Pilling et al. 2020: Vitamin D & delirium

Supplementary Information

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Supplementary Methods

Mendelian randomization analysis

Mendelian randomization (MR) analyses are used to determine whether an association between a risk factor (such as vitamin D) and an outcome (such as delirium) may share a causal pathway. If individuals carrying more vitamin D-increasing genetic variants have greater risk of delirium, this supports the hypothesis that there is a shared causal pathway. We previously applied these methods to an earlier version of the UK Biobank data (Bowman *et al.*, 2019) and extend the analysis using the longer follow-up now available (*n*=3,405 delirium cases, up from 544 in our previous work).

Known genetic variants associated with circulating 25[OH]D concentration were extracted from a large meta-analysis by Jiang et al. (Jiang *et al.*, 2018) of 79,366 Europeans that did not include UK Biobank participants. Only one variant from each locus was included, and only if the final meta-analysis p value was <5 × 10−8; all included variants had sufficient imputation quality [>0.4] and were in Hardy-Weinberg equilibrium [p >1× 10−6] in the UK Biobank participants.

The genome-wide association study described above was performed in individuals of European descent; we therefore restricted the genetic analysis in this study to UK Biobank participants of European descent (n=451,367 of 487,442 with complete genetics data), the derivation of which is described previously(Thompson *et al.*, 2019). 326,558participants met the above criteria for analysis (i.e. with complete vitamin D and delirium incidence data), had complete genetic data, and were of European ancestry.

The primary MR analysis used 6 genetic variants from the larger Jiang et al. (Jiang *et al.*, 2018) meta-analysis to determine whether there was evidence for a causal effect of vitamin D on delirium risk. A genetic risk score was computed by summing the number of vitamin D-increasing alleles each participant had, weighted by the reported effect on vitamin D levels (Jiang *et al.*, 2018). R (v4.0.2) package `MendelianRandomization` (v0.4.2) was used in the primary analysis to apply two-sample Mendelian Randomization analysis methods to test the association between serum vitamin D and delirium risk. This package tests whether the result is robust to several assumptions of the different models. First, penalized robust inverse-variance weighted (IVW) regression assumes there is no unbalanced horizontal pleiotropy. Secondly, the penalized weighted median estimate assumes less than 50% of the weight in the analysis comes from invalid instruments. Thirdly, penalized robust MR-Egger regression assumes the genetic variants' effect is not correlated with any pleiotropic effect on the outcome. Lastly, the intercept from the MR-Egger regression, unlike IVW the MR-Egger

intercept, is not fixed, and deviation from the null is used to test for possible horizontal pleiotropy. We used the output of the penalized robust IVW regression result as the primary analysis, checked the others for consistency of effect, and observed the MR-Egger intercept for evidence of no significant deviation from the null. In secondary analysis we examined whether the large effect vitamin D SNP rs3755967 was disproportionately affecting the results in two ways: firstly, R package `RadialMR` (v0.4)(Bowden *et al.*, 2018) was used to apply Radial IVW analysis (using default options) to investigate whether any SNPs were outliers, and secondly we repeated the primary IVW analysis excluding rs3755967.

To convert the IVW estimate to units of delirium HR per standard deviation (SD) change in genetically instrumented vitamin D, we used the mean (3.802) and SD (0.464) for logged vitamin D in the 351,320 UK Biobank participants. We multiplied the IVW estimate and CIs by 0.464, then exponentiated to get the estimate on the HR scale (logHR is required for MR analysis).

Additional and sensitivity analyses

The time-to-event analysis between baseline risk factors and incident delirium were repeated using Fine and Gray competing risks regression(Jason P. Fine, 1999) to account for the competing risk of mortality: the same adjustments and specifications were used as described above in each case.

We performed additional analyses with adjustment for self-reported smoking status (at baseline) and highest education level attained to determine whether the association was confounded by tobacco exposure or socio-economic status (using education as a proxy).

