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Article

ARTICLE

Association of Circulating Vitamin D With Colorectal Cancer Depends on Vitamin D–Binding Protein Isoforms: A Pooled, Nested, Case-Control Study

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Abstract

Background: Higher circulating 25-hydroxyvitamin-D [25(OH)D] concentrations are consistently inversely associated with colorectal cancer (CRC) risk in observational studies. However, it is unknown whether this association depends on the functional GC-rs4588*A (Thr436Lys) variant encoding the vitamin D–binding protein-2 (DBP2) isoform, which may affect vitamin D status and bioavailability.
Methods: We analyzed data from 1710 incident CRC cases and 1649 incidence-density–matched controls nested within three prospective cohorts of mostly Caucasians. Study-specific incidence rate ratios (RRs) for associations of prediagnostic, season-standardized 25(OH)D concentrations according to DBP2 isoform with CRC were estimated using multivariable unconditional logistic regression and were pooled using fixed-effects models. All statistical significance tests were two-sided.
Results: The odds of having 25(OH)D concentrations less than 50 nmol/L (considered insufficient by the Institute of Medicine) were 43% higher for each DBP2-encoding variant (rs4588*A) inherited (per DBP2 odds ratio [OR] = 1.43, 95% confidence interval [CI] = 1.27 to 1.62, $P_{trend} = 1.2 \times 10^{-8}$). The association of 25(OH)D concentrations with CRC risk differed by DBP2: 25(OH)D concentrations considered sufficient (≥ 50 nmol/L), relative to deficient (< 30 nmol/L), were associated with a 53% lower CRC risk among individuals with the DBP2 isoform (RR = 0.47, 95% CI = 0.33 to 0.67), but with a non–statistically significant 12% lower risk among individuals without it (RR = 0.88, 95% CI = 0.61 to 1.27) ($P_{heterogeneity} = .01$).
Conclusions: Our results suggest that the 25(OH)D-CRC association may differ by DBP isoform, and those with a DBP2-encoding genotype linked to vitamin D insufficiency may particularly benefit from adequate 25(OH)D for CRC prevention.

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Colorectal cancer (CRC) is the second leading cause of cancer-related death and the third most common cause of cancer among men and women globally (1). Strong experimental evidence supports that vitamin D may prevent colorectal carcinogenesis via several mechanisms, including increasing bile acid catabolism, decreasing inflammation and angiogenesis, and direct effects on cellular proliferation, differentiation, and apoptosis (2,3). Although higher circulating 25-hydroxyvitamin D [25(OH)D] concentrations—used clinically to assess vitamin D status—are inversely associated with CRC risk in observational studies (4), randomized clinical trials of the efficacy of vitamin D supplementation in preventing colorectal neoplasms were largely null (5–7). Various limitations of these trials, including sample size, dosing, trial duration, timing of supplementation in the natural history of the disease, and compliance, that may have contributed to these null findings have been described (5–7). Additionally, the effects of vitamin D supplementation and circulating 25(OH)D concentrations on vitamin D metabolism may differ by functional genetic variants, such as those in the vitamin D–binding protein (DBP) gene, formerly known as group component (*GC*) (8,9). However, whether the 25(OH)D-CRC risk association differs by functional *GC* variants is unknown. Addressing this is relevant to the National Institute of Health's Precision Medicine Initiative aimed at tailoring health care recommendations based on individual characteristics such as genotypes (10).

Nearly 90% of circulating 25(OH)D is bound to the DBP, which maintains stable serum vitamin D stores and regulates free 25(OH)D available to target tissues (11). DBP may also play a role in fatty acid binding, actin scavenging, and complement-mediated immune cell chemotaxis (12). Two *GC* missense variants (rs7041 and rs4588) determine three common DBP protein "isoforms" (DBP1s, DBP1f, and DBP2, also known as Gc1s, Gc1f, and Gc2), which are associated with differences in vitamin D status and vitamin D pathway induction (13,14). Moreover, the association of 25(OH)D with, and the effects of vitamin D supplementation on, colorectal adenoma risk were reported to be stronger among those with the DBP2-encoding variant than among those without it, but whether there is a similar pattern of effect modification in relation to CRC risk is unknown (8,15).

