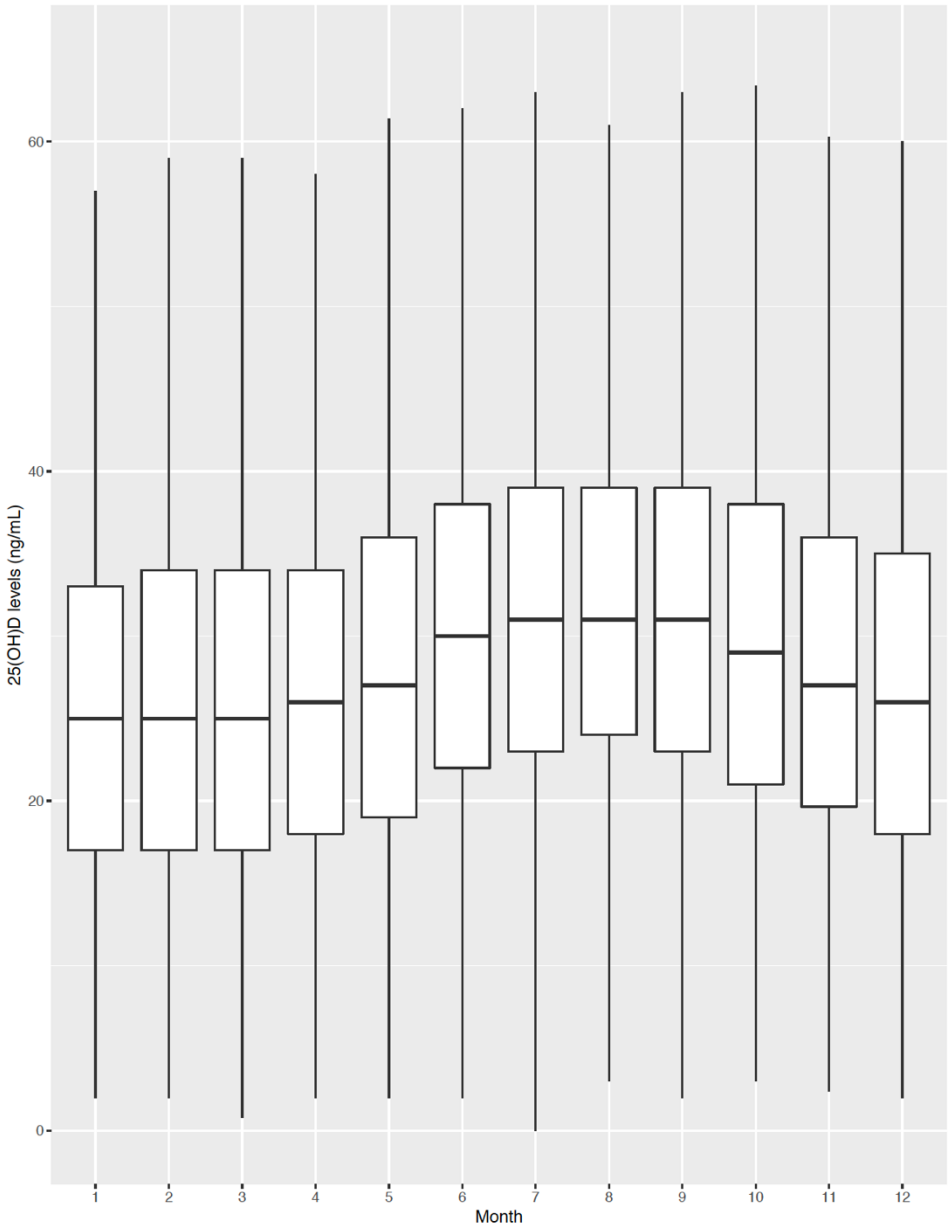
$$Bias\ factor = \frac{\sum_z RR_{dz0} P_{z1}}{\sum_z RR_{dz0} P_{z0}} \qquad (1)$$

Supplementary References

1. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373-383.
2. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol. 1992;45:613-619.
3. Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, Januel JM, Sundararajan V. Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol. 2011;173:676-682.
4. Quan H, Sundararajan V, Halfon P, Fong A, Burnand B, Luthi JC, Saunders LD, Beck CA, Feasby TE, Ghali WA. Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. Med Care. 2005;43:1130-1139.
5. Team RC: R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing; 2013.
6. McCormick P: medicalrisk: Calculating risk and comorbidities from ICD-9-CM codes. in R-project. 1.2 ed2016.
7. Ananthakrishnan AN, Cheng SC, Cai T, Cagan A, Gainer VS, Szolovits P, Shaw SY, Churchill S, Karlson EW, Murphy SN, Kohane I, Liao KP. Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol. 2014;12:821-827.
8. Kennel KAD, M.T.; Hurley, D.L. Vitamin D Deficiency in Adults: When to Test and How to Treat. Mayo Clin Proc. 2010;85:752-758.
9. Laboratories A: Vitamin D Testing. Edited by Utah Uo. Salt Lake City, UT, University of Utah; 2013.
10. Holick MFB, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; Murad, M.H.; Weaver, C.M. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism. 2011;96:1911-1930.
11. K. GUK. Influence of drugs on vitamin D and calcium metabolism. Dermato-Endocrinology. 2012;4:158-166.
12. Barnett AG, Dobson AJ: Analysing seasonal health data. Berlin ; London, Springer; 2010.
13. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253.



Supplemental Figure 1.1 Study design scheme



Supplemental Figure 1.2 Patterns of 25(OH)D levels by month in the study population

Supplemental Table 1.1 Association between mean annual serum 25(OH)D levels and incident major depressive disorder using Cox regression models

Serum 25(OH)D	n	MDD cases (%)	Model 1		Model 2		Model 3	
			HR (95 % CI)	p	HR (95 % CI)	p	HR (95 % CI)	p
Continuous (per 10 ng/mL increase)	233,970	10,152 (4.3)	0.87 (0.85, 0.88)	<0.0001	0.88 (0.86, 0.90)	<0.0001	0.89 (0.87, 0.90)	<0.0001
Conventional Clinical Cut-offs								
Normal (≥30 ng/mL)	104,483	4,104 (3.9)	ref	ref	ref	ref	ref	ref
Insufficient (20-29.9 ng/mL)	71,466	3,154 (4.4)	1.19 (1.14, 1.25)	<0.0001	1.19 (1.13, 1.25)	<0.0001	1.18 (1.13, 1.24)	<0.0001
Deficient (<20 ng/mL)	58,021	2,894 (5.0)	1.50 (1.42, 1.58)	<0.0001	1.43 (1.36, 1.51)	<0.0001	1.41 (1.34, 1.48)	<0.0001
Insufficient vs. Deficient								
Insufficient (20-29.9 ng/mL)	71,466	3,154 (4.4)	ref	ref	ref	ref	ref	ref
Deficient (<20 ng/mL)	58,021	2,894 (5.0)	1.26 (1.19, 1.32)	<0.0001	1.20 (1.15, 1.27)	<0.0001	1.19 (1.13, 1.26)	<0.0001

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; HR: hazard ratio; MDD: major depressive disorder
Model 1: Adjusted for age, sex, race/ethnicity, assay, season
Model 2: Model 1 covariates + healthcare use intensity + vitamin D supplementation + Charlson-Deyo comorbidity index
Model 3: Model 2 covariates + medical conditions and therapies indicating vitamin D testing

Supplemental Table 1.2 Distribution of confounders in the Partners Healthcare Biobank sub-cohort

	N (%)
Total moderate-intense physical activity (hours/week)	
Quantile 1 (<0.4)	377 (34.6)
Quantile 2 (0.4-2.49)	382 (35.0)
Quantile 3 (≥2.5)	332 (30.4)
BMI (kg/m^2)	
Non-obese (<30)	833 (74.2)
Obese (≥30)	286 (25.8)
Smoking	
Current	67 (6.0)
Past	405 (36.4)
Never	642 (57.6)
Alcohol (drinks/week)	
<1	482 (43.7)
1-6	483 (43.8)
7-12	139 (12.5)
Employment in past 5 years	
Yes	893 (79.8)
No	226 (20.2)
Highest educational attainment	
High school or less	113 (10.2)
Some college	101 (9.0)
College or graduate	898 (80.8)

Supplemental Table 1.3 Bias factor-corrected hazard ratios (HR) due to potential confounding by physical activity, smoking, alcohol consumption, and SES characteristics

Vitamin D status[1]	Original aHR (95% CI)	Bias-corrected aHR (95% CI)[2]					
		Adjusted for physical activity (BF1=1.09; BF2=1.17)	Adjusted for obesity (BF1=0.99; BF2=0.98)	Adjusted for smoking (BF1=0.98; BF2= 0.87)	Adjusted for alcohol (BF1=0.99; BF2= 0.99)	Adjusted for education (BF1=1.01; BF2=1.02)	Adjusted for occupation (BF1= 0.98; BF2= 0.96)
Normal	ref	ref	ref	ref	ref	ref	ref
Insufficient	1.17 (1.12, 1.23)	1.07 (1.03, 1.13)	1.18 (1.13, 1.24)	1.19 (1.14, 1.26)	1.18 (1.13, 1.24)	1.16 (1.11, 1.22)	1.19 (1.14, 1.26)
Deficient	1.37 (1.30, 1.45)	1.17 (1.11, 1.24)	1.40 (1.33, 1.48)	1.57 (1.49, 1.67)	1.38 (1.31, 1.46)	1.34 (1.27, 1.42)	1.43 (1.35, 1.51)

Abbreviations: BF = bias factor; BF1 = bias factor for insufficient vitamin D category; BF2 = bias factor for deficient vitamin D category; CI = confidence interval; aHR = adjusted hazard ratio.

[1] Vitamin D status defined by the following 25(OH)D cut-offs: Deficient, <20 ng/mL; Insufficient, 20-29.9 ng/mL; Normal, ≥30 ng/mL

[2] Bias-corrected aHR= (Original aHR)/(BF)

Chapter 2. Investigating the causal relationship between serum 25-hydroxyvitamin D and depression: A two-sample bidirectional Mendelian randomization study

Min-Jung Wang[1, *], Erin C. Dunn[2, 3, 4, 5], Olivia I. Okereke[1, 3, 6], Peter Kraft[1, 7], Chia-Yen Chen[2,3,4,8], Karmel W. Choi[1, 2, 3, 4], David M. Howard[9], Andrew M. McIntosh[9, 10], Jordan W. Smoller[1, 2, 3, 4]

[1] Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA
[2] Psychiatric and Neurodevelopmental Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA
[3] Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA
[4] Stanley Center for Psychiatric Research, The Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA
[5] Henry and Allison McCance Center for Brain Health, Massachusetts General Hospital, Boston, MA, USA
[6] Department of Medicine, Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA
[7] Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[8] Analytic and Translational Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA
[9] Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK
[10] Centre for Cognitive Ageing and Cognitive Epidemiology, University of Edinburgh, Edinburgh, UK

Abstract

Objectives: Evidence from observational studies regarding the association between serum 25-hydroxyvitamin D [25(OH)D] levels and depression has been inconsistent. Further, the causality and directionality of the relationship remains unclear. To address this gap, we conducted a two-sample bidirectional Mendelian randomization (MR) using summary-level data from the largest genome-wide association studies to date for serum 25(OH)D and depression.

Methods: Six genome-wide significant single-nucleotide polymorphisms (SNPs) associated with 25(OH)D levels in 79,366 individuals were used as genetic instruments to investigate the causal effect of serum 25(OH)D on depression, while 81 SNPs that were genome-wide significantly associated with depression in 807,553 individuals were used to assess the causal effect of depression on serum 25(OH)D levels. MR effect estimates were combined using inverse-variance weighted (IVW) meta-analysis, and other methods, including weighted median, likelihood, and MR Egger.

Results: We did not find evidence for an effect of 25(OH)D on depression, with an IVW odds ratio of 0.98 (95% CI: 0.95, 1.01; p=0.12) for depression per standard deviation increase in serum 25(OH)D. There was also no evidence for the effect of depression on 25(OH)D levels (Change in 25(OH)D levels comparing depression cases to controls: β=-0.008, 95% CI: -0.03, 0.01, p=0.50). Results using the likelihood, MR Egger, and weighted median methods yielded consistent estimates.

Conclusion: The current MR study provides no evidence for a strong causal relationship between lifelong circulating 25(OH)D levels and risk of depression. However, we cannot rule out the possibility of weaker causal effects and non-linear associations.

Keywords: Vitamin D, depression, Mendelian randomization

Introduction

Depression is a multifactorial disorder that likely results from a combination of genetic, physiologic, and/or environmental factors, such as life stressors, childhood adversities, inflammation, and lack of physical activity (1-5). Growing evidence suggests that vitamin D deficiency, defined by circulating 25-hydroxyvitamin D [25(OH)D], may be implicated in the etiology of depression (6, 7), given reported associations with other neuropsychiatric disorders (8, 9). Vitamin D is a neurosteroid hormone that has important effects on the brain, spanning neurological development and memory-related cognitive functions (10-13). Vitamin D receptors are also present in subcortical limbic brain regions that are implicated in depression risk (8, 14-16). Furthermore, neuromodulating and neuroprotective effects of vitamin D have been reported in animal studies, which reveal plausible biological pathways through which vitamin D may influence vulnerability to depression (8, 14, 16).

In humans, observational studies have generally found that vitamin D deficiency is associated with an increased risk for depression, with hazard ratios ranging between 1.42 and 1.80 (6, 17). However, a number of observational studies have also failed to detect associations (18-20), which suggests that other factors may explain some of the previously observed associations. First, the possibility of residual confounding cannot be excluded. That is, important factors related to both 25(OH)D and depression, including socioeconomic status, diet, physical activity, and other lifestyle characteristics, may not have been fully accounted for in prior studies. Second, some of the true associations may in fact be in the reverse direction. For instance, individuals with depression may change their outdoor activity and/or dietary habits, which may lower their 25(OH)D levels. Curiously, results from published randomized controlled trials do not support the use of vitamin D supplementation as a strategy to reduce depressive symptoms among adults (6, 7, 21, 22). However, these studies have been criticized for having relatively small sample sizes, low doses, and short treatment durations, making it difficult to draw more definitive conclusions about the relationship between vitamin D status and depression risk (6).

Mendelian randomization (MR) has emerged in recent years as a method that can strengthen causal inference and may serve as a bridge between observational and interventional studies. MR is an instrumental variable approach that uses genetic variants, typically single-nucleotide polymorphisms (SNPs), as proxies for the exposure of interest to infer causal relationships between two phenotypes (23). MR analysis is based on the principle that genetic variants are randomly assorted at conception and as a result, two common issues in observational studies can be circumvented, including (1) residual confounding, since genetic variants are unlikely associated with potential exposure-outcome confounding factors, and (2) reverse causation, as bi-directional MR allows assessment of the directionality of effects. A handful of published MR studies have been performed to understand the causal role of 25(OH)D on a range of physical and mental health outcomes (24). These studies have shown that circulating 25(OH)D levels may be causally associated with multiple sclerosis (25, 26), cancers (27, 28), and Alzheimer's disease (29), but do not appear to causally impact risk for schizophrenia (30) or Parkinson's disease (31).

In the current study, we performed a two-sample bidirectional MR analysis to determine whether there is a causal relationship between 25(OH)D levels and risk for depression. Summary-level data for these analyses came from two of the largest genome-wide association study (GWAS) meta-analyses to date: (1) the Study of Underlying Genetic Determinants of Vitamin D and Highly Related Traits (SUNLIGHT) Consortium (32) provided GWAS summary statistics for 25(OH)D; (2) the Howard *et al*. (33) study provided GWAS summary statistics for depression, based on a meta-analysis of results from the Psychiatric Genomics Consortium (PGC) (34), UK Biobank (35), and 23andMe (36). This study expands upon a recent MR analysis of vitamin D and major depressive disorder (MDD) (37) using summary data for depression from a substantially larger cohort (n=807,553 vs. n=173,005) and also examines the effect of depression on 25(OH)D levels.

Methods

The current analysis was based solely on GWAS summary statistics from previously reported studies that have respectively obtained appropriate ethical approval (32, 34), and no individual-level data was used. Therefore, additional Institutional Review Board approval was not required for this project.

Data Sources and SNP selection

Serum 25(OH)D. Summary-level data was obtained from the SUNLIGHT GWAS meta-analysis (32). This GWAS for 25(OH)D levels comprised 79,366 individuals of European ancestry and identified six independent, genome-wide significant (GWS, $p<5x10^{-8}$) SNPs, which together explained ~2.84% of serum 25(OH)D variation (30). Four of these variants are located in genes that were previously implicated in the synthesis, metabolism, or transport of vitamin D (38).

Depression. Summary-level statistics were obtained from a meta-analysis of the three largest GWAS of depression (33), performed by the Psychiatric Genetics Consortium (PGC-MDD) (34), UK Biobank (35), and 23andMe (36). The study, which included 246,363 depression cases and 561,190 controls, identified 102 SNPs that were GWS-associated with depression. The GWS SNPs, combined in a polygenic risk score (PRS), explained up to 3% of the depression variation on the liability scale. Several of the identified SNPs have previously been linked to neuropsychiatric traits or disorders.

For exposure-related SNPs that were unavailable in the GWAS summary statistics for the outcome, proxy SNPs in high linkage disequilibrium ($R^2>0.8$) were identified using LDlink, a web-based platform that utilizes data from the 1000 Genomes Project Phase 3 to explore genomic structures (39). For the 25(OH)D to depression analysis, all 6 SNPs were available in the depression GWAS. For the depression to 25(OH)D analysis, 46 of 102 SNPs were identified, 42 SNPs were matched to proxy SNPs (R^2: 0.80-1.00), and 14 SNPs were excluded due to a lack of suitable proxies in the 25(OH)D GWAS. An

additional 7 strand ambiguous SNPs (i.e., A/T or G/C SNPs), for which the forward strand could not be inferred based on the minor allele frequency (MAF), were excluded. 81 variants remained for the depression to 25(OH)D MR analysis.

SNP validation

SNPs used as instrumental variables (IVs) for our MR analysis were validated by testing the following three MR assumptions: (1) strong association between the IV and exposure; (2) IV is independent of confounders of the exposure-outcome association; and (3) absence of horizontal pleiotropy, meaning that the IVs exert effects on the outcome only through the exposure and no other independent biological pathways (40, 41). By selecting genetic variants that were GWS-associated with the exposure, we ensured that the first assumption was fulfilled.

To evaluate the second assumption, we examined linkage disequilibrium (LD) patterns between all SNPs selected as genetic instruments for the analysis in 1000 Genomes Project European (CEU) samples (42), recognizing that SNPs highly correlated with other risk loci could lead to confounding (43). For any SNP-pairs with evidence of LD (r^2>0.1), the variant with the strongest association (i.e., smallest p-value) with the exposure was retained for analysis.

To verify the third MR assumption, we assessed for potential horizontal pleiotropic effects of the selected SNPs that may arise from their effects on confounders of the exposure-outcome association. Pleiotropy is less likely for the selected 25(OH)D IVs, most of which map strongly to 25(OH)D pathways. In addition, prior work in the 1958 British Birth Cohort found no associations between four of the 25(OH)D-related SNPs and potential pleiotropic pathways such as physical activity, sun exposure, and smoking habits (all p>0.05) (44). We also performed a search of associations between the selected IVs and other health outcomes/traits and potential confounders using PhenoScanner, a curated database of a publicly available GWAS data (45) (**Supplemental Tables 2.1 and 2.2**). Additional analyses were performed, as summarized below, to evaluate the effects on the results of removing SNPs with any

evidence of having pleiotropic pathways and evaluate the robustness of our findings to violations of the pleiotropy assumption.

Statistical Analysis

All analyses were performed using the *TwoSampleMR* (version 0.4.16) package in R version 3.3.1 (46). The primary MR analysis was performed using the inverse-variance weighted (IVW) meta-analysis, which takes a weighted mean of ratio estimates from multiple IVs (47). For the 25(OH)D to depression MR analysis, the ratio estimate ($\exp(\hat{\beta})$) is interpreted as the odds ratio (OR) of depression per standard deviation (SD) increase in ln(25(OH)D) concentrations. The SD (1 SD=0.33 nmol/L on the log-transformed scale) was derived from a population-based Swedish Mammography cohort (48), and has been used in published 25(OH)D MR analyses (49). For the depression to serum 25(OH)D MR analysis, $\hat{\beta}$ is interpreted as the difference in ln(25(OH)D) levels comparing cases of depression to controls.

The Cochran's Q statistic was calculated to test for statistical heterogeneity, which could indicate the presence of horizontal pleiotropy (50, 51). To evaluate the robustness of the causal estimates, the weighted median, MR-Egger regression, and likelihood methods were also employed (50, 52, 53). Briefly, the weighted median ratio estimate is the median of the weighted empirical distribution function of all SNP ratio estimates (53); this method provides an unbiased estimate of the causal effect even when up to half of the SNPs are invalid instruments. MR Egger involves fitting a weighted linear regression of the SNP-outcome against SNP-exposure effect estimates (50) and provides an unbiased causal effect estimate by adjusting for average pleiotropic bias. The likelihood-based approach calculates a maximum likelihood estimate (MLE), with the SNP-exposure and SNP-outcome associations jointly modeled under a bivariate normal distribution. This method produces valid estimates even when the SNP-exposure and SNP-outcome associations are correlated (47). MR-PRESSO was also implemented to detect and remove horizontal pleiotropic outliers (54).

We also performed sensitivity analyses by excluding SNPs that may potentially have pleiotropic effects. For the 25(OH)D to depression analysis, analyses were performed removing the following SNPs: (1) rs12785878 (*DHCR7*), found to be strongly associated with ancestry (44); (2) rs3755967 (*GC*), which could affect depression through opposing effects on the bioavailability of vitamin D, rather than influencing 25(OH)D levels (55). For the depression to 25(OH)D analysis, we performed additional analyses excluding SNPs that were GWS-associated with potential confounders of the association between 25(OH)D levels and depression, such as educational attainment and body mass index (**Supplemental Table 2.2**).

Power Calculations

Statistical power for the bidirectional MR analysis was estimated using the online mRnd tool (http://cnsgenomics.com/shiny/mRnd/), developed by Brion and colleagues (56). Power estimations were made for a range of effect estimates (OR=0.75-0.98 per SD increase in ln(25(OH)D); beta=0.01-2.0 comparing depression case to control status), with type I error fixed at α=0.05 and assuming no heterogeneity. The study had approximately 80% power to detect an effect size of OR=0.93 for the risk of depression per 1 SD increase in ln(25(OH)D) (nmol/L), and 80% power to detect an effect size of beta=2.0 for the difference in ln(25(OH)D) comparing depression cases to controls (**Supplemental Figure 2.1**).

