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Vitamin D-Related Genes, Blood Vitamin D Levels and Colorectal Cancer Risk in Western European Populations

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Abstract: Higher circulating 25-hydroxyvitamin D levels (25(OH)D) have been found to be associated with lower risk for colorectal cancer (CRC) in prospective studies. Whether this association is modified by genetic variation in genes related to vitamin D metabolism and action has not been well studied in humans. We investigated 1307 functional and tagging single-nucleotide polymorphisms (SNPs; individually, and by gene/pathway) in 86 vitamin D-related genes in 1420 incident CRC cases matched to controls from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. We also evaluated the association between these SNPs and circulating 25(OH)D in a subset of controls. We confirmed previously reported CRC risk associations between SNPs in the *VDR*, *GC*, and *CYP27B1* genes. We also identified additional associations with 25(OH)D, as well as CRC risk, and several potentially novel SNPs in genes related to vitamin D transport and action (*LRP2*, *CUBN*, *NCOA7*, and *HDAC9*). However, none of these SNPs were statistically significant after Benjamini–Hochberg (BH) multiple testing correction. When assessed by a priori defined functional pathways, tumor growth factor β (TGF β) signaling was associated with CRC risk ($P \leq 0.001$), with most statistically significant genes being *SMAD7* ($P_{BH} = 0.008$) and *SMAD3* ($P_{BH} = 0.008$), and 18 SNPs in the vitamin D receptor (VDR) binding sites ($P = 0.036$). The 25(OH)D-gene pathway analysis suggested that genetic variants in the genes related to VDR complex formation and transcriptional activity are associated with CRC depending on 25(OH)D levels (interaction $P = 0.041$). Additional studies in large populations and consortia, especially with measured circulating 25(OH)D, are needed to confirm our findings.

Keywords: single nucleotide polymorphism (SNP); vitamin D; colorectal neoplasms; incidence

1. Introduction

Colorectal cancer (CRC) is the second most common cancer in men and women combined, with approximately 1.4 million new cases diagnosed in 2012 worldwide [1]. There is compelling observational evidence that low circulating vitamin D concentrations are associated with increased risk of incident CRC [2,3]. However, other human evidence is less convincing. A few Mendelian randomization (MR) studies did not support an association between vitamin D genetic score and CRC risk, but the genetic contribution to 25(OH)D is relatively small (7.5% as estimated based on genome-wide association studies (GWAS) on common SNPs [4]), possibly explaining the null findings [5,6]. Also, the relatively few randomized clinical trials (RCTs) of vitamin D supplementation and colorectal neoplasms have not shown statistically significant effects, but sample size, duration and timing of supplementation, issues with compliance and choice of study population, and the limited range of vitamin D exposures assessed may have contributed to the null results [7–9]. Finally, the benefits from vitamin D supplementation for the prevention of colorectal neoplasms may vary according to genetic variation in the vitamin D-related genes (e.g., vitamin D receptor (VDR) [10]).

Anti-neoplastic effects of vitamin D on colorectal tissue are also supported by the fact that the normal colorectal epithelium expresses the vitamin D receptor (VDR) and vitamin D metabolizing enzymes (CYP27B1 and CYP24A1) and, therefore, can locally produce and degrade the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)₂D), from 25-hydroxyvitamin D (25(OH)D) [11–13]. In the colorectum, the active metabolite of vitamin D, 1,25(OH)₂D, exerts its anti-neoplastic effects by genomic (mediated by the VDR) and non-genomic mechanisms [14], including the regulation of over 200 vitamin D-responsive genes and rapid activation of intracellular signaling pathways, resulting in modulation of the cell cycle, bile acid degradation, immune response, growth factor signaling, and anti-inflammation [15].