Accordingly, we hypothesized that higher 25(OH)D concentrations would be more strongly inversely associated with CRC risk among individuals with the DBP2 isoform than among those without it. We investigated this hypothesis in three prospective case-control studies nested within cohort studies conducted in the United States and Europe.

Methods

Study Population

We conducted an individual-participant, pooled analysis of data from three prospective cohort studies (1): the European Prospective Investigation into Cancer and Nutrition (EPIC), which recruited men and women from the general population in 10 Western European countries (1992–1998) (16); (2) the Cancer Prevention Study-II Nutrition Cohort (CPS-II), which recruited men and women from 21 US states (1992–1993) (17); and (3) the Nurses' Health Study (NHS), which recruited female nurses in the United States (1976) (18). Previously, 1914 incident CRC cases within these cohorts were identified, and 2249 controls were matched using incidence density sampling with regards to age, sex (except NHS), and date of blood draw (4,19,20). Additional details regarding case ascertainment and matching criteria, available in the Supplementary Methods (available online), were published previously for EPIC (4,20), CPS-II (4), and NHS (4,19). Of the combined matched set, 1710 cases and 1649 controls had relevant genotyping information and were included in this analysis. Each participating cohort was approved by its respective institutional review board, and written informed consent was obtained from each participant.

25(OH)D Assays

Total 25(OH)D (D_2 and D_3) was measured using the US Food and Drug Administration–approved DiaSorin LIAISON chemiluminescence immunoassay (CLIA) in CPS-II (Heartland Assays, Ames, IA), the OCTEIA enzyme immunoassay (Immuno Diagnostic Systems, Boldon, UK) in EPIC (20), and a radioimmunoassay at the laboratory of Dr B.W. Hollis (The Medical University of South Carolina, Charleston, SC) and the Heartland Laboratory (Heartland Assays, Ames, IA) in NHS (19). Because 25(OH)D measurements may vary by assay, a subset of control samples from EPIC and NHS in each 25(OH)D decile were reassayed using the DiaSorin CLIA at Heartland Assays and were used to calibrate 25(OH)D to this standard assay using the robust linear regression described previously (21) and in the Supplementary Methods (available online). The intra-assay coefficient of variance was 4.5% for EPIC, 5.2% for CPS-II, and 13.5% for NHS.

Genotyping

Genotyping was performed using a custom GoldenGate Universal-plex assay kit (Illumina, CA) in EPIC (22); a custom Affymetrix genome-wide platform, the Axiom Correct Set (Affymetrix, CA) in CPS-II (23); and the OmniExpress platform in NHS (Illumina, CA) (23). Genotyping quality control for CPS-II and NHS samples was described previously (23). In EPIC, all *GC* genotyping was conducted using standard quality control: The lowest reproducibility frequency across 62 replicate samples was 0.98; call rates were greater than 95% for all samples and single-nucleotide polymorphisms.

Individuals with the *GC*-rs4588*A allele (CA or AA) were classified as having the DBP2 isoform, whereas those without the A allele (CC) were classified as having only DBP1 isoforms (13,24). The two DBP1 (1f and 1s) isoforms, distinguished by *GC*-rs7041, were combined in this analysis based on previous studies' effect-modification findings and our hypothesis (8,15,25,26). These genotypes perfectly predict the expected amino acid changes of the circulating protein isoforms as determined in previous proteomic analyses (24). *GC* rs3755967 (G > A) was used as a proxy rs4588 in EPIC; these single-nucleotide polymorphisms are in complete linkage disequilibrium ($r^2 = 1.0$) in the HapMap Spanish and British populations (1000 Genomes Project Phase 3, LD link, National Cancer Institute, Washington, DC). *GC* rs3755967 and rs4588 were in Hardy-Weinberg equilibrium ($P > .05$) in each study.