Results

Effect of 25(OH)D on Depression

Of the six 25(OH)D variants included in the MR analysis, the SNP in the *GC* gene (rs3755967) had the strongest association with 25(OH)D levels (β=0.09 per-allele difference in ln(25(OH)D), $p=4.74x10^{-343}$) (**Supplemental Table 2.3**). None of the 25(OH)D SNPs were GWS-associated with depression (all $p \geq 0.01$) (**Supplemental Table 2.3**). There was no evidence for an association between

25(OH)D levels on depression using the IVW model (OR of depression per 1 SD increase in 25(OH)D levels=0.98; 95% CI: 0.95, 1.01; p=0.12) (**Table 2.1, Figure 2.1**).

Table 2.1 MR estimates for the effect of 25(OH)D levels on risk for depression

Method[a]	No. SNPs	OR[b]	95% CI	p-value
IVW	6	0.98	0.95, 1.01	0.12
Maximum likelihood	6	0.98	0.96, 1.00	0.08
Weighted median	6	0.98	0.96, 1.01	0.19
MR Egger	6	0.98	0.93, 1.04	0.59

IVW: Inverse-variance weighted; MR: Mendelian randomization; OR: odds ratio; SNPs: single-nucleotide polymorphism.
[a]No MR-PRESSO outliers detected
[b]OR=odds ratio for depression per 1 SD increase in ln(25(OH)D) (nmol/L)

(a)

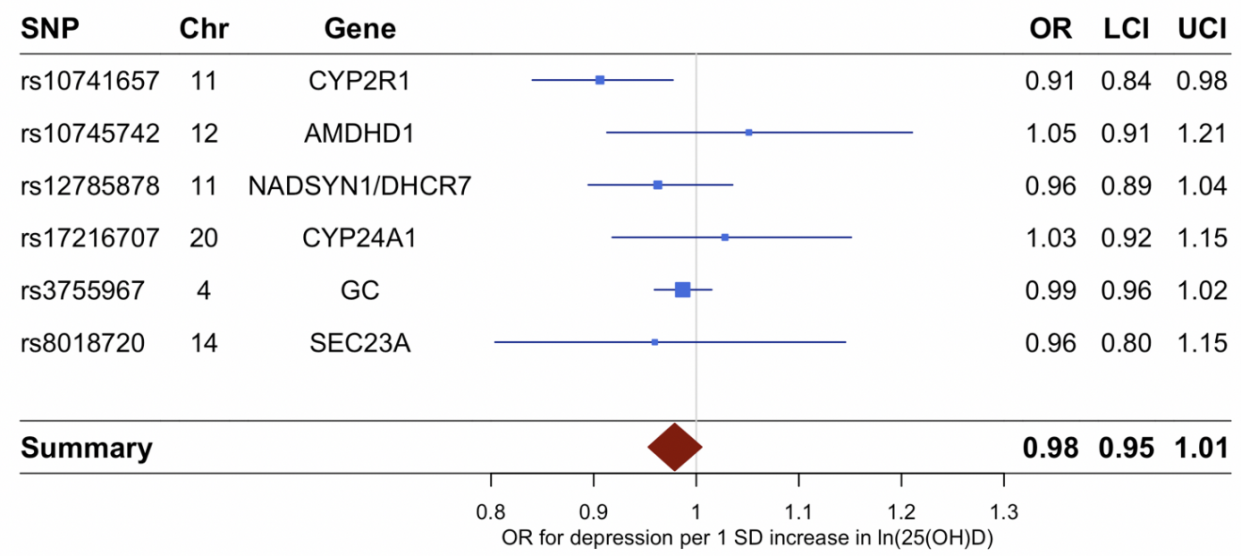


Figure 2.1 Mendelian randomization (MR) plots for the association between 25(OH)D levels and the risk for depression. (a) Forest plot for the individual and combined SNP effects using the inverse-variance weighted (IVW) model. (b) Scatterplot for the effect of SNPs on ln(25(OH)D) and depression with the slope representing the MR effect estimate for each method.

(b)

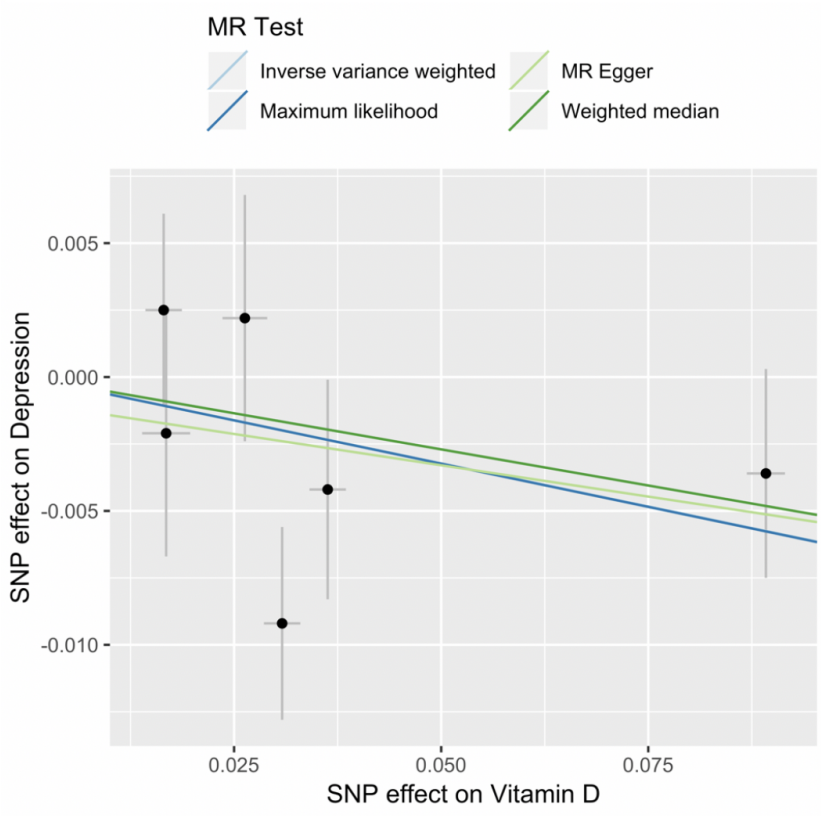


Figure 2.1 (continued)

There was no evidence of horizontal pleiotropy based on the tests of heterogeneity (Q=6.27, p=0.28) and Egger regression (Intercept=1.00; 95% CI: 0.99, 1.01; p=0.80). MR-PRESSO also did not detect any outliers (p=0.31). The causal effect estimates were similar using the likelihood (OR= 0.98; 95% CI: 0.96, 1.00; p=0.08), weighted median (OR=0.98; 95% CI: 0.96, 1.01; p=0.19), and MR Egger (OR=0.98; 95% CI: 0.93, 1.04; p=0.59) methods (**Table 2.1**). Sensitivity analyses excluding the rs12785878 (*DHCR7*) and rs3755967 (*GC*) SNPs did not substantially change the MR effect estimates (**Supplemental Table 2.4**).

Effect of Depression on 25(OH)D

Among the 81 SNPs included in this MR analysis, the SNP that had the strongest association with depression was rs2568958 (OR=1.04; $p=8.47x10^{-25}$), which is found in the uncharacterized RNA gene

LOC105378797 (**Supplemental Table 2.5**). Twelve SNPs (rs301799, rs7685686, rs11135349, rs3823624, rs3793577, rs58621819, rs57344483, rs4772087, rs9592461, rs7193263, rs143186028, and rs5995992) were nominally associated with serum 25(OH)D ($p<0.05$), but none reached GWS (**Supplemental Table 2.5**). There was no evidence for causal effects of depression on 25(OH)D levels using the IVW model (β=-0.008; 95% CI: -0.03, 0.01; p=0.50) (**Table 2.2, Figure 2.2**).

Table 2.2 MR estimates for the effect of depression case status on 25(OH)D levels.

Method[a]	No. SNPs	Beta[b]	95% CI	p-value
IVW	81	-0.008	-0.03, 0.01	0.50
Maximum likelihood	81	-0.008	-0.03, 0.01	0.45
Weighted median	81	0.008	-0.02, 0.04	0.59
MR Egger	81	-0.027	-0.16, 0.11	0.70

IVW: Inverse-variance weighted; MR: Mendelian randomization; SNPs: single-nucleotide polymorphism.
[a]No MR-PRESSO outliers detected
[b]Beta=Difference in ln(25(OH)D) comparing depression cases to controls

(a)

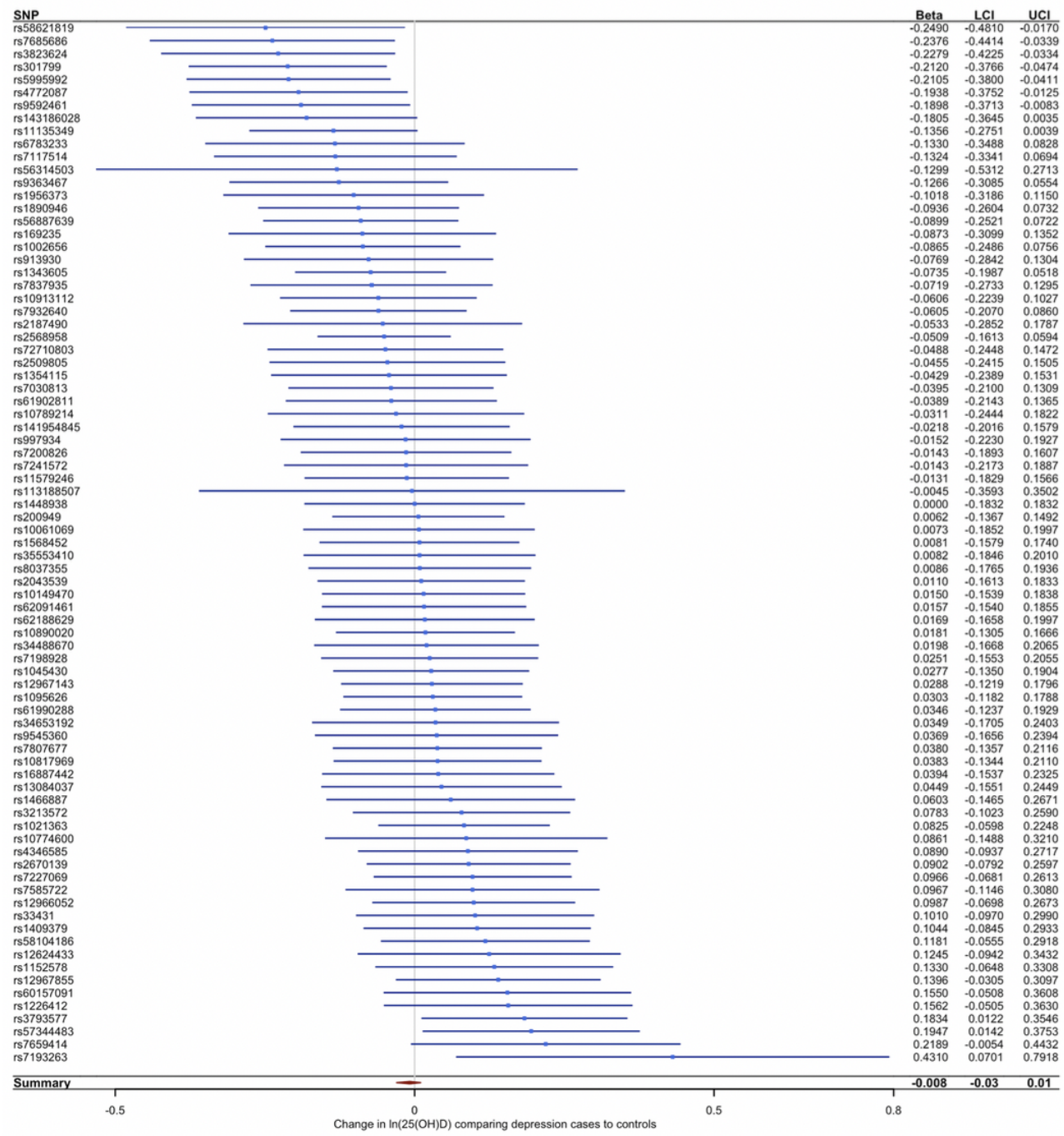


Figure 2.2 MR plots for the association between depression case-control status and ln(25(OH)D levels. (a) Forest plot for the individual and combined SNP effects using the IVW model. (b) Scatterplot for the effect of SNPs on depression and ln(25(OH)D) with the slope representing the MR effect estimate for each method.

(b)

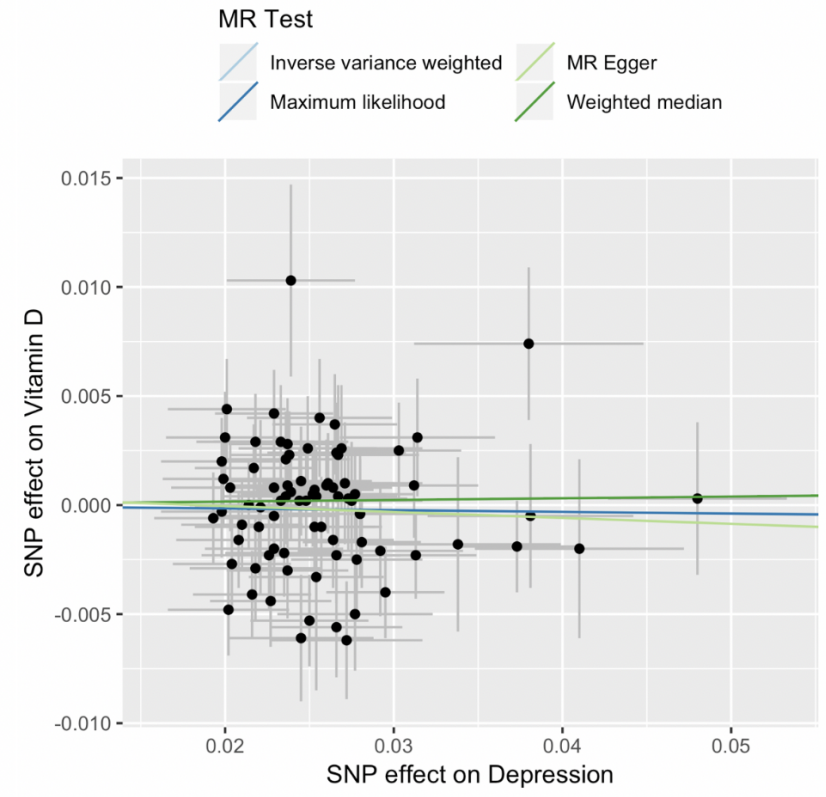


Figure 2.2 (continued)

There was little evidence for horizontal pleiotropy, with moderate heterogeneity across SNPs (Q=100.77; p=0.06) and an Egger regression intercept close to the null (Intercept=0.001; 95% CI: -0.006, 0.008; p=0.78). MR-PRESSO also did not detect any outliers (p=0.09). The causal effect estimates yielded from the likelihood (β=-0.008; 95% CI: -0.03; 0.01; p=0.45), weighted median (β=0.008; 95% CI: -0.02, 0.04; p=0.59), and MR egger (β=-0.027; 95% CI: -0.16, 0.11; p=0.70) approaches yielded similar results (**Table 2.2**). Sensitivity analysis excluding the 12 SNPs that were associated with potential confounding factors resulted did not change the pattern of effects (β=-0.002; 95% CI: -0.03, 0.02; p=0.85) (**Supplemental Table 2.6**).

Discussion

Both depression and low vitamin D levels are rising public health issues worldwide, yet their association remains controversial. Understanding whether vitamin D is a causal risk factor for depression would have important implications for depression prevention efforts, since vitamin D levels are easily modifiable through vitamin D supplementation, and lifestyle and/or dietary changes. As such, we performed a MR analysis to examine the causal relationship between 25(OH)D levels and risk for depression. Using genetic instruments from the largest published GWAS meta-analysis for 25(OH)D and depression to date, we employed various MR methods including IVW, likelihood-based MR, weighted median, and MR Egger to explore this association. We did not find evidence for a causal effect of lifelong average 25(OH)D levels on the risk for depression or causal effects in the reverse direction.

Our results are consistent with those reported in a previous MR analysis, which showed no association between lifelong 25(OH)D levels and MDD in European adults (37). The present study also corroborates the findings of a recent longitudinal study that assessed the association between continuous circulating 25(OH)D and incident MDD (19) in older adults, where the authors found a non-significant inverse effect of serum 25(OH)D levels on incident MDD that was of comparable magnitude (HR=0.95, 95% CI: 0.86, 1.05) to that found in the current study (19). Using a bidirectional MR, we were also able to conduct a formal test of whether depression influences 25(OH)D levels, an important analysis in light of concerns about reverse causation in observational studies. While this study may have lacked sufficient power to detect weaker effects, our results suggest that depression did not have substantial effects on 25(OH)D levels, and that prior cross-sectional vitamin D-depression associations may not be explained by the direct effects of depression on vitamin D levels. These results together suggest that modest and incremental changes in 25(OH)D levels are unlikely to reduce the risk for depression.

However, these results should be interpreted in the context of several factors. MR is a powerful statistical method that can be used to establish or refute observational results, but there are important differences between the hypotheses tested under the MR framework compared to those tested in

traditional epidemiological cohort studies. First, 25(OH)D-related SNPs influence lifelong exposures to circulating vitamin D, therefore the MR results are consistent with the lack of a causal relationship between lower lifelong 25(OH)D levels and depression risk. On the other hand, observational studies have conventionally investigated the association between depression and more proximal 25(OH)D levels (i.e., incidence of depression within 1-5 years of vitamin D measurement (57, 58)). Hence, it remains possible that more recent exposures to vitamin D, rather than average lifetime exposures, have causal effects on depression risk. Second, we cannot rule out the existence of specific sensitive periods during which vitamin D levels may have stronger effects on depression risk. Prior studies have found that prenatal 25(OH)D exposures are associated with mental health outcomes, such as schizophrenia (59-61) and neuropsychological scores (62), and that childhood 25(OH)D levels are associated with adolescent depression (63). Therefore, it is possible that early life exposures to 25(OH)D during important periods of brain development have stronger effects on the risk for depression, compared to average lifelong exposures. Third, observational studies have primarily reported the impact of vitamin D deficiency on depression risk; it is plausible that levels below a certain threshold, but not incremental linear changes in circulating vitamin D levels, that are causally related to depression risk (17). Indeed, in a meta-analysis of cohort studies, authors found an increased risk of depression comparing those in the highest vs. lowest vitamin D categories, but no association for 20 nmol/L differences in vitamin D levels (17). While some genetic instruments may be associated with vitamin D deficiency (64), such causal threshold effects could not be tested with currently available MR methods and GWAS data. Further studies are needed to elucidate these potentially complex relationships.

A major strength of this MR study is that we used the largest available GWAS summary statistics for both depression and 25(OH)D levels at the time of writing. Furthermore, participants in both study populations were of European ancestry, reducing potential bias due to population stratification. However, our analysis is not without limitations. First, while the sample sizes were large, the SNPs that we selected as genetic instruments accounted for a relatively small proportion of variation in both 25(OH)D (~2.8%) (32) and depression (~3%) (34). The study therefore lacked statistical power to detect weaker causal

relationships between serum 25(OH)D and depression risk. Second, given unknown functional roles of these tested SNPs, we could not completely eliminate the possibility of horizontal pleiotropy, which could bias the analyses. However, our findings were robust to multiple sensitivity analyses and there was no strong evidence, quantitatively or qualitatively, for the presence of pleiotropic effects. Third, due to a lack of available methods, we could not investigate either non-linear associations between circulating vitamin D and risk of depression nor threshold effects based on conventional clinical cut-offs for vitamin D deficiency (16). MR methods that enable testing for non-linear associations between the exposure and outcome are currently under development (65). Fourth, we could not assess the role of the biologically active form of vitamin D (1,25-dihydroxyvitamin D (1,25(OH)D), for which GWAS have not yet been performed. Therefore, conclusions cannot be drawn about lifelong average levels of 1,25(OH)D on depression risk. Fifth, our null results may be explained by canalization, which are compensatory feedback mechanisms that could buffer harmful effects of lowered vitamin D levels and could bias the association toward the null (43, 66). Lastly, the study was mainly restricted to European adults; thus, results may be less readily generalizable to younger or non-European populations with different prevalence of depression and distributions of circulating vitamin D.

In summary, this MR study does not provide evidence for a causal relationship between lifelong 25(OH)D levels and the risk for depression among individuals of European descent. However, we cannot preclude the possibility of smaller causal associations, sensitive periods, threshold effects, or effects in specific sub-populations. The largest RCT to date (VITAL-DEP), evaluating the effect of vitamin D supplements on preventing depression among approximately 25,000 older adults, is underway and will provide further evidence for the causal effects of vitamin D on depression (67). Future well-designed studies are warranted to determine evidence-based cut-offs for vitamin D deficiency, potentially allowing for stronger conclusions about the vitamin D-depression association. Similar analyses in other ethnic/racial samples could also elucidate the benefit of vitamin D supplementation in other sub-populations.

Acknowledgments

We would like to thank the participants, employees, and research teams behind the SUNLIGHT Consortium, the Psychiatric Genomics Consortium, the UK Biobank, and 23andMe.

References

1. Harvey SB, Overland S, Hatch SL, Wessely S, Mykletun A, Hotopf M. Exercise and the Prevention of Depression: Results of the HUNT Cohort Study. Am J Psychiatry. 2018;175:28-36.
2. Kendler KS, Kuhn JW, Prescott CA. Childhood sexual abuse, stressful life events and risk for major depression in women. Psychol Med. 2004;34:1475-1482.
3. Liu RT. Childhood Adversities and Depression in Adulthood: Current Findings and Future Directions. Clin Psychol (New York). 2017;24:140-153.
4. Musliner KL, Seifuddin F, Judy JA, Pirooznia M, Goes FS, Zandi PP. Polygenic risk, stressful life events and depressive symptoms in older adults: a polygenic score analysis. Psychol Med. 2015;45:1709-1720.
5. Cepeda MS, Stang P, Makadia R. Depression Is Associated With High Levels of C-Reactive Protein and Low Levels of Fractional Exhaled Nitric Oxide: Results From the 2007-2012 National Health and Nutrition Examination Surveys. J Clin Psychiatry. 2016;77:1666-1671.
6. Okereke OI, Singh A. The role of vitamin D in the prevention of late-life depression. J Affect Disord. 2016;198:1-14.
7. Parker GB, Brotchie H, Graham RK. Vitamin D and depression. J Affect Disord. 2017;208:56-61.
8. Eyles DW, Burne TH, McGrath JJ. Vitamin D, effects on brain development, adult brain function and the links between low levels of vitamin D and neuropsychiatric disease. Front Neuroendocrinol. 2013;34:47-64.
9. Belvederi Murri M, Respino M, Masotti M, Innamorati M, Mondelli V, Pariante C, Amore M. Vitamin D and psychosis: mini meta-analysis. Schizophr Res. 2013;150:235-239.
10. Annweiler C, Allali G, Allain P, Bridenbaugh S, Schott AM, Kressig RW, Beauchet O. Vitamin D and cognitive performance in adults: a systematic review. Eur J Neurol. 2009;16:1083-1089.
11. Kesby JP, Eyles DW, Burne TH, McGrath JJ. The effects of vitamin D on brain development and adult brain function. Mol Cell Endocrinol. 2011;347:121-127.
12. Holick MF. Vitamin D and brain health: the need for vitamin D supplementation and sensible sun exposure. J Intern Med. 2015;277:90-93.
13. Miller JW, Harvey DJ, Beckett LA, Green R, Farias ST, Reed BR, Olichney JM, Mungas DM, DeCarli C. Vitamin D Status and Rates of Cognitive Decline in a Multiethnic Cohort of Older Adults. JAMA Neurol. 2015;72:1295-1303.
14. Berridge MJ. Vitamin D cell signalling in health and disease. Biochem Biophys Res Commun. 2015;460:53-71.

15. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. J Chem Neuroanat. 2005;29:21-30.
16. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-281.
17. Anglin RE, Samaan Z, Walter SD, McDonald SD. Vitamin D deficiency and depression in adults: systematic review and meta-analysis. Br J Psychiatry. 2013;202:100-107.
18. Chan R, Chan D, Woo J, Ohlsson C, Mellstrom D, Kwok T, Leung P. Association between serum 25-hydroxyvitamin D and psychological health in older Chinese men in a cohort study. J Affect Disord. 2011;130:251-259.
19. Jovanova O, Aarts N, Noordam R, Carola-Zillikens M, Hofman A, Tiemeier H. Vitamin D serum levels are cross-sectionally but not prospectively associated with late-life depression. Acta Psychiatr Scand. 2017;135:185-194.
20. Toffanello ED, Sergi G, Veronese N, Perissinotto E, Zambon S, Coin A, Sartori L, Musacchio E, Corti MC, Baggio G, Crepaldi G, Manzato E. Serum 25-hydroxyvitamin d and the onset of late-life depressive mood in older men and women: the Pro.V.A. study. J Gerontol A Biol Sci Med Sci. 2014;69:1554-1561.
21. Gowda U, Mutowo MP, Smith BJ, Wluka AE, Renzaho AM. Vitamin D supplementation to reduce depression in adults: meta-analysis of randomized controlled trials. Nutrition. 2015;31:421-429.
22. Shaffer JA, Edmondson D, Wasson LT, Falzon L, Homma K, Ezeokoli N, Li P, Davidson KW. Vitamin D supplementation for depressive symptoms: a systematic review and meta-analysis of randomized controlled trials. Psychosom Med. 2014;76:190-196.
23. Sheehan NA, Didelez V, Burton PR, Tobin MD. Mendelian randomisation and causal inference in observational epidemiology. PLoS Med. 2008;5:e177.
24. Jiang X, Kiel DP, Kraft P. The genetics of vitamin D. Bone. 2018.
25. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, Leong A, Greenwood CM, Thanassoulis G, Richards JB. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. PLoS Med. 2015;12:e1001866.
26. Rhead B, Baarnhielm M, Gianfrancesco M, Mok A, Shao X, Quach H, Shen L, Schaefer C, Link J, Gyllenberg A, Hedstrom AK, Olsson T, Hillert J, Kockum I, Glymour MM, Alfredsson L, Barcellos LF. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. Neurol Genet. 2016;2:e97.
27. Ong JS, Cuellar-Partida G, Lu Y, Australian Ovarian Cancer S, Fasching PA, Hein A, Burghaus S, Beckmann MW, Lambrechts D, Van Nieuwenhuysen E, Vergote I, Vanderstichele A, Anne Doherty J, Anne Rossing M, Chang-Claude J, Eilber U, Rudolph A, Wang-Gohrke S, Goodman MT, Bogdanova N, Dork T, Durst M, Hillemanns P, Runnebaum IB, Antonenkova N, Butzow R, Leminen A, Nevanlinna H,

Pelttari LM, Edwards RP, Kelley JL, Modugno F, Moysich KB, Ness RB, Cannioto R, Hogdall E, Hogdall CK, Jensen A, Giles GG, Bruinsma F, Kjaer SK, Hildebrandt MA, Liang D, Lu KH, Wu X, Bisogna M, Dao F, Levine DA, Cramer DW, Terry KL, Tworoger SS, Stampfer M, Missmer S, Bjorge L, Salvesen HB, Kopperud RK, Bischof K, Aben KK, Kiemeney LA, Massuger LF, Brooks-Wilson A, Olson SH, McGuire V, Rothstein JH, Sieh W, Whittemore AS, Cook LS, Le ND, Gilks CB, Gronwald J, Jakubowska A, Lubinski J, Kluz T, Song H, Tyrer JP, Wentzensen N, Brinton L, Trabert B, Lissowska J, McLaughlin JR, Narod SA, Phelan C, Anton-Culver H, Ziogas A, Eccles D, Campbell I, Gayther SA, Gentry-Maharaj A, Menon U, Ramus SJ, Wu AH, Dansonka-Mieszkowska A, Kupryjanczyk J, Timorek A, Szafron L, Cunningham JM, Fridley BL, Winham SJ, Bandera EV, Poole EM, Morgan TK, Risch HA, Goode EL, Schildkraut JM, Pearce CL, Berchuck A, Pharoah PD, Chenevix-Trench G, Gharahkhani P, Neale RE, Webb PM, MacGregor S. Association of vitamin D levels and risk of ovarian cancer: a Mendelian randomization study. Int J Epidemiol. 2016;45:1619-1630.

28. Takahashi H, Cornish AJ, Sud A, Law PJ, Kinnersley B, Ostrom QT, Labreche K, Eckel-Passow JE, Armstrong GN, Claus EB, Ll'yasova D, Schildkraut J, Barnholtz-Sloan JS, Olson SH, Bernstein JL, Lai RK, Schoemaker MJ, Simon M, Hoffmann P, Nothen MM, Jockel KH, Chanock S, Rajaraman P, Johansen C, Jenkins RB, Melin BS, Wrensch MR, Sanson M, Bondy ML, Turnbull C, Houlston RS. Mendelian randomisation study of the relationship between vitamin D and risk of glioma. Sci Rep. 2018;8:2339.

29. Larsson SC, Traylor M, Markus HS, Michaelsson K. Serum Parathyroid Hormone, 25-Hydroxyvitamin D, and Risk of Alzheimer's Disease: A Mendelian Randomization Study. Nutrients. 2018;10.

30. Taylor AE, Burgess S, Ware JJ, Gage SH, Richards JB, Davey Smith G, Munafo MR. Investigating causality in the association between 25(OH)D and schizophrenia. Sci Rep. 2016;6:26496.

31. Larsson SC, Singleton AB, Nalls MA, Richards JB, International Parkinson's Disease Genomics C. No clear support for a role for vitamin D in Parkinson's disease: A Mendelian randomization study. Mov Disord. 2017;32:1249-1252.

32. Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, Ingelsson E, Karasik D, Pilz S, Berry D, Kestenbaum B, Zheng J, Luan J, Sofianopoulou E, Streeten EA, Albanes D, Lutsey PL, Yao L, Tang W, Econs MJ, Wallaschofski H, Volzke H, Zhou A, Power C, McCarthy MI, Michos ED, Boerwinkle E, Weinstein SJ, Freedman ND, Huang WY, Van Schoor NM, van der Velde N, Groot L, Enneman A, Cupples LA, Booth SL, Vasan RS, Liu CT, Zhou Y, Ripatti S, Ohlsson C, Vandenput L, Lorentzon M, Eriksson JG, Shea MK, Houston DK, Kritchevsky SB, Liu Y, Lohman KK, Ferrucci L, Peacock M, Gieger C, Beekman M, Slagboom E, Deelen J, Heemst DV, Kleber ME, Marz W, de Boer IH, Wood AC, Rotter JI, Rich SS, Robinson-Cohen C, den Heijer M, Jarvelin MR, Cavadino A, Joshi PK,

Wilson JF, Hayward C, Lind L, Michaelsson K, Trompet S, Zillikens MC, Uitterlinden AG, Rivadeneira F, Broer L, Zgaga L, Campbell H, Theodoratou E, Farrington SM, Timofeeva M, Dunlop MG, Valdes AM, Tikkanen E, Lehtimaki T, Lyytikainen LP, Kahonen M, Raitakari OT, Mikkila V, Ikram MA, Sattar N, Jukema JW, Wareham NJ, Langenberg C, Forouhi NG, Gundersen TE, Khaw KT, Butterworth AS, Danesh J, Spector T, Wang TJ, Hypponen E, Kraft P, Kiel DP. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nat Commun. 2018;9:260.

33. Howard DM, Adams MJ, Clarke T-K, Hafferty JD, Gibson J, Shirali M, Coleman JRI, Ward J, Wifmore EM, Aolloza C, Shen X, Barbu MC, Xu EY, Whalley HC, Marioni RE, Porteous DM, Davies G, Dreary IJ, Hermani G, Tian C, Hinds DA, Team aR, Consortium MDDWGotPG, Trzaskowski M, Byrne EM, Ripke S, Smith DJ, Sullivan PF, Wray NR, Breen G, Lewis CM, McIntosh AM. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. bioRxiv preprint. 2019.

34. Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A, Adams MJ, Agerbo E, Air TM, Andlauer TMF, Bacanu SA, Baekvad-Hansen M, Beekman AFT, Bigdeli TB, Binder EB, Blackwood DRH, Bryois J, Buttenschon HN, Bybjerg-Grauholm J, Cai N, Castelao E, Christensen JH, Clarke TK, Coleman JIR, Colodro-Conde L, Couvy-Duchesne B, Craddock N, Crawford GE, Crowley CA, Dashti HS, Davies G, Deary IJ, Degenhardt F, Derks EM, Direk N, Dolan CV, Dunn EC, Eley TC, Eriksson N, Escott-Price V, Kiadeh FHF, Finucane HK, Forstner AJ, Frank J, Gaspar HA, Gill M, Giusti-Rodriguez P, Goes FS, Gordon SD, Grove J, Hall LS, Hannon E, Hansen CS, Hansen TF, Herms S, Hickie IB, Hoffmann P, Homuth G, Horn C, Hottenga JJ, Hougaard DM, Hu M, Hyde CL, Ising M, Jansen R, Jin F, Jorgenson E, Knowles JA, Kohane IS, Kraft J, Kretzschmar WW, Krogh J, Kutalik Z, Lane JM, Li Y, Li Y, Lind PA, Liu X, Lu L, MacIntyre DJ, MacKinnon DF, Maier RM, Maier W, Marchini J, Mbarek H, McGrath P, McGuffin P, Medland SE, Mehta D, Middeldorp CM, Mihailov E, Milaneschi Y, Milani L, Mill J, Mondimore FM, Montgomery GW, Mostafavi S, Mullins N, Nauck M, Ng B, Nivard MG, Nyholt DR, O'Reilly PF, Oskarsson H, Owen MJ, Painter JN, Pedersen CB, Pedersen MG, Peterson RE, Pettersson E, Peyrot WJ, Pistis G, Posthuma D, Purcell SM, Quiroz JA, Qvist P, Rice JP, Riley BP, Rivera M, Saeed Mirza S, Saxena R, Schoevers R, Schulte EC, Shen L, Shi J, Shyn SI, Sigurdsson E, Sinnamon GBC, Smit JH, Smith DJ, Stefansson H, Steinberg S, Stockmeier CA, Streit F, Strohmaier J, Tansey KE, Teismann H, Teumer A, Thompson W, Thomson PA, Thorgeirsson TE, Tian C, Traylor M, Treutlein J, Trubetskoy V, Uitterlinden AG, Umbricht D, Van der Auwera S, van Hemert AM, Viktorin A, Visscher PM, Wang Y, Webb BT, Weinsheimer SM, Wellmann J, Willemsen G, Witt SH, Wu Y, Xi HS, Yang J, Zhang F, eQtlgen, andMe, Arolt V, Baune BT, Berger K, Boomsma DI, Cichon S, Dannlowski U, de Geus ECJ, DePaulo JR, Domenici E, Domschke K, Esko T, Grabe HJ,

Hamilton SP, Hayward C, Heath AC, Hinds DA, Kendler KS, Kloiber S, Lewis G, Li QS, Lucae S, Madden PFA, Magnusson PK, Martin NG, McIntosh AM, Metspalu A, Mors O, Mortensen PB, Muller-Myhsok B, Nordentoft M, Nothen MM, O'Donovan MC, Paciga SA, Pedersen NL, Penninx B, Perlis RH, Porteous DJ, Potash JB, Preisig M, Rietschel M, Schaefer C, Schulze TG, Smoller JW, Stefansson K, Tiemeier H, Uher R, Volzke H, Weissman MM, Werge T, Winslow AR, Lewis CM, Levinson DF, Breen G, Borglum AD, Sullivan PF, Major Depressive Disorder Working Group of the Psychiatric Genomics C. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat Genet. 2018;50:668-681.

35. Howard DM, Adams MJ, Shirali M, Clarke TK, Marioni RE, Davies G, Coleman JRI, Alloza C, Shen X, Barbu MC, Wigmore EM, Gibson J, andMe Research T, Hagenaars SP, Lewis CM, Ward J, Smith DJ, Sullivan PF, Haley CS, Breen G, Deary IJ, McIntosh AM. Genome-wide association study of depression phenotypes in UK Biobank identifies variants in excitatory synaptic pathways. Nat Commun. 2018;9:1470.

36. Hyde CL, Nagle MW, Tian C, Chen X, Paciga SA, Wendland JR, Tung JY, Hinds DA, Perlis RH, Winslow AR. Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. Nat Genet. 2016;48:1031-1036.

37. Michaelsson K, Melhus H, Larsson SC. Serum 25-Hydroxyvitamin D Concentrations and Major Depression: A Mendelian Randomization Study. Nutrients. 2018;10.

38. Dastani Z, Li R, Richards B. Genetic regulation of vitamin D levels. Calcif Tissue Int. 2013;92:106-117.

39. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics. 2015;31:3555-3557.

40. Angrist JD IG, Rubin DB. Identification of causal effects using instrumental variables. Am Stat Assoc. 1996;9:444-455.

41. Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. Stat Methods Med Res. 2007;16:309-330.

42. Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012;491:56-65.

43. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008;27:1133-1163.

44. Berry DJ, Vimaleswaran KS, Whittaker JC, Hingorani AD, Hypponen E. Evaluation of genetic markers as instruments for Mendelian randomization studies on vitamin D. PLoS One. 2012;7:e37465.

45. Staley JR, Blackshaw J, Kamat MA, Ellis S, Surendran P, Sun BB, Paul DS, Freitag D, Burgess S, Danesh J, Young R, Butterworth AS. PhenoScanner: a database of human genotype-phenotype associations. Bioinformatics. 2016;32:3207-3209.
46. Team RC: R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing; 2013.
47. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013;37:658-665.
48. Michaelsson K, Wolk A, Byberg L, Mitchell A, Mallmin H, Melhus H. The seasonal importance of serum 25-hydroxyvitamin D for bone mineral density in older women. J Intern Med. 2017;281:167-178.
49. Larsson SCT, M.; Mishra, A.; Howson, J.M.M; Michaëlsson, K.; Markus, H.S.; MEGASTROKE Project of the International Stroke Genetics Consortium. Serum 25-Hydroxyvitamin D Concentrations and Ischemic Stroke and Its Subtypes: A Mendelian Randomization Study. Stroke. 2018;49:2508-2511.
50. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44:512-525.
51. Greco MFD MC, Sheehan NA, Thompson JF. Detecting pleiotropy in mendelian rnaomdization studies with summary data and a continuous outcome. Stat Med. 2015;34:2926-2940.
52. Burgess S, Dudbridge F, Thompson SG. Combining information on multiple instrumental variables in Mendelian randomization: comparison of allele score and summarized data methods. Stat Med. 2016;35:1880-1906.
53. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016;40:304-314.
54. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018;50:693-698.
55. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, Tamez H, Zhang D, Bhan I, Karumanchi SA, Powe NR, Thadhani R. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013;369:1991-2000.
56. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. Int J Epidemiol. 2013;42:1497-1501.
57. May HT, Bair TL, Lappe DL, Anderson JL, Horne BD, Carlquist JF, Muhlestein JB. Association of vitamin D levels with incident depression among a general cardiovascular population. Am Heart J. 2010;159:1037-1043.

58. Milaneschi Y, Shardell M, Corsi AM, Vazzana R, Bandinelli S, Guralnik JM, Ferrucci L. Serum 25-hydroxyvitamin D and depressive symptoms in older women and men. J Clin Endocrinol Metab. 2010;95:3225-3233.
59. McGrath JJ, Burne TH, Feron F, Mackay-Sim A, Eyles DW. Developmental vitamin D deficiency and risk of schizophrenia: a 10-year update. Schizophr Bull. 2010;36:1073-1078.
60. McGrath JJ, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, Norgaard-Pedersen B, Hougaard DM, Mortensen PB. Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. Arch Gen Psychiatry. 2010;67:889-894.
61. Darryl W. Eyles MT, Anna A. E. Vinkhuyzen, Manuel Mattheisen, Sandra Meier, Helen Gooch, Victor Anggono, Xiaoying Cui, Men Chee Tan, Thomas H. J. Burne, Se Eun Jang, David Kvaskoff, David M. Hougaard, Bent Nørgaard-Pedersen, Arieh Cohen, Esben Agerbo, Carsten B. Pedersen, Anders D. Børglum, Ole Mors, Pankaj Sah, Naomi R. Wray, Preben B. Mortensen, John J. McGrath. The association between neonatal vitamin D status and risk of schizophrenia. Sci Rep. 2018;8.
62. Morales E, Romieu I, Guerra S, Ballester F, Rebagliato M, Vioque J, Tardon A, Rodriguez Delhi C, Arranz L, Torrent M, Espada M, Basterrechea M, Sunyer J, Project I. Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. Epidemiology. 2012;23:64-71.
63. Tolppanen AM, Sayers A, Fraser WD, Lewis G, Zammit S, Lawlor DA. The association of serum 25-hydroxyvitamin D3 and D2 with depressive symptoms in childhood--a prospective cohort study. J Child Psychol Psychiatry. 2012;53:757-766.
64. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Jarvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hypponen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010;376:180-188.
65. Burgess S, Davies NM, Thompson SG, Consortium EP-I. Instrumental variable analysis with a nonlinear exposure-outcome relationship. Epidemiology. 2014;25:877-885.
66. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23:R89-98.

67. Okereke OI, Reynolds CF, 3rd, Mischoulon D, Chang G, Cook NR, Copeland T, Friedenberg G, Buring JE, Manson JE. The VITamin D and OmegA-3 TriaL-Depression Endpoint Prevention (VITAL-DEP): Rationale and design of a large-scale ancillary study evaluating vitamin D and marine omega-3 fatty acid supplements for prevention of late-life depression. Contemporary clinical trials. 2018;68:133-145.

Appendix

Supplemental Table 2.1 Association between 25(OH)D SNPs and other phenotypes as identified by genome-wide association studies

SNP	Chr	Gene	Associated traits in European populations (p<5E-08)
rs3755967	4	GC	Blood cell counts (white blood cell, myeloid white cell, basophil, eosinophil, neutrophil, granulocyte)
rs10741657	11	CYP2R1	Height
rs12785878	11	NADSYN1/ DHCR7	-
rs17216707	20	CYP24A1	Glomerular filtration rate, creatinine
rs10745742	12	AMDHD1	-
rs8018720	14	SEC23A	-

Supplemental Table 2.2 Association between depression SNPs and other phenotypes as identified by genome-wide association studies

SNP	Chr	Gene	Associated traits in European populations (p<5E-08)
rs2568958	1	LOC105378797	BMI, obesity, overweight, weight, gene expression, age at menarche, arm fat, basal metabolic weight, impedance of leg, leg fat, education, self-reported non-cancer illness, trunk fat, sodium in urine, seen doctor for nerves/anxiety/depression
rs10890020	1	LOC105378800	-
rs10913112	1	RFWD2	-
rs301799	1	RERE	Eosinophil/basophil/lymphocyte count, neutrophil granulocytes, allergic disease, arm fat, asthma, body fat, DBP/SBP, allergy, bone mineral density, leg fat, hypertension, trunk fat, vascular/heart problems
rs1002656	1	-	Neuroticism
rs1890946	1	NRDC	-
rs72710803	1	-	-
rs11579246	1	ELAVL4	-
rs113188507	1	-	Body fat, leg fat, trunk fat, waist circumference
rs169235	1	CACNA1E	Neuroticism
rs1466887	1	-	-
rs10789214	1	SGIP1	-
rs1568452	2	LOC105377628	Impedance of leg
rs62188629	2	-	No medication for pain relief, constipation, heartburn
rs1226412	2	LINC01876	Neuroticism
rs7585722	2	RNF103-CHMP3	-
rs1095626	3	RSRC1	BMI, forced vital capacity (FVC), height
rs4346585	3	-	-
rs141954845	3	FHIT	-
rs13084037	3	KLHDC8B	Age at menarche, hemoglobin, frequency of tiredness/lethargy, impedance of arm/whole body, miserableness, overall health rating, pulse rate, education

Supplemental Table 2.2 (continued)

SNP	Chr	Gene	Associated traits in European populations (p<5E-08)
rs6783233	3	LOC101926953	Age at menarche, seen doctor for nerves/anxiety/tension/depression
rs35553410	4	-	-
rs7659414	4	-	Impedance of leg
rs7685686	4	HTT	Alcohol intake frequency, tiredness/lethargy frequency
rs11135349	5	-	Hip circumference
rs10061069	5	FAM172A	-
rs60157091	5	-	Mean platelet volume
rs200949	6	HIST1H1B	Blood cell counts (eosinophil, granulocyte, hematocrit, reticulocyte, lymphocyte, monocyte, myeloid white cell, neutrophil, basophil, neutrophil, white blood cell), hemoglobin concentration, IgA deficiency, DBP, FEV-1, FVC, guilty feelings, hearing problems, intestinal malabsorption, medication for pain relief/constipation/heart burn, dental problems, potassium in urine, seen doctor/psychiatrist for nerves/anxiety/tension/depression, thyroid disorders, sarcoidosis, treatment with insulin
rs9363467	6	-	-
rs2043539	7	TMEM106B	Miserableness, mood swings, neuroticism, seen doctor for nerves/anxiety/tension/depression
rs58104186	7	-	Tiredness/lethargy frequency
rs7807677	7	CTTNBP2	Smoking
rs3823624	7	MAD1L1	Age at first live birth, arm/leg fat, body fat, BMI, impedance of leg, irritability, miserableness, self-reported non-cancer illnesses, overall health rating, education, prescription medications, trunk fat, physical activity
rs16887442	7	-	-
rs7837935	8	CYP7B1	-
rs7030813	9	PAX5	-
rs10817969	9	ASTN2	-

Supplemental Table 2.2 (continued)