Observational and RCT data suggest a potential vitamin D-colorectal neoplasms risk association is modified by polymorphisms in the vitamin D receptor (VDR) [10,16,17] and the vitamin D-binding protein gene (GC) [18]; however, only a few single nucleotide polymorphisms (SNPs) and a limited number of related pathways were considered. Novel evidence highlights a wide array of VDR binding sites across the human genome [19], and multiple pathways related to vitamin D effects [20]. Thus, it is plausible that the vitamin D–CRC risk association may be modulated by variation in a broad array of genes related to vitamin D metabolism (e.g. absorption, endogenous synthesis, transport, activation, and deactivation) and action (including transcriptional activity/post-transcriptional effects). All of these genes are polymorphic, but no studies to date have comprehensively investigated their individual and collective associations with CRC risk or circulating vitamin D levels. In consideration of these points, we investigated whether variation in genes related to vitamin D metabolism and transcriptional activity is related to circulating blood vitamin D levels, and whether genetic variation at the SNP, pathway and gene level, alone and in combination with circulating vitamin D levels, is associated with CRC risk in a large Western European prospective cohort study.

2. Materials and Methods

2.1. Study Population

We used a case-control design nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, a large prospective study with over 520,000 men and women aged 35–70 years enrolled from 23 centers in 10 Western European countries (Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom). The methods of the EPIC study have been described in detail elsewhere [21,22]. Individuals who were eligible for the study were selected from the general population of a specific geographical area, town, or province. Exceptions included the French sub-cohort, which is based on members of the health insurance system or state-school employees, and the Utrecht (Netherlands) sub-cohort, which is based on women who underwent screening for breast cancer. Between 1992 and 1998, standardized lifestyle and personal

history information, anthropometrics, and blood samples were collected from most participants at recruitment. Diet over the previous 1 year was measured at baseline by validated country-specific dietary questionnaires developed to ensure high compliance and better measures of local dietary habits [21]. Blood samples were stored at the International Agency for Research on Cancer (Lyon, France; -196°C , in liquid nitrogen) for all countries except Denmark (-150°C , in nitrogen vapor) and Sweden (in -80°C freezers). The EPIC study was approved by the Ethical Review Board of the International Agency for Research on Cancer (IARC) and the Institutional Review Board of each participating EPIC center. Written consent was obtained from all EPIC participants at enrolment into the study.

2.2. Cancer Incidence and Vital Status Follow-Up

Cancer incidence was determined through record linkages with regional cancer registries (Denmark/Italy/the Netherlands/Norway/Spain/Sweden/United Kingdom; complete up to December 2006) or via a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries, and active follow-up through study subjects and their next-of-kin (France/Germany/Naples/Greece; complete up to June 2010).

Vital status follow-up (98.5% complete) was collected by record linkage with regional and/or national mortality registries in all countries except France, Germany, and Greece, where data are collected through an active follow-up. Censoring dates for complete follow-up were between June 2005 and June 2009 in Denmark, the Netherlands, Spain, the United Kingdom, Sweden, Norway, and Italy. In Germany, Greece, and France follow-up was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. In these centers, the end of follow-up was defined as the last known date of contact, or the date of death whichever came first. The last update of endpoint information occurred between December 2007 and December 2009.

2.3. Nested Case-Control Design and Participant Selection

2.3.1. Case Ascertainment and Selection

CRC cases were selected among participants who developed colon (C18.0–C18.7, according to the ICD-10), rectum (C19–C20), and overlapping/unspecified origin tumors (C18.8 and C18.9). Cancers of the anus were excluded. CRC is defined as the combination of the colon and rectal cancers.

A total of 1420 first-time previously cancer-free colorectal cancer cases (colon cancer = 900; rectal cancer = 520) were identified. Cases were not selected from Norway (blood samples only recently collected; few colorectal cancers diagnosed after blood donation) and the Malmö center of Sweden. The number of cases for gene-environment analyses was 1176 because of missing, previously collected 25(OH)D measurements [23] (France = 6, Italy = 49, Spain = 30, UK = 27, The Netherlands = 8, Greece = 18, Germany = 21, and Sweden = 16).

2.3.2. Control Selection

Controls were selected (1:1) by incidence density sampling from all cohort members alive and not having a reported cancer at the time of diagnosis of the cases and were matched by age (± 6 months at recruitment), sex, study center, time of the day at blood collection, and fasting status at the time of blood collection (less than three hours, three to six hours, and more than six hours). Women were further matched by menopausal status (pre-/post-/peri-menopausal, and unknown) and for pre-menopausal women, phase of menstrual cycle at time of blood collection and usage of postmenopausal hormone therapy at time of blood collection (yes/no, regardless of menopausal status). The additional matching criteria for women were required for other studies that were being carried out using the same matched case-control sets. One control sample failed the genotyping and was not included in the analysis, resulting in a total of 1419 controls. The number of controls for analyses involving 25(OH)D was 764

because of missing or unobtainable, previously collected 25(OH)D measurements [23] (France = 18, Italy = 69, Spain = 48, UK = 62, The Netherlands = 41, Greece = 23, Germany = 60, Sweden = 49, and Denmark = 328).