Sensitivity analysis excluded a subset of participants (n=30,956, 8.8%) reporting taking vitamin D or multivitamin supplements at the baseline, either over the counter (data field 6155 (Vitamin and mineral supplements), using the code 4 (Vitamin D)) or prescribed (data field 20003 (Vitamin and mineral supplements), using the codes 1140852766 1140852948 1140870954 1140877612 1140852976 1140876592 1140909766 1141164602).

Serum calcium measurement (in mmol/L) was performed by Beckman Coulter AU5800 analyzer, with data available on 321,224 of the 351,320 participants eligible for the described analyses.

Increased rates of delirium diagnosis observed with vitamin D levels may be secondary to the effect of vitamin D deficiency on increased hospitalization from specific, relevant conditions. Sensitivity analyses excluded the following hospital-recorded diagnoses: bone fractures, chronic kidney disease

(CKD), dementia, liver disease (all known causes of delirium (Fong, Tulebaev and Inouye, 2009)), and Parkinson's (patients with Parkinson's are known to be at increased risk of delirium (Ebersbach *et al.*, 2019), and have lower bone mineral density with higher incidence of hip fractures (Critchley *et al.*, 2015)). See Supplementary Table S1 for ICD-10 codes.

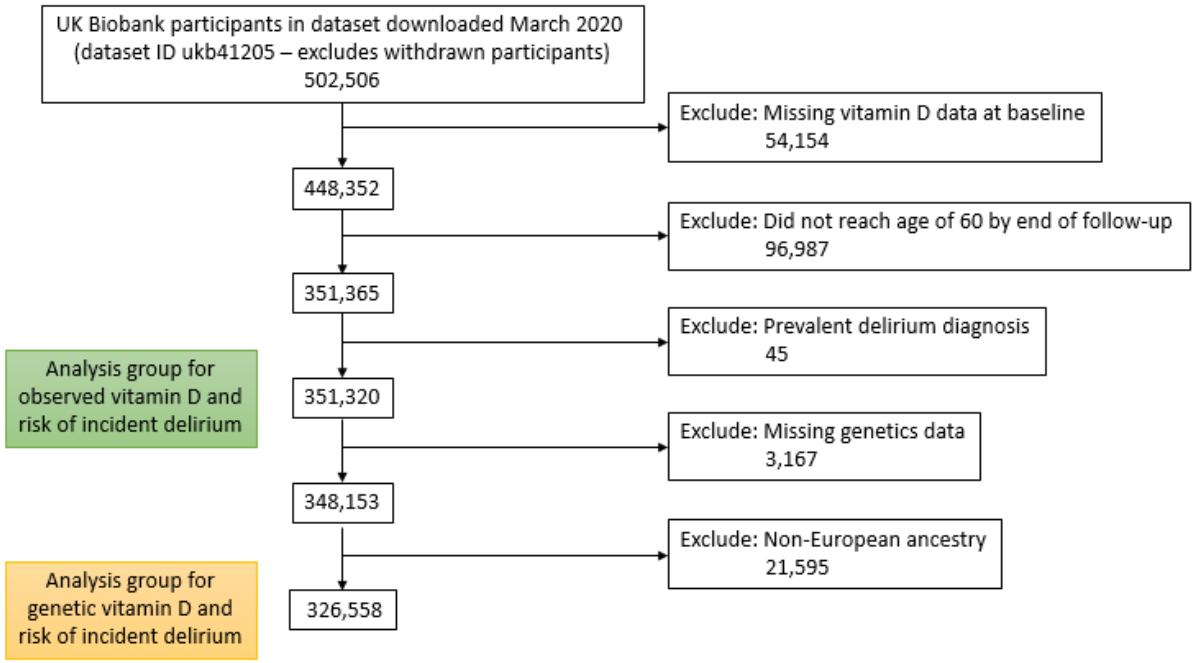
Frail individuals are known to have increased risk of delirium (Quinlan *et al.*, 2011) so we utilized a validated Frailty Index (FI) for the UK Biobank (Williams *et al.*, 2019). The FI is a 49-item count of health deficits including disease diagnoses and disabilities, mental health, and wellbeing. Of these, 286,616 of 351,320 had complete data for the FI. We included FI as a continuous covariate in sensitivity analysis.