SNP	Chr	Gene	Associated traits in European populations (p<5E-08)
rs3793577	9	ELAVL2	Depressed mood, guilty feelings, neuroticism, waist circumference
rs2670139	9	DENND1A	-
rs34653192	9	LOC105376009	-
rs1354115	9	-	-
rs913930	9	-	-
rs1021363	10	SORCS3	Seen doctor for nerves/anxiety/tension/depression
rs997934	10	LOC105376345	-
rs7932640	11	GRM5	Seen doctor for nerves/anxiety/tension/depression
rs61902811	11	LOC105369501	Guilty feelings, miserableness, mood swings, neuroticism, seen doctor for nerves/anxiety/tension/depression, worry, neuroticism
rs1448938	11	DCDC1	Red blood cell count, impedance of arm/whole body
rs7117514	11	SHANK2	-
rs2509805	11	-	Red wine intake, walking, neuroticism, physical activity, worry
rs58621819	11	LTBP3	Platelet count, red cell distribution, arm/leg/trunk fat/mass, basal metabolic rate, body fat percentage, BMI, bone mineral density, impedance of arm, pulse rate, waist circumference, weight
rs57344483	11	-	-
rs2187490	11	-	-
rs56314503	12	LOC107984536	-
rs3213572	12	SPPL3	Allergic disease, height
rs10774600	12	ATP2A2	Red blood cell count, eosinophil/basophil counts
rs1343605	13	-	Sleeplessness/insomnia
rs4772087	13	STK24	-
rs9592461	13	PCDH9	-
rs1409379	13	-	-

Supplemental Table 2.2 (continued)

SNP	Chr	Gene	Associated traits in European populations (p<5E-08)
rs9545360	13	-	-
rs10149470	14	-	Monocyte count, height, cognitive ability, intelligence, trunk mass
rs61990288	14	LRFN5	-
rs1045430	14	AREL1	Nervous feelings, neuroticism, worrier/anxious feelings
rs1152578	14	ESR2	-
rs1956373	14	RTN1	-
rs8037355	15	-	-
rs34488670	15	SEMA6D	Tobacco smoking, education
rs56887639	16	-	Depressive symptoms
rs7200826	16	SHISA9	-
rs7198928	16	RBFOX1	Neuroticism, worrying
rs7193263	16	RBFOX1	Depressive symptoms
rs12967143	18	TCF4	Height, irritability, neuroticism, seen doctor for nerves/anxiety/tension/depression
rs12967855	18	CELF4	Fed-up feelings, guilty feelings, mood swings, neuroticism, overall health, education, seen doctor for nerves/anxiety/tension/depression, sensitivity/hurt feelings, TV watching, worrying
rs12966052	18	LOC105372125	Mood swings
rs7227069	18	DCC	Fed-up feelings, depressed mood, tiredness/lethargy, low enthusiasm/disinterest, miserableness, mood swings, neuroticism, sitting height, tense/highly strung
rs7241572	18	KCNG2	Wheeze or whistling in chest
rs62091461	18	RAB27B	Arm/leg mass, basal metabolic rate, BMI, body size, hip circumference, weight, body fat
rs33431	19	ZNF536	-
rs143186028	20	EMILIN3	-

Supplemental Table 2.2 (continued)

SNP	Chr	Gene	Associated traits in European populations (p<5E-08)
rs12624433	20	SLC12A5	Rheumatoid arthritis
rs5995992	22	EP300	Irritability, mood swings, neuroticism, neutrophil percentage of granulocytes

Supplemental Table 2.3 Characteristics of genome-wide significant 25(OH)D SNPs used as genetic instruments in the 25(OH)D to depression MR analysis

SNP	Chr	Gene	Vitamin D-related pathway	EA[c]	Serum 25(OH)D results[a]		Depression results[b]	
					Effect on ln(25OHD) (nmol/L)	p-value	OR[d]	p-value
rs3755967	4	GC	Metabolism	C	0.089	4.74E-343	1.00	0.36
rs10741657	11	CYP2R1	Synthesis	A	0.031	2.05E-46	0.99	0.01
rs12785878	11	NADSYN1/ DHCR7	Synthesis	T	0.036	3.80E-62	1.00	0.31
rs17216707	20	CYP24A1	Metabolism	T	0.026	8.14E-23	1.00	0.64
rs10745742	12	AMDHD1	-	T	0.017	1.88E-14	1.00	0.49
rs8018720	14	SEC23A	-	G	0.017	4.72E-09	1.00	0.65

25(OH)D: 25-hydroxyvitamin D; Chr: chromosome; EA: effect allele; OR: odds ratio of depression per effect allele increase; SNP: single-nucleotide polymorphism.
[a] Summary statistics obtained from the SUNLIGHT GWAS meta-analysis (n=79,366)
[b] Summary statistics obtained from the PGC-MDD2, UKB, 23andMe GWAS meta-analysis (Howard *et al.*; n=807,553)
[c] 25(OH)D-increasing allele
[d] Effect size estimates represents the OR of depression per effect allele, based on the results from the PGC-MDD2, UKB, 23andMe GWAS meta-analysis.

Supplemental Table 2.4 Sensitivity analyses MR estimates for the effect of 25(OH)D levels on the risk of depression

IVW Model	No. SNPs	OR	95% CI	p-value
Excluding rs12785878 (*DHCR7*)	5	0.98	0.95, 1.01	0.22
Excluding rs3755967 (*GC*)	5	0.96	0.91, 1.01	0.11

IVW: Inverse-variance weighted; OR: odds ratio for depression per 1 SD increase in ln(25(OH)D); SNPs: single-nucleotide polymorphism

Supplemental Table 2.5 Characteristics of genome-wide significant depression SNPs used as genetic instruments in the depression to 25(OH)D MR analysis

SNP	Chr	Gene	Depression results[a]				Serum 25(OH)D results[b]					
			EA[c]	EAF	OR	p	Proxy SNP[d]	R2	EA[e]	Effect on ln(25(OH)D)[f]	SE	p
rs2568958	1	LOC105378797	A	0.62	1.04	8.47E-25	-	-	-	-0.002	0.00	0.37
rs10890020	1	LOC105378800	A	0.52	0.97	4.03E-15	rs10789363	1.00	A	-5.00E-04	0.00	0.82
rs10913112	1	RFWD2	T	0.38	0.97	3.40E-13	-	-	-	0.002	0.00	0.47
rs301799	1	RERE	T	0.57	0.98	1.36E-12	-	-	-	0.005	0.00	0.01
rs1002656	1	-	T	0.70	0.97	3.74E-12	-	-	-	0.002	0.00	0.29
rs1890946	1	NRDC	T	0.47	0.98	2.68E-11	-	-	-	0.002	0.00	0.28
rs72710803	1	-	A	0.91	0.96	5.29E-11	rs11579578	0.96	T	-0.002	0.00	0.63
rs11579246	1	ELAVL4	A	0.91	1.04	5.71E-10	-	-	-	-5.00E-04	0.00	0.87
rs113188507	1	-	A	0.28	1.02	1.87E-08	rs35020936	0.95	A	1.00E-04	0.00	0.98
rs169235	1	CACNA1E	A	0.75	0.98	2.98E-08	rs199930	0.92	T	-0.002	0.00	0.46
rs1466887	1	-	T	0.55	0.98	4.12E-08	rs1542886	0.80	A	-0.001	0.00	0.57
rs10789214	1	SGIP1	T	0.57	1.02	4.44E-08	-	-	-	-6.00E-04	0.00	0.79
rs1568452	2	LOC105377628	T	0.39	1.03	8.12E-12	-	-	-	2.00E-04	0.00	0.91
rs62188629	2	-	A	0.31	1.02	7.13E-10	rs7586277	0.99	A	4.00E-04	0.00	0.84
rs1226412	2	LINC01876	T	0.79	1.03	3.46E-09	rs2697360	0.98	A	0.004	0.00	0.13
rs7585722	2	RNF103-CHMP3	T	0.85	0.97	2.68E-08	-	-	-	-0.003	0.00	0.37
rs1095626	3	RSRC1	T	0.58	0.97	7.13E-14	rs827113	1.00	T	8.00E-04	0.00	0.69
rs4346585	3	-	T	0.70	0.98	7.13E-10	rs13058822	1.00	A	0.002	0.00	0.35

Supplemental Table 2.5 (continued)

SNP	Chr	Gene	Depression results[a]				Serum 25(OH)D results[b]					
			EA[c]	EAF	OR	p	Proxy SNP[d]	R2	EA[e]	Effect on ln(25(OH)D)[f]	SE	p
rs141954845	3	FHIT	A	0.39	1.02	8.15E-10	rs1916801	0.99	A	5.00E-04	0.00	0.81
rs13084037	3	KLHDC8B	A	0.77	0.98	7.08E-09	-	-	-	-0.001	0.00	0.65
rs6783233	3	LOC101926953	T	0.28	1.02	2.90E-08	-	-	-	-0.003	0.00	0.22
rs35553410	4	-	T	0.75	0.98	1.42E-09	rs1022079	0.97	A	2.00E-04	0.00	0.94
rs7659414	4	-	A	0.58	0.98	1.20E-08	-	-	-	-0.004	0.00	0.06
rs7685686	4	HTT	A	0.58	1.02	2.57E-08	-	-	-	-0.005	0.00	0.02
rs11135349	5	-	A	0.47	0.97	6.04E-17	rs10035449	0.82	T	0.004	0.00	0.05
rs10061069	5	FAM172A	C	0.22	0.97	8.15E-11	-	-	-	-2.00E-04	0.00	0.93
rs60157091	5	-	T	0.52	1.02	1.42E-08	rs10939933	1.00	C	0.003	0.00	0.14
rs200949	6	HIST1H1B	A	0.87	1.05	2.53E-19	-	-	-	3.00E-04	0.00	0.94
rs9363467	6	-	T	0.60	1.02	6.44E-11	rs9351533	0.93	A	0.003	0.00	0.18
rs2043539	7	TMEM106B	A	0.42	1.03	9.89E-15	-	-	-	3.00E-04	0.00	0.91
rs58104186	7	-	A	0.47	1.02	1.82E-11	rs11561993	0.99	T	0.003	0.00	0.18
rs7807677	7	CTTNBP2	T	0.55	1.02	1.82E-11	-	-	-	9.00E-04	0.00	0.66
rs3823624	7	MAD1L1	T	0.81	1.03	1.99E-09	-	-	-	-0.006	0.00	0.02
rs16887442	7	-	T	0.43	1.02	8.62E-09	rs4556040	1.00	T	-8.00E-04	0.00	0.70
rs7837935	8	CYP7B1	T	0.15	0.97	3.34E-09	-	-	-	0.002	0.00	0.48

Supplemental Table 2.5 (continued)

SNP	Chr	Gene	Depression results[a]				Serum 25(OH)D results[b]					
			EA[c]	EAF	OR	p	Proxy SNP[d]	R2	EA[e]	Effect on ln(25(OH)D)[f]	SE	p
rs7030813	9	PAX5	T	0.37	1.03	3.07E-12	rs1329572	0.94	A	-0.001	0.00	0.66
rs10817969	9	ASTN2	T	0.72	1.03	3.11E-11	rs2418449	1.00	T	0.001	0.00	0.67
rs3793577	9	ELAVL2	A	0.47	0.98	8.41E-11	-	-	-	-0.004	0.00	0.03
rs2670139	9	DENND1A	T	0.76	0.97	1.21E-10	-	-	-	-0.002	0.00	0.30
rs34653192	9	LOC105376009	C	0.32	0.98	2.23E-09	rs1414019	0.91	A	8.00E-04	0.00	0.75
rs1354115	9	-	A	0.62	1.02	7.08E-09	-	-	-	-9.00E-04	0.00	0.68
rs913930	9	-	A	0.64	0.98	2.42E-08	-	-	-	0.002	0.00	0.45
rs1021363	10	SORCS3	A	0.35	1.03	4.41E-16	-	-	-	0.003	0.00	0.24
rs997934	10	LOC105376345	T	0.38	1.02	4.81E-08	-	-	-	-3.00E-04	0.00	0.90
rs7932640	11	GRM5	T	0.44	1.03	1.62E-15	-	-	-	-0.002	0.00	0.41
rs61902811	11	LOC105369501	A	0.37	0.97	1.40E-12	rs17602038	0.98	T	-0.001	0.00	0.67
rs1448938	11	DCDC1	A	0.42	1.02	1.30E-09	-	-	-	0.000	0.00	0.99
rs7117514	11	SHANK2	A	0.54	0.98	7.29E-09	rs11237238	0.97	A	0.003	0.00	0.20
rs2509805	11	-	T	0.32	1.02	9.17E-09	rs682503	0.81	C	-0.001	0.00	0.64
rs58621819	11	LTBP3	A	0.79	0.98	1.57E-08	rs947791	0.99	A	-0.006	0.00	0.04
rs57344483	11	-	A	0.93	0.96	1.82E-08	rs7115915	1.00	T	-0.007	0.00	0.04
rs2187490	11	-	T	0.91	0.97	3.82E-08	-	-	-	0.002	0.00	0.66

Supplemental Table 2.5 (continued)

SNP	Chr	Gene	Depression results[a]				Serum 25(OH)D results[b]					
			EA[c]	EAF	OR	p	Proxy SNP[d]	R2	EA[e]	Effect on ln(25(OH)D)[f]	SE	p
rs56314503	12	LOC107984536	T	0.75	0.97	2.95E-10	rs41466449	0.96	A	0.003	0.01	0.53
rs3213572	12	SPPL3	A	0.47	1.02	7.61E-10	-	-	-	0.002	0.00	0.39
rs10774600	12	ATP2A2	T	0.17	0.97	3.39E-08	rs1265775	0.96	A	-0.002	0.00	0.49
rs1343605	13	-	A	0.38	1.03	6.23E-18	rs2806949	0.99	A	-0.002	0.00	0.26
rs4772087	13	STK24	T	0.37	1.02	3.91E-10	-	-	-	-0.004	0.00	0.03
rs9592461	13	PCDH9	A	0.49	1.02	9.10E-10	-	-	-	-0.004	0.00	0.04
rs1409379	13	-	T	0.76	1.03	1.67E-09	-	-	-	0.003	0.00	0.27
rs9545360	13	-	A	0.18	0.97	5.02E-09	rs9531035	0.99	A	-0.001	0.00	0.73
rs10149470	14	-	A	0.49	0.97	3.72E-14	-	-	-	-4.00E-04	0.00	0.85
rs61990288	14	LRFN5	A	0.51	0.97	1.68E-13	rs17781288	0.99	C	9.00E-04	0.00	0.66
rs1045430	14	AREL1	T	0.48	0.98	7.31E-13	-	-	-	-7.00E-04	0.00	0.72
rs1152578	14	ESR2	T	0.44	0.98	6.36E-10	-	-	-	-0.003	0.00	0.18
rs1956373	14	RTN1	T	0.74	0.98	2.06E-08	-	-	-	0.002	0.00	0.35
rs8037355	15	-	T	0.56	0.98	3.94E-11	rs16964908	0.88	A	-2.00E-04	0.00	0.92
rs34488670	15	SEMA6D	T	0.79	0.98	6.03E-09	rs12901436	1.00	C	-5.00E-04	0.00	0.83
rs56887639	16	-	A	0.73	0.97	1.51E-12	rs4456522	0.93	A	-0.003	0.00	0.28
rs7200826	16	SHISA9	T	0.26	1.03	3.74E-12	rs12921037	0.84	C	4.00E-04	0.00	0.87
rs7198928	16	RBFOX1	T	0.62	1.02	4.45E-11	rs3785238	0.91	A	-6.00E-04	0.00	0.78

Supplemental Table 2.5 (continued)

SNP	Chr	Gene	Depression results[a]				Serum 25(OH)D results[b]					
			EA[c]	EAF	OR	p	Proxy SNP[d]	R2	EA[e]	Effect on ln(25(OH)D)[f]	SE	p
rs7193263	16	RBFOX1	A	0.67	0.98	4.33E-10	rs7188257	0.88	T	-0.010	0.00	0.02
rs12967143	18	TCF4	C	0.70	0.97	3.70E-16	-	-	-	-9.00E-04	0.00	0.70
rs12967855	18	CELF4	A	0.33	1.03	1.18E-12	-	-	-	0.004	0.00	0.10
rs12966052	18	LOC105372125	C	0.18	0.97	1.25E-11	-	-	-	-0.003	0.00	0.25
rs7227069	18	DCC	A	0.43	1.02	1.50E-11	rs7232543	0.96	A	-0.002	0.00	0.25
rs7241572	18	KCNG2	A	0.20	1.03	2.70E-10	-	-	-	-4.00E-04	0.00	0.88
rs62091461	18	RAB27B	T	0.23	0.97	1.95E-09	rs12456731	0.81	T	-4.00E-04	0.00	0.85
rs33431	19	ZNF536	T	0.61	1.02	4.81E-08	-	-	-	0.002	0.00	0.34
rs143186028	20	EMILIN3	T	0.18	1.03	2.29E-09	rs6065338	0.95	A	-0.005	0.00	0.05
rs12624433	20	SLC12A5	A	0.26	1.02	7.44E-09	-	-	-	0.003	0.00	0.26
rs5995992	22	EP300	T	0.72	0.97	1.30E-11	rs11090039	0.85	A	-0.006	0.00	0.02

25(OH)D: 25-hydroxyvitamin D; Chr: chromosome; EA: effect allele; OR: odds ratio of depression per effect allele increase; SNP: single-nucleotide polymorphism.

[a] Summary statistics obtained from the PGC-MDD2, UKB, 23andMe GWAS meta-analysis (Howard *et al.*; n=807,553)

[b] Summary statistics acquired from the SUNLIGHT GWAS meta-analysis (n=79,366)

[c] Allele associated with the increased risk of depression

[d] Proxy SNP in SUNLIGHT GWAS

[e] Effect allele in the SUNLIGHT GWAS (prior to data harmonization)

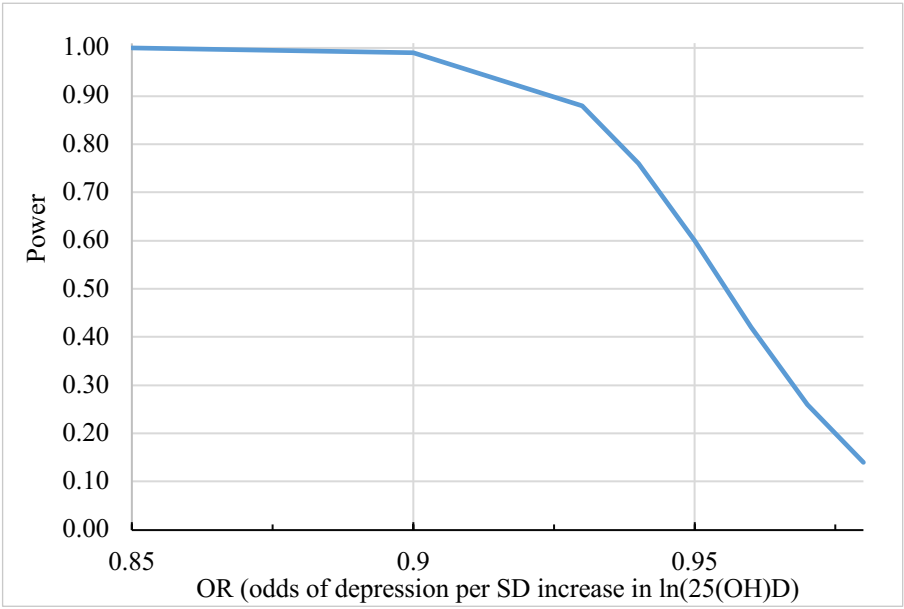
[f] Effect size estimates represent the change in log-transformed 25(OH)D (nmol/L) per effect allele, based on the beta coefficients in the SUNLIGHT GWAS

Supplemental Table 2.6 Sensitivity analyses MR estimates for the effect of depression case status on 25(OH)D levels

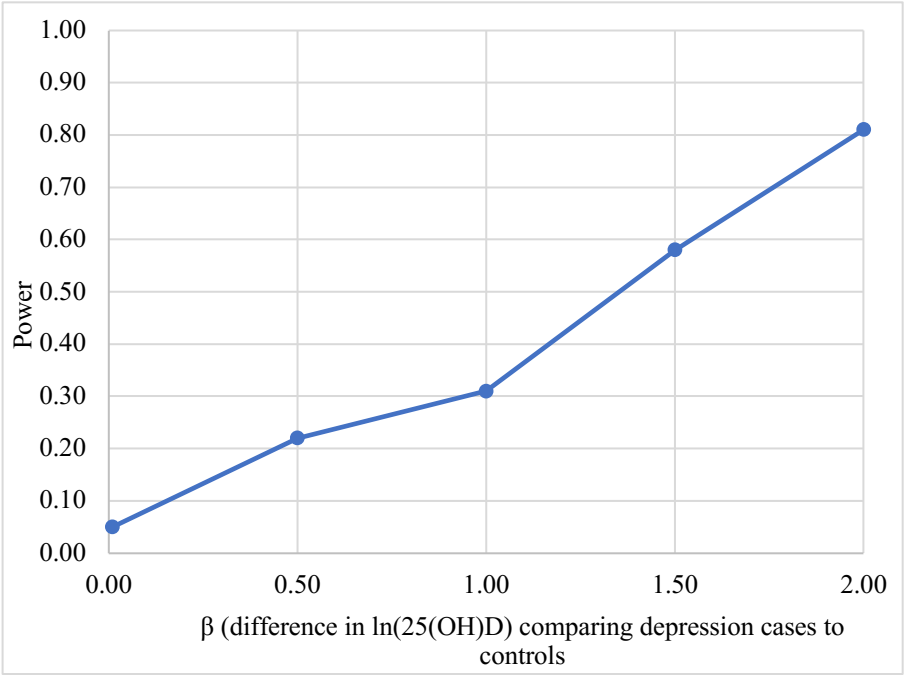
IVW Model	No. SNPs	Beta[a]	95% CI	p-value
Excluding 12 SNPs with potential associations with confounding factors[b]	69	-0.002	-0.03, 0.02	0.85

CI: confidence interval; IVW: inverse-variance weighted; SNPs: single-nucleotide polymorphism.
[a] Beta: Change in ln(25(OH)D) comparing depression case to control status
[b] Excluded SNPs: rs2568958, rs301799, rs113188507, rs1095626, rs13084037, rs7807677, rs3823624, rs2509805, rs58621819, rs34488670, rs12967855, and rs62091461

(a)



(b)



Supplemental Figure 2.1 Power calculation plots for varying effect estimates for the MR analyses.
(a) ln(25(OH)D) (nmol/L) to depression and (b) depression to ln(25(OH)D) (nmol/L).