Statistical Analyses

Calibrated 25(OH)D measurements were season standardized using a cos/sin function described previously (21) and in the Supplementary Methods (available online). The season-standardized value may be interpreted as a participant's predicted 25(OH)D concentration averaged over the entire year, accounting for study-specific seasonal variation in 25(OH)D (21).

We estimated the association of DBP2 inheritance (*GC*-rs4588 genotype) with 25(OH)D concentrations less than 50 nmol/L, using unconditional logistic regression; 50 nmol/L is considered the cut point for vitamin D sufficiency by the Institute of Medicine (IOM, now the National Academy of Medicine). A two-stage approach was used to estimate summary odds ratios (ORs): study-specific odds ratios were calculated in separate unconditional logistic regression models, and then combined using fixed-effects models (in sensitivity analyses, the use of mixed-effects models did not materially affect the results). All study-specific odds ratios were adjusted for age, sex, and case-control status; EPIC models were further adjusted for study center. Study-specific mean 25(OH)D concentrations among DBP1-1, DBP1-2, and DBP2-2 participants were calculated using general linear regression models adjusted for the same covariates.

We estimated the association of 25(OH)D concentrations, categorized using IOM-recommended cut points, with CRC risk using unconditional logistic regression models stratified by DBP2 isoform inheritance (ie, *GC*-rs4588 using a dominant inheritance model). We report associations as incidence rate ratios (RRs), which are estimated by odds ratios in nested case-control studies in which controls are selected using incidence density sampling (20). Conditional logistic regression necessitated excluding participants in matched pairs who were discordant on DPB2-encoding genotypes; however, in sensitivity analyses, the results from conditional and unconditional logistic regression did not materially differ, so unconditional logistic regression was chosen to maximize our sample size and statistical power. A dominant inheritance model was chosen based on previous findings of effect modification by DBP2 for the association of 25(OH)D with colorectal adenoma risk (15) and to maximize statistical efficiency given the rarity of the DBP2-2 genotype, especially in the smaller CPS-II and NHS studies. A two-stage approach was used to estimate summary relative risks: study-specific relative risks were calculated in separate logistic regression models, and then combined using fixed-effects models (in sensitivity analyses, the use of mixed-effects models did not materially affect the results). Study-specific relative risks were adjusted for study-specific matching factors (Supplementary Methods, available online), body mass index (BMI) (continuous, kg/m^2), and physical activity (combined recreational and household activity metabolic equivalent hours per week, quartiles). Potential covariates, chosen based on biological plausibility and previous literature, included education, smoking, and total dietary intakes of energy, calcium (from food and supplements), fruits and vegetables, red and processed meats, and alcohol; of these, only those that affected the relative risks by 10% or greater were included in the final models (see the Tables' footnotes). Between-study heterogeneity was evaluated using the I^2 statistic. Effect modification of the RRs by DBP2 was evaluated using meta-regression (27).

Because the cut points for vitamin D status are debated, in separate analyses we included an additional upper category ($\geq$ 75 nmol/L) and collapsed the lower IOM categories ($<$ 50 nmol/L) because other professional societies use these values to define vitamin D sufficiency and deficiency, respectively (28). In all models, the lowest 25(OH)D category was used as the reference. To assess the significance of trend in CRC risk across the three- and four-level 25(OH)D categories, participants were assigned the study-specific median value of their respective 25(OH)D category, and the study-specific coefficients were pooled using fixed-effects models (27).

All statistical tests were two-sided; a *P* less than .05 or a 95% confidence interval (CI) that excluded 1.0 was considered statistically significant. Analyses were performed using SAS version 9.3 (Cary, NC), except for the meta-analyses performed in STATA version 12.1 (College Station, TX).

Results

Selected characteristics of the study participants, by cohort and case-control status, are summarized in Table 1; tumor characteristics (site and stage) of CRC cases are presented in Supplementary Table 1 (available online). In EPIC, CPS-II, and NHS, the median ages at blood draw were 59, 75, and 59 years; the median times from blood draw to CRC diagnosis were 3.6, 3.2, and 9.6 years; and the frequencies of the DBP2-encoding allele were 0.29, 0.26, and 0.28, respectively.

