Research Article

Genetic Predictors of Circulating 25-Hydroxyvitamin D and Risk of Colorectal Cancer

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Abstract

Background: Experimental evidence has demonstrated an antineoplastic role for vitamin D in the colon, and higher circulating 25-hydroxyvitamin D [25(OH)D] levels are consistently associated with a lower risk of colorectal cancer. Genome-wide association studies have identified loci associated with levels of circulating 25(OH)D. The identified single-nucleotide polymorphisms (SNPs) from four gene regions collectively explain approximately 5% of the variance in circulating 25(OH)D.

Methods: We investigated whether five polymorphisms in GC, CYP2R1, CYP24A1, and DHCR7/NADSYN1, genes previously shown to be associated with circulating 25(OH)D levels, were associated with colorectal cancer risk in 10,061 cases and 12,768 controls drawn from 13 studies included in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and Colon Cancer Family Registry (CCFR). We conducted a meta-analysis of crude and multivariate-adjusted logistic regression models to calculate odds ratios and associated confidence intervals for SNPs individually, SNPs simultaneously, and for a vitamin D additive genetic risk score (GRS).

Results: We did not observe a statistically significant association between the 25(OH)D-associated SNPs and colorectal cancer marginally, conditionally, or as a GRS, or for colon or rectal cancer separately.

Conclusions: Our findings do not support an association between SNPs associated with circulating 25(OH)D and risk of colorectal cancer. Additional work is warranted to investigate the complex relationship between 25(OH)D and colorectal cancer risk.

Impact: There was no association observed between genetic markers of circulating 25(OH)D and colorectal cancer. These genetic markers account for a small proportion of the variance in 25(OH)D. Cancer Epidemiol Biomarkers Prev; 22(11): 2037–46. ©2013 AACR.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Introduction

Colorectal cancer is the second leading cause of cancer death in men and women in the United States. It is estimated that in 2013, a total of 142,820 U.S. men and women will be diagnosed with cancer of the colon and rectum (1). Family history is a strong risk factor for colorectal cancer (2, 3), which is consistent with the existence of shared etiologic and genetic determinants among relatives. Known genetic mutations account for about 30% to 50% of the familial risk (4); much of the remaining familial aggregation is unexplained. Genome-wide association studies (GWAS) of sporadic colorectal cancer have identified at least 20 independent loci statistically significantly associated with lower circulating 25(OH)D levels in four single-nucleotide polymorphisms (SNPs) significantly contributing substantially to circulating vitamin D levels, with known genetic variants cumulatively explaining only a very small fraction of colorectal cancer risk (13).

Beyond inherited risk, there is a large body of evidence supporting the role of nongenetic factors, including vitamin D status, in the etiology of colorectal cancer. The first human evidence to suggest an association between vitamin D and colorectal cancer was the ecologic study by Garland and Garland based on data collected in the 1950’s and 60’s which showed a strong inverse association between colon cancer mortality and solar UVB radiation exposure in the United States. (14). Since then, most but not all case–control and cohort studies have found an inverse association between intake of vitamin D (both diet and supplements) and colorectal cancer risk (15–18), with even stronger associations observed using directly measured circulating 25(OH)D (19–22), an integrated biomarker of vitamin D status (23). Randomized clinical trials of vitamin D supplementation, including the Women’s Health Initiative (24) and the British Oxford Trial (25), have not showed reductions in colorectal cancer incidence. However, these trials have generally tested low doses of vitamin D and each included less than 7 years of follow-up, which is likely insufficient to show an effect on cancer incidence, particularly in light of the long latency of disease. Similarly, large meta-analyses of clinical trials have not shown robust evidence for a protective role of vitamin D in the development of colorectal cancer (26) despite some indication of a preventative role in the development of adenomas (27).

Circulating 25(OH)D levels are a function of dietary sources and exposure of the skin to sunlight, specifically UVB rays. In addition to environmental determinants, twin and family studies suggest that genetic factors contribute substantially to circulating vitamin D levels, with heritability estimates ranging from 43% to 80% (28–31).