Chapter 3. Maternal vitamin D status during pregnancy and childhood/adolescent depression: Results from the Avon Longitudinal Study of Parents and Children (ALSPAC)

Min-Jung Wang[1], Erin C. Dunn[2, 3, 4, 5], Peter Kraft[1, 6], Olivia I. Okereke[1, 3, 7], Yiwen Zhu[2], Jordan W. Smoller[1, 2, 3, 4]

[1] Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA
[2] Psychiatric and Neurodevelopmental Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA
[3] Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA
[4] Stanley Center for Psychiatric Research, The Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA
[5] Henry and Allison McCance Center for Brain Health, Massachusetts General Hospital, Boston, MA, USA
[6] Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[7] Department of Medicine, Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, Massachusetts, USA

Abstract

Background: Low maternal vitamin D levels [serum 25-hydroxyvitamin D (25(OH)D)] during pregnancy have been linked to offspring neuropsychiatric outcomes such as schizophrenia and autism, but studies on depression are lacking. This study examined the association between maternal vitamin D status during pregnancy and offspring depression during childhood and adolescence, and whether any associations were modified by offspring genetic risk for depression.

Methods: Mother-singleton offspring pairs in the Avon Longitudinal Study of Parents and Children (ALSPAC) that had maternal 25(OH)D measurements, offspring genetic data, and offspring depression measures collected in childhood (mean age=10.6 years; n=2938) and/or adolescence (mean age=13.8 years; n=2485) were included in the analyses. Using multivariable logistic regression models, we assessed relations of maternal vitamin D status and offspring polygenic risk score (PRS) for depression to childhood/adolescent depression risk.

Results: There was little evidence for an association between maternal vitamin D status during pregnancy and offspring depression in childhood (p-trend=0.72) or adolescence (p-trend=0.07), after adjusting for potential confounders. Offspring depression PRS were independently associated with childhood depression (p-trend=0.003) but did not interact with maternal vitamin D status. These results were robust to adjustments for potential confounders and different cut-offs for vitamin D insufficiency/deficiency.

Conclusion: These findings suggest that maternal vitamin D status during pregnancy does not affect the offspring's risk for early life depression.

Keywords: Vitamin D, polygenic risk, gene-environment interactions, depression

Introduction

Vitamin D deficiency among pregnant women is highly prevalent globally, and particularly in geographical regions at higher latitudes, such as Northern Europe and North America (1, 2), where it affects up to 80% of dark-skinned women and 47% of light-skinned women (2-5). Maintaining sufficient vitamin D levels during pregnancy is important, given that it is the sole source of fetal vitamin D, which may play a crucial role in fetal programming and brain development. Since vitamin D levels can be easily increased by lifestyle modifications and supplementation, understanding the influence of maternal vitamin D levels on offspring health outcomes can inform early prevention efforts.

Possible mechanisms linking gestational vitamin D exposure to neurodevelopment include the role of vitamin D in neuronal growth and the signaling and regulation of endocrine functions (6). Animal studies reported that vitamin D deficiency during pregnancy resulted in alterations in brain morphology and functioning of rat offspring with long-lasting effects on behavior spanning into adulthood (6-9). Epidemiological research in humans has provided some evidence suggesting that maternal vitamin D status, defined by circulating levels of 25-hydroxyvitamin D [25(OH)D], during gestation could influence neurocognitive and mental health outcomes in offspring (10-13) and the subsequent development of neuropsychiatric disorders including schizophrenia, autism, and attention deficit/hyperactivity disorder (ADHD) (9, 14-17). As such, early life vitamin D deficiency in offspring may also be a risk factor for the subsequent onset of depression. In a prospective cohort study of children, higher serum 25(OH)D levels during childhood were associated with lower depressive symptoms in adolescence (18). However, studies examining the relationship between maternal 25(OH)D levels during gestation and offspring depression are lacking. To our knowledge, only one study has assessed the effect of low gestational vitamin D levels on the risk of offspring depression (13). However, the relatively small sample size of 850 mother-child pairs and relatively crude definition for depression rendered findings inconclusive.

It is well-established that genetic factors contribute to the risk for depression in both children and young adults (19, 20). Understanding the interactions between maternal vitamin D status during pregnancy and polygenic risk scores (PRS) for depression could potentially have important implications on public health interventions. For instance, the presence or absence of gene-environment interactions (GxE) could inform whether preventive strategies should take on a targeted or universal approach (21).

In the current study, we used data from the Avon Longitudinal Study of Parents and Children study (ALSPAC) (22), a birth cohort study based in South West England, to examine the prospective association between maternal vitamin D status during pregnancy and offspring depression in childhood and adolescence, and to investigate whether the effects of maternal vitamin D status were modified by offspring genetic risk for depression.

Methods

Study Population

ALSPAC is an ongoing population-based birth cohort from South West England. Detailed information about ALSPAC has been described previously (22, 23) and is available on the study website (http://www.bristol.ac.uk/alspac), which also includes a searchable data dictionary. In brief, the study enrolled 14,541 pregnant women with estimated delivery dates between April 1, 1991 and December 31, 1992, which resulted in 14,062 live births (24). Ethical approval was obtained from the ALSPAC Law and Ethics Research Committee and National Health Service (NHS) local research ethics committee, and all participants provided written informed consent at enrolment. Data on health, physical, and psychological development were collected from the parents and children at regular clinic visits and via self-reported questionnaires.

The eligible study sample included mother-offspring pairs with singleton births and complete data on maternal 25(OH)D levels during pregnancy, offspring genetic data, and offspring depressive

symptoms collected during childhood and/or adolescence. Since less than 3% of the ALSPAC cohort were of black or mixed race/ethnicity (25), only white participants were included. Mothers were excluded if their total 25(OH)D levels were three standard deviations (SD) above or below the study population mean (n=39). This yielded analytic sample sizes of 2,938 (child subsample) and 2,485 (adolescent subsample) (**Figure 3.1**).

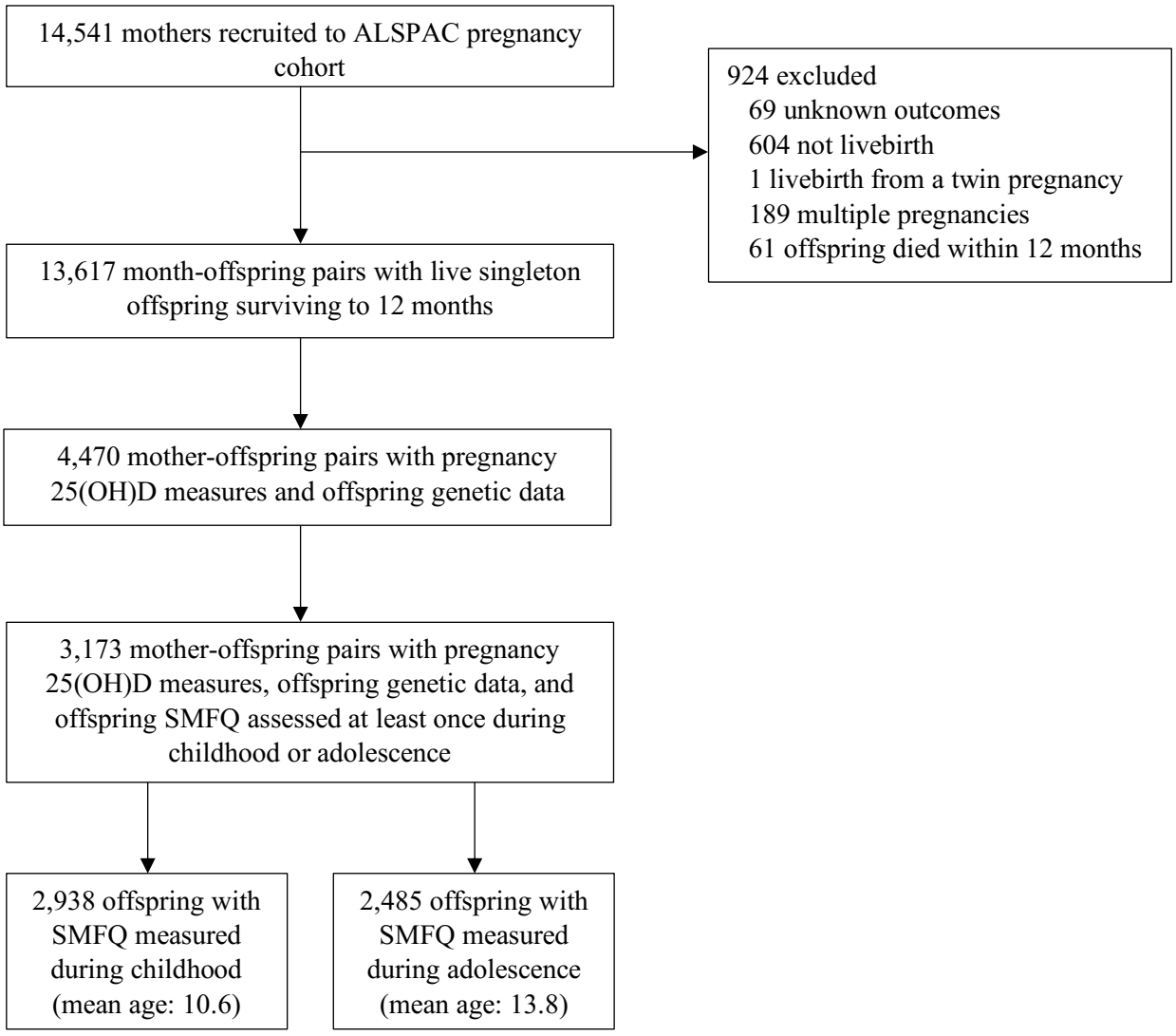


Figure 3.1 Study sample flow chart

Offspring Depression

Depressive symptoms were measured using the Short Moods and Feelings Questionnaire (SMFQ) by a trained interviewer during childhood (mean: 10.62; SD: 0.25) and adolescence (mean: 13.83; SD: 0.21). The SMFQ consists of 13 items that ascertain depressive symptoms in the past 2 weeks. Total SMFQ scores were obtained by summing across all items (possible score range: 0-26), with higher scores corresponding to higher depressive symptoms. The SMFQ has been validated in individuals aged between 6 and 18, and correlates highly with both the Children's Depression Inventory scores and past-year Diagnostic Interview for Children depression scores (26). Since the raw SMFQ scores were positively skewed (skewness: 1.36 for childhood subsample, 1.46 for adolescent sample) and remained skewed after log-transformations, the SMFQ was dichotomized, with depression defined as SMFQ≥11. This threshold has been shown to have high sensitivity and specificity for depression defined by the revised Diagnostic and Statistical Manual of Mental Disorders, Third Edition (DSM-III-R) (27), and has been used in prior ALSPAC studies of depression (28, 29).

Serum 25(OH)D Measurements

Maternal serum 25(OH)D_2 and 25(OH)D_3, were measured on non-fasting blood samples taken for routine pregnancy tests at any point during pregnancy. Blood samples were assayed using high-performance liquid chromatography tandem mass spectrometry (HPLC/MS) in accordance with Vitamin D External Quality Assessment Scheme requirements. Details about sampling, storage, and processing are described in detail elsewhere (30). For the few mothers who had multiple measurements taken during pregnancy (4.8%) (31), the last result available was used, in line with similar studies that have assessed the influence of maternal 25(OH)D concentrations during pregnancy on child health outcomes (30, 32). Total 25(OH)D was calculated by summing 25(OH)D_2 and 25(OH)D_3 levels.

As total maternal 25(OH)D levels displayed sinusoidal seasonal variation (**Supplemental Figure 3.1**), a seasonality-adjusted 25(OH)D variable was derived to represent mean annual levels of 25(OH)D. Similar trigonometric models have been previously applied to adjust for seasonal variation in 25(OH)D in ALSPAC (30, 32), and has been shown to have good validity (33). Further details of the method are described in the **Supplementary Methods**. Briefly, 25(OH)D was modeled against sine and cosine transformations of month of blood sampling in a linear regression model. The mean annual 25(OH)D concentration for each mother was then estimated using the residuals of the model. The seasonality-adjusted and unadjusted 25(OH)D levels were strongly correlated (Spearman's r=0.88).

Genetic Data

9,912 children in the ALSPAC cohort were genotyped on the Illumina HumanHap550-Quad genome-wide single nucleotide polymorphisms (SNPs) genotyping platform. Standard quality control (QC) measures were performed to exclude individuals on the basis of gender mismatch, minimal or excessive heterozygosity, individual genotyping call rates<97%, cryptic relatedness (IBD>10%), and non-European ancestry (assessed using multidimensional scaling analysis and compared to HapMap II). SNPs were excluded based on the following criteria: minor allele frequency (MAF)<1%; missing rate>5%; and significant deviation from Hardy-Weinberg Equilibrium (HWE) ($p<5x10^{-7}$). 500,527 directly genotyped SNPs and 8,365 children remained after quality control (QC). Imputation was conducted using Impute V2.2.2 (34) against the 1000 genomes reference panel (Phase 1, version 3) (35), with 2186 reference haplotypes (including non-Europeans).

Polygenic Risk Scores (PRS)

PRS for depression were generated using methods described by Purcell *el al.* in PLINK v.1.90 (36). The Psychiatric Genomics Consortium wave 2 (PGC-MDD2) (37) was used as the discovery sample

(meta-analytic subsample excluding 23andMe: n=173,005) and the ALSPAC cohort was used as the training sample. Additional details about the data sources and QC procedures prior to PRS construction are provided in the **Supplementary Methods**. We generated a single PRS for each offspring based on a threshold of $p<0.05$, which was the threshold that maximized the variance explained in major depression (37). PRS was generated by summing the risk alleles (0, 1, or 2) for each SNP, weighted by the natural log-transformed odds ratio (OR) for its association with major depressive disorder in the PGC-MDD2. PRS scores were then standardized using the z-transformation and categorized into three risk groups (low risk: quartile 1; medium risk: quartiles 2 and 3; high risk: quartile 4) (**Supplemental Figure 3.2**).

Missing Data

In the analytic samples, a substantial proportion of participants had missing data on one or more of the covariates. For any single covariate, the amount of missingness ranged between 0.3% and 20.9%, and 71.2% and 72.9% had complete covariate data for the child and adolescent subsamples, respectively. Missing covariate information was imputed using multiple imputation by chained equations (MICE) in the *mice* (version 2.30) R package (38) (**Supplementary Methods**). The distribution of covariates with missing data in the observed data was similar to that in the imputed data (**Supplemental Table 3.1**). All primary analyses were performed in the imputed data sets and effect estimates were pooled across the 20 generated imputed data sets using Rubin's rule (39).

Statistical Analysis

Comparisons of covariate distributions were made across categories of maternal 25(OH)D during pregnancy using the analysis of variance (ANOVA), Kruskal-Wallis rank sum test, or χ^2 test. Similar comparisons were made between the eligible sample and the excluded sample with maternal 25(OH)D and offspring genetic data, but missing offspring depression measures.

Since continuous maternal 25(OH)D was non-linearly associated with the log odds of depression at both time points (**Supplemental Figure 3.3**), 25(OH)D was modeled as a categorical variable, based on clinical cut-offs defined by the Endocrine Society (i.e., deficient <20 ng/mL; insufficient 20-29.9 ng/mL; normal ≥30 ng/mL) (4). PRS was modeled categorically by polygenic risk groups, as described above, with the reference group set as low PRS. Logistic regression models were used to assess: (1) the main effects of PRS and maternal 25(OH)D on offspring depression during childhood and/or adolescence, separately (models 1-2) and together (model 3); (2) the interaction effect between PRS and maternal 25(OH)D on offspring depression (model 4). All models were adjusted for gestational age at 25(OH)D measurement, maternal age at delivery, maternal pre-pregnancy BMI, maternal education, maternal occupation, parity, smoking during the first trimester, maternal depression during pregnancy, and child sex (**Supplementary Methods**). Models including PRS were additionally adjusted for the top three principal components (PCs); additional details about the population structure analysis are provided in the **Supplementary Methods**. Models with multiplicative interaction terms were further adjusted for all significant covariate*maternal 25(OH)D and covariate*PRS interactions, based on recommendations by Keller *et al.* (40). Effect estimates were presented as odds ratios (ORs) and 95% confidence intervals (CI). Multiplicative interaction terms between maternal 25(OH)D and PRS were tested separately using the Wald test and globally using the likelihood ratio test. Statistical significance was set at two-tailed $p<0.05$ for all analyses. All analyses were performed in R (version 3.3.1) (41).

Based on the power calculations (**Supplementary Methods**), the study had 80% power to detect OR=1.4-1.7 for the association between vitamin D deficiency and child/adolescent depression, and sufficient power to detect GxE effects of magnitudes greater than OR_{gxe}=1.7.

Additional Analysis

We conducted several sensitivity analyses to assess the robustness of the findings. First, we fit logistic regression models that further adjusted for covariates that may be affected by or affect 25(OH)D levels during pregnancy, including vitamin D intake, calcium intake, vitamin D supplementation, and oily fish intake at 32 weeks of gestation. As the cut-offs for vitamin D deficiency remain controversial, we additionally conducted analyses using lower cut-offs set by the Institute of Medicine (i.e., deficient <10 ng/mL; insufficient 10-19.9 ng/mL; normal ≥20 ng/mL) (44). Also, given that vitamin D may have different effects on fetal brain development at different stages of gestation, we repeated the analyses stratified by the trimester of 25(OH)D measurement. Finally, we performed a complete case analysis on the subsample of participants with complete observed data on all variables (child subsample: n=2,091, adolescent subsample: n=1,812).

We also examined the prospective association between maternal vitamin D status and the risk of offspring depressive symptoms using negative binomial regression models. Negative binomial regression allows for the modeling of the highly skewed and over-dispersed counts of depressive symptoms and could potentially provide greater statistical power to detect associations (45). Estimates were presented as rate ratios (RR) and 95% CI, representing the association between maternal vitamin D status, PRS, and the risk for offspring depressive symptoms.

Results

Among the eligible mother-offspring pairs (n=3,173), the mean maternal 25(OH)D was 27.38 ng/mL (SD=11.97), and 63.5% of mothers were vitamin D insufficient or deficient during pregnancy based on clinical cut-offs defined by the Endocrine Society. There were no significant differences between the number of 25(OH)D measurements taken across the seasons (Winter: 23.9%; Spring: 28.7%; Summer: 24.6%; Fall: 22.8%). The median gestational week at 25(OH)D measurement was 29.4 weeks

(IQR: 12.7, 33.1), with most available measurements collected in the third trimester (58.5%). **Table 3.1** displays the distribution of mother and offspring characteristics by maternal vitamin D status during pregnancy.

Table 3.1 Maternal and offspring characteristics by maternal vitamin D status during pregnancy, among eligible mother-offspring pairs with complete data on maternal 25(OH)D, offspring genetic data, and at least one SMFQ measure

	All (n=3173)	Maternal 25(OH)D[1]			
		<20 ng/mL (n=988)	20-29.9 ng/mL (n=1027)	≥30 ng/mL (n=1158)	p[2]
Maternal age at delivery, n (%)					
15-19	46 (1.4)	18 (39.1)	18 (39.1)	10 (21.7)	**0.02**
20-35	2857 (90.0)	906 (31.7)	915 (32.0)	1036 (36.3)	
>35	270 (8.5)	64 (23.7)	94 (34.8)	112 (41.5)	
Maternal BMI (kg/m^2), median [IQR]	22.18 [20.53, 24.38]	22.18 [20.53, 24.52]	22.35 [20.53, 24.51]	22.02 [20.47, 23.88]	
Maternal education, n (%)					
Lower than O-levels	577 (19.3)	203 (52.3)	180 (31.2)	194 (33.8)	0.09
O-levels	1080 (36.1)	331 (30.6)	359 (33.2)	390 (36.1)	
Higher than O-levels	1332 (44.6)	390 (29.3)	428 (32.1)	514 (38.6)	
Maternal occupation, n (%)					
Manual	427 (16.0)	154 (36.1)	131 (30.7)	142 (33.2)	**0.03**
Non-manual	2237 (84.0)	659 (29.5)	747 (33.4)	831 (37.1)	
Parity, n (%)					
0	1446 (46.8)	482 (33.3)	477 (33.0)	487 (33.7)	**0.007**
≥1	1647 (53.2)	483 (29.3)	524 (31.8)	640 (38.9)	
Gestational week of 25(OH)D measurement, median [IQR]	29.43 [12.71, 33.14]	28.29 [11.29, 32.86]	28.71 [11.43, 32.86]	31.14 [18.75, 33.29]	**<0.001**
Season of 25(OH)D measurement, n (%)					
Winter	758 (23.9)	343 (45.3)	256 (33.8)	159 (20.9)	**<0.001**
Spring	912 (28.7)	412 (45.2)	297 (32.6)	203 (22.3)	
Summer	781 (24.6)	100 (12.8)	218 (27.9)	463 (59.3)	
Fall	722 (22.8)	133 (18.4)	256 (35.5)	333 (46.1)	
Tobacco use during 1st trimester, n (%)					
No	2569 (82.2)	752 (29.3)	831 (32.4)	984 (38.3)	**<0.001**
Yes	557 (17.8)	221 (39.7)	181 (32.5)	155 (27.8)	

Table 3.1 (continued)

	All (n=3173)	Maternal 25(OH)D[1]			
		<20 ng/mL (n=988)	20-29.9 ng/mL (n=1027)	≥30 ng/mL (n=1158)	p[2]
Oily fish intake at 32 weeks, n (%)					
≥1 times/week	813 (26.8)	214 (26.3)	261 (32.1)	338 (41.6)	**<0.001**
<1 times/week	1045 (34.5)	318 (30.4)	341 (32.6)	386 (37.0)	
Never/rarely	1173 (38.7)	408 (34.8)	379 (32.3)	386 (32.9)	
Vitamin D supplementation at 32 weeks, n (%)					
No	3035 (95.7)	961 (31.7)	985 (32.4)	1089 (35.9)	**0.001**
Yes	138 (4.3)	27 (19.6)	42 (30.4)	69 (50.0)	
Vitamin D intake at 32 weeks (ug), median [IQR]	3.43 [2.44, 5.40]	3.21 [2.28, 4.84]	3.39 [2.46, 5.46]	3.69 [2.59, 5.72]	**<0.001**
Calcium intake at 32 weeks (mg), median [IQR]	938.54 [762.25, 1131.15]	935.50 [762.92, 1129.98]	940.01 [763.07, 1129.33]	939.08 [759.17, 1134.02]	0.87
Maternal depression during pregnancy[3], n (%)					
No	2066 (75.6)	637 (30.8)	642 (31.1)	787 (38.1)	0.10
Yes	667 (24.4)	211 (31.6)	231 (34.6)	225 (33.8)	
Breastfeeding, n (%)					
No	1067 (34.9)	366 (34.3)	356 (33.4)	345 (32.3)	**0.001**
Yes	1994 (65.1)	585 (29.4)	633 (31.7)	776 (38.9)	
Child sex, n (%)					
Male	1592 (50.2)	483 (30.3)	520 (32.7)	589 (37.0)	0.62
Female	1581 (49.8)	505 (31.9)	507 (32.1)	569 (36.0)	

[1] Unadjusted 25(OH)D levels

[2] P-value calculated using the Chi-squared test for categorical variables and the ANOVA or Kruskal-Wallis rank sum test for normally and non-normally distributed continuous variables, respectively

[3] Maternal depression at 18 and/or 32 weeks of gestation

Maternal age at delivery, oily fish intake, and vitamin D intake during pregnancy increased with maternal 25(OH)D; the proportion of mothers with non-manual occupations, more than one child, were non-smokers during the first trimester, were on vitamin D supplementation, and breastfed increased from lower to higher maternal 25(OH)D levels. 6.6% and 13.0% of the offspring were classified as depression cases during childhood and adolescence, respectively.