Since it is known that vitamin D levels are lower in non-white individuals but bioavailable levels are unchanged, current cut-offs for deficiency may have less significance in e.g. blacks compared to whites (Powe *et al.*, 2013). We therefore repeated the analysis on the 335,517 participants (96%) who self-reported as white.

Supplementary Table S1: ICD-10 codes for clinical conditions from the hospital inpatient data

Outcome	**ICD-10 code(s)**
Delirium	F05*
Bone fracture	S02*; S12*; S22*; S32*; S42*; S52*; S62*; S72*; S82*; S92*; T02*; T08*; T10*; T12*; T14.2
Chronic kidney disease	N18; N183; N184; N185; Y841
Dementia	F00*; F01*; F02*; F03*; G30*
Liver disease	K70-K77*
Parkinson's disease	G20; F02.3

Supplementary Figure S1: Cohort flowchart



Supplementary Results

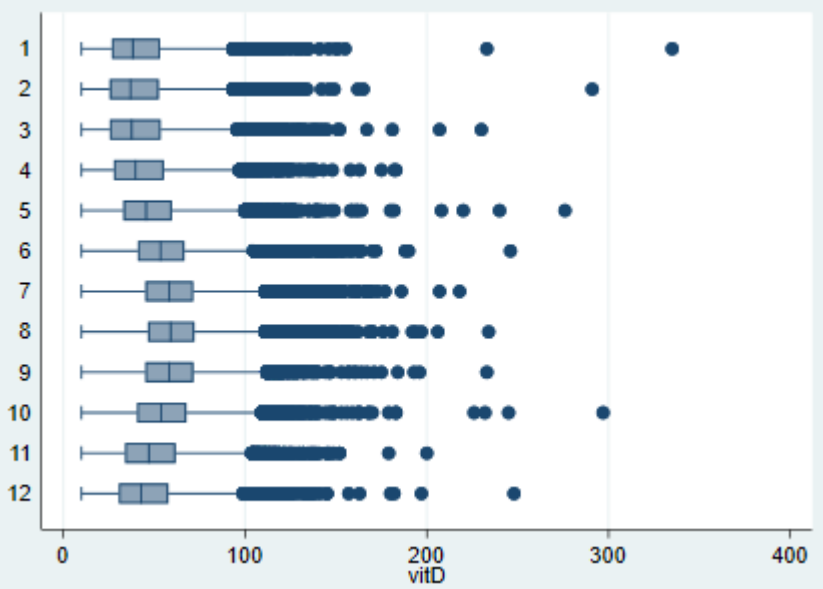
Seasonal variation in vitamin D

Supplementary Table S2: vitamin D levels associated with assessment month

Assessment month	N	%	Coef.	95% CIs		p
Jan	23,878	6.8	*ref*			
Feb	27,788	7.91	-0.806	-1.099	-0.512	7.5*10-8
Mar	33,856	9.64	-0.531	-0.813	-0.250	2.1*10-4
Apr	30,502	8.68	1.648	1.360	1.935	2.7*10-29
May	36,213	10.31	6.236	5.958	6.514	<1*10-324
Jun	36,666	10.44	13.607	13.327	13.887	<1*10-324
Jul	30,817	8.77	18.188	17.897	18.479	<1*10-324
Aug	27,516	7.83	19.346	19.045	19.647	<1*10-324
Sep	25,268	7.19	18.823	18.519	19.127	<1*10-324
Oct	30,251	8.61	13.958	13.667	14.250	<1*10-324
Nov	29,106	8.28	7.747	7.456	8.038	<1*10-324
Dec	19,459	5.54	4.173	3.853	4.494	3.0*10-143

