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Supplementary information

The health effects of vitamin D supplementation: evidence from human studies

In the format provided by the authors and unedited

Supplementary Box 1. Overview of the recent major megatrials on vitamin D.

The largest trial to date is the **VITAL study** [1] that recruited more than 25,000 adults from 44 centers in the USA (Table 1). The trial had a two-by-two factorial design to evaluate the potential benefits of vitamin D_3 (2000 IU/d) or omega-3 marine fatty acids (1g/d). Adult males (>50 yrs.) or females (>55 yrs.) were followed for a mean duration of 5.3 yrs. The primary end points were invasive cancer (any type) and major cardiovascular (CV) events (a composite of myocardial infarction, stroke or death from CV events). Many secondary events were also examined. The mean baseline vitamin D status (serum 25OHD concentration of 30.8±10 ng/ml) was higher than that of the average American adult (based on comparison with the latest NHANES data). The Vitamin D Assessment Study **(ViDA study)** [2] evaluated the effects of monthly high dose vitamin D supplementation in more than 5000 New Zealand adults followed for a mean duration of 3.3 yrs. (Table 1). Subjects were randomized to receive either placebo or a dose of 200,000 IU of vitamin D at the start of the study followed by a monthly dose of 100,000 IU (~equivalent of about 3300 IU/d). Most of the participants had a normal vitamin D status at baseline (mean serum 25OHD of 26 ng/ml). The primary aim of the **D2d study** was to evaluate the effects of a daily dose of vitamin D (4000 IU/d) for a mean duration of 2.5 yrs. on the conversion of prediabetes to type 2 diabetes mellitus [3]. The criteria used to define prediabetes or type 2 diabetes were in line with the American Diabetes Association guidelines. Most study participants were vitamin D replete at baseline as their mean baseline serum 25OHD concentration was about 28 ng/ml [3]. The DO-HEALTH study [4] was a complex 2x2x2 factorial RCT evaluating vitamin D (2000 IU/d), omega-3 fatty acids and strength exercise program in elderly European subjects without major comorbidities. These subjects were less vitamin D replete than in the other megatrials. The **Calgary study** is not really a megatrial as it included "only" 311 Canadian adults and intended to explore the effects and safety of daily high dose vitamin D (4000 and 10,000 IU versus 400 IU) for 3 yrs. with bone structure and quality (measured by HRpQCT) [5,6].

Supplementary Box 2. Strengths and weaknesses of Mendelian Randomization studies to evaluate the long-term risks of vitamin D deficiency on a variety of health outcomes.

This approach has several advantages. First, genetic variants are randomly assigned at conception and this process, somewhat analogous to randomization in an RCT, breaks association with confounding factors. This is particularly helpful in the case of 25OHD, since it is associated with many factors that may confound the relationship between 25OHD and disease - such as educational attainment, body mass index (BMI) and smoking. Second, since alleles are inherited at conception, which always precedes disease onset, MR is free of bias due to reverse causation, where disease can influence 25OHD levels. This is particularly relevant since many diseases can lead individuals to stay inside more often, have less sun exposure and therefore lowered 25OHD levels. Third, MR studies can provide an assessment of the lifetime effects of lowered 25OHD levels, since alleles are inherited at conception. Despite these advantages, MR studies also have important limitations, which arise mostly from the assumptions inherent in such studies.

The first assumption is that the genetic variants are associated with 25OHD levels. This can be largely be overcome by utilizing only genetic variants that have been associated at a genome-wide significant level ($P < 5 \times 10^{-8}$) with 25OHD.

The second assumption is that the genetic variants are not associated with factors that confound the relationship between the exposure and the outcome. Studies that have explored the relationship between the alleles that lower 25OHD and potential confounders have demonstrated a lack of association [7].

The third assumption is most problematic. In order to properly assess the effect of lowered 25OHD on disease outcomes, the alleles that influence 25OHD levels must not influence risk of disease independently of 25OHD levels. While other assumptions are required and some types of MR analyses allow for the relaxation of these assumptions, it is important to bear in mind these assumptions, as they directly affect the validity of MR studies.