Compared to the excluded participants, the eligible sample comprised mothers with higher 25(OH)D levels during pregnancy and offspring with lower PRS (**Supplemental Table 3.2**). Further, mothers in the eligible sample also tended to be older when they gave birth, had higher educational attainment, were in non-manual occupations, had no previous pregnancies, were non-smokers during the first trimester, and showed no evidence of depression during pregnancy. However, the absolute difference between maternal 25(OH)D (i.e., 1.3 ng/mL higher in the eligible sample) and offspring PRS (i.e., 0.07 standardized units lower in the eligible sample) in the eligible and excluded samples was small, hence the results are unlikely to be affected by selection bias.

Maternal Vitamin D Status and PRS on Offspring Depression

Associations between maternal 25(OH)D during pregnancy, PRS, and offspring depression are presented in **Table 3.2**. There were no associations between maternal 25(OH)D and offspring depression during childhood, although there was suggestive evidence that offspring exposed to deficient vitamin D levels during gestation had higher odds of depression during adolescence, compared to offspring exposed to normal vitamin D levels (OR=1.32; 95% CI: 0.98, 1.79; p=0.07).

Table 3.2 Association between maternal vitamin D status during pregnancy, offspring polygenic risk scores (PRS), and offspring depression during childhood or adolescence

	Childhood depression (n=2938)					Adolescent depression (n=2485)				
	n	**OR**	**95% CI**	**p**	**p-trend**	**n**	**OR**	**95% CI**	**p**	**p-trend**
Model 1: Maternal 25(OH)D only										
Normal (≥30 ng/mL)	985	*ref*	*ref*	*ref*		841	*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	1090	1.02	0.71, 1.48	0.90	0.72	907	1.12	0.84, 1.51	0.44	0.07
Deficient (<20 ng/mL)	863	1.07	0.73, 1.58	0.72		737	1.32	0.98, 1.79	0.07	
Model 2: PRS only										
PRS-Low	735	*ref*	*ref*	*ref*		622	*ref*	*ref*	*ref*	
PRS-Intermediate	1471	1.37	0.90, 2.09	0.14	**0.003**	1242	0.99	0.73, 1.34	0.93	0.06
PRS-High	732	**1.94**	**1.24, 3.03**	**0.004**		621	1.34	0.96, 1.87	0.09	
Model 3: PRS and Maternal 25(OH)D (Main effects)										
Normal (≥30 ng/mL)	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/mL)	-	1.03	0.71, 1.49	0.87	0.70	-	1.13	0.84, 1.51	0.43	0.07
Deficient (<20 ng/mL)	-	1.08	0.73, 1.59	0.70		-	1.33	0.98, 1.81	0.06	
PRS-Low	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
PRS-Intermediate	-	1.36	0.90, 2.09	0.15	**0.003**	-	0.98	0.73, 1.33	0.92	0.07
PRS-High	-	**1.94**	**1.24, 3.03**	**0.004**		-	1.34	0.96, 1.87	0.09	

Table 3.2 (continued)

	Childhood depression (n=2938)					Adolescent depression (n=2485)				
	n	**OR**	**95% CI**	**p**	**p-inter.**	**n**	**OR**	**95% CI**	**p**	**p-inter.**
Model 4: PRS and Maternal 25(OH)D (Interaction)										
Normal (≥30 ng/mL)	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	-	0.65	0.29, 1.45	0.29	-	-	1.15	0.62, 2.11	0.66	-
Deficient (<20 ng/mL)	-	0.38	0.13, 1.06	0.06		-	1.45	0.78, 2.69	0.24	
PRS-Low	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
PRS-Intermediate	-	0.74	0.63, 1.46	0.38	-	-	1.21	0.71, 2.06	0.49	-
PRS-High	-	1.26	0.37, 2.53	0.52		-	1.06	0.58, 1.96	0.84	
Insufficient*PRS-Intermediate	-	1.97	0.74, 5.26	0.18		-	0.80	0.38, 1.66	0.54	
Deficient*PRS-Intermediate	-	4.09	1.26, 13.3	0.02	0.19	-	0.68	0.32, 1.45	0.32	0.36
Insufficient*PRS-High	-	1.65	0.59, 4.58	0.34		-	1.38	0.61, 3.15	0.44	
Deficient*PRS-High	-	2.92	0.86, 10.0	0.09		-	1.39	0.60, 3.22	0.44	

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; CI: confidence intervals; OR: odds ratio; p-inter.: p for interaction
Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal occupation, parity, smoking during 1st trimester, maternal depression during pregnancy, child sex
Model 2: Model 1 confounders + 3 PCs
Model 3: Model 2 confounders
Model 4: Model 3 confounders + significant PRS*covariate, maternal 25(OH)D*covariate interactions

Offspring PRS was positively associated with the odds of depression during childhood (p for trend=0.003), with high PRS conferring an approximately 2-fold higher odds of childhood depression compared to low PRS (OR=1.94; 95% CI: 1.24, 3.03; p=0.0004); higher PRS was associated with non-significantly higher odds of depression during adolescence (p for trend=0.06) (model 2). When modeled together (model 3), the effect estimates for both maternal vitamin D status and PRS were almost identical to those yielded from their separate models. In model 4, there was no evidence for significant interactions between maternal vitamin D status and PRS on childhood (p=0.19) or adolescent (p=0.36) depression.

Additional Analyses

Further adjustments for nutritional intake at 32 weeks of gestation (model 5) (**Supplemental Table 3.3**) resulted in estimates similar to those in the main analyses. We also investigated the robustness of the results to alternative, lower cut-offs for vitamin D insufficiency and deficiency. These lower cut-offs resulted in large imbalances in the numbers across categories, resulting in a small number of mothers classified as vitamin D deficient during pregnancy (n=55-71). Nonetheless, the direction and magnitude of effects were generally similar to those using higher cut-offs for deficiency (**Supplemental Table 3.4**). The results did not change substantially when stratified by trimester of 25(OH)D measurement, although some of the effect directions were reversed in the small subsamples with measurements in the second trimester (n=381-438) (**Supplemental Table 3.5**). Complete case analysis also showed similar patterns of trends to those from the primary analyses on the imputed data (**Supplemental Table 3.6**), but the associations between PRS and childhood/adolescent depression were attenuated to non-significance, likely due to large reductions in sample sizes.

Results from the negative binomial regression models showed similar associational patterns as those produced from the logistic regression models (**Supplemental Table 3.7**). There were no significant main effects of maternal 25(OH)D nor interactions with PRS on the risk of offspring depressive

symptoms during childhood or adolescence. The only notable difference was the presence of a statistically significant positive association between PRS and the risk of depressive symptoms during adolescence (p-trend=0.03).

Discussion

In this large, prospective birth cohort, we found little evidence for an association between maternal pregnancy vitamin D status (i.e., blood 25(OH)D level) and offspring depression in childhood or adolescence. In this population, PRS was positively associated with risk for offspring depression at both time points but did not interact with maternal 25(OH)D. These findings were robust to adjustments for a range of potential confounders and different cut-offs for vitamin D insufficiency/deficiency.

Although several studies have reported the influence of maternal and/or cord blood 25(OH)D on the offspring's risk for neuropsychiatric or neurodevelopmental disorders/traits, including schizophrenia (16), ADHD (17), and autism-related traits (46), the same effects do not appear to be present for depression. Results from the current study are consistent with the only existing study, to our knowledge, that assessed the association between maternal 25(OH)D during pregnancy and offspring depression. In the Strom *et al.* study (13), maternal vitamin D deficiency during pregnancy was not associated with offspring risk for depression over 22 years of follow-up. Studies that assessed features similar to depression in childhood, such as emotional problems and/or internalizing symptoms, also did not report significant associations with maternal 25(OH)D (11, 47, 48). Taken together, these findings do not support influences of maternal vitamin D status during pregnancy on offspring depression or related symptoms in early life.

It has been postulated that there may be sensitive developmental periods during which the effects of fetal 25(OH)D on neurodevelopment are particularly salient (9). The precise timing of the

neurodevelopmental effects of vitamin D is unclear, but may have importance at any stage of pregnancy: early gestation, when structures important for behavioral regulation and dopaminergic neurons develop (47, 49); mid-gestation, which is characterized by neural circuit formation and myelination (50, 51); late gestation, when prolific brain development and growth occurs (52-54). When we stratified our analysis by trimester of 25(OH)D measurement, the overall results did not differ from those yielded from the primary analysis in the full cohort. However, the stratified analyses had limited statistical power given the small sample sizes within some of the subgroups. Hence, the possibility for sensitive periods for vitamin D effects remains, and longitudinal studies sampling 25(OH)D in all three trimesters are needed to establish stronger conclusions. Alternatively, the lack of gestational 25(OH)D effects on child depression may be due to critical window(s) of vulnerability to vitamin D exposure occurring postnatally. In the Tolpannen *et al.* study (18), similarly based on children in the ALSPAC cohort, childhood 25(OH)D_3 levels were found to be associated with adolescent depressive symptoms. It is plausible that the protective effects of vitamin D on depression and mental health are exerted through neuroprotective actions, such as moderating inflammatory processes or modulating neurotrophic factors, after birth. Further studies are necessary to characterize the precise mechanisms of vitamin D on the developing brain.

Our study has notable strengths. First, this study was conducted in a large, population-based sample that was more than three times larger than the previous study examining the same associations. Second, the longitudinal study design allowed us to prospectively investigate the effects of maternal 25(OH)D on offspring depression measured during both childhood and adolescence, while adjusting for a wide range of potential confounding factors.

The study also has some limitations. First, only one measurement of 25(OH)D, taken at any time during pregnancy, was available for all mothers in the study sample, which may not have been representative of average 25(OH)D levels for the duration of the pregnancy. However, studies have shown that single measurements may be reasonable proxies for vitamin D status throughout pregnancy, given the strong correlation in 25(OH)D concentrations over time (55, 56). Second, the SMFQ only

captures depressive symptoms in the past 2 weeks, which may not represent depression over longer periods. This may have led to outcome misclassification, particularly for adolescent depression, which has been shown to be highly episodic in nature (57); such misclassification may have biased the results towards the null. Third, it is possible that an association between low maternal vitamin D levels and offspring depression is only detectable at very low levels of maternal 25(OH)D, and that the distribution of 25(OH)D levels in this population was not sufficiently wide to capture this. Compared to prior studies that detected significant associations (16, 46), mothers in the current study were less diverse ethnically/racially and/or socioeconomically, and on average, had higher 25(OH)D levels (27.38 ng/mL compared to 15.3-23.6 ng/mL in other studies) with only a very small proportion having 25(OH)D levels <10 ng/mL (2%). Fourth, as with most longitudinal studies, there was considerable attrition over time, which could introduce bias when both maternal 25(OH)D and offspring depression were associated with loss to follow-up. Nevertheless, since the difference between the average maternal 25(OH)D levels in the excluded and included samples was small in magnitude, we do not expect attrition in this case to substantially influence the results. Fifth, the study was based in a white population, thus the results may not be generalizable to other racial/ethnic groups or populations with different prevalence of vitamin D deficiency and/or depression. Finally, we may have lacked power to detect weaker effects of maternal 25(OH)D on offspring depression and had even lower power to detect GxE. Larger sample sizes are needed to definitively rule out the presence of smaller associations and gene-environment interactions.

In sum, our findings do not support an association between maternal vitamin D status during pregnancy and offspring depression during childhood and adolescence. This suggests that interventions aimed at increasing 25(OH)D levels during pregnancy are unlikely to reduce the risk of offspring depression during childhood or adolescence, although such efforts may still be beneficial for other child health outcomes (58). Larger prospective studies in more racially/ethnically diverse populations with lower average values and/or broader ranges of levels of 25(OH)D during pregnancy are needed to confirm the current findings.

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. ALSPAC children were genotyped using the Illumina HumanHap550 quad chip genotyping platforms by Sample Logistics and Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America), using support from 23andMe. The UK Medical Research Council and Wellcome (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). This research was specifically funded by: The UK Medical Research Council provided funds to ALSPAC for completion of the 25(OH)D assays used in this paper (grant no. G0701603).

References

1. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr. 2007;137:447-452.
2. Eggemoen AR, Falk RS, Knutsen KV, Lagerlov P, Sletner L, Birkeland KI, Jenum AK. Vitamin D deficiency and supplementation in pregnancy in a multiethnic population-based cohort. BMC Pregnancy Childbirth. 2016;16:7.
3. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. J Clin Endocrinol Metab. 2007;92:3517-3522.
4. Dror DK, Allen LH. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. Nutr Rev. 2010;68:465-477.
5. Holmes VA, Barnes MS, Alexander HD, McFaul P, Wallace JM. Vitamin D deficiency and insufficiency in pregnant women: a longitudinal study. Br J Nutr. 2009;102:876-881.
6. Eyles DW, Feron F, Cui X, Kesby JP, Harms LH, Ko P, McGrath JJ, Burne TH. Developmental vitamin D deficiency causes abnormal brain development. Psychoneuroendocrinology. 2009;34 Suppl 1:S247-257.
7. Eyles D, Brown J, Mackay-Sim A, McGrath J, Feron F. Vitamin D3 and brain development. Neuroscience. 2003;118:641-653.
8. O'Loan J, Eyles DW, Kesby J, Ko P, McGrath JJ, Burne TH. Vitamin D deficiency during various stages of pregnancy in the rat; its impact on development and behaviour in adult offspring. Psychoneuroendocrinology. 2007;32:227-234.
9. Pet MA, Brouwer-Brolsma EM. The Impact of Maternal Vitamin D Status on Offspring Brain Development and Function: a Systematic Review. Adv Nutr. 2016;7:665-678.
10. Darling AL, Rayman MP, Steer CD, Golding J, Lanham-New SA, Bath SC. Association between maternal vitamin D status in pregnancy and neurodevelopmental outcomes in childhood: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Br J Nutr. 2017;117:1682-1692.
11. Keim SA, Bodnar LM, Klebanoff MA. Maternal and cord blood 25(OH)-vitamin D concentrations in relation to child development and behaviour. Paediatr Perinat Epidemiol. 2014;28:434-444.
12. Morales E, Guxens M, Llop S, Rodriguez-Bernal CL, Tardon A, Riano I, Ibarluzea J, Lertxundi N, Espada M, Rodriguez A, Sunyer J, Project I. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics. 2012;130:e913-920.

13. Strom M, Halldorsson TI, Hansen S, Granstrom C, Maslova E, Petersen SB, Cohen AS, Olsen SF. Vitamin D measured in maternal serum and offspring neurodevelopmental outcomes: a prospective study with long-term follow-up. Ann Nutr Metab. 2014;64:254-261.
14. Agarwal S, Kovilam O, Agrawal DK. Vitamin D and its impact on maternal-fetal outcomes in pregnancy: A critical review. Crit Rev Food Sci Nutr. 2018;58:755-769.
15. Magnusson C, Lundberg M, Lee BK, Rai D, Karlsson H, Gardner R, Kosidou K, Arver S, Dalman C. Maternal vitamin D deficiency and the risk of autism spectrum disorders: population-based study. BJPsych Open. 2016;2:170-172.
16. Eyles DW, Trzaskowski M, Vinkhuyzen AAE, Mattheisen M, Meier S, Gooch H, Anggono V, Cui X, Tan MC, Burne THJ, Jang SE, Kvaskoff D, Hougaard DM, Norgaard-Pedersen B, Cohen A, Agerbo E, Pedersen CB, Borglum AD, Mors O, Sah P, Wray NR, Mortensen PB, McGrath JJ. The association between neonatal vitamin D status and risk of schizophrenia. Sci Rep. 2018;8:17692.
17. Morales E, Julvez J, Torrent M, Ballester F, Rodriguez-Bernal CL, Andiarena A, Vegas O, Castilla AM, Rodriguez-Dehli C, Tardon A, Sunyer J. Vitamin D in Pregnancy and Attention Deficit Hyperactivity Disorder-like Symptoms in Childhood. Epidemiology. 2015;26:458-465.
18. Tolppanen AM, Sayers A, Fraser WD, Lewis G, Zammit S, Lawlor DA. The association of serum 25-hydroxyvitamin D3 and D2 with depressive symptoms in childhood--a prospective cohort study. J Child Psychol Psychiatry. 2012;53:757-766.
19. Rice F. Genetics of childhood and adolescent depression: insights into etiological heterogeneity and challenges for future genomic research. Genome Med. 2010;2:68.
20. Xia L, Yao S. The Involvement of Genes in Adolescent Depression: A Systematic Review. Front Behav Neurosci. 2015;9:329.
21. Carey G, Crammond B. A glossary of policy frameworks: the many forms of 'universalism' and policy 'targeting'. J Epidemiol Community Health. 2017;71:303-307.
22. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol. 2013;42:111-127.
23. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A, Ring S, Nelson SM, Lawlor DA. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol. 2013;42:97-110.
24. Golding J, Pembrey M, Jones R, Team AS. ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. Paediatr Perinat Epidemiol. 2001;15:74-87.
25. Matijasevich A, Victora CG, Lawlor DA, Golding J, Menezes AM, Araujo CL, Barros AJ, Santos IS, Barros FC, Smith GD. Association of socioeconomic position with maternal pregnancy and infant health

outcomes in birth cohort studies from Brazil and the UK. J Epidemiol Community Health. 2012;66:127-135.
26. Angold A, Erkanli A, Silberg J, Eaves L, Costello EJ. Depression scale scores in 8-17-year-olds: effects of age and gender. J Child Psychol Psychiatry. 2002;43:1052-1063.
27. Thapar A, McGuffin P. Validity of the shortened Mood and Feelings Questionnaire in a community sample of children and adolescents: a preliminary research note. Psychiatry Res. 1998;81:259-268.
28. Joinson C, Kounali D, Lewis G. Family socioeconomic position in early life and onset of depressive symptoms and depression: a prospective cohort study. Soc Psychiatry Psychiatr Epidemiol. 2017;52:95-103.
29. Pearson RM, Heron J, Button K, Bentall RP, Fernyhough C, Mahedy L, Bowes L, Lewis G. Cognitive styles and future depressed mood in early adulthood: the importance of global attributions. J Affect Disord. 2015;171:60-67.
30. Lawlor DA, Wills AK, Fraser A, Sayers A, Fraser WD, Tobias JH. Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. Lancet. 2013;381:2176-2183.
31. Wills AK, Shaheen SO, Granell R, Henderson AJ, Fraser WD, Lawlor DA. Maternal 25-hydroxyvitamin D and its association with childhood atopic outcomes and lung function. Clin Exp Allergy. 2013;43:1180-1188.
32. Williams DM, Fraser A, Fraser WD, Hypponen E, Davey Smith G, Deanfield J, Hingorani A, Sattar N, Lawlor DA. Associations of maternal 25-hydroxyvitamin D in pregnancy with offspring cardiovascular risk factors in childhood and adolescence: findings from the Avon Longitudinal Study of Parents and Children. Heart. 2013;99:1849-1856.
33. Sachs MC, Shoben A, Levin GP, Robinson-Cohen C, Hoofnagle AN, Swords-Jenny N, Ix JH, Budoff M, Lutsey PL, Siscovick DS, Kestenbaum B, de Boer IH. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2013;97:1243-1251.
34. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet. 2007;39:906-913.
35. Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, McVean GA. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012;491:56-65.
36. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559-575.

37. Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A, Adams MJ, Agerbo E, Air TM, Andlauer TMF, Bacanu SA, Baekvad-Hansen M, Beekman AFT, Bigdeli TB, Binder EB, Blackwood DRH, Bryois J, Buttenschon HN, Bybjerg-Grauholm J, Cai N, Castelao E, Christensen JH, Clarke TK, Coleman JIR, Colodro-Conde L, Couvy-Duchesne B, Craddock N, Crawford GE, Crowley CA, Dashti HS, Davies G, Deary IJ, Degenhardt F, Derks EM, Direk N, Dolan CV, Dunn EC, Eley TC, Eriksson N, Escott-Price V, Kiadeh FHF, Finucane HK, Forstner AJ, Frank J, Gaspar HA, Gill M, Giusti-Rodriguez P, Goes FS, Gordon SD, Grove J, Hall LS, Hannon E, Hansen CS, Hansen TF, Herms S, Hickie IB, Hoffmann P, Homuth G, Horn C, Hottenga JJ, Hougaard DM, Hu M, Hyde CL, Ising M, Jansen R, Jin F, Jorgenson E, Knowles JA, Kohane IS, Kraft J, Kretzschmar WW, Krogh J, Kutalik Z, Lane JM, Li Y, Li Y, Lind PA, Liu X, Lu L, MacIntyre DJ, MacKinnon DF, Maier RM, Maier W, Marchini J, Mbarek H, McGrath P, McGuffin P, Medland SE, Mehta D, Middeldorp CM, Mihailov E, Milaneschi Y, Milani L, Mill J, Mondimore FM, Montgomery GW, Mostafavi S, Mullins N, Nauck M, Ng B, Nivard MG, Nyholt DR, O'Reilly PF, Oskarsson H, Owen MJ, Painter JN, Pedersen CB, Pedersen MG, Peterson RE, Pettersson E, Peyrot WJ, Pistis G, Posthuma D, Purcell SM, Quiroz JA, Qvist P, Rice JP, Riley BP, Rivera M, Saeed Mirza S, Saxena R, Schoevers R, Schulte EC, Shen L, Shi J, Shyn SI, Sigurdsson E, Sinnamon GBC, Smit JH, Smith DJ, Stefansson H, Steinberg S, Stockmeier CA, Streit F, Strohmaier J, Tansey KE, Teismann H, Teumer A, Thompson W, Thomson PA, Thorgeirsson TE, Tian C, Traylor M, Treutlein J, Trubetskoy V, Uitterlinden AG, Umbricht D, Van der Auwera S, van Hemert AM, Viktorin A, Visscher PM, Wang Y, Webb BT, Weinsheimer SM, Wellmann J, Willemsen G, Witt SH, Wu Y, Xi HS, Yang J, Zhang F, eQtlgen, andMe, Arolt V, Baune BT, Berger K, Boomsma DI, Cichon S, Dannlowski U, de Geus ECJ, DePaulo JR, Domenici E, Domschke K, Esko T, Grabe HJ, Hamilton SP, Hayward C, Heath AC, Hinds DA, Kendler KS, Kloiber S, Lewis G, Li QS, Lucae S, Madden PFA, Magnusson PK, Martin NG, McIntosh AM, Metspalu A, Mors O, Mortensen PB, Muller-Myhsok B, Nordentoft M, Nothen MM, O'Donovan MC, Paciga SA, Pedersen NL, Penninx B, Perlis RH, Porteous DJ, Potash JB, Preisig M, Rietschel M, Schaefer C, Schulze TG, Smoller JW, Stefansson K, Tiemeier H, Uher R, Volzke H, Weissman MM, Werge T, Winslow AR, Lewis CM, Levinson DF, Breen G, Borglum AD, Sullivan PF, Major Depressive Disorder Working Group of the Psychiatric Genomics C. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat Genet. 2018;50:668-681.