2.3.3. Blood 25-(OH)-Vitamin D Assessment

We previously measured blood concentrations of 25(OH)D using a commercially available enzyme immunoassay kit (OCTEIA 25-(OH)D Kit, Immuno Diagnostic Systems, Boldon, UK) at the Laboratory for Health Protection Research, National Institute for Public Health and the Environment, the Netherlands [23]. The kit is specific for 100% of 25-(OH)-vitamin D₃ form and 75% of 25-(OH)-vitamin D₂ form. The inter-assay coefficient of variation as determined with two kit control samples was minimal (5.9% at the level of 20.3 nmol/L and 5.4% at the level of 77.4 nmol/L). No significant between-day drift, time shifts, or other trends were observed and the percentage of variance attributable to batch-to-batch differences was 4.5%. For all analyses, laboratory technicians were blinded to the case-control status of the samples.

2.3.4. SNP Selection, Genotyping, and Quality Control

Genomic DNA was extracted from whole blood samples using conventional methods. We used the custom GoldenGate Universal-32 3072-plex assay kit (Illumina, CA, USA) to genotype 1716 genetic variants within the genes known and proposed to be involved in (1) vitamin D metabolism (*DHCR7*, *GC*, *CYP3A4*, *CYP2R1*, *CYP24A1*, *CUBN*, and *LRP2*), (2) mineral homeostasis and endocrine regulations of 1,25(OH)₂D synthesis (*CASR*, *PTH*, *TRPV5*, and *TRPV6*), (3) vitamin D genomic effects (*VDR*, *RXRA*, *RXRB*, and *RXRG*), (4) formation of the *VDR* complex (co-activators and co-regulators *ACTL6A*, *ARID1A*, *BAZ1B*, *CARM1*, *CHAF1A*, *CREBBP*, *EP300*, *HDAC9*, *MED1*, *NCOA1*, *NCOA2*, *NCOA3*, *NCOA7*, *NCOR1*, *NCOR2*, *PCAF/KAT2B*, *PRMT1*, *SMARCA2*, *SMARCA4*, *SMARCC1*, *SMARCD1*, *SMARCE1*, *SNW1*, *SUPT16H*, *TOP2B*, and *TSC2*), and (5) vitamin D post-transcriptional response (tumor growth factor β (TGF β)-signaling, inflammation, oxidative stress, insulin growth factor (IGF) signaling, cell cycle, and *VDR* binding sites; please see Supplementary Table S1 for a complete list of genes and SNPs). The custom GoldenGate assay was designed using the Illumina online Assay Design Tool in May 2012. SNP genotype dataset for CEU population (Utah residents with Northern and Western European ancestry; HapMap Data Rel 28 Phase II + III, August 10, on NCBI B36 assembly, dbSNP b126) were loaded in the Haploview program (Broad Institute, MIT and Harvard, Cambridge, MA, USA) and SNPs with minor allele frequencies (MAFs) greater than 5% and the r^2 linkage disequilibrium (LD) statistic of 0.8 were selected as tagging SNPs (tagSNPs). Additionally, we searched published literature for previously reported functional and regulatory SNPs in the genes of interest and included them in genotyping irrespective of MAFs or r^2 with other SNPs. Genotyping was performed by the Genetics Laboratory at Imperial College London. After excluding 409 SNPs [247 (14.4%) that failed genotyping, 54 (3.1%) that failed to satisfy the Hardy-Weinberg criterion (Supplementary Table S1), 98 (5.7%) missing in more than 20% of genotyped samples, and 10 (0.6%) that were monomorphic], a total of 1307 SNPs were included in the analysis. All genotyping underwent standard quality control including concordance checks for blinded duplicates and examination of sample and SNP call rates. The lowest reproducibility frequency across 62 replicate samples was 0.98. The call rate was 95% for all samples and 95% for all SNPs.