A recent review [8] summarized the results of 38 Mendelian Randomization trials. Four MR studies of autoimmune diseases showed that subjects with genetically lower serum 25OHD concentrations (throughout life) had a significantly higher risk for multiple sclerosis. The results of newer MR will be discussed later according to the type of disease studied.

MR studies have strengths that cannot be replicated by RCTs as they estimate the effect of lifelong differences in vitamin D status based on allelic variants. They also have limitations as most polymorphisms could, in combination, only predict less than 5% variation in serum 25OHD level and cannot detect non-linear effects. However, this low variance explained does not impact the validity of the results, but rather influences the statistical power of such studies. This statistical power can be best assessed by examining the 95% confidence intervals of the effect estimates. If these confidence intervals exclude clinically relevant effects, then the null findings are likely to be clinically meaningful. Nevertheless, the discovery of new polymorphisms has recently increased the predictability of serum 25OHD into the 10% range, more than double in comparison with the original polymorphisms found in 4 genes canonical to the vitamin D synthesis, transport and metabolism pathways [9,10]. Therefore, it is likely that in the near future new disease outcomes will be re-evaluated with greater statistical power to detect the potential lifelong effects of differences in vitamin D status.

Supplementary Box 3: vitamin D status and type 2 diabetes mellitus: E*vidence from Mendelian randomization:*

Study	Genetic Instruments	No/No of Events	Outcomes	Estimate of effect (95% CI)	*P* value	Unit of estimated effect
Ye 2015 [11]	*CYP2R1, DHCR7, DBP and CYP24A1*	104 488/28 144	T2D	1.01 (0.75, 1.36)	0.94	1 SD decrease in 25OHD level
		46 368	Fasting glucose	-0.02 (-0.04, 0.01)	0.28	mmol/L per SD decrease in 25OHD level
		46 368	2-h glucose	0.08 (-0.06, 0.22)	0.25	mmol/L per SD decrease in 25OHD level
		46 368	Fasting insulin	-1.04 (-3.91, 1.83)	0.48	% difference per SD decrease in 25OHD level
		46 368	HbA1c	0.01 (-0.04, 0.05)	0.80	% difference per SD decrease in 25OHD level
Lu 2018 [12]	DHCR7, CYP2R1	58 312/370 592	Diabetes	0.86 (0.77, 0.97)	0.01	25 nmol/L high 25OHD as determined by genetic variants
	DHCR7, CYP2R1, GC, CYP24A1	32 796/248 629	Diabetes	0.92 (0.84, 1.01)	0.07	
Chen 2019 [13]	DHCR7, CYP2R1, GC, CYP24A1	10 655 Chinese adults	Fasting plasma glucose	-0.186(-0.399, 0.026)		per 10 nmol/L GRS synthesis determined increase of 25(OH) D levels
Yuan 2019 [14]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	898 130/74 124	T2D	0.94 (0.88, 0.99)	0.03	1 SD increment of serum 25OHD levels
Meng 2019 [15]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	339 256/15 958	T2D	0.97 (0.85,1.12)	0.62	1 SD increase of the log-transformed 25(OH)D level
Wang 2020 [16]	DHCR7, CYP2R1, GC, and CYP24A1	14 570/3 915 Chinese	prediabetes	0.982 (0.948, 1.016)		1 unit increase in the natural-log-transformed vitamin D level
		12 220/1 565 Chinese	T2D	0.985 (0.940, 1.032)		
		16 135 Chinese	Fasting plasma glucose	− 0.015 (− 0.035, 0.006)		
		16 135 Chinese	hbA1c	− 0.003 (− 0.017, 0.011)		
Revez 2020 [10]	165 25OHD SNPs - Conditioned on BMI (mtCOJO)		Type II Diabetes	-0.02 (-0.08,0.03)	0.44	per unit increase in rank-based inverse-normal transformed 25OHD level

	155 25OHD SNPs- No adjustment for BMI		Type II Diabetes	-0.04 (-0.09,0.02)	0.20	
	161 25OHD SNPs-With BMI covariate		Type II Diabetes	-0.004 (-0.06,0.05)	0.88	