Individuals with the DBP2 isoform were more likely than those with DBP1 isoforms to have 25(OH)D concentrations less than 50 nmol/L (per DBP2 OR = 1.43, 95% CI: 1.27 to 1.62, $P_{trend} = 1.2 \times 10^{-8}$) (Table 2). Mean 25(OH)D concentrations were lower in EPIC (DBP1-1: 43.1, DBP2-2: 40.8, DBP2-2: 37.5 nmol/L) than in NHS (DBP1-1: 69.2, DBP1-2: 55.5, DBP2-2: 63.6 nmol/L) or CPS-II (DBP1-1: 62.3, DBP1-2: 61.5, DBP2-2: 64.3 nmol/L) (Supplementary Table 2, available online).

Higher 25(OH)D concentrations were more strongly associated with lower CRC risk among individuals with the DBP2 isoform than among those with only DBP1 isoforms (Table 3). Among those with DBP2, 25(OH)D concentrations of 30 to 49, 50 to 74, and 75 or greater nmol/L, relative to less than 30 nmol/L, were associated with statistically significant 31%, 56%, and 60% lower risk of CRC, respectively ($P_{trend} = 5.8 \times 10^{-5}$). Among those with only DBP1 isoforms, the corresponding RRs for CRC risk were 20% higher, 8% lower, and 34% lower (for concentrations of 30 to 49, 50 to 74, and 75 or greater nmol/L, relative to less than 30 nmol/L [$P_{trend} = .01$; $P_{heterogeneity\ for\ DBP2} = .02$, .02, and .21], respectively). Concentrations of 50 or greater nmol/L relative to less than 30 nmol/L were associated with a statistically significant 53% lower CRC risk among those with DBP2 ($P_{trend} = .0001$), and non–statistically significant 12% lower risk among individuals with only DBP1 isoforms ($P_{trend} = .09$; $P_{heterogeneity\ by\ DBP2} = .01$).

The pattern of effect modification by DBP2 was most pronounced in the larger EPIC study (Supplementary Table 3, available online), but there was no evidence of statistically significant study heterogeneity in the meta-analyses (I^2 = 0.0–20.1%, $P_{heterogeneity\ by\ study} > .28$ for all meta-estimates [Supplementary Table 4, available online]). Our findings did not substantially differ by BMI or follow-up time between blood draw and CRC diagnosis (stratified at study-specific means), tumor site (colon or rectum), or sex (in EPIC and CPS-II; results not shown).

Discussion

Our findings suggest that associations of 25(OH)D concentrations with CRC risk differ by common, inherited vitamin D–binding protein isoforms, and that individuals with DBP2—who may be predisposed to vitamin D insufficiency relative to individuals with DBP1 isoforms—may particularly benefit from maintaining sufficient vitamin D concentrations for CRC prevention. To our knowledge, this is the first study to report that the 25(OH)D-CRC association differs by DBP isoform.

DBP2 is encoded by the functional *GC*-rs4588 polymorphism (C > A) resulting in a Thr (DBP1) → Lys (DBP2) amino acid substitution at residue 436 (29,30). Although the physiologic consequences of the isoforms have not been fully elucidated, consistent with previous studies (31–33), the DBP2-encoding variant was strongly

Table 1. Selected characteristics of participants in three case-control studies nested within the EPIC, CPS-II, and NHS cohorts*