Two published GWAS of 25(OH)D have uncovered single-nucleotide polymorphisms (SNPs) significantly associated with lower circulating 25(OH)D levels in four gene regions that seem to have functional relevance: GC (group-specific component vitamin D–binding protein); CYP2R1 (cytochrome P450, family 2, subfamily R, polypeptide 1), encoding C-25 hydroxylase that converts vitamin D to the active ligand for the vitamin D receptor; DHCR7 / NADSYN1 [7-dehydrocholesterol (7-DHC) reductase/nicotinamide adenine dinucleotide synthetase] (32) with roles in the synthetic vitamin D pathway (33, 34); and CYP24A1 (encoding 24-hydroxylase involved in the degradation of both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D; refs. 33, 34). Both the GC SNP rs2282679 and the DHCR7/NADSYN1 SNPs are located in intronic regions, with the GC SNP showing the largest magnitude of association with 25(OH)D < 75 nmol/L [odds ratios (OR), 1.63; 95% confidence intervals (CI), 1.53–1.73; ref. 34]. The SNP rs10741657 is proximal to the CYP2R1 gene and rs6013897 is proximal to CYP24A1 yet the precise associations with gene expression are yet to be determined.

We investigated the association between these SNPs previously identified as associated with 25(OH)D and risk of colorectal cancer in 13 cohorts that are part of the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and the Colon Cancer Family Registry (CCFR). Within a subset of participants from the Nurses’ Health Study (NHS), Health Professionals Follow-up Study (HPFS), and Physician’s Health Study (PHS) with measured prediagnostic plasma 25(OH)D levels, we also examined the joint effects of plasma 25(OH)D and 25(OH)D–associated SNPs on colorectal cancer risk.

Materials and Methods

Study population

The analysis included a total of 10,061 cases and 12,768 controls of European ancestry drawn from 13 studies within GECCO and CCFR. Details on the studies are provided in Table 1, and are described in detail in the Supplementary Data and Supplementary Table S1. In brief, each study defined colorectal cancer cases as colorectal adenocarcinoma, confirmed by medical records, pathologic reports, or death certificates. All participants provided informed consent and studies were approved by their respective Institutional Review Boards. None of the studies included in GECCO or CCFR contributed subjects to the any previous GWAS of 25(OH)D with the exception of a subset of the NHS subjects (n = 1,342) who participated in the validation stage of one study (33).

Genotyping, quality assurance/quality control, and imputation

We used genotype data from GECCO and CCFR. GECCO consisted of participants within the French Association Study Evaluating RISK for sporadic colorectal cancer (ASTERISK); Hawaii Colorectal Cancer Studies 2 and 3 (Colo2&3); Darmkrebs: Chancen der Verhütung durch Screening (DACHS); Diet, Activity, and Lifestyle Study (DALS); HPFS; Multiethnic Cohort (MEC); NHS; Ontario Familial Colorectal Cancer Registry (OFCCR); PHS; Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial (PLCO); Vitamins And Lifestyle (VITAL); and the Women’s Health Initiative (WHI). Phase I genotyping on a total of 1,709 colon cancer cases and
4,214 controls from PLCO, WHI, and DALS (PLCO Set 1, WHI Set 1, and DALS Set 1) was conducted using Illumina HumanHap 550 K, 610 K, or combined Illumina 300 K and 240 K, and has been described previously (12). A total of 650 colorectal cancer cases and 522 controls from OFCCR are included in GECCO from previous genotyping using Affymetrix platforms (35). A total of 5,540 colorectal cancer cases and 5,425 controls from ASTERISK, Colo2&3, DACHS, DALS Set 2, MEC, PMH, PLCO Set 2, VITAL, and WHI Set 2 were successfully genotyped using Illumina HumanCytoSNP. A total of 2,004 colorectal cancer cases and 2,244 controls from HPFS (2 sets), NHS (2 sets), and PHS (2 sets) were successfully genotyped using Illumina HumanOmniExpress. The CCFR included a population-based case–control set of participants from sites in the United States, Canada, and Australia successfully genotyped using Illumina Human1M or Human1M-Duo (36).