Since 2015, 5 large MR studies investigated the causal effect of genetically altered 25OHD levels on risk of T2DM and related traits (Table 2). In the earliest study [11], instruments to infer 25OHD levels were four SNPs within or near genes related to 25OHD synthesis and metabolism (*DHCR7*-rs12785878, related to vitamin D synthesis; *CYP2R1*-rs10741657, the hepatic 25-hydroxylase; *GC/DBP* -rs2282679 involved in 25OHD transport; and *CYP24A1*-rs6013897 involved in catabolism), explaining 2.4% of the variance in 25OHD levels. Each SNP was assessed for association with risk of T2DM in 28,144 cases and 76,344 controls of European descent, and with glycemic traits among 46,368 individuals. These associations were then combined to estimate the causal association of 25OHD concentration with the above outcomes. The MR OR for T2DM was 1.01 (95% CI 0.75, 1.36) per 25 nmol/L (equivalent to 1 SD) lower 25OHD concentration. The MR-derived 95% confidence intervals for estimates for glycemic traits also included the null. In another MR study [12] using 4 SNPs in the same vitamin D genes, the authors studied the association of these SNPs with T2DM in a meta-analysis totaling 32,796 T2DM cases of European and Chinese ancestry. A 25 nmol/l higher genetically instrumented 25OHD concentration was associated with an MR OR of 0.86 (95% CI 0.77, 0.97) for T2DM using the 2 synthesis SNPs. An equivalent difference in 25OHD using all 4 SNPs was not associated with T2DM. These discordant results are difficult to interpret, but the absence of causal association based on the 4 SNP- score is notable, given that the 4 SNPs explain a larger portion of the variance in 25OHD levels. In 2019, a large MR study [15] investigated associations of 25OHD levels and multiple traits in 339,256 White British from UK Biobank, among which T2DM status. In this study, 6 SNPs were used as instruments to infer 25OHD levels, including the 4 previous SNPs and two newly identified SNPs in *SEC23A* and *AMDHD1,* both genes without clear role in the vitamin D metabolic pathway. These 6 SNPs explain ~5.3% of the variance in 25OHD levels. This substantially powered MR analysis identified no change in the odds of T2DM per SD increase in the log-transformed 25OHD level. An MR analysis on an even larger population of 898,130 Europeans[14] (74,124 T2DM cases) used SNPs in the same 6 vitamin D genes, and a low-frequency variant in *CYP2R1* (rs10741657). They demonstrated that for 1 SD increment of 25OHD levels, the resultant MR OR for T2DM was

0.94 (95% CI 0.88, 0.99). Finally, a recent MR study [17] used up to 285 25OHD-related SNPs, explaining more than 10.5% of the variance in 25OHD levels, to investigate the causal role of vitamin D in multiple outcomes in 417,580 White British from UK Biobank. Using sensitivity analyses excluding SNPs with possible pleiotropic effects, and adjusting, or not, for BMI, this MR analysis did not support an association between lowered 25OHD levels and T2DM (all MR ORs between 0.96 and 1.05, with tight 95% CI including the 1).

In non-European, an MR study [16] used 4 SNPs in *DHCR7*, *CYP2R1*, *GC* and *CYP24A1* and included up to 16,135 genotyped Chinese subjects with information on T2DM status and glycemic traits. The MR ORs for risk of T2DM (in 1,565 cases and 10,655 controls) and prediabetes (amongst 3,915 cases and 10,655 controls) were 0.99 (95% CI 0.94, 1.03) and 0.98 (95% CI 0.95, 1.02), respectively. The MR-derived estimates for fasting plasma glucose and HbA_{1c} were also not significant.