38. van Buuren SG-O, K. mice: Multivariate Imputation by Chained Equations in R. Journal of Statistical Software. 2011;45:1-67.

39. Marshall A, Altman DG, Holder RL, Royston P. Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice and guidelines. BMC Med Res Methodol. 2009;9:57.

40. Keller MC. Gene x environment interaction studies have not properly controlled for potential confounders: the problem and the (simple) solution. Biol Psychiatry. 2014;75:18-24.
41. Team RC: R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing; 2013.
42. Demidenko E. Sample size determination for logistic regression revisited. Stat Med. 2007;26:3385-3397.
43. Demidenko E. Sample size and optimal design for logistic regression with binary interaction. Stat Med. 2008;27:36-46.
44. Ross AC, Institute of Medicine (U. S.). Committee to Review Dietary Reference Intakes for Vitamin D and Calcium.: Dietary reference intakes : calcium, vitamin D. Washington, DC, National Academies Press; 2011.
45. Agresti A: Categorical data analysis. 3rd ed. Hoboken, NJ, Wiley; 2013.
46. Vinkhuyzen AAE, Eyles DW, Burne THJ, Blanken LME, Kruithof CJ, Verhulst F, Jaddoe VW, Tiemeier H, McGrath JJ. Gestational vitamin D deficiency and autism-related traits: the Generation R Study. Mol Psychiatry. 2018;23:240-246.
47. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C, Princess Anne Hospital Study G. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr. 2008;62:68-77.
48. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Kusel MM, Hart PH. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics. 2012;129:485-493.
49. Almqvist PM, Akesson E, Wahlberg LU, Pschera H, Seiger A, Sundstrom E. First trimester development of the human nigrostriatal dopamine system. Exp Neurol. 1996;139:227-237.
50. Keunen K, Counsell SJ, Benders M. The emergence of functional architecture during early brain development. Neuroimage. 2017;160:2-14.
51. Kinney HC, Brody BA, Kloman AS, Gilles FH. Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. J Neuropathol Exp Neurol. 1988;47:217-234.
52. Clouchoux C, Guizard N, Evans AC, du Plessis AJ, Limperopoulos C. Normative fetal brain growth by quantitative in vivo magnetic resonance imaging. Am J Obstet Gynecol. 2012;206:173 e171-178.
53. Kostovic I, Jovanov-Milosevic N. The development of cerebral connections during the first 20-45 weeks' gestation. Semin Fetal Neonatal Med. 2006;11:415-422.
54. Bouyssi-Kobar M, du Plessis AJ, McCarter R, Brossard-Racine M, Murnick J, Tinkleman L, Robertson RL, Limperopoulos C. Third Trimester Brain Growth in Preterm Infants Compared With In Utero Healthy Fetuses. Pediatrics. 2016;138.

55. Hofmann JN, Yu K, Horst RL, Hayes RB, Purdue MP. Long-term variation in serum 25-hydroxyvitamin D concentration among participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Cancer Epidemiol Biomarkers Prev. 2010;19:927-931.
56. Major JM, Graubard BI, Dodd KW, Iwan A, Alexander BH, Linet MS, Freedman DM. Variability and reproducibility of circulating vitamin D in a nationwide U.S. population. J Clin Endocrinol Metab. 2013;98:97-104.
57. Holsen IK, P.; Vitterso, J. Stability in depressed mood in adolescence: Results from a 6-year longitudinal panel study. Journal of Youth and Adolescence. 2000;29:61-78.
58. Wagner CL, Hollis BW. The Implications of Vitamin D Status During Pregnancy on Mother and her Developing Child. Front Endocrinol (Lausanne). 2018;9:500.

Appendix

Supplementary Methods

Derivation of seasonality-adjusted maternal 25(OH)D measurements

The cosinor model is a linear regression model that models 25(OH)D against sine and cosine transformations of time (i.e., months) (1). The transformed model coefficients can be used to derive the amplitude (distance between mean to peak or trough location of the curve) and phase shift (location of peak and trough on time axis) of the sine curve. The coefficient for the model intercept is used to obtain the mean annual 25(OH)D concentration in the study population, and the residuals are used to derive the mean annual 25(OH)D levels for each individual. Full details of this method has been described elsewhere (1).

Polygenic risk score construction

Polygenic risk scores (PRS) for major depressive disorder (MDD) were constructed using summary-level data from the GWAS meta-analysis of MDD from the Psychiatric Genomics Consortium wave 2 (PGC-MDD2; 130,664 cases and 330,470 controls) (2). The PGC-MDD2 consisted of 35 cohorts, including the 29 PGC-MDD anchor cohorts (16,823 cases, 25,632 controls) (2) and 6 additional cohorts: GERA (7,162 cases, 38,307 controls) (3), deCODE (1,980 cases, 9,536 controls) (2), GenScotland (997 cases, 6,358 controls) (4, 5), iPsych (18,629 cases, 17,841 controls) (2), UK Biobank (14,260 cases, 15,480 controls) (6, 7), and 23andMe (75,607 cases, 231,747 controls) (8). Due to general access constraints, full summary statistics were only available for the meta-analytic subsample (n=173,005) excluding the 23andMe cohort. Prior to score construction, SNPs with imputation quality metric score (INFO)<0.8, MAF<1%, call rate<95%, and HWE $p<1x10^{-6}$ were filtered out. SNPs were pruned for linkage disequilibrium (LD) using p-value informed clump-based pruning in PLINK version 1.90 (https://www.cog-genomics.org/plink2) using the following parameters: --clump-p1 1 --clump-p2 1 --

clump-r2 0.2 --clump-kb 500). Within a specified window, LD clumping preferentially keeps the most significant SNPs and removes all other SNPs in high LD.

Assessment of covariates

Data on maternal pre-pregnancy body mass index (BMI, kg/m^2), parity, maternal socioeconomic position indicators, tobacco use, nutritional intake, and vitamin D supplementation were provided by mothers through self-administered questionnaires at 18 and/or 32 weeks of pregnancy. Maternal depression during pregnancy was derived using the Edinburgh Postnatal Depression Scale (9), with depressed mood defined as scores ≥13. Maternal age at delivery, month/season and gestational age at 25(OH)D measurement (weeks) were obtained from obstetric records. Child sex was obtained from birth records, while information on breastfeeding was collected postnatally from mother-completed questionnaires.

Maternal pre-pregnancy BMI and gestational age at 25(OH)D measurement were included in the models as continuous variables. All other variables were included as categorical variables: maternal age at child birth (age 15-19 years, 20-35 years, or >35 years), education (less than O-levels or equivalent, O-levels, or higher than O-levels), occupation (manual or non-manual), parity (0 or ≥1 child), first trimester tobacco use (yes or no), oily fish intake at 32 weeks of gestation (≥once/week, <once/week, or never/rarely), vitamin D supplementation at 32 weeks of gestation (yes or no), vitamin D intake at 32 weeks of gestation (ug), calcium intake at 32 weeks of gestation (mg), maternal depression during pregnancy (yes or no), breastfeeding (yes or no), child sex (male or female). The reference group for all categorical variables were chosen on the basis of sample size and consistency with the literature.

Population structure analysis

Principal components (PCs) reflect a population's genetic structure and can be used to adjust for population stratification (10). Using genome-wide SNPs in the directly genotyped data, the top 20 PCs

were estimated in PLINK using methods described by Price and colleagues (10). In line with the PGC ricopili pipeline (https://sites.google.com/a/broadinstitute.org/ricopili/pca) the following quality control (QC) procedures were performed: MAF>5%; HWE>1.0E-03; missing rate<2%; no strand ambiguous SNPs (AT/GC); no MHC (6:25-35Mb); no Chromosome 8 inversions (8:7-13Mb). 462,838 SNPs remained after QC and were pruned if their r2>0.2, within a 200 SNP window. After pruning, 90,414 SNPs were available for principal component analysis. 20 PCs were generated from the analysis and the top 3 PCs were included in the analytic models based on examination of the eigenvalues and a scree plot.

Multivariable multiple imputation

All exposure, covariate, and outcome variables that were included in the final analysis were included in the imputation model for imputing covariate data, since failure to do so may result in a biased analytic model (11). Variables that were strongly correlated with the covariates, such as home crowding and home ownership, were also included in the imputation model because this has been shown to reduce bias and improve precision (11). For continuous variables, predictive mean matching was used for imputation, while logistic and polynomial regression was used to impute dichotomous and categorical variables, respectively. Twenty imputed data sets were generated using this method.

Several diagnostic tests were performed to assess proper imputation. First, convergence plots were inspected to determine whether convergence was achieved. Second, density plots were examined to compare the distribution of the imputed data to observed data. **Table S1** presents the percentage of missing data for each covariate, as well as a comparison of the distribution of the variables in the observed data versus imputed data.

Power analysis

Power calculations were performed for both the main effects of maternal 25(OH)D and GxE on offspring depression. Power analysis for the main effects of maternal 25(OH)D on offspring depression, was implemented in an online power calculator for logistic regression

(http://www.dartmouth.edu/~eugened/power-samplesize.php), developed using algorithms derived by Demidenko (12, 13). Power analysis for GxE was performed using the *powerGWASinteraction* package in R. All power calculations assumed α=0.05 and a two-sided test; other parameters were derived from the literature or directly estimated in the study cohort.

Supplementary References

1. Barnett AG, Dobson AJ: Analysing seasonal health data. Berlin ; London, Springer; 2010.
2. Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A, Adams MJ, Agerbo E, Air TM, Andlauer TMF, Bacanu SA, Baekvad-Hansen M, Beekman AFT, Bigdeli TB, Binder EB, Blackwood DRH, Bryois J, Buttenschon HN, Bybjerg-Grauholm J, Cai N, Castelao E, Christensen JH, Clarke TK, Coleman JIR, Colodro-Conde L, Couvy-Duchesne B, Craddock N, Crawford GE, Crowley CA, Dashti HS, Davies G, Deary IJ, Degenhardt F, Derks EM, Direk N, Dolan CV, Dunn EC, Eley TC, Eriksson N, Escott-Price V, Kiadeh FHF, Finucane HK, Forstner AJ, Frank J, Gaspar HA, Gill M, Giusti-Rodriguez P, Goes FS, Gordon SD, Grove J, Hall LS, Hannon E, Hansen CS, Hansen TF, Herms S, Hickie IB, Hoffmann P, Homuth G, Horn C, Hottenga JJ, Hougaard DM, Hu M, Hyde CL, Ising M, Jansen R, Jin F, Jorgenson E, Knowles JA, Kohane IS, Kraft J, Kretzschmar WW, Krogh J, Kutalik Z, Lane JM, Li Y, Li Y, Lind PA, Liu X, Lu L, MacIntyre DJ, MacKinnon DF, Maier RM, Maier W, Marchini J, Mbarek H, McGrath P, McGuffin P, Medland SE, Mehta D, Middeldorp CM, Mihailov E, Milaneschi Y, Milani L, Mill J, Mondimore FM, Montgomery GW, Mostafavi S, Mullins N, Nauck M, Ng B, Nivard MG, Nyholt DR, O'Reilly PF, Oskarsson H, Owen MJ, Painter JN, Pedersen CB, Pedersen MG, Peterson RE, Pettersson E, Peyrot WJ, Pistis G, Posthuma D, Purcell SM, Quiroz JA, Qvist P, Rice JP, Riley BP, Rivera M, Saeed Mirza S, Saxena R, Schoevers R, Schulte EC, Shen L, Shi J, Shyn SI, Sigurdsson E, Sinnamon GBC, Smit JH, Smith DJ, Stefansson H, Steinberg S, Stockmeier CA, Streit F, Strohmaier J, Tansey KE, Teismann H, Teumer A, Thompson W, Thomson PA, Thorgeirsson TE, Tian C, Traylor M, Treutlein J, Trubetskoy V, Uitterlinden AG, Umbricht D, Van der Auwera S, van Hemert AM, Viktorin A, Visscher PM, Wang Y, Webb BT, Weinsheimer SM, Wellmann J, Willemsen G, Witt SH, Wu Y, Xi HS, Yang J, Zhang F, eQtlgen, andMe, Arolt V, Baune BT, Berger K, Boomsma DI, Cichon S, Dannlowski U, de Geus ECJ, DePaulo JR, Domenici E, Domschke K, Esko T, Grabe HJ, Hamilton SP, Hayward C, Heath AC, Hinds DA, Kendler KS, Kloiber S, Lewis G, Li QS, Lucae S, Madden PFA, Magnusson PK, Martin NG, McIntosh AM, Metspalu A, Mors O, Mortensen PB, Muller-

Myhsok B, Nordentoft M, Nothen MM, O'Donovan MC, Paciga SA, Pedersen NL, Penninx B, Perlis RH, Porteous DJ, Potash JB, Preisig M, Rietschel M, Schaefer C, Schulze TG, Smoller JW, Stefansson K, Tiemeier H, Uher R, Volzke H, Weissman MM, Werge T, Winslow AR, Lewis CM, Levinson DF, Breen G, Borglum AD, Sullivan PF, Major Depressive Disorder Working Group of the Psychiatric Genomics C. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat Genet. 2018;50:668-681.

3. Banda Y, Kvale MN, Hoffmann TJ, Hesselson SE, Ranatunga D, Tang H, Sabatti C, Croen LA, Dispensa BP, Henderson M, Iribarren C, Jorgenson E, Kushi LH, Ludwig D, Olberg D, Quesenberry CP, Jr., Rowell S, Sadler M, Sakoda LC, Sciortino S, Shen L, Smethurst D, Somkin CP, Van Den Eeden SK, Walter L, Whitmer RA, Kwok PY, Schaefer C, Risch N. Characterizing Race/Ethnicity and Genetic Ancestry for 100,000 Subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. Genetics. 2015;200:1285-1295.

4. Fernandez-Pujals AM, Adams MJ, Thomson P, McKechanie AG, Blackwood DH, Smith BH, Dominiczak AF, Morris AD, Matthews K, Campbell A, Linksted P, Haley CS, Deary IJ, Porteous DJ, MacIntyre DJ, McIntosh AM. Epidemiology and Heritability of Major Depressive Disorder, Stratified by Age of Onset, Sex, and Illness Course in Generation Scotland: Scottish Family Health Study (GS:SFHS). PLoS One. 2015;10:e0142197.

5. Smith BH, Campbell A, Linksted P, Fitzpatrick B, Jackson C, Kerr SM, Deary IJ, Macintyre DJ, Campbell H, McGilchrist M, Hocking LJ, Wisely L, Ford I, Lindsay RS, Morton R, Palmer CN, Dominiczak AF, Porteous DJ, Morris AD. Cohort Profile: Generation Scotland: Scottish Family Health Study (GS:SFHS). The study, its participants and their potential for genetic research on health and illness. Int J Epidemiol. 2013;42:689-700.

6. Smith DJ, Nicholl BI, Cullen B, Martin D, Ul-Haq Z, Evans J, Gill JM, Roberts B, Gallacher J, Mackay D, Hotopf M, Deary I, Craddock N, Pell JP. Prevalence and characteristics of probable major depression and bipolar disorder within UK biobank: cross-sectional study of 172,751 participants. PLoS One. 2013;8:e75362.

7. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. 2015;12:e1001779.

8. Hyde CL, Nagle MW, Tian C, Chen X, Paciga SA, Wendland JR, Tung JY, Hinds DA, Perlis RH, Winslow AR. Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. Nat Genet. 2016;48:1031-1036.

9. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. Br J Psychiatry. 1987;150:782-786.
10. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;38:904-909.
11. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. Stat Med. 2011;30:377-399.
12. Demidenko E. Sample size determination for logistic regression revisited. Stat Med. 2007;26:3385-3397.
13. Demidenko E. Sample size and optimal design for logistic regression with binary interaction. Stat Med. 2008;27:36-46.

Supplemental Table 3.1 Comparison of the distribution of main covariates in the observed data set and the imputed data set, for mother-offspring pairs with (A) SMFQ in childhood; (B) SMFQ in adolescence

(A)

Covariates with missing data	N missing (%)	Mean (SD) or n (%) in sample	
		Observed data	Imputed data
Maternal pre-pregnancy BMI (kg/m^2)	264 (5.9)	22.87 (3.63)	22.84 (3.58)
Maternal education	166 (3.7)		
<O-level		534 (19.0)	570 (19.2)
O-level		1016 (36.2)	1077 (36.2)
>O-level		1259 (44.8)	1328 (44.6)
Parity	71 (1.6)		
0		1347 (46.4)	1382 (46.5)
1+		1557 (53.6)	1593 (53.5)
Maternal occupation	464 (10.4)		
Manual		398 (15.9)	504 (16.9)
Non-manual		2113 (84.1)	2471 (83.1)
Tobacco use during the first trimester	40 (0.9)		
No		2434 (82.9)	2466 (82.9)
Yes		501 (17.1)	509 (17.1)
Maternal depression during pregnancy	405 (9.1)		
No		1960 (76.3)	2266 (76.2)
Yes		610 (23.7)	709 (23.8)

(B)

Covariates with missing data	N missing (%)	Mean (SD) or n (%) in sample	
		Observed data	Imputed data
Maternal pre-pregnancy BMI (kg/m^2)	191 (7.6)	22.89 (3.74)	22.89 (3.74)
Maternal education	116 (4.6)		
<O-level		417 (17.4)	438 (17.4)
O-level		848 (35.4)	891 (35.5)
>O-level		1130 (47.2)	1182 (47.1)
Parity	57 (2.3)		
0		1200 (48.9)	1226 (48.8)
1+		1254 (51.1)	1285 (51.2)
Maternal occupation	353 (14.1)		
Manual		322 (14.9)	409 (16.3)
Non-manual		1846 (85.1)	2102 (83.7)
Tobacco use during the first trimester	32 (1.3)		
No		2072 (83.6)	2101 (83.7)
Yes		1254 (16.4)	410 (16.3)
Maternal depression during pregnancy	320 (12.7)		
No		1677 (76.5)	1910 (76.1)
Yes		514 (23.5)	601 (23.9)

Supplemental Table 3.2 Comparison of covariate distribution among eligible sample and participants excluded due to missing offspring SMFQ

	Excluded sample (n=1297)	Eligible sample (n=3173)	p[1]
Maternal 25(OH)D (ng/mL), median [IQR][2]	24.14 [17.68, 32.82]	25.45 [18.89, 33.42]	**0.003**
Standardized MDD PRS, mean (SD)	0.03 (0.99)	-0.04 (1.01)	**0.04**
Maternal age at birth (years), n (%)			
15-19	77 (5.9)	46 (1.4)	**<0.001**
20-35	1150 (88.7)	2857 (90.0)	
>35	70 (5.4)	270 (8.5)	
Maternal BMI (kg/m^2), median [IQR]	22.02 [20.47, 24.51]	22.18 [20.53, 24.38]	0.79
Maternal education, n (%)			
Lower than O-levels	301 (29.7)	577 (19.3)	**<0.001**
O-levels	390 (38.5)	1080 (36.1)	
Higher than O-levels	323 (31.9)	1332 (44.6)	
Maternal occupation, n (%)			
Manual	195 (22.4)	427 (16.0)	**<0.001**
Non-manual	676 (77.6)	2237 (84.0)	
Parity, n (%)			
0	500 (41.9)	1446 (46.8)	**0.005**
≥1	693 (58.1)	1647 (53.2)	
Gestational week of 25(OH)D measurement, median [IQR]	29.71 [13.00, 33.29]	29.43 [12.71, 33.14]	0.26
Season of 25(OH)D measurement, n (%)			
Winter	303 (23.4)	758 (23.9)	0.78
Spring	381 (29.4)	912 (28.7)	
Summer	305 (23.5)	781 (24.6)	
Fall	308 (23.7)	722 (22.8)	
Tobacco use during 1st trimester, n (%)			
No	832 (68.5)	2569 (82.2)	**<0.001**
Yes	383 (31.5)	557 (17.8)	
Maternal depression during pregnancy, n (%)			
No	656 (70.0)	2066 (75.6)	**0.001**
Yes	281 (30.0)	667 (24.4)	

[1] P-value calculated using Chi-squared test for categorical variables and the student's t-test or Kruskall-Wallis test for continuous variables

[2] Mean annual 25(OH)D levels

Supplemental Table 3.3 Association between maternal vitamin D status during pregnancy, PRS, and child/adolescent depression, additionally adjusting for other covariates (Model 5)

	Childhood depression (n=2938)				Adolescent depression (n=2485)			
	OR	**95% CI**	**p**	**p-interaction**	**OR**	**95% CI**	**p**	**p-interaction**
Model 5: PRS and Maternal 25(OH)D								
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	0.63	0.28, 1.42	0.27	-	1.14	0.62, 2.10	0.66	-
Deficient (<20 ng/mL)	0.35	0.12, 0.99	0.05		1.46	0.79, 2.71	0.23	
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	0.74	0.37, 1.48	0.40	-	1.22	0.72, 2.08	0.46	-
PRS-High	1.28	0.64, 2.59	0.48		1.09	0.59, 2.00	0.79	
Insufficient*PRS-Intermediate	1.94	0.73, 5.19	0.19		0.80	0.38, 1.67	0.55	
Insufficient*PRS-High	4.06	1.25, 13.19	0.02	0.20	0.68	0.32, 1.44	0.31	0.37
Deficient*PRS-Intermediate	1.61	0.86, 4.48	0.36		1.38	0.60, 3.15	0.44	
Deficient*PRS-High	2.95	0.58, 10.12	0.08		1.37	0.59, 3.16	0.46	

Abbreviations: CI: confidence intervals; OR: odds ratio; PRS: polygenic risk score

Model 5: Model 4 covariates + oily fish intake + vitamin D supplementation + vitamin D intake + Calcium intake

Supplemental Table 3.4 Association between maternal vitamin D status during pregnancy, PRS, and child/adolescent depression, using thresholds of <10, 10-19.99, and ≥20 ng/mL