DNA was extracted from samples of white blood cells or, in the case of a subset of NHS, HPFS, DACHS, MEC, and PLCO samples, and all VITAL samples from buccal cells using conventional methods (37). All studies included 1% to 6% blinded duplicates to monitor quality of the genotyping. All individual-level genotype data were managed centrally at University of Southern California (Los Angeles, CA; CCFR), the Ontario Institute for Cancer Research (Toronto, Ontario, Canada; OFCCR), the University of Washington (Seattle, WA; HPFS, NHS, and PHS), or the GECCO and CCFR Coordinating Center (CC) at the Fred Hutchinson Cancer Research Center (all other studies) to ensure consistent quality assurance and quality control and statistical analysis. Details on the quality assurance and quality control can be found in Supplementary Table S2. In brief, samples were excluded on the basis of call rate, heterozygosity, unexpected duplicates, gender discrepancy, and unexpectedly high identity-by-descent or unexpected concordance (>65%) with another individual. For missing SNP data, all GECCO studies were imputed to HapMap II release 24, with the exception of OFCCR, which was imputed to HapMap II release 22. CCFR was imputed using IMPUTE (38), OFCCR was imputed using BEAGLE (39), and all other studies were imputed using MACH (40). All SNPs met quality-control measures for Hardy–Weinberg equilibrium in controls (HWE; \( P < 10^{-5} \)) and minor allele frequency (MAF ≥ 1%) or imputation \( R^2 > 0.3 \).

**Dietary and lifestyle factors**

Dietary information, including calcium, folate, fiber, and alcohol intake, was available for Colo2&3, DALS, HPFS, MEC, NHS, PLCO I, PLCO II, VITAL, WHI; calcium, folate, and alcohol was available in PHS; and calcium and alcohol in ASTERISK and DACHS. Regular use of nonsteroidal anti-inflammatory drugs (NSAID) was

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**Table 1. Characteristics of patients with colorectal cancer within included study populations**

<table>
<thead>
<tr>
<th>Study</th>
<th>Abbreviation</th>
<th>Number of cases</th>
<th>Number of controls</th>
<th>% Female</th>
<th>Mean age diagnosis</th>
<th>% Colorectal cancer family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontario Familial Colorectal</td>
<td>OFCCR</td>
<td>650</td>
<td>522</td>
<td>52</td>
<td>62.0</td>
<td>20.1</td>
</tr>
<tr>
<td>Cancer Registry</td>
<td>CCFR</td>
<td>1,171</td>
<td>983</td>
<td>50</td>
<td>54.2</td>
<td>16.9</td>
</tr>
<tr>
<td>Colon Cancer Family Registry</td>
<td>DALS</td>
<td>1,116</td>
<td>1,174</td>
<td>45</td>
<td>63.9</td>
<td>13.3</td>
</tr>
<tr>
<td>Diet, Activity, and Lifestyle Study</td>
<td>COLO 2&amp;3</td>
<td>87</td>
<td>125</td>
<td>45</td>
<td>65.2</td>
<td>11.8</td>
</tr>
<tr>
<td>A case–control study from the University of Hawaii</td>
<td>MEC</td>
<td>328</td>
<td>346</td>
<td>46</td>
<td>63.0</td>
<td>12.2</td>
</tr>
<tr>
<td>Multiethnic Cohort</td>
<td>DACHS</td>
<td>1,710</td>
<td>1,708</td>
<td>41</td>
<td>68.6</td>
<td>12.5</td>
</tr>
<tr>
<td>Darmkrebs: Chancen der Verhütung durch Screening</td>
<td>PLCO</td>
<td>1,019</td>
<td>2,391</td>
<td>31</td>
<td>64.0</td>
<td>11.4</td>
</tr>
<tr>
<td>Prostate, Lung, Colorectal, and Ovarian Cancer Initiative</td>
<td>WHI</td>
<td>1,476</td>
<td>2,538</td>
<td>100</td>
<td>65.8</td>
<td>17.6</td>
</tr>
<tr>
<td>Women’s Health Initiative</td>
<td>ASTERISK</td>
<td>892</td>
<td>947</td>
<td>41</td>
<td>65.2</td>
<td>NA</td>
</tr>
<tr>
<td>Association Study Evaluation RISK for</td>
<td>VITAL</td>
<td>285</td>
<td>288</td>
<td>48</td>
<td>66.5</td>
<td>13.6</td>
</tr>
<tr>
<td>sporadic colorectal cancer</td>
<td>HPFS</td>
<td>403</td>
<td>402</td>
<td>0</td>
<td>65.2</td>
<td>17.3</td>
</tr>
<tr>
<td>Vitamins And Lifestyle Study</td>
<td>NHS</td>
<td>549</td>
<td>955</td>
<td>100</td>
<td>59.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Nurses’ Health Study</td>
<td>PHS</td>
<td>375</td>
<td>389</td>
<td>0</td>
<td>58.9</td>
<td>NA</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>10,061</td>
<td>12,768</td>
<td>53</td>
<td>64.0</td>
<td>13.0</td>
</tr>
</tbody>
</table>