Supplementary Box 4: Effects of vitamin D supplementation on cancer mortality

Author	N Age	Baseline 25OHD (ng/ml)	Final* 25OHD (ng/ml)	Daily dose (equivalent) (IU)	Duration (yrs.)	comments	Cancer incidence	Cancer mortality
Manson [1]	25,871 67	31	42	2,000	5.3	Primary outcome: invasive cancer of any type	Overall Placebo: 824 D_3: 793 →HR 0.96 (NS) After exclusion of first 2yrs follow-up Placebo: 528 D_3: 429 →HR 0.94 (NS)	Overall Placebo: 187 D_3: 154 →HR 0.83 (0.67-1.02) After exclusion of first 2 yrs. follow-up Placebo: 149 D_3: 112 →HR 0.75 (0.59-0.96)*
Scragg [18]	5,108 66	25	48-54	One loading dose of 200,000 IU and then 10,000/m ≈3.300 IU/d	3.3	Primary invasive and in situ malignant neoplasms (excluding non-melanoma skin cancer)	Placebo: 6.4% D_3: 6.3% →HR 1.01 (NS)	Placebo: 45 D_3: 44 →RR 0.97 (NS)
Avenell (RECORD) [19]	5,292 77	16	25	800 IU + 1g Ca	2-5		Overall Placebo: 315 D_3: 338 →HR 1.07 (NS)	Placebo: 178 D_3: 151 →RR 0.85 (0.68-1.06)
Wactawski Wende [20]	36,282 62	17	21	400 IU + 1g Ca	7	Postmenopausal women; incidence of colorectal, breast or overall invasive cancer	Overall study colorectal cancer →HR 1.08 (NS) subjects with baseline 25OHD < 12 ng/ml →HR 0.75 (0.39-1.48) Overall cancer incidence →RR 1.09 (NS)	Death from colorectal cancer →HR 0.82 (0.52-1.29)
Trivedi [21]	2,686 65-85	NA	30 (vs 21 in controls)	100,000 IU/4m ≈833 IU /d	5			Overall cancer mortality Placebo: 72 D_3: 63 →RR 0.86 (0.61-1.20)

*Final 25OHD concentration in vitamin D supplemented group. Modified from Keum et al [22] and Manson et al [23].

Supplementary Box 5: vitamin D status and cancer: E*vidence from Mendelian randomization:*

Study	Genetic Instruments	No/No of Events	Outcomes	Estimate of effect (95% CI)	*P* value	Unit of estimated effect
Ong 2016 [24]	*DHCR7*, *CYP2R1* and *GC*	31 719/10 065	All ovarian cancer	1.27 (1.06, 1.51)	NA	20 nmol/L lower 25OHD
		31 15/86	Serous subtype of ovarian cancer	1.54 (1.19, 2.01)	NA	
Theodoratou 2012 [25]	*DHCR7*, *CYP2R1*, *GC*, and *CYP24A1*	4238/2001	Colorectal cancer	1.16 (0.60, 2.23)	NA	per unit increase in log 25OHD level
Dudding 2018 [26]	GC, CYP2R1, DHCR7, CYP24A1, PDE3B	5 133/5 984	Oral cancer	0.86 (0.68, 1.09)	0.22	per standard deviation increase in log transformed 25OHD
Chandler 2018 [27]	DHCR7, CYP2R1, GC	3 985/23 294 women	Incident total cancer	1.10 (0.96, 1.25)	0.17	20 nmol/L higher 25OHD level as determined by genetic variants
		1 560	Incident breast cancer	1.14 (0.92, 1.41)	0.22	NA
		329	Incident colorectal cancer	1.54 (0.96, 2.46)	0.07	NA
		330	Incident lung cancer	0.96 (0.55, 1.68)	0.89	NA
		770	Total cancer death	0.98 (0.73, 1.32)	0.90	NA
Dimitrakopoulou 2017 [28]	DHCR7, CYP2R1, GC, CYP24A1	11 488	Colorectal cancer	0.92 (0.76, 1.10)	0.36	
		15 748	Breast cancer	1.05 (0.89, 1.24)	0.59	
		22 898	Prostate cancer	0.89 (0.77, 1.02)	0.08	
		4 369	Ovarian cancer	1.12 (0.86, 1.47)	0.40	per 25 nmol/L increase in genetically determined 25OHD level
		12 537	Lung cancer	1.03 (0.87, 1.23)	0.72	
		1 896	Pancreatic cancer	1.36 (0.81, 2.27)	0.25	
		1 627	Neuroblastoma cancer	0.76 (0.47, 1.21)	0.24	
Sun 2018 [29]	GC,DHCR7 and CYP2R1	54 580 /676	Lung cancer	0.96 (0.54,1.69)	0.88	25 nmol/L increase in 25OHD
Ong 2018 [30]	GC, CYP2R1, DHCR7, and CYP24A1	438 870/46 155	Cancer	0.97 (0.91,1.04)	0.40	20 nmol/L increase in 25OHD
		438 870/6 998	Cancer mortality	0.97 (0.84,1.11)	0.54	
He 2018 [31]	DHCR7, CYP2R1, GC, CYP24A1, SEC23A, AMDHD1	48 168/18 967	Colorectal cancer	0.91 (0.69, 1.19)	0.48	per unit log transformed 25OHD change determiend by genetic variants