Variable	EPIC		CPS-II		NHS	
	Cases (n = 1106)	Controls (n = 719)	Cases (n = 246)	Controls (n = 217)	Cases (n = 358)	Controls (n = 713)
Circulating 25(OH)D, nmol/L, mean (SD)†	40.7 (16.0)	42.9 (14.6)	59.4 (20.5)	63.5 (21.6)	62.8 (27.3)	67.3 (27.0)
Vitamin D – binding protein (DBP) isoforms (rs4588 genotype)						
DBP1-1 (CC), %	52	50	54	58	56	52
DBP1-2 (CA), %	39	41	40	34	37	38
DBP2-2 (AA), %	9	9	6	8	7	10
Age, mean (SD), y	58.6 (7.1)	58.7 (8.0)	74.6 (5.7)	75.0 (5.7)	58.7(6.7)	58.7(6.7)
Female,%	50	52	53	52	100	100
Educational level						
None/primary, %	38	46	4	4	0	0
Secondary (high school), %	15	12	25	23	0	0
Technical/professional, %	26	22	8	5	70§	66§
University or higher, %	18	17	63	69	27	30
Missing, %	3	3	1	0	4	4
Body mass index, kg/m^2, mean (SD)	26.7 (4.2)	26.3 (3.7)	26.4 (5.0)	25.8 (4.2)	25.3(4.4)	24.7(4.3)
Smoking status						
Never smokers, %	41	45	45	47	42	44
Former smokers, %	33	32	46	45	44	43
Current smokers, %	25	21	4	2	14	12
Missing, %	1	1	5	6	1	0
Physical activity, MET-h/wk‡						
Median (IQR)	73.5 (44.5–120.6)	88.0 (48.8–126.0)	13.5 (6.8–23.0)	13.4 (7.0–22.0)	10.8 (4.2–19.4)	10.4 (4.2–20.7)
Quartile 1, %	24	20	24	24	24	25
Quartile 2, %	24	17	25	24	25	25
Quartile 3, %	21	23	25	23	26	25
Quartile 4, %	26	32	24	26	24	25
Missing	6	8	2	2	1	1
Menopausal status‖						
Premenopausal, %l	9	12	0	0	13	12
Postmenopausal, %	12	10	100	100	87	88
Perimenopausal/unknown, %	80	79	0	0	0	0
Hormone replacement therapy at time of blood draw‖						
No, %	83	83	61	45	65	57
Yes, %	14	12	35	48	31	40
Unknown, %	3	4	4	7	4	3
Dietary intakes						
Total energy, kcal/day mean (SD)	2149 (681)	2065 (621)	1729 (463)	1774 (606)	1711 (461)	1708.6 (442.4)
Total fruits, g/day, median (IQR)¶	178 (93–288)	207 (117–328)	160 (105–238)	161 (97–247)	2.2 (1.5–2.9)	2.2 (1.5–2.9)
Total vegetables, g/day, median (IQR)¶	153 (97–227)	161 (102–255)	175 (124–235)	186 (113–252)	2.8 (2.2–3.5)	2.9 (2.1–3.7)
Total red and processed meats, g/day, median (IQR)¶	48 (25–79)	38 (20–64)	45 (31–67)	41 (30–60)	0.9 (0.6–1.3)	0.8 (0.6–1.2)
Total alcohol, g/day, median (IQR)	9.1 (1.4–24.2)	6.1 (0.9–16.3)	1.6 (0–8.0)	1.6 (0–10.7)	2.2 (0.4–8.3)	2.3 (0.4–9.0)

(continued)

Table 1. (continued)

Variable	EPIC		CPS-II		NHS	
	Cases (n = 1106)	Controls (n = 719)	Cases (n = 246)	Controls (n = 217)	Cases (n = 358)	Controls (n = 713)
Total vitamin D, IU/day, median (IQR)	137 (93–199)	128 (84–188)	361 (177–565)	454 (174–575)	270 (178–413)	299 (199–457)
Total calcium, mg/day, median (IQR)	930 (716–1219)	948 (724–1206)	1011 (665–1419)	1, 067 (732–1559)	853 (662–1079)	896 (717–1165)

25(OH)D = 25-hydroxyvitamin D; CPS-II = Cancer Prevention Study-II; DBP = vitamin D–binding protein; EPIC = European Prospective Investigation into Cancer and Nutrition; IQR = interquartile range; MET = metabolic equivalents of task; NHS = Nurses' Health Study.