*DALS Set 2, Colo2&3, DACHS, MEC, PLCO Set 2, WHI Set 2, ASTERISK, and VITAL were genotyped on the Illumina CytoSNP BeadChip. WHI Set 1 was genotyped using Illumina 550 K and 550 K duo platforms; PLCO Set 1 was genotyped using Illumina 550 K and 610 K platforms; DALS set 1 was genotyped using Illumina 610 K and 550 K platforms; OFCCR was genotyped using Affymetrix GeneChip Human Mapping 100 K and 500 K Array Set and a 10 K nonsynonymous SNP chip; CCFR was genotyped using Illumina 1M, 1Mduo, and 1M-Omni platforms; and HPFS, NHS, and PHS were genotyped on the Illumina HumanOmniExpress platform.
available for CCFR, Colo2&3, DACHS, HPFS, MEC, NHS, and VITAL. All studies collected data on smoking status, red meat consumption, physical activity, body mass index (BMI), and hormone replacement therapy in postmenopausal women with the exception of ASTERISK. ASTERISK was restricted to cases with no family history of colorectal cancer. We adopted a flexible approach to retrospective covariate harmonization as previously described (41, 42).

**Laboratory assessment of 25(OH)D**

In previous studies, we measured plasma levels of 25(OH)D in a subset of the cases and controls with genetic data that were nested within the NHS, HPFS, and PHS (total cases \( n = 672 \) and total controls \( n = 909 \)) using a radioimmunosorbent assay in the laboratory of Dr. Bruce W. Hollis (Medical University of South Carolina, Charleston, SC). The median intra-assay coefficient of variation from blinded quality-control samples was 11.8% in NHS, 10.1% in HPFS, and 13.8% in PHS. Cases and their controls were analyzed in the same batch and laboratory personnel were blinded to case, control, and quality-control status (21, 43, 44).

**Statistical analyses**

The statistical analyses of the GECCO and CCFR samples were conducted at a central data analysis center on individual-level data to ensure a consistent analytic approach. For each study, we estimated the association between each SNP and risk for colorectal cancer by calculating \( \beta \) ORs, standard errors (SEs), 95% CIs, and \( P \) values using log-additive genetic models relating the genotype dose (0, 1, or 2 copies of the allele) to risk of colorectal cancer. For imputed SNPs, we used the dosage (expected number of copies of the minor allele) when testing associations, which has been shown to give unbiased estimates (45). We also created a genetic risk score (GRS), composed of four SNPs from four distinct gene regions to ensure no single gene was overrepresented in the score using an allelic scoring system based on summing the number of risk alleles [previously associated with lower 25(OH)D], yielding a possible range of 0 to 8 alleles to derive estimates of allelic OR.

Minimally adjusted models included covariates for age, sex (when appropriate), center (when appropriate), batch effects (ASTERISK only), and the first three principal components from EIGENSTRAT to account for population substructure. Multivariate models were additionally adjusted for family history of colorectal cancer, BMI, NSAID use, smoking status, alcohol use, dietary calcium, folate and red meat intake, sedentary status, and hormone replacement therapy based on covariate availability. We repeated the minimally adjusted model analyses stratified by anatomic site (colon and rectum).

We conducted inverse-variance weighted, fixed-effects meta-analysis to combine \( \beta \) estimates and SEs from log-additive models across individual studies. We chose to focus on fixed-effects to improve power and assessed heterogeneity across studies utilizing random effects models (46).