Note: Linear regression models with independent variables age, sex, assessment centre, assessment month and ethnicity

Supplementary Figure S2: Boxplot of vitamin D by assessment month



Vitamin D variation and assessment centre

Supplementary Table S3: Vitamin D variation by assessment centre

Assessment centre	N	%	Coef.	95% CIs		p
Leeds	31,595	8.99	*ref*			
Manchester	10,167	2.89	-0.891	-1.275	-0.507	5.4E-06
Oxford	11,000	3.13	1.078	0.694	1.461	3.5E-08
Cardiff	11,637	3.31	2.324	1.974	2.675	1.4E-38
Glasgow	12,167	3.46	-4.756	-5.104	-4.409	3.0E-158
Edinburgh	11,165	3.18	-4.210	-4.570	-3.850	3.0E-116
Stoke	14,710	4.19	0.393	0.052	0.734	2.4E-02
Reading	21,448	6.1	1.861	1.562	2.159	2.8E-34
Bury	21,589	6.15	0.293	-0.008	0.594	5.6E-02
Newcastle	27,215	7.75	-1.064	-1.345	-0.783	1.1E-13
Bristol	29,751	8.47	2.174	1.903	2.445	9.9E-56
Barts	8,116	2.31	-1.598	-2.003	-1.193	1.0E-14
Nottingham	24,667	7.02	0.886	0.598	1.175	1.7E-09
Sheffield	21,183	6.03	1.593	1.294	1.891	1.3E-25
Liverpool	23,496	6.69	1.300	1.009	1.591	2.1E-18
Middlesborough	15,072	4.29	1.397	1.063	1.732	2.6E-16
Hounslow	19,329	5.5	0.866	0.562	1.170	2.4E-08
Croydon	18,606	5.3	0.335	0.026	0.645	3.4E-02
Birmingham	16,706	4.76	-0.351	-0.668	-0.034	3.0E-02
Swansea	1,315	0.37	1.895	1.014	2.776	2.5E-05
Wrexham	386	0.11	1.199	-0.376	2.773	1.4E-01

Note: Linear regression models with independent variables age, sex, assessment centre, assessment month and ethnicity

Vitamin D variation and self-reported ethnic group

Supplementary Table S4: UK Biobank ethnic group coding

Ethnic background	UKB code	Collapsed groups for analysis
Missing	.	*Missing*
Prefer not to answer	-3	*Missing*
Do not know	-1	*Missing*
White	1	White
Mixed	2	Mixed
Asian or Asian British	3	Asian
Black or Black British	4	Black
Chinese	5	Asian
Other ethnic group	6	Other
British	1001	White
Irish	1002	White
Any other white background	1003	White
White and Black Caribbean	2001	Mixed
White and Black African	2002	Mixed
White and Asian	2003	Mixed
Any other mixed background	2004	Mixed
Indian	3001	Asian
Pakistani	3002	Asian
Bangladeshi	3003	Asian
Any other Asian background	3004	Asian
Caribbean	4001	Black
African	4002	Black
Any other Black background	4003	Black