*Percentages given for categorical variables; may not sum to 100 because of rounding. Mean and SD given for normally distributed continuous variables; median and IQR given for non–normally distributed continuous variables.

†25(OH)D blood concentrations in EPIC and NHS were calibrated to the assay used for the CPS-II cohort; all 25(OH)D blood concentrations were seasonally adjusted.

‡MET-hours/week calculated from self-reported combined recreational and household activity in the EPIC study, and leisure time recreational physical activity in the CPS-II and NHS studies.

§Includes nurses who checked *registered nurse* (RN) as highest completed degree.

‖Among women.

¶Presented in servings per day for the NHS Cohort.

associated with lower circulating 25(OH)D concentrations and higher odds of vitamin D insufficiency in our study population. This association may be due to differences in circulating DBP concentrations (20–30% lower among DBP2 homozygotes relative to DBP1 homozygotes were reported in studies that did not use the isoform-biased monoclonal R&D assay (24,34–37)) because DBP mediates the renal reabsorption of 25(OH)D and prolongs its circulating half-life (25,29,38). Some studies suggest that the DBP2 isoform also has the lowest binding affinity to 25(OH)D, which, in addition to lower DBP concentrations, could lead to higher levels of free 25(OH)D (11,24,39,40). This may underlie the higher induction of vitamin D target genes by 25(OH)D in cultured monocytes and colon cancer cell lines with DBP2 relative to cells cultured with DBP1 isoforms (41,42). Normal and neoplastic colon tissues express the vitamin D-receptor (VDR) and are able to locally convert 25(OH)D to the VDR-activating 1, 25$(OH)_2$D form, which may play an important role in colorectal carcinogenesis via modulating cell growth, inflammation, angiogenesis, and apoptosis (43,44). Taken together, we hypothesize that individuals with the DBP2 isoform may particularly benefit from higher 25(OH)D concentrations because these concentrations may lead to higher vitamin D–pathway activation and may be needed to compensate for DBP2 individuals' reduced capacity to otherwise maintain adequate 25(OH)D concentrations.

Supporting this hypothesis are findings from other observational studies and randomized, controlled trials (RCT) that reported similar patterns of effect modification by DBP2. In a US case-control study of individuals of European ancestry, 25(OH)D concentrations of 50 or greater relative to less than 50 nmol/L were associated with lower risk of incident, sporadic colorectal adenoma among those with DBP2 (OR = 0.51, 95% CI = 0.33 to 0.81), but not among those without DBP2 (OR = 1.11, 95% CI = 0.68 to 1.92) ($P_{interaction}$ = .05) (15). Findings from two other observational studies (including an NHS study that used the same matched case-control set used in our analysis) suggest that the 25(OH)D-CRC risk association is stronger among those with DBP concentrations less than the median, which provides indirect support of our findings, given the strong association of DBP2 with lower DBP concentrations (19,45). Additionally, although the reported effects of vitamin D supplementation on colorectal neoplasm prevention in RCTs have largely been null (7,46), it is possible that the effects of vitamin D supplementation on 25(OH)D concentrations and colorectal neoplasm prevention may also depend on the functional DBP2 isoform (8,9). In two trials, vitamin D supplementation increased 25(OH)D concentrations more among those with the DBP2-encoding relative to DBP1-encoding genotypes (9,47). Moreover, in a large RCT (n = 2259) (8), the "interaction relative risk"—ratio of the vitamin D supplementation RR per DBP2-encoding minor allele divided by that for the DBP1-encoding major allele—was 0.82 (95% CI = 0.69 to 0.98), indicating that the effect of vitamin D supplementation on reducing adenoma recurrence was statistically significantly stronger with each DBP2-encoding variant inherited ($P_{interaction}$ = .03).