2.3.5. Statistical Analysis

The season adjustment of 25(OH)D was carried out by the week of blood draw using the sine curve method [24]. The associations between season-adjusted 25(OH)D concentrations and genetic variants (coded as 0, 1, 2 corresponding to the number of minor alleles) were assessed among controls using linear regression models adjusted for age, sex, and center. Further adjustment for BMI, smoking status, and physical activity did not change the results substantially. We used unconditional logistic regression analysis to assess the association of individual SNPs with CRC risk, adjusting for age (continuous), sex, and study center. Results were similar when we used conditional logistic regression on 1331 complete case-matched sets. We assumed a log-additive genetic model, but also tested dominant and recessive models as the underlying genetic model for these SNPs is unknown. Further adjustment for body mass index (BMI; continuous), smoking status (never, former, current smokers, missing), physical activity (active, moderately active, moderately inactive, and inactive), alcohol intake (continuous), hormone therapy, and menopausal status did not substantially change the results, and thus these variables were not included in the final statistical model. Subgroup analyses were conducted by sex and tumor location (colon vs. rectum).

To examine the associations between genes (a combination of SNPs) and genetic pathways (a combination of genes) and CRC risk, we used the Adaptive Rank Truncated Product (ARTP) method [25] as implemented in the first step (no interaction) of the R package PIGE (<http://cran.r-project.org/web/packages/PIGE/index.html>). This method can combine associations of SNPs in each gene (or from the genes in a pathway) to provide a *P*-value at the gene or pathway level, respectively. Genetic markers in high LD ($r^2 \geq 0.8$) were excluded using the AdaJoint R package (<https://cran.r-project.org/web/packages/ARTP2>). To investigate the multiplicative interaction between the genes and genetic pathways with 25(OH)D on CRC risk, we used the modified ARTP method as implemented in the R package PIGE. The *P*-values at the SNP and the gene levels were corrected for multiple testing for the number of SNPs and for the number of genes, respectively, using the false discovery rate (Benjamini–Hochberg or BH) method [26]. Furthermore, we used traditional methods to assess potential interactions between SNPs and 25(OH)D stratifying by categories of 25(OH)D concentrations and assuming a log-additive model for genetic markers. Also, we assessed the association of 25(OH)D (per 24.96 nmol/L = 10 ng/mL) with CRC risk by genotype.

All statistical tests were two-sided with *P*-values < 0.05 considered statistically significant (SAS software, version 9.2; SAS Institute, Cary/NC; R, R Foundation for Statistical Computing, Vienna/Austria).

3. Results

3.1. Baseline Characteristics of Cases and Controls

Selected baseline characteristics of the CRC cases and matched controls are shown in Table 1. The mean age at blood donation of cases and controls was 58 years. On average, CRC cases had 4 years between blood donation and the time of diagnosis. The dataset included 520 rectal cancer cases and 900 colon cancer cases.

Table 1. Selected baseline characteristics of incident colorectal cancer (CRC) cases and their matched controls, the European Prospective Investigation into Cancer and Nutrition (EPIC) study, 1992–2003.

| Baseline Characteristic | Cases | | Controls | |
|--|----------|--------|----------|--------|
| | n = 1420 | | n = 1419 | |
| Women, N (%) | 705 | (49.6) | 701 | (49.4) |
| Mean age at blood collection, (SD) years | 58.5 | (7.3) | 58.6 | (7.3) |
| Mean years of follow-up, (SD) years | 4.1 | (2.3) | — | |
| Smoking status, N (%) ^a | | | | |
| Never | 580 | (40.8) | 594 | (41.9) |
| Former | 476 | (33.5) | 460 | (32.4) |
| Current | 346 | (24.4) | 349 | (24.6) |
| Physical activity, N (%) | | | | |
| Inactive | 202 | (14.2) | 183 | (12.9) |
| Moderately inactive | 402 | (28.3) | 367 | (25.9) |
| Moderately active | 583 | (41.1) | 612 | (43.1) |
| Active | 130 | (9.2) | 148 | (10.4) |
| BMI, (SD) kg/m ² | 26.8 | (4.2) | 26.3 | (3.8) |
| 25-(OH)-vitamin D measurement, N (%) | 1,176 | (82.8) | 764 | (53.8) |
| 25-(OH)-vitamin D, mean (SD) nmol/L ^b | 58.5 | (25.6) | 62.0 | (25.4) |
| Country, N (%) | | | | |
| France | 28 | (2.0) | 29 | (2.0) |
| Italy | 202 | (14.2) | 198 | (14.0) |
| Spain | 146 | (10.3) | 141 | (9.9) |
| United Kingdom | 240 | (16.9) | 250 | (17.6) |
| The Netherlands | 153 | (10.8) | 158 | (11.1) |
| Greece | 46 | (3.2) | 48 | (3.4) |
| Germany | 179 | (12.6) | 169 | (11.9) |
| Sweden | 88 | (6.2) | 86 | (6.1) |
| Denmark | 338 | (23.8) | 340 | (24.0) |