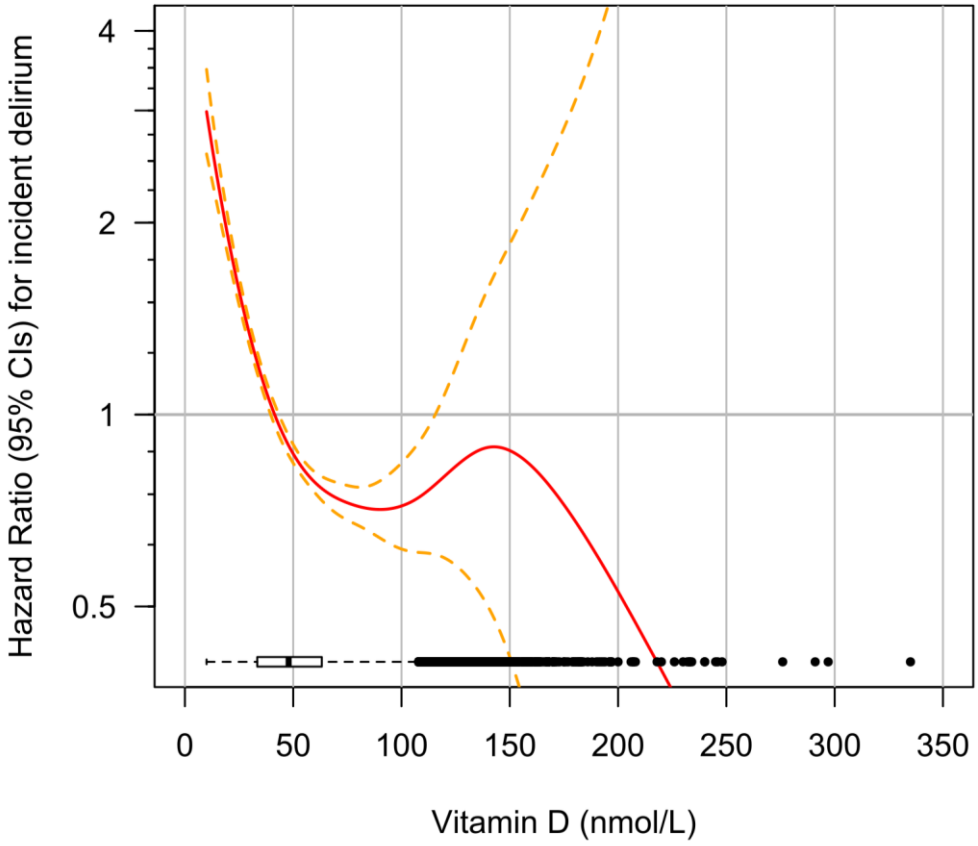
Supplementary Table S5: collapsed groups and associations with vitamin D levels

Ethnic background, collapsed groups	N	%	Coef.	95% CIs		p
White	335,517	95.5	*ref*			
Asian	6,135	1.8	-20.88	-21.28	-20.48	<1*10-324
Black	4,121	1.2	-14.49	-14.95	-14.04	<1*10-324
Other	2,486	0.7	-13.45	-14.05	-12.84	<1*10-324
Mixed	1,469	0.4	-8.67	-9.41	-7.94	8.0*10-118
Prefer not to answer/Do not know/Missing	1,592	0.5	-8.31	-9.13	-7.48	7.5*10-87

Note: Linear regression models with independent variables age, sex, assessment centre, assessment month and ethnicity