For analyses of the joint effect of plasma 25(OH)D and our GRS composed of 25(OH)D-associated SNPs, we included the 672 cases and 909 controls in NHS, HPFS, and PHS among whom we had previously measured prediagnostic levels of 25(OH)D and also had genotype data (21, 44). We calculated ORs and 95% CI for colorectal cancer comparing extreme quartiles of 25(OH)D and also had genotype data (21, 44). We calculated ORs and 95% CI for colorectal cancer comparing extreme quartiles of 25(OH)D defined according to cohort-specific cutoff points determined by the distribution in controls (44). We compared the GRS-associated risk for colorectal cancer across categories of

### Table 2. Association between five 25(OH)D-associated SNPs and colorectal cancer among 10,061 cases and 12,768 controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (nearest gene)</th>
<th>Major/Minor allele</th>
<th>Minor allele frequency</th>
<th>Age, sex, and PCA-adjusted OR (95% CI)</th>
<th>Multivariable-adjusted OR (95% CI)</th>
<th>N studies genotyped</th>
<th>Mean R²</th>
<th>( P_{\text{heterogeneity}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2282679</td>
<td>GC</td>
<td>T/G</td>
<td>0.29</td>
<td>1.03 (0.99-1.08)</td>
<td>0.14 (0.77-1.09)</td>
<td>0.14 (0.99-0.95)</td>
<td>11</td>
<td>1.00 (0.55)</td>
</tr>
<tr>
<td>rs10741657</td>
<td>CY2PR1</td>
<td>G/A</td>
<td>0.39</td>
<td>0.98 (0.94-1.02)</td>
<td>0.22 (0.93-1.02)</td>
<td>0.22 (0.93-1.02)</td>
<td>0</td>
<td>0.99 (0.73)</td>
</tr>
<tr>
<td>rs12785878</td>
<td>DHCIR7/NADSYN1</td>
<td>T/G</td>
<td>0.26</td>
<td>1.03 (0.99-1.08)</td>
<td>0.15 (0.99-1.10)</td>
<td>0.15 (0.99-1.10)</td>
<td>0</td>
<td>0.99 (0.19)</td>
</tr>
<tr>
<td>rs11234027</td>
<td>DHCIR7/NADSYN1</td>
<td>G/A</td>
<td>0.16</td>
<td>1.01 (0.96-1.07)</td>
<td>0.63 (0.97-1.10)</td>
<td>0.63 (0.97-1.10)</td>
<td>9</td>
<td>0.99 (0.18)</td>
</tr>
<tr>
<td>rs6013987</td>
<td>CY2PR1</td>
<td>T/A</td>
<td>0.21</td>
<td>0.97 (0.92-1.02)</td>
<td>0.30 (0.92-1.04)</td>
<td>0.30 (0.92-1.04)</td>
<td>0</td>
<td>0.88 (0.74)</td>
</tr>
</tbody>
</table>

Abbreviations: N studies genotyped, number of studies with directly genotyped in all the studies; mean \( R² \), average imputation \( R² \) value across all the studies, which the SNP was imputed.

\( ^a \)Allele associated with decreased circulating 25(OH)D in prior GWAS [Ahn and colleagues (33); Wang and colleagues (34)].

\( ^b \)OR calculated in reference to the allele associated with decreased 25(OH)D. Multivariable models included available covariates age, sex (when appropriate), center (when appropriate), smoking status, batch effects, three principal components from EIGENSTRAT, family history of colorectal cancer, BMI, NSAID use, alcohol use, dietary calcium, folate and red meat intake, sedentary status, and hormone replacement therapy.

\( ^c \)Number of studies directly genotyping and imputing SNPs varies for each SNP.

\( ^d \)\( P_{\text{heterogeneity}} \) across study in the multivariable-adjusted models.
high versus low vitamin D levels and quartiles of vitamin D, as well as tested for multiplicative interactions between GRS and a 1 ng/mL increase in 25(OH)D and high/low vitamin D using a product term in the model and assessing its significance by the Wald method.

We used PLINK, R (47, 48), and SAS 9.2 (SAS Institute, Inc.) to conduct the statistical analyses. We estimated our power to detect an association between a GRS and colorectal cancer using the method of Tosteson and colleagues. (49). These calculations account for the strength of association between the vitamin D SNPs and circulating 25(OH)D.