^a Percent missing is not shown. Therefore the total percentages do not add up to 100%. ^b Season standardized using the sine-curve method [25].

3.2. SNPs in the Genes Related to Vitamin D Metabolism/Transcriptional Activity and 25(OH)D

Thirty-seven SNPs in the genes related to vitamin D metabolism, formation of the VDR complex, and VDR transcriptional activity were associated with season-adjusted 25(OH)D concentrations with unadjusted $P \leq 0.05$ among controls (Supplementary Table S2). The top 10 SNPs are shown in Table 2. Of the 37, 17 SNPs were in the genes involved in vitamin D metabolism, and 20 SNPs in the genes involved in vitamin D transcriptional activity. None of these SNPs were statistically significantly associated with 25(OH)D after BH correction. The associations of all SNPs with 25(OH)D among controls only are shown in Supplementary Table S3A, and among cases and controls combined in Supplementary Table S3B.

Table 2. Top 10 single-nucleotide polymorphisms (SNPs) in the genes related to vitamin D metabolism and transcriptional activity associated with season-adjusted 25(OH)D concentrations among controls only, the EPIC study, 1992–2003 ^a.

| Gene ^b | SNP | N | 25(OH)D, β (95% CI) | P | P _{BH} ^c |
|-------------------|------------|-----|---------------------------|-------|------------------------------|
| VDR | rs2239182 | 742 | −3.82 (−6.15, −1.49) | 0.001 | 0.949 |
| LRP2 | rs2673170 | 747 | −4.43 (−7.27, −1.58) | 0.002 | 0.949 |
| NCOA7 | rs579477 | 758 | −3.57 (−6.07, −1.07) | 0.005 | 0.949 |
| GC | rs1352844 | 747 | 5.26 (1.63, 8.88) | 0.005 | 0.949 |
| GC | rs188812 | 752 | 5.38 (1.57, 9.20) | 0.006 | 0.949 |
| GC | rs2298849 | 757 | 4.26 (1.11, 7.41) | 0.008 | 0.949 |
| CUBN | rs4525114 | 750 | 7.11 (1.94, 12.29) | 0.007 | 0.949 |
| CYP27B1 | rs4646536 | 751 | 3.65 (0.93, 6.37) | 0.009 | 0.949 |
| CYP27B1 | rs10877013 | 764 | 3.42 (0.73, 6.12) | 0.013 | 0.974 |
| HDAC9 | rs212669 | 753 | −8.12 (−14.34, −1.90) | 0.011 | 0.974 |

^a Adjusted for age at blood collection, sex, and center. ^b Genes related to vitamin D metabolism and transcriptional activity. ^c P after Benjamini–Hochberg (BH) multiple testing correction.

3.3. SNPs in the Genes Related to Vitamin D Metabolism/Function and CRC Risk

We examined the associations between SNPs in the genes involved in vitamin D metabolism (genes = 9, SNPs = 274), mineral homeostasis and endocrine regulation of 1,25(OH)₂D synthesis (genes = 5, SNPs = 58), vitamin D genomic effects including the VDR complex co-activators and co-regulators (genes = 30, SNPs = 538), and two SNPs in the intergenic regions previously associated with circulating 25(OH)D [27] and CRC risk (Supplementary Table S4). In Table 3, we show the top fifteen statistically significant SNPs associated with CRC risk defined by $P_{unadjusted} < 0.01$. However, after BH correction, none of the associations remained statistically significant (all $P_{BH} > 0.2$). The results did not differ by tumor location (Table 3 and Supplementary Table S4) or sex (Supplementary Table S5).