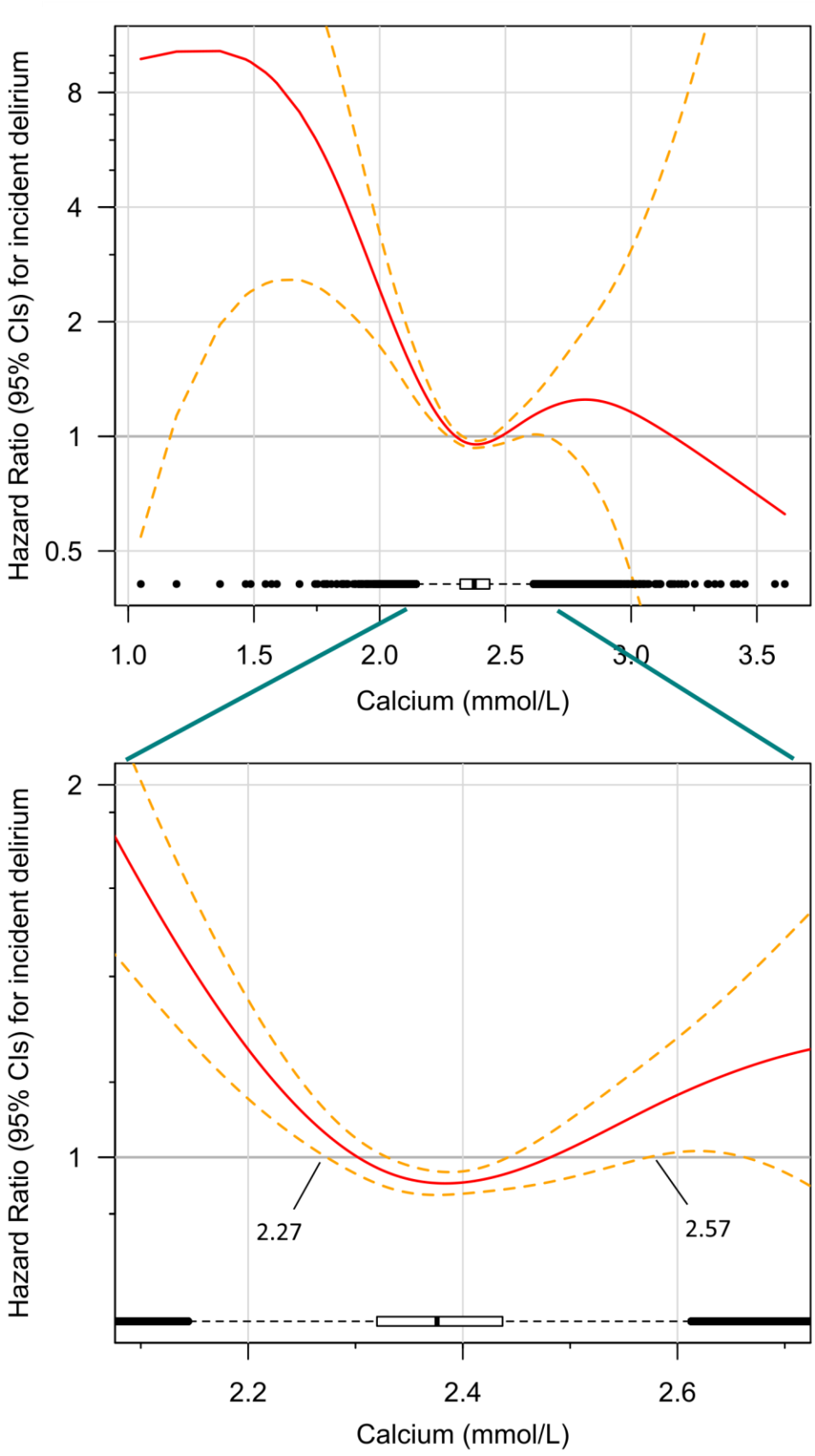
Non-linear analysis of serum vitamin D and rates of incident delirium diagnosis