Results

Our study included 10,061 colorectal cancer cases and 12,768 controls. Overall 53% were female and the mean age at colorectal cancer diagnosis was 64 years (±9.6 SD), 55.0% past/current smokers and with risk allele frequencies ranging from 16% to 61%. Table 1 summarizes the characteristics of the studies included in the analyses. Analyses of each individual SNP in models first minimally and then fully adjusted, did not show a statistically significant association with colorectal cancer risk (Table 2). In analyses stratified by anatomic site, each of the five SNP associations remained nonsignificant (Table 3), in tests of associations with cancers of the colon and rectum.

We considered the possibility that a combination of SNPs associated with circulating 25(OH)D in prior GWAS may be associated with risk of colorectal cancer. However, an analysis of a GRS composed of the risk alleles from the four SNPs associated with plasma 25(OH)D and colorectal cancer risk did not show any significant association (Table 4). The Fig. 1 forest plot depicts the ORs and 95% CIs of the colorectal cancer association of the individual SNP and the best integrated biomarker of vitamin D status, and risk of colorectal cancer. However, contrary to expectation, we did not observe a statistically significant association between SNPs associated with circulating 25(OH)D and colorectal cancer, marginally or in an additive GRS.

A number of epidemiologic studies have reported inverse associations between 25(OH)D and colorectal cancer. A meta-analysis of five nested case–control studies reported a pooled OR of 0.49 (95% CI, 0.35–0.68) for colorectal cancer comparing the highest quintile (median 37 ng/mL) of 25(OH)D with the lowest (6 ng/mL) (19). Another recent systematic review of nine studies observed a pooled relative risk (RR) for colorectal cancer of 0.67 (95% CI, 0.54–0.80) comparing extreme quintiles of 25(OH)D (22). Overall, the estimated OR of colorectal cancer for a 10 ng/mL increase in circulating 25(OH)D was 0.74 (95% CI, 0.63–0.89) with the relationship appearing approximately linear (22). Several mechanisms could explain an anticancer benefit for vitamin D: reduction of cell proliferation; inhibition of angiogenesis; promotion of cell differentiation; and stimulation of apoptosis (23, 50–57). Vitamin D also has an anti-inflammatory effect, reducing PTGS-2 (COX-2) expression and decreasing levels of the inflammatory marker C-reactive protein (58–60).

Prior clinical trials testing the association between vitamin D and cancer have been null. In a 5-year British placebo-controlled trial with cancer assessed as a secondary outcome, 100,000 IU of vitamin D3 every 4 months over 5 years was not associated with colorectal cancer incidence (RR = 1.02; 95% CI, 0.60–1.74; 25). Similarly,

### Table 3. Association between five 25(OH)D-associated SNPs and colorectal cancer stratified by site

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (nearest gene)</th>
<th>Colon cancer cases only</th>
<th>Rectal cancer cases only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age, sex, and PCA-adjusted OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>P</td>
<td>N studies&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs2282679</td>
<td>GC</td>
<td>1.03 (0.98–1.08)</td>
<td>0.23</td>
</tr>
<tr>
<td>rs10741657</td>
<td>CYP2R1</td>
<td>0.98 (0.94–1.02)</td>
<td>0.35</td>
</tr>
<tr>
<td>rs12785878</td>
<td>DHCR7/NADSYN1</td>
<td>1.05 (1.00–1.10)</td>
<td>0.06</td>
</tr>
<tr>
<td>rs11234027</td>
<td>DHCR7/NADSYN1</td>
<td>1.02 (0.97–1.08)</td>
<td>0.30</td>
</tr>
<tr>
<td>rs6013897</td>
<td>CYP24A1</td>
<td>0.96 (0.91–0.2)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

<sup>a</sup>A total of 7,220 colon cancer and 2,308 rectal cancer cases were included in each site-specific analysis with 12,768 controls.

<sup>b</sup>OR calculated in reference to the allele associated with decreased 25(OH)D.
among 36,000 women in the WHI calcium–vitamin D trial, a combination of calcium (1,000 mg/day) plus low-dose vitamin D₃ (400 IU/day) for a mean of 7 years did not reduce colorectal cancer incidence (RR = 1.08; 95% CI, 0.86–1.34; 24). However, the interpretation of these null results is tempered by several important limitations. First, the relatively low doses of vitamin D used were probably inadequate to yield a substantial contrast between the treatment and placebo groups. Second, the duration of follow-up was probably too short to observe an influence on incidence of cancer. Observational data suggest that any influence of calcium and vitamin D intake on colorectal cancer risk could require at least 10 years to emerge, consistent with our understanding of the prolonged dwell time of the adenoma–carcinoma pathway (18). On the other hand, a Nebraska population-based placebo-controlled trial of calcium alone or calcium plus vitamin D₃ (1,100 IU/day) observed a significantly lower cancer incidence among those supplemented with calcium and vitamin D over just 4 years of treatment (61). However, follow-up of total cancers was a secondary outcome and there were only a small number of colorectal cancer cases, limiting the interpretation of these results.