Our findings may help explain certain inconsistencies in the literature regarding vitamin D concentrations, *GC* genotypes, and CRC risk. In a recent international pooling project of 17 cohorts, the study-specific RRs for CRC with each 25-nmol/L increase in 25(OH)D were mostly inverse, but they varied from 1.17 to 0.63, with only five being statistically significant (4). In addition to differences in sample size that may affect the precision of the estimates, this heterogeneity may, in part, be due to differences in DBP2 frequency in different study populations because DBP2 frequency varies by geographic area and ethnicity

Table 2. Study-specific and summary associations of vitamin D–binding protein isoforms with vitamin D nonsufficiency* in the EPIC, CPS-II, and NHS cohorts

Study DBP isoform (rs4588 genotype)	<50 nmol/L (nonsufficient)	≥50 nmol/L (sufficient)	<50 vs. ≥50 nmol/LOR (95% CI)†	P_{trend}
EPIC				
DBP1-1 (CC)	674	254	1.00 (Referent)	
DBP1-2 (CA)	560	173	1.41 (1.11 to 1.78)	
DBP2-2 (AA)	138	26	2.59 (1.63 to 4.42)	
Per DBP2 isoform (per A allele)	1372	453	1.52 (1.26 to 1.82)	8.7×10^{-6}
CPS-II				
DBP1-1 (CC)	78	180	1.00 (Referent)	
DBP1-2 (CA)	53	120	1.03 (0.67 to 1.57)	
DBP2-2 (AA)	12	20	1.47 (0.68 to 3.18)	
Per DBP2 isoform (per A allele)	143	320	1.13 (0.82 to 1.55)	.42
NHS				
DBP1-1 (CC)	149	421	1.00 (Referent)	
DBP1-2 (CA)	120	282	1.20 (0.91 to 1.60)	
DBP2-2 (AA)	47	52	2.55 (1.65 to 3.95)	
Per DBP2 isoform (per A allele)	316	755	1.46 (1.20 to 1.77)	.0002
All studies‡				
DBP1-1 (CC)	901	855	1.00 (Referent)	
DBP1-2 (CA)	733	575	1.27 (1.08 to 1.50)	
DBP2-2 (AA)	197	98	2.36 (1.74 to 3.19)	
Per DBP2 isoform (per A allele)	912	2447	1.43 (1.27 to 1.62)	1.2×10^{-8}

25(OH)D = 25-hydroxyvitamin D; CI = confidence interval; CPS-II = Cancer Prevention Study-II; DBP = vitamin D–binding protein; EPIC = European Prospective Investigation into Cancer and Nutrition; NHS = Nurses' Health Study; OR = odds ratio.
*According to 2011 Institute of Medicine recommendations based on circulating 25(OH)D concentrations; 25(OH)D blood concentrations were calibrated to the same assay and seasonally adjusted using the method described by Gail et al. (21).
†Odds ratio and 95% confidence interval estimated in logistic regression models adjusted for age (continuous), sex, study center (for EPIC models), and case-control status.
‡Odds ratios and 95% confidence intervals estimated in fixed-effects meta-analyses ($I^2 = 0.0$ to 22.1; $P_{heterogeneity\ by\ study} > .25$ for all summary estimates).

Table 3. Summary incidence rate ratios (RR) of colorectal cancer according to vitamin D status and functional vitamin D–binding protein (DBP) isoforms in the EPIC, CPS-II, and NHS cohorts