Supplementary Figure S3: Time-to-event model with smoothing spline function



Non-linear analysis of serum calcium and rates of incident delirium diagnosis

Supplementary Figure S4: Time-to-event model with smoothing spline function



Mendelian Randomization full results

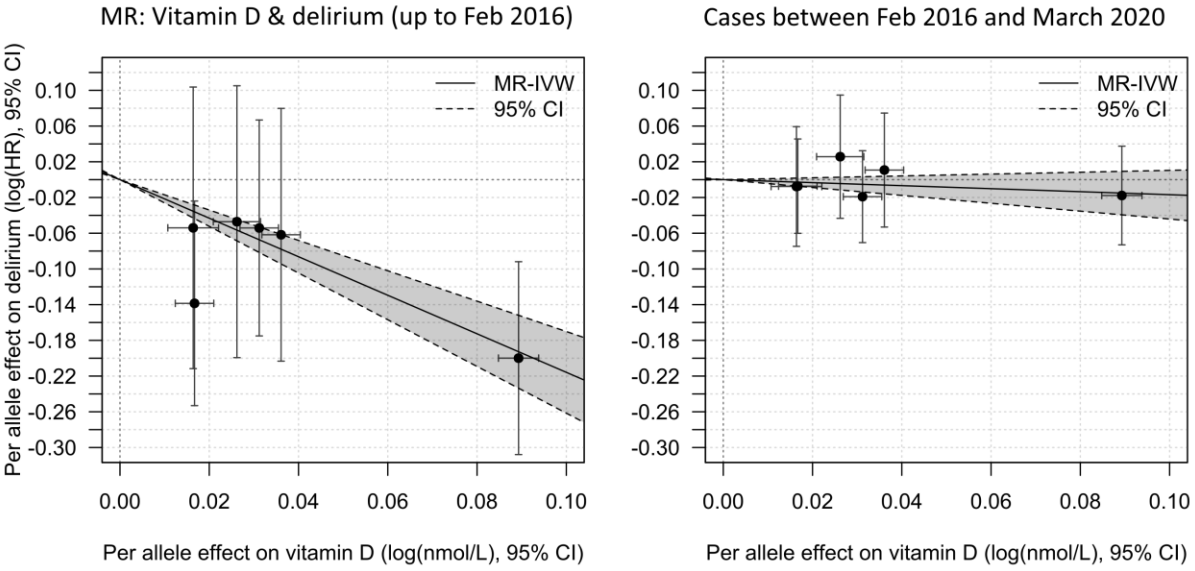
Supplementary Table S6: Expanded version of Table 3.

R package	Method	Estimate	Std Error	95% CI		P-value
MendelianRandomization	Simple median	-0.6382	0.4181	-1.4578	0.1814	1.27E-01
	Weighted median	-0.5098	0.2578	-1.0150	-0.0046	4.79E-02
	Penalized weighted median	-0.5098	0.2578	-1.0150	-0.0046	4.79E-02
	IVW	-0.4802	0.2420	-0.9546	-0.0059	4.72E-02
	Penalized IVW	-0.4802	0.2420	-0.9546	-0.0059	4.72E-02
	Robust IVW	-0.4837	0.0924	-0.6648	-0.3027	1.64E-07
	Penalized robust IVW	-0.4837	0.0924	-0.6648	-0.3027	1.64E-07
	MR-Egger	-0.4167	0.4234	-1.2466	0.4132	3.25E-01
	(intercept)	-0.0036	0.0195	-0.0418	0.0346	8.55E-01
	Penalized MR-Egger	-0.4167	0.4234	-1.2466	0.4132	3.25E-01
	(intercept)	-0.0036	0.0195	-0.0418	0.0346	8.55E-01
	Robust MR-Egger	-0.4146	0.1609	-0.7300	-0.0992	9.98E-03
	(intercept)	-0.0040	0.0128	-0.0291	0.0211	7.55E-01
	Penalized robust MR-Egger	-0.4146	0.1609	-0.7300	-0.0992	9.98E-03
	(intercept)	-0.0040	0.0128	-0.0291	0.0211	7.55E-01
RadialMR	Effect (Mod.2nd)	-0.4802	0.1449	-0.7642	-0.1961	9.23E-04
	Iterative	-0.4802	0.1449	-0.7642	-0.1961	9.23E-04
	Exact (FE)	-0.4805	0.2422	-0.9553	-0.0057	4.73E-02
	Exact (RE)	-0.4805	0.1665	-0.8069	-0.1542	3.43E-02
	Radial MR-Egger	-0.4868	0.2681	-1.0124	0.0388	1.44E-01
	Radial MR-Egger (intercept)	0.0140	0.4519	-0.8717	0.8998	9.77E-01
MendelianRandomization	Simple median	-0.7715	0.6416	-2.0289	0.4860	2.29E-01
(excluding rs3755967)	Weighted median	-0.4262	0.5423	-1.4892	0.6367	4.32E-01
	Penalized weighted median	-0.4262	0.5423	-1.4892	0.6367	4.32E-01
	IVW	-0.4121	0.4690	-1.3313	0.5071	3.80E-01
	Penalized IVW	-0.4121	0.4690	-1.3313	0.5071	3.80E-01
	Robust IVW	-0.4146	0.3043	-1.0111	0.1819	1.73E-01
	Penalized robust IVW	-0.4146	0.3043	-1.0111	0.1819	1.73E-01
	MR-Egger	0.6809	1.5899	-2.4352	3.7969	6.68E-01
	(intercept)	-0.0303	0.0421	-0.1127	0.0522	4.72E-01
	Penalized MR-Egger	0.6809	1.5899	-2.4352	3.7969	6.68E-01
	(intercept)	-0.0303	0.0421	-0.1127	0.0522	4.72E-01
	Robust MR-Egger	0.6903	0.5999	-0.4856	1.8662	2.50E-01
	(intercept)	-0.0307	0.0134	-0.0569	-0.0044	2.19E-02
	Penalized robust MR-Egger	0.6903	0.5999	-0.4856	1.8662	2.50E-01
	(intercept)	-0.0307	0.0134	-0.0569	-0.0044	2.19E-02