Winslow 2018 [32]	DHCR7, CYP2R1	1 569/103 084	Non-melanoma skin cancer	1.11 (0.91, 1.35)	NA	20 nmol/L higher 25OHD level as determined by genetic variants
Dong 2019 [33]	GC,CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	23 326/6 167	Esophageal adenocarcinoma	0.68 (0.39,1.19)	0.18	20 nmol/L increase in 25OHD
		23 326/4 112	Barrett's esophagus	1.21 (0.77,1.92)	0.41	
Liyanage 2020 [34]	DHCR7, CYP2R1, GC, and CYP24A1	36 077/12 874	melanoma	1.06 (0.95-1.19)		20 nmol L-1 decrease in 25(OH)D
Jiang 2019 [35]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	122 977	breast cancer	1.02 (0.97-1.08)	0.47	Per 25 nmol/L increase in 25OHD
		79 148	prostate cancer	1.00 (0.93-1.07)	0.99	

The Ovarian Cancer Association (OCA) Consortium (10,065 cases, 21,654 controls) [24], reported a 27% increase in the risk of **epithelial ovarian cancer** per 20 nmol/L decrease in genetically determined 25OHD serum concentration (OR=1.27 95%CI: 1.06 to 1.51). However, the results were not corroborated by Dimitrakopulou et al [28] (4,369 cases, 84,418 controls) which reported a non-significant OR of 1.12 (95% CI:0.86 to 1.47) per 25 nmol/L increase in genetically determined 25OHD levels. The discrepancy could be result of the difference in power between the studies as well as the different source of the effect estimates of the association between the SNP and 25OHD concentrations. The study by Dimitrakapoulos et al also showed no evidence of an association between genetically determined 25OHD concentrations and risk of **colorectal** (OR=0.92 95%CI: 076 to 1.10), **breast** (OR=1.05 95%CI: 0.89 to 1.24), **prostate** (OR=0.89 95%CI: 0.77-1.02), **lung** (OR=1.03 95%CI: 0.87 to 1.23),**pancreatic** (OR=1.36, 95%CI: 0.81-2.27) and **neuroblastoma** (OR=0.76 95%CI: 0.47 to 1.21) cancer. Similar findings were reported by Chandler et al [27] in relation with total incident cancer (n=3,985, OR=1.10 95%CI: 0.96 to 1.25) and cancer subtypes such as **breast** (n=1,560, OR=1.14 95%CI 0.92–1.41), **colorectal** (n=329, OR=1.54 95%CI:0.96–2.46), and **lung** (n=330, OR=0.96 95%CI: 0.55to 1.68) cancer in 23,294 women. The null effect on colorectal carcinoma was confirmed by He et al [31] after including two additional SNPs. Similarly, a large-scale two sample MR study (breast cancer cases = 122,977 and prostate cancer cases=79,148) did not show an effect of 25OHD concentrations on breast and prostate cancer risk (Table 3). Dong et al [33] demonstrated that 25OHD concentrations were also not associated with the risk of esophageal adenocarcinoma. Finally,

the OR per 20 nmol/L increase in genetically estimated 25OHD concentration was 1.11 (95%:0.91 to 1.35) for non-melanoma skin cancer [32] and 0.94 (95%:0.87 to 1.05) for melanoma.