More than 90% of circulating 25(OH)D is protein-bound with the GC encoded vitamin D–binding protein being the major carrier of 25(OH)D. Less than 1% of vitamin D circulates in its unbound form (62). Vitamin D–binding protein is a multifunctional protein that also binds fatty acids and may have immune functions independent of its role as a carrier of vitamin D (63). Prior studies have observed that unbound 25(OH)D was more strongly related to bone mineral density (64) and parathyroid hormone levels among hemodialized patients (65), than total 25(OH)D, thereby implicating a role for vitamin D–binding protein in modifying the biologic activity of circulating vitamin D. The available estimates of the association between 25(OH)D and colorectal cancer, as well as genetic markers of 25(OH)D, are based solely on total circulating 25(OH)D levels (19, 22, 33, 34). It is unclear how these estimates might change when accounting for vitamin D–binding protein levels or by individually examining free and protein-bound 25(OH)D.

Prior studies have examined individual SNPs in CYP24A1 or GC (66, 67) in association with risk of colorectal cancer. A prior DALS multicenter population-based case–control study of 1,600 colorectal cancer cases found a statistically significant association between one CYP24A1 polymorphism and overall risk of colon cancer, particularly for proximal colon cancer, as well as an association between three CYP24A1 polymorphisms and distal colon cancer (67). However, the correlation between these CYP24A1 genetic markers investigated by the DALS study and the CYP24A1 marker examined in this full combined analysis study is very low ($r^2 < 0.1$). Because our a priori hypothesis was that SNPs most strongly associated with 25(OH)D levels would be associated with colorectal cancer, we did not consider total genetic variation in CYP24A1 with colorectal cancer risk. It is possible that alternative CYP24A1 SNPs may be associated with colorectal cancer through mechanisms independent of 25(OH)D levels. A prior study of the CCFR cohort of 1,750 sibships found no evidence for associations between GC and colon cancer.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position (nearest gene)</th>
<th>N cases/N controls</th>
<th>Major/minor allele</th>
<th>Age, sex, and PCA-adjusted OR (95% CI)$^b$</th>
<th>P</th>
<th>N studies</th>
<th>P heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2282679</td>
<td>GC</td>
<td>10,128/12,768</td>
<td>T/G$^a$</td>
<td>1.03 (0.99–1.08)</td>
<td>0.13</td>
<td>13</td>
<td>0.20</td>
</tr>
<tr>
<td>rs10741657</td>
<td>CYP2R1</td>
<td>10,128/12,768</td>
<td>G/A$^a$</td>
<td>0.98 (0.94–1.02)</td>
<td>0.22</td>
<td>13</td>
<td>0.77</td>
</tr>
<tr>
<td>rs1278578</td>
<td>DHCR7/NADSYN1</td>
<td>10,128/12,768</td>
<td>T/G$^a$</td>
<td>1.03 (0.99–1.08)</td>
<td>0.16</td>
<td>13</td>
<td>0.10</td>
</tr>
<tr>
<td>rs6013897</td>
<td>CYP24A1</td>
<td>10,128/12,768</td>
<td>T/A$^a$</td>
<td>0.97 (0.93–1.03)</td>
<td>0.33</td>
<td>13</td>
<td>0.89</td>
</tr>
<tr>
<td>Score</td>
<td>—</td>
<td>10,128/12,768</td>
<td>—</td>
<td>1.00 (0.98–1.03)</td>
<td>0.72</td>
<td>13</td>
<td>0.11</td>
</tr>
</tbody>
</table>

$^a$Allele associated with decreased circulating 25(OH)D in prior GWAS [Ahn and colleagues (33); Wang and colleagues (34)].

$^b$OR calculated in reference to the allele associated with decreased 25(OH)D.