Mendelian Randomization stratified by date of diagnosis

Although the effect of vitamin D-associated variants on risk of delirium overall is consistent with our previous report using hospital admissions data up to Feb 2016 (Bowman *et al.*, 2019), in sensitivity analysis using only the "new" diagnoses up to March 2020 not included in the previous analysis the association is attenuated (n cases old hes = 544, n cases new hes = 2,861).

Supplementary Figure S5: Mendelian Randomization stratified by date of diagnosis



Supplementary Table S7: Mendelian Randomization stratified by date of diagnosis

	HES up to Feb 2016				HES Feb 2016 to March 2020			
Method	**Estimate**	**95% CI**		**P-value**	**Estimate**	**95% CI**		**P-value**
Simple median	-2.02	-4.06	0.02	5.2E-02	-0.32	-1.24	0.61	5.0E-01
Weighted median	-2.16	-3.29	-1.03	1.8E-04	-0.20	-0.77	0.36	4.8E-01
Penalized weighted median	-2.16	-3.29	-1.03	1.8E-04	-0.20	-0.77	0.36	4.8E-01
IVW	-2.31	-3.37	-1.24	2.3E-05	-0.16	-0.69	0.37	5.5E-01
Penalized IVW	-2.31	-3.37	-1.24	2.3E-05	-0.16	-0.69	0.37	5.5E-01
Robust IVW	-2.16	-2.62	-1.70	2.2E-20	-0.17	-0.44	0.10	2.3E-01
Penalized robust IVW	-2.16	-2.62	-1.70	2.2E-20	-0.17	-0.44	0.10	2.3E-01
MR-Egger	-1.62	-3.49	0.25	9.0E-02	-0.20	-1.12	0.73	6.8E-01
(intercept)	-0.04	-0.13	0.05	3.8E-01	0.00	-0.04	0.04	9.3E-01
Penalized MR-Egger	-1.62	-3.49	0.25	9.0E-02	-0.20	-1.12	0.73	6.8E-01
(intercept)	-0.04	-0.13	0.05	3.8E-01	0.00	-0.04	0.04	9.3E-01
Robust MR-Egger	-2.31	-3.20	-1.43	3.2E-07	-0.19	-0.44	0.05	1.3E-01
(intercept)	0.01	-0.06	0.08	7.6E-01	0.00	-0.02	0.03	9.1E-01
Penalized robust MR-Egger	-2.31	-3.20	-1.43	3.2E-07	-0.19	-0.44	0.05	1.3E-01
(intercept)	0.01	-0.06	0.08	7.6E-01	0.00	-0.02	0.03	9.1E-01

Results from `MendelianRandomization` R package

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