Supplementary Box 6: vitamin D status and health outcomes other than type 2 diabetes mellitus, cancer and multiple sclerosis: E*vidence from Mendelian randomization:*

Study	Genetic Instruments	No/No of Events	Outcomes	Estimate of effect (95% CI)	*P* value	Unit of estimated effect
Vim 2014 [36]	*CYP2R1* and *DHCR7*	146 581	SBP	-0.37 (-0.73, 0.003)	0.05	mm Hg per 10% increase in 25OHD level
		142 255	DBP	-0.29 (-0.52, -0.07)	0.01	mm Hg per 10% increase in 25OHD level
		142 255	Risk of hypertension	0.92 (0.87, 0.97)	0.00	per 10% increase in 25OHD level
Manousaki 2016 [37]	*DHCR7*, *CYP2R1*, *GC*, and *CYP24A1*	86 995/22 233	Coronary artery disease	0.99 (0.84, 1.17)	0.93	1 SD decrease in log-transformed 25OHD level
Afzal 2014 [38]	*DHCR7* and *CYP2R1*	95 766/10 349	All-cause mortality	1.3 (1.05, 1.61)	NA	20 nmol/L lower 25OHD
		95 23/97	Cardiovascular mortality	0.77 (0.55, 1.08)	NA	
		95 17/63	Cancer mortality	1.43 (1.02, 1.99)	NA	
		95 8/27	Other mortality	1.44 (1.01, 2.04)	NA	
Li 2016 [39]	*DHCR7*, *CYP2R1*, *GC*, and *CYP24A1*	1824	Lumar 1-4 BMD	-0.048 (-0.158, 0.062)	0.38	g/cm^2 per unit increase in log-transformed 25OHD
		1824	Femoral neck BMD	-0.044 (-0.120, 0.032)	0.26	g/cm^2 per unit increase in log-transformed 25OHD
		1824	Total hip BMD	-0.041 (-0.123, 0.041)	0.33	g/cm^2 per unit increase in log-transformed 25OHD
		1824	PTH	0.088 (-0.034, 0.210)	0.15	pg/mL per unit increase in log-transformed 25OHD
		1824	P1NP	-0.099 (-0.291, 0.093)	0.31	g/L per unit increase in log-transformed 25OHD
Hysinger 2016 [40]	*CYP2R1* and *GC*	5080/1203	Paediatric asthma	-0.0000351 (NA, NA)	0.85	NA
		NA	Severe asthma exacerbations	-0.00833 (NA, NA)	0.86	NA
Ooi 2014 [41]	DHCR7 and CYP2R1	79 743	Remnant cholesterol	4.0 (-2.4, 11)	0.22	% per 50% decrease in 25OHD level
		79 812	LDL cholesterol	2.2 (-1.7, 6.2)	0.28	
		85 363	HDL cholesterol	-6.0 (-10, -2.3)	0.00	
Larsson 2018 [42]		32 965	Femoral neck BMD	0.02 (-0.03, 0.07)	0.37	