	Childhood depression (n=2938)					Adolescent depression (n=2485)				
	n	**OR**	**95% CI**	**p**	**p-trend**	**n**	**OR**	**95% CI**	**p**	**p-trend**
Model 1: Maternal 25(OH)D										
Normal (≥20 ng/mL)	2075	*ref*	*ref*	*ref*		1748	*ref*	*ref*	*ref*	
Insufficient (10-19.9 ng/mL)	792	1.08	0.77, 1.51	0.66	0.83	682	1.26	0.97, 1.64	0.09	0.14
Deficient (<10 ng/mL)	71	0.87	0.31, 2.46	0.80		55	1.05	0.46, 2.39	0.92	
Model 2: PRS										
PRS-Low	735	*ref*	*ref*	*ref*		622	*ref*	*ref*	*ref*	
PRS-Intermediate	1471	1.37	0.90, 2.09	0.14	**0.003**	1242	0.99	0.73, 1.34	0.93	0.06
PRS-High	732	**1.94**	**1.24, 3.03**	**0.004**		621	1.34	0.96, 1.87	0.09	
Model 3: PRS and Maternal 25(OH)D (Main effects)										
Normal (≥20 ng/mL)	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
Insufficient (10-19.9 ng/mL)	-	1.08	0.77, 1.51	0.67	0.82	-	1.27	0.97, 1.65	0.08	0.14
Deficient (<10 ng/mL)	-	0.89	0.32, 2.50	0.82		-	1.04	0.45, 2.38	0.93	
PRS-Low	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
PRS-Intermediate	-	1.37	0.90, 2.09	0.15	**0.003**	-	0.99	0.73, 1.34	0.93	0.07
PRS-High	**-**	**1.93**	**1.23, 3.03**	**0.004**		-	1.34	0.96, 1.87	0.09	

Supplemental Table 3.4 (continued)

	Childhood depression (n=2938)					Adolescent depression (n=2485)				
	n	**OR**	**95% CI**	**p**	**p-trend**	**n**	**OR**	**95% CI**	**p**	**p-trend**
Model 4: PRS and Maternal 25(OH)D (Interaction)										
Normal (≥20 ng/mL)	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
Insufficient (10-19.9 ng/mL)	-	0.40	0.14, 1.17	0.10	-	-	1.41	0.82, 2.41	0.21	-
Deficient (<10 ng/mL)	-	1.16	0.15, 9.26	0.89		-	0.70	0.09, 5.60	0.74	
PRS-Low	-	*ref*	*ref*	*ref*		-	*ref*	*ref*	*ref*	
PRS-Intermediate	-	1.04	0.64, 1.69	0.86	-	-	1.07	0.74, 1.55	0.71	-
PRS-High	-	1.60	0.96, 2.67	0.07		-	1.27	0.84, 1.92	0.25	
Insufficient*PRS-Intermediate	-	3.46	1.08, 11.07	0.04		-	0.72	0.37, 1.39	0.32	
Deficient*PRS-Intermediate	-	0.71	0.06, 8.97	0.79	0.25	-	2.03	0.20, 20.32	0.55	0.39
Insufficient*PRS-High	-	2.68	0.79, 9.08	0.11		-	1.18	0.58, 2.42	0.64	
Deficient*PRS-High	-	0.72	0.04, 13.52	0.83		-	0.75	0.04, 14.63	0.85	

Abbreviations: CI: confidence intervals; OR: odds ratio; p-inter.: p for interaction; PRS: polygenic risk score
Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal occupation, parity, smoking during 1st trimester, maternal depression during pregnancy, and child sex
Model 2: Model 1 confounders + 3 PCs
Model 3: Model 2 confounders
Model 4: Model 3 confounders + significant PRS*covariate, maternal 25(OH)D*covariate interactions

Supplemental Table 3.5 Association between maternal vitamin D status during pregnancy, PRS, and child/adolescent depression, stratified by trimester of 25(OH)D measurement

	Trimester 1									
	Childhood depression (n=781)					Adolescent depression (n=652)				
	OR	LCI	UCI	p	p-trend	OR	LCI	UCI	p	p-trend
Model 1: Maternal 25(OH)D										
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	1.56	0.69	3.52	0.28	0.34	1.12	0.61	2.05	0.71	0.09
Deficient (<20 ng/ml)	1.55	0.67	3.56	0.30		1.62	0.90	2.92	0.11	
Model 2: PRS										
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	1.40	0.64	3.08	0.40	0.72	0.97	0.53	1.77	0.93	0.27
PRS-High	1.19	0.46	3.07	0.72		1.39	0.71	2.70	0.34	
Model 3: PRS and Maternal 25(OH)D (Main effects)										
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	1.58	0.70	3.57	0.27	0.34	1.14	0.62	2.08	0.68	0.09
Deficient (<20 ng/ml)	1.55	0.68	3.58	0.30		1.64	0.91	2.98	0.10	
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	1.42	0.64	3.14	0.38	0.70	0.97	0.53	1.77	0.93	0.29
PRS-High	1.22	0.47	3.14	0.69		1.40	0.71	2.73	0.33	
Model 4: PRS and Maternal 25(OH)D (Interaction)	**OR**	**LCI**	**UCI**	**p**	**p-inter.**	**OR**	**LCI**	**UCI**	**p**	**p-inter.**
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	0.51	0.10	2.71	0.43	-	0.65	0.15	2.84	0.57	-
Deficient (<20 ng/ml)	0.54	0.10	3.03	0.49		2.38	0.65	8.69	0.19	
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	0.43	0.09	2.08	0.29	-	1.01	0.29	3.49	0.99	-
PRS-High	0.54	0.08	3.51	0.52		1.30	0.33	5.16	0.71	
Insufficient*PRS-Intermediate	5.00	0.65	38.35	0.12		1.69	0.31	9.22	0.54	
Deficient*PRS-Intermediate	4.30	0.53	34.61	0.17	0.62	0.65	0.14	3.01	0.58	0.46
Insufficient*PRS-High	2.90	0.25	33.14	0.39		2.56	0.40	16.29	0.32	
Deficient*PRS-High	3.00	0.25	35.22	0.38		0.56	0.10	3.17	0.51	

Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal social class, parity, smoking during 1st trimester, maternal psychopathology during pregnancy, child sex
Model 2: Model 1 confounders + 3 PCs
Model 3: Model 2 confounders
Model 4: Model 3 confounders + any significant PRS*covariate and maternal 25(OH)D*covariate interactions

Supplemental Table 3.5 (continued)

	Trimester 2									
	Childhood depression (n=438)					Adolescent depression (n=381)				
	OR	**LCI**	**UCI**	**p**	**p-trend**	**OR**	**LCI**	**UCI**	**p**	**p-trend**
Model 1: Maternal 25(OH)D										
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	0.78	0.30	2.03	0.61	0.05	1.30	0.63	2.66	0.48	0.91
Deficient (<20 ng/ml)	0.21	0.04	1.03	0.05		1.03	0.48	2.19	0.95	
Model 2: PRS										
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	1.41	0.36	5.51	0.62	0.14	0.64	0.30	1.36	0.24	0.71
PRS-High	2.60	0.65	10.43	0.18		0.79	0.34	1.84	0.59	
Model 3: PRS and Maternal 25(OH)D (Main effects)										
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	0.74	0.28	1.98	0.55	0.04	1.33	0.64	2.77	0.44	0.80
Deficient (<20 ng/ml)	0.20	0.04	0.95	0.04		1.10	0.51	2.37	0.81	
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	1.38	0.34	5.55	0.65	0.12	0.64	0.30	1.36	0.25	0.64
PRS-High	1.76	0.67	11.33	0.16		0.80	0.35	1.87	0.61	
Model 4: PRS and Maternal 25(OH)D (Interaction)*	**OR**	**LCI**	**UCI**	**p**	**p-inter.**	**OR**	**LCI**	**UCI**	**p**	**p-inter.**
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	-	-	-	-	-	-	-	-	-	-
Deficient (<20 ng/ml)	-	-	-	-		-	-	-	-	
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	-	-	-	-	-	-	-	-	-	-
PRS-High	-	-	-	-		-	-	-	-	
Insufficient*PRS-Intermediate	-	-	-	-		-	-	-	-	
Deficient*PRS-Intermediate	-	-	-	-		-	-	-	-	
Insufficient*PRS-High	-	-	-	-		-	-	-	-	
Deficient*PRS-High	-	-	-	-		-	-	-	-	

Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal social class, parity, smoking during 1st trimester, maternal psychopathology during pregnancy, child sex
Model 2: Model 1 confounders + 3 PCs
Model 3: Model 2 confounders
Model 4: Insufficient sample size to run GxE analysis

Supplemental Table 3.5 (continued)

	Trimester 3									
	Childhood depression (n=1719)					Adolescent depression (n=1452)				
	OR	**LCI**	**UCI**	**p**	**p-trend**	**OR**	**LCI**	**UCI**	**p**	**p-trend**
Model 1: Maternal 25(OH)D										
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	0.91	0.56	1.47	0.71	0.53	1.13	0.76	1.66	0.55	0.19
Deficient (<20 ng/ml)	1.17	0.72	1.92	0.52		1.31	0.87	1.98	0.19	
Model 2: PRS										
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	1.27	0.73	2.21	0.39	**0.004**	1.12	0.75	1.69	0.58	0.06
PRS-High	**2.15**	**1.22**	**3.78**	**0.008**		1.54	0.98	2.40	0.06	
Model 3: PRS and Maternal 25(OH)D (Main effects)										
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	0.92	0.57	1.49	0.74	0.50	1.10	0.75	1.63	0.62	0.23
Deficient (<20 ng/ml)	1.20	0.73	1.96	0.47		1.30	0.86	1.96	0.22	
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	1.27	0.73	2.21	0.40	**0.005**	1.12	0.74	1.69	0.58	0.06
PRS-High	**2.15**	**1.22**	**3.79**	**0.008**		1.54	0.98	2.41	0.06	
Model 4: PRS and Maternal 25(OH)D (Interaction)*	**OR**	**LCI**	**UCI**	**p**	**p-inter.**	**OR**	**LCI**	**UCI**	**p**	**p-inter.**
Normal (≥30 ng/ml)	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
Insufficient (20-30 ng/ml)	0.71	0.26	1.95	0.51	-	1.12	0.51	2.45	0.78	-
Deficient (<20 ng/ml)	0.26	0.06	1.22	0.09		1.10	0.48	2.54	0.82	
PRS-Low	*ref*	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	*ref*	
PRS-Intermediate	0.72	0.30	1.72	0.46	-	1.31	0.67	2.56	0.43	-
PRS-High	1.35	0.57	3.17	0.50		1.00	0.45	2.21	0.99	
Insufficient*PRS-Intermediate	1.28	0.36	4.61	0.70		0.78	0.30	2.03	0.61	
Deficient*PRS-Intermediate	7.25	1.31	40.10	0.02	0.10	0.80	0.28	2.24	0.67	0.24
Insufficient*PRS-High	1.61	0.46	5.71	0.46		1.48	0.80	4.39	0.48	
Deficient*PRS-High	4.64	0.81	26.50	0.08		2.47	0.50	7.69	0.12	

Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal social class, parity, smoking during 1st trimester, maternal psychopathology during pregnancy, child sex
Model 2: Model 1 confounders + 3 PCs
Model 3: Model 2 confounders
Model 4: Model 3 confounders + any significant PRS*covariate and maternal 25(OH)D*covariate interactions

Supplemental Table 3.6 Complete case analysis for the association between maternal vitamin D status during pregnancy, PRS, and child/adolescent depression

	Childhood depression (n=2091)				Adolescent depression (n=1812)			
	OR	**95% CI**	**p**	**p-trend**	**OR**	**95% CI**	**p**	**p-trend**
Model 1: Maternal 25(OH)D								
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref* *ref*	*ref*	
Insufficient (20-29.9 ng/mL)	1.19	0.77, 1.84	0.43	0.57	1.01	0.71, 1.43	0.97	0.15
Deficient (<20 ng/mL)	1.14	0.72, 1.80	0.57		1.30	0.91, 1.85	0.14	
Model 2: PRS								
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	1.39	0.89, 2.24	0.16	0.12	0.85	0.60, 1.20	0.34	0.10
PRS-High	1.51	0.90, 2.56	0.12		1.36	0.93, 1.99	0.11	
Model 3: PRS and Maternal 25(OH)D (Main effects)								
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	1.19	0.77, 1.84	0.44	0.55	1.03	0.73, 1.47	0.86	0.15
Deficient (<20 ng/mL)	1.14	0.72, 1.80	0.58		1.32	0.92, 1.88	0.13	
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	1.38	0.88, 2.23	0.17	0.12	0.85	0.60, 1.20	0.34	0.10
PRS-High	1.51	0.90, 2.56	0.12		1.36	0.93, 2.00	0.11	
Model 4: PRS and Maternal 25(OH)D (Interaction)	**OR**	**95% CI**	**p**	**p-inter.**	**OR**	**95% CI**	**p**	**p-inter.**
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	0.49	0.17, 1.32	0.16	-	0.71	0.35, 1.43	0.35	-
Deficient (<20 ng/mL)	0.29	0.09, 0.84	0.03		1.28	0.65, 2.54	0.47	
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	0.74	0.36, 1.57	0.43	-	4.50	1.19, 17.7	0.03	-
PRS-High	0.61	0.24, 1.47	0.28		3.73	0.77, 19.3	0.11	
Insufficient*PRS-Intermediate	2.70	0.90, 0.87	0.08		0.85	0.35, 2.05	0.72	
Insufficient*PRS-High	2.89	0.92, 0.99	0.08	0.12	0.72	0.31, 1.66	0.44	0.18
Deficient*PRS-Intermediate	4.70	1.35, 1.78	0.02		2.36	0.90, 6.30	0.08	
Deficient*PRS-High	3.39	0.88, 1.41	0.08		1.47	0.57, 3.86	0.43	

Abbreviations: CI: confidence intervals; OR: odds ratio; p-inter.: p for interaction; PRS: polygenic risk score

Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal occupation, parity, smoking during 1st trimester, maternal depression during pregnancy, and child sex

Model 2: Model 1 confounders + 3 PCs

Model 3: Model 2 confounders

Model 4: Model 3 confounders + significant PRS*covariate, maternal 25(OH)D*covariate interactions

Supplemental Table 3.7 Negative binomial regression results for the association between maternal vitamin D status during pregnancy, PRS, and child/adolescent depressive symptoms

	Childhood depression (n=2938)				Adolescent depression (n=2485)			
	RR	**95% CI**	**p**	**p-trend**	**RR**	**95% CI**	**p**	**p-trend**
Model 1: Maternal 25(OH)D								
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	1.02	0.94, 1.10	0.68	0.48	1.02	0.94, 1.11	0.63	0.15
Deficient (<20 ng/mL)	1.03	0.95, 1.12	0.48		1.07	0.98, 1.17	0.15	
Model 2: PRS								
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	1.10	1.01, 1.18	0.03	**0.002**	1.01	0.93, 1.10	0.86	**0.03**
PRS-High	1.15	1.06, 1.26	0.002		1.11	1.01, 1.23	0.04	
Model 3: PRS and Maternal 25(OH)D (Main effects)								
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	1.02	0.94, 1.10	0.68	0.49	1.02	0.94, 1.11	0.63	0.15
Deficient (<20 ng/mL)	1.03	0.95, 1.11	0.50		1.07	0.98, 1.17	0.15	
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	1.09	1.01, 1.18	0.03	**0.002**	1.01	0.92, 1.10	0.87	**0.04**
PRS-High	1.15	1.06, 1.26	0.002		1.11	1.01, 1.23	0.04	
Model 4: PRS and Maternal 25(OH)D (Interaction)*	**RR**	**95% CI**	**p**	**p-inter.**	**RR**	**95% CI**	**p**	**p-inter.**
Normal (≥30 ng/mL)	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
Insufficient (20-29.9 ng/mL)	0.95	0.82, 1.11	0.52	-	1.06	0.89, 1.26	0.49	-
Deficient (<20 ng/mL)	0.94	0.80, 1.11	0.49		0.99	0.83, 1.19	0.94	
PRS-Low	*ref*	*ref*	*ref*		*ref*	*ref*	*ref*	
PRS-Intermediate	1.02	0.90, 1.17	0.72	-	1.03	0.88, 1.19	0.72	-
PRS-High	1.08	0.93, 1.26	0.32		1.04	0.88, 1.24	0.63	
Insufficient*PRS-Intermediate	1.09	0.91, 1.32	0.34		0.91	0.74, 1.12	0.39	
Deficient*PRS-Intermediate	1.11	0.91, 1.36	0.28	0.79	1.05	0.84, 1.31	0.66	0.41
Insufficient*PRS-High	1.09	0.88, 1.34	0.43		1.02	0.81, 1.30	0.84	
Deficient*PRS-High	1.13	0.90, 1.42	0.28		1.21	0.94, 1.56	0.14	

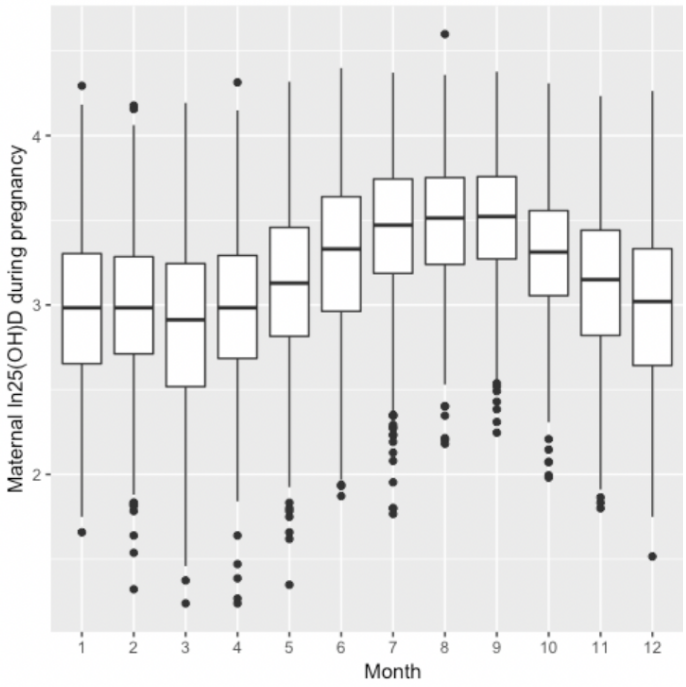
Abbreviations: CI: confidence intervals; RR: risk ratio; p-inter.: p for interaction; PRS: polygenic risk score

Model 1: Adjusted for gestational age of 25(OH)D measurement, maternal age, maternal pre-pregnancy BMI, maternal education, maternal occupation, parity, smoking during 1st trimester, maternal depression during pregnancy, and child sex

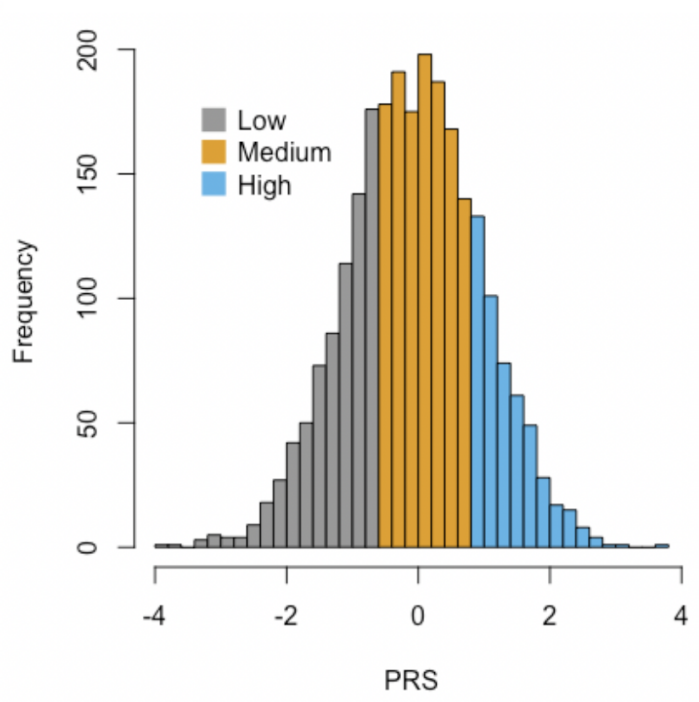
Model 2: Model 1 confounders + 3 PCs

Model 3: Model 2 confounders

Model 4: Model 3 confounders + significant PRS*covariate, maternal 25(OH)D*covariate interactions

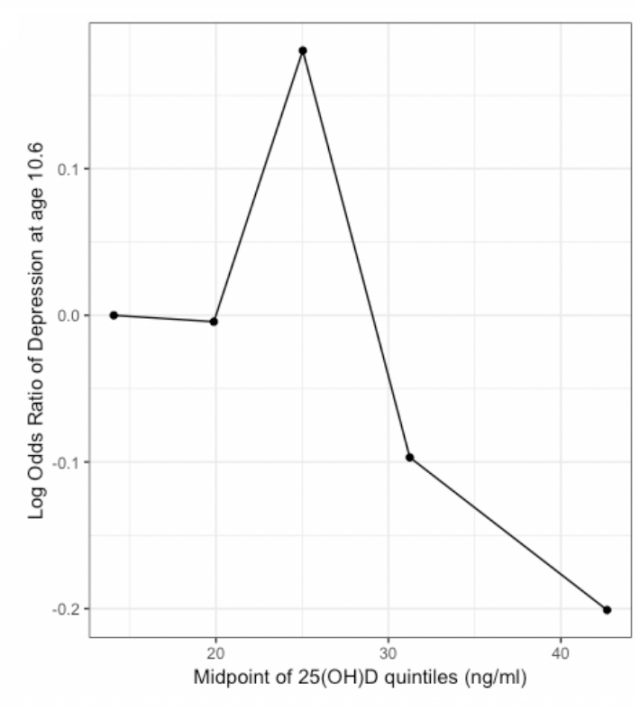


Supplemental Figure 3.1 Patterns of maternal 25(OH)D levels during pregnancy by month in the study population

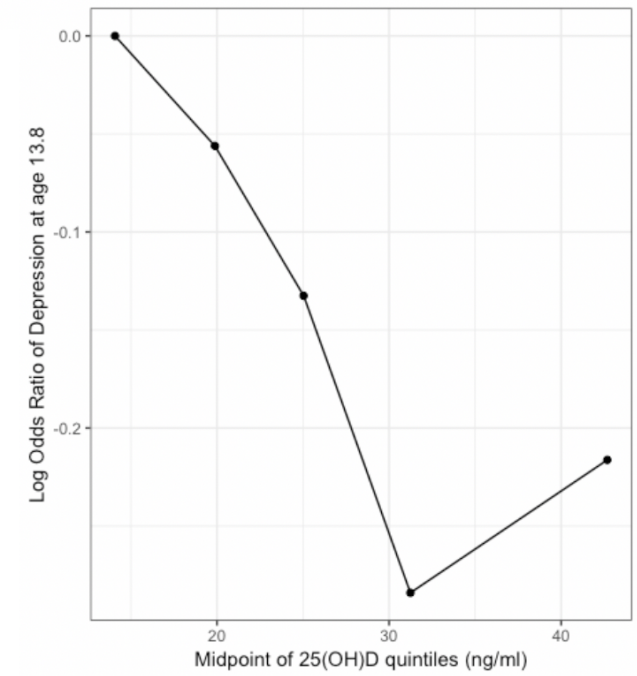


Supplemental Figure 3.2 Distribution of offspring MDD PRS and categorizations (low, medium, and high polygenic risk) in the study population

(A)



(B)



Supplemental Figure 3.3 The association between quintiles of maternal 25(OH)D, compared to the lowest quintile, and the log odds of offspring depression in (A) childhood and (B) adolescence