Table 4. Association between four mutually adjusted 25(OH)D-associated SNPs, a composite SNP additive GRS and colorectal cancer

Figure 1. Forest plot of the GRS and colorectal cancer for individual studies and meta-analysis of all studies (allelic OR, 95% CI).

Figure 1. Forest plot of the GRS and colorectal cancer for individual studies and meta-analysis of all studies (allelic OR, 95% CI).
the risk of colorectal cancer and no evidence for modification of the association by calcium and/or vitamin D intake (66). Studies of additional genes that are not significantly associated with circulating 25(OH)D but are implicated in the vitamin D pathway, including the vitamin D receptor (VDR) SNPs FokI and BsmI, have yielded inconsistent results (68–71).

The lack of association that we observed between genetic markers associated with circulating 25(OH)D and colorectal cancer is consistent with prior clinical trials of vitamin D and colorectal cancer and would, at least initially, seem to argue against a causal association between vitamin D and colorectal cancer (72). However, prior work has showed that these 4 SNPs, although correlated with circulating 25(OH)D, explain only a small fraction (5%) of the variance in circulating 25(OH)D (73). Recently, a Scottish case–control study observed a significant association between direct plasma measurements of 25(OH)D and colorectal cancer risk, yet failed to replicate the association using an instrumental-variable method of Mendelian randomization with the same four genome-wide significant risk loci examined in our analysis (74). The investigators attributed these inconsistent results to a presumed weak correlation between these SNPs and 25(OH)D, as well as a limited sample size of 2,001 cases of colorectal cancer and 2,237 controls. Given our significantly larger sample size of approximately 10,000 cases and 12,500 controls and assuming a correlation between our GRS and 25(OH)D of \( r = 0.17 \), with a 10 ng/mL increase in 25(OH)D associated with OR = 0.74 for colorectal cancer (22), our power to detect a one-allele change in our GRS is 96% (significance level of 0.05). However, if the true magnitude of association with a 10 ng/mL increase in 25(OH)D is in fact an OR of 0.85 for colorectal cancer, we would have only had 56% power to detect a one-allele change in our GRS.

We are not certain of the precise pathway or biologic mediators by which 25(OH)D influences colorectal cancer risk. Our GRS assumes that each included SNP would be associated with increased colorectal cancer risk according to their observed association with lower 25(OH)D. If this assumption is invalid, combining the alleles into this GRS would reduce our power to detect associations with colorectal cancer. If we remove the GC SNP and repeat our power calculation, our observed correlation between our GRS and 25(OH)D becomes \( r = 0.11 \), resulting in 68% power to detect a one-allele change in our proxy score.

We acknowledge some limitations. First, our study includes only populations of European descent, which limits the generalizability of our findings. However, the circulating 25(OH)D SNPs that we examined were identified in GWAS of populations of European descent and so the underlying genetic associations should hold in our study population. Moreover, limiting our analysis to European descent populations minimizes the potential for confounding by population structure. Second, if these SNPs are correlated with another locus that influences the risk of colorectal cancer, this could confound our results (72). Third, despite our large sample size, we had limited power to detect associations between individual SNPs and risk of colorectal cancer.

In conclusion, our findings do not support an association between SNPs associated with circulating 25(OH)D and risk of colorectal cancer. This may be due to the fact that these SNPs account for only a small portion of the variance observed in circulating 25(OH)D levels and that those alleles associated with low-circulating levels of 25(OH)D may not affect colorectal cancer risk in the same direction. Future studies are needed to examine the role of unbound and protein-bound 25(OH)D, along with other biomarkers of the vitamin D pathway, in the development of colorectal cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Writing, review, and/or revision of the manuscript: L.T. Hiraki, C. Qu, C.M. Hutter, D.V. Conti, C.S. Fuchs, E. Giovannucci, A. Hazra, J.L. Hopper, M. Lemire, J.E. Manson, H. Nan, P.A. Newcomb, K. Ng, F. Schumacher, K. Wu, P. Kraft, U. Peters, A.T. Chan
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.T. Hiraki, C. Qu, T.A. Harrison, M. Hoffmeister, S. Kury, L. Le Marchand, J.E. Manson, F. Schumacher, P. Kraft, U. Peters, A.T. Chan
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