	DHCR7, CYP2R1, GC, CYP24A1	32 965 142 487	Lumbar spine BMD Estimated BMD	0.02 (-0.04, 0.08) -0.03 (-0.05, -0.01)	0.49 0.02	1 SD increment of 25(OH)D determined by genetic variants
Sun 2019 [43]	DHCR7, CYP2R1, GC, CYP24A1, SEC23A, AMDHD1	66 628	Total boday BMD	0.92 (0.82, 1.04)	0.17	NA
Aspelund 2019 [44]	DHCR7, CYP2R1	10 501/4 003 10 501/4 003	All-cause mortality All-cause mortality	1.32 (0.80, 2.24) 1.35 (0.81, 2.37)	NA NA	per 20 nmol/L decrease in genetically determined 25OHD level NA
Gianfrancesco 2017 [45]	GC, CYP2R1, DHCR7	17 15/34	Paediatric onset multiple sclerosis	0.72 (0.55, 0.94)	0.02	NA
Manousaki 2017 [46]	GC, CYP2R1, DHCR7, and CYP24A1	146 761/25 109 15 008/7 047 40 835/10 788 12 853	Asthma Childhood onset asthma Atopic dermatitis Elevated IgE level	1.03 (0.90,1.19) 0.95 (0.69,1.31) 1.12 (0.92,1.37) −0.40 (−1.65,0.85)	0.63 0.76 0.27 0.54	1 SD decrease in log-transformed 25OHD level
Bae 2018 [47]	SSTR4, GC, and NADSYN1	4 744/2 104 41 282/12 307	Systemic lupus erythematosus Rheumatoid arthritis	0.032 (-0.201,0.265) 0.026 (-0.094,0.146)	0.79 0.66	NA NA
Magnus 2018 [48]	GC, CYP2R1, DHCR7, and CYP24A1	9 447 9 447	Gestational hypertension Preeclampsia	0.90 (0.78,1.03) 0.98 (0.89,1.07)	NA NA	10% decrease in 25OHD
Lund-Nielsen 2018 [49]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	115 16/95 115 110/1 265	Crohn disease Ulcerative colitis	0.98 (0.94 ,1.03) 1.01 (0.97,1.05)	NA NA	1.4-nmol/L increase in 25OHD 1.4-nmol/L increase in 25OHD
Mai 2019 [50]	GC, CYP2R1, DHCR7, and CYP24A1	56 435 56 435 56 435	High-density lipoprotein Total cholesterol Non-HDL cholesterol	2.52 (0.79,4.25) 0.60 (- 0.73,1.94) - 2.74 (- 6.16, 0.67)	0.00 0.38 0.96	25 nmol/L increase in 25(OH)D
Trajanoska 2018 [51]	DHCR7, CYP2R1, GC, CYP24A1	562258/185057	Fracture risk	0.84 （0.70, 1.02）	0.07	per SD decrease of genetically determined 25OHD level

Jacobs 2020 [52]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	41 505/14 802	Multiple sclerosis	0.57 (0.41-0.81)	0.00	1 unit increase in the natural-log-transformed vitamin D level
Kämpe 2019 [53]	GC and CYP2R1 (haplotype analyses)	648 children	pQCT BMD pQCT BMC pQCT cross-sectional area pQCT cortical density pQCT cortical content		0.08 0.79 0.03 0.01 0.05	haplotype combination analysis*
Kwak 2019 [54]	DHCR7, CYP2R1, GC, CYP24A1	2 591 Korean adults	SBP DBP hypertension	-0.42 (-1.51, 0.67) 0.001 (-0.67, 0.67) 1.04 (0.91, 1.19)	0.45 0.99 0.60	unit change in polygenic risk score *
Chen 2019 [13]	DHCR7, CYP2R1, GC, CYP24A1	10 655 Chinese adults	metabolic syndrome Waist circumference ln(triglycerides) High-density lipoprotein Systolic blood pressure Diastolic blood pressure	0.977 (0.966, 1.030) 0.403(-0.854, 1.659) -0.026(-0.099, 0.047) -0.010(-0.053, 0.033) -0.198(-3.032, 2.637) 0.061(-1.776, 1.899)		per 10 nmol/L increase of 25(OH) D levels
Meng 2019 [15]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	319 778 319 779 339 256/106 405 339 256/28 337 338 172 339 256/23 294 339 256/23 603 339 256/9830	SBP DBP hypertension Ischemic heart disease BMI Depression non-vertebral fracture All-cause mortality	–0.65 (-1.53, 0.07) –0.12 (-0.36, 0.12) 0.97 (0.91,1.04) 1.02 (0.92,1.14) 0.13(-0.11,0.37) 0.91 (0.82,1.02) 0.97 (0.87,1.08) 1.03 (0.87,1.22)	0.21 0.66 0.34 0.65 0.33 0.09 0.50 0.67	1 SD increase of the log-transformed 25(OH)D level
Milaneschi 2019 [55]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	480 359/135 458	Major depression	0.03 (-0.07,0.13)	0.50	1 unit increase in the natural-log-transformed vitamin D level