	DBP1-1 (rs4588 CC)†				DBP1-2 or DBP2-2 (rs4588 CA or AA)‡				
25(OH)D concentration (IOM-defined vitamin D status)*	No. cases	No. controls	RR (95% CI)§	P_{trend}	No. cases	No. controls	RR (95% CI)§	P_{trend}	$P_{heterogeneity\ by\ DBP2}$
< 30 nmol/L (deficient)*	144	104	1.00 (Referent)		218	107	1.00 (Referent)		
30 to < 50 nmol/L (insufficient)	386	267	1.20 (0.86 to 1.67)		320	285	0.69 (0.51 to 0.95)		.02
50 to < 75 nmol/L (sufficient)	266	288	0.92 (0.63 to 1.34)		191	266	0.44 (0.27 to 0.73)		.02
≥ 75 nmol/L (beyond sufficient)	105	196	0.66 (0.37 to 1.16)	0.01	80	136	0.40 (0.23 to 0.68)	5.8×10^{-5}	.21
< 30 nmol/L (deficient)	144	104	1.00 (Referent)		218	107	1.00 (Referent)		
30 to < 50 nmol/L (insufficient)	386	267	1.19 (0.85 to 1.66)		320	285	0.69 (0.50 to 0.94)		.02
≥ 50 nmol/L (sufficient)	371	484	0.88 (0.61 to 1.27)	0.09	271	402	0.47 (0.33 to 0.67)	.0001	.01
< 50 nmol/L (nonsufficient)	530	371	1.00 (Referent)		538	392	1.00 (Referent)		
≥ 50 nmol/L (sufficient)	371	484	0.79 (0.63 to 1.00)		271	402	0.60 (0.47 to 0.76)		.10

25(OH)D = 25-hydroxyvitamin D; CI = confidence interval; CPS-II = Cancer Prevention Study-II; DBP = vitamin D–binding protein; EPIC = European Prospective Investigation into Cancer and Nutrition; IOM = Institute of Medicine; NHS = Nurses' Health Study; RR = incidence rate ratio.
*The 25(OH)D blood concentrations were calibrated to the same assay and seasonally adjusted using the method described by Gail et al. (21).
†Participants with no minor allele at GC-rs4588 (rs4588*CC genotype) were defined as not having the DBP2 isoform (or only DBP1 isoforms).
‡Participants with a minor allele at GC-rs4588 (rs4588*CA or rs4588*AA genotypes) were defined as having the DBP2 isoform.

(from 0.01 to 0.41 internationally, and from 0.21 to 0.41 in European and white American populations) (48). Additionally, although the DBP2-encoding GC-rs4588 variant is associated with lower 25(OH)D concentrations, it was not associated with CRC risk in genome-wide association or Mendelian randomization studies (49–51). The potential interaction between 25(OH)D concentrations and DBP2 in relation to CRC risk could contribute to these null findings.

Strengths of our study include the use of data from three prospective cohorts in the United States and Europe, with participants from geographically diverse areas. We also used season-adjusted 25(OH)D concentrations, thereby reducing misclassification of vitamin D status, which may vary throughout the year and in study populations living at different latitudes. Given that 25(OH)D measurements may vary by assay type, harmonization of 25(OH)D levels to a standard assay is another

strength of this study, providing more reliable meta-estimates and the ability to assess 25(OH)D using absolute clinical cut points—a limitation in most prior meta-analyses.

Our study also has several limitations. IOM cut points for vitamin D status are based on skeletal health research because their guidelines currently cite insufficient evidence to inform recommendations for nonskeletal health outcomes (28,52). Larger studies are needed to investigate more precise categories of 25(OH)D that may be relevant to CRC risk. Data for certain potential confounding factors (eg, aspirin or multivitamin use) were not available in EPIC; however, adjusting for these covariates in the CPS-II and NHS models did not materially affect the results. Additionally, prediagnostic 25(OH)D was measured only once, although it may still have been a relatively good indicator of long-term vitamin D status given that previous studies estimated within-person correlations between 0.53 and 0.81 for repeated 25(OH)D measures taken 1 to 11 years apart (53,54). Although our meta-estimates were largely driven by the estimates from EPIC because of its much larger sample size—especially for those with lower 25(OH)D concentrations—between-study heterogeneity was minimal. Last, because the frequency and effects of DBP isoforms may differ by race or ethnicity (29,48), our findings may not be generalizable to other populations.

In conclusion, our findings suggest that the association of circulating vitamin D concentration with CRC risk may differ by common, inherited genotypes encoding vitamin D–binding protein isoforms. Individuals with the DBP2 isoform—linked to vitamin D insufficiency—may particularly benefit from maintaining adequate vitamin D concentrations for CRC prevention.

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