Huang 2019 [56]	CYP2R1 and DHCR7	99 012 Chinese adults 106 911 Danish adults	Cardiovascular disease Myocardial infraction Stroke Ischemic stroke Intracerebral hemorrhage Ischemic heart disease	1.01 (0.99, 1.02) 1.00 (0.97,1.03) 1.00 (0.99, 1.01) 1.00 (0.98,1.02) 1.01 (0.98,1.04) 1.00 (0.98-1.01)	0.18 0.54 0.68 0.69 0.86 0.54	25 nmol/L increase in 25(OH)D concentrations
Liyanage 2020 [34]	DHCR7, CYP2R1, GC, and CYP24A1	36 077/12 874	melanoma	1.06 (0.95-1.19)		20 nmol L-1 decrease in 25(OH)D
Manousaki 2021 [57]	69 conditionally independent common 25OHD SNPs	24 063/9358	Type 1 diabetes	1.09 (0.86-1.40)	0.48	29 nmol/L decrease in 25(OH)D
Butler-Laporte 2020 [58]	81 conditionally independent common 25OHD SNPs	1 299 010/ 14 134 908,494/6 406 628 238/4 336	COVID-19 susceptibility COVID-19 hospitalization COVID-19 severity	0.97 (0.95-1.10) 1.11 (0.91-1.35) 0.93 (0.73-1.17)	0.60 0.30 0.53	1 SD increase of the log-transformed 25(OH)D level
Amin 2020 [59]	CYP2R1, DHCR7, CYP24A1, SEC23A, and AMDHD1	127 637 /11 181 7 268 /1 389	COVID-19 susceptibility COVID-19 severity	ln(OR) 0.17 (−0.22 - 0.57) ln(OR)=0.36 (−0.89 - 1.61)	0.39 0.57	
Revez 2020 [10]	275 25OHD - Conditioned on BMI (mtCOJO)		Allergic rhinitis	0.03 (-0.02,0.09)	0.23	per unit increase in rank-based inverse-normal transformed 25(OH)D level
	268 25OHD - No adjustment for BMI		Allergic rhinitis	0.04 (-0.02,0.09)	0.17	
	276 25OHD - With BMI covariate		Allergic rhinitis	0.03 (-0.02,0.09)	0.21	
	242 25OHD - Conditioned on BMI (mtCOJO)		Coronary Artery Disease	-0.02 (-0.06,0.02)	0.26	
	232 25OHD - No adjustment for BMI		Coronary Artery Disease	-0.02 (-0.06,0.02)	0.26	

	242 25OHD - With BMI covariate		Coronary Artery Disease	-0.02 (-0.05,0.02)	0.35	
	230 25OHD - Conditioned on BMI (mtCOJO)		Dyslipidemia	-0.05 (-0.08,-0.01)	0.01	
	220 25OHD - No adjustment for BMI		Dyslipidemia	-0.06 (-0.09,-0.02)	1.16E-03	
	230 25OHD - With BMI covariate		Dyslipidemia	-0.04 (-0.08,-0.01)	0.01	
	251 25OHD - Conditioned on BMI (mtCOJO)		Hypertension	-0.01 (-0.05,0.02)	0.39	
	246 25OHD - No adjustment for BMI		Hypertension	-0.03 (-0.06,0.001)	0.06	
	252 25OHD - With BMI covariate		Hypertension	-0.01 (-0.05,0.02)	0.41	
	276 25OHD - Conditioned on BMI (mtCOJO)		Inflammatory Bowel Disease	-0.08 (-0.18,0.03)	0.15	
	270 25OHD - No adjustment for BMI		Inflammatory Bowel Disease	-0.08 (-0.19,0.02)	0.12	
	279 25OHD - With BMI covariate		Inflammatory Bowel Disease	-0.07 (-0.18,0.03)	0.18	
	225 25OHD - Conditioned on BMI (mtCOJO)		Rheumatoid Arthritis	0.03 (-0.20,0.25)	0.82	
	218 25OHD - No adjustment for BMI		Rheumatoid Arthritis	-0.01 (-0.24,0.22)	0.93	
	223 25OHD - With BMI covariate		Rheumatoid Arthritis	0.04 (-0.19,0.26)	0.76	

For details see the main text of the manuscript.

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