3.4. SNPs in the Vitamin D-Responsive Genes and CRC Risk

We also examined the associations between 434 SNPs in the genes responsive to vitamin D, including the genes in the TGF β and IGF signaling pathways, inflammation, oxidative stress, cell cycle, and 19 SNPs located in the VDR binding sites as previously published [19] (Supplementary Table S6). Twenty-five SNPs were significantly associated with CRC risk at $P < 0.01$. However, after BH correction, none of the associations (except for SMAD3 rs7180244; SMAD7 rs11874392, rs12953717 and rs4939827) remained statistically significant (Supplementary Table S7). Interestingly, three SNPs (rs3197999, rs3802842, rs762421) in previously identified VDR binding sites were associated with CRC risk. The results did not differ by tumor location (Supplementary Tables S6 and S7) or sex (Supplementary Table S8).

3.5. Vitamin D Genes/Pathways and CRC Risk

At the pathway level, the VDR binding sites and TGF β signaling pathway were statistically significantly associated with CRC risk ($P < 0.04$; Table 4). For colon cancer, in addition to the VDR binding sites ($P = 0.008$) and TGF β signaling pathway ($P = 0.0001$), an association with cell cycle pathway was observed ($P = 0.03$). The TGF β ($P = 0.0001$) and IGF ($P = 0.007$) signaling pathways, but not the VDR binding sites ($P = 0.256$), were statistically significantly associated with rectal cancer risk.

At the gene level, several genes (CHAF1A, SMARCE1, SMAD7, SMAD3, BMP2, and C-MYC region) were associated with CRC risk at unadjusted $P < 0.05$. However, all of them except SMAD7 ($P_{BH} = 0.008$) and SMAD3 ($P_{BH} = 0.008$) were not statistically significant after BH correction. The SMAD7, SMAD3, BMP2, and C-MYC regions were associated with colon cancer; however, after BH correction, only SMAD7 ($P_{BH} = 0.04$) and SMAD3 ($P_{BH} = 0.009$) remained statistically significant. In addition to SMAD7 ($P = 0.0005$) and SMAD3 ($P = 0.0003$), several other genes or genetic regions (CYP2R1, CHAF1A, CREBBP, IL10, SNPs identified in genome-wide association studies (GWAS) to be associated with IGF

levels, *IGFBP2/IGFBP5*, *IGFBP3*, and *C-MYC* region) were associated with rectal cancer. However, after BH correction, only *SMAD7* ($P = 0.02$) and *SMAD3* ($P = 0.02$) remained statistically significant.

3.6. 25(OH)D-Gene and 25(OH)D Pathway Interactions and CRC Risk

At the pathway level, the *VDR* complex and its transcriptional co-regulators and co-activators demonstrated a potential interaction with 25(OH)D concentrations in the association with CRC risk ($P = 0.04$; Table 4). Within this pathway, the interaction P -values of <0.05 were observed for *ARID1A*, *CARM1*, *CHAF1A*, and *SMARCA2*, but none were statistically significant after BH correction. Similar associations were observed for colon cancer, but not rectal cancer (P for interaction for the *VDR* complex and its transcriptional co-regulators and co-activators were 0.105 and 0.727, respectively).

At the gene level, the interaction P -values of <0.05 were observed for *CYP27B1* and *GC* (vitamin D metabolism) and *IL10* (inflammation) for CRC and colon cancer. Also, the interaction between 25(OH)D and *IGFBP2/IGFBP5* was statistically significant for colon cancer. For rectal cancer, the interaction P -values of <0.05 were observed for *CYP24A1* (vitamin D metabolism) and *BMP2* (TGF signaling). None of the gene-25(OH)D interactions were statistically significant after BH correction.

Next, we assessed the associations between 25(OH)D (per 24.96 nmol/L) with CRC risk, stratified by genotypes of SNPs in the genes that were identified in the step above as potentially modifying the association of 25(OH)D with CRC risk (*CYP27B1*, *GC*, *ARID1A*, *CARM1*, *CHAF1A*, *SMARCA2*, and *IL10*; Supplementary Table S9). Sixteen SNPs in these genes with P for interaction <0.05 are presented in Table 5. None were statistically significant after BH correction. Several SNPs had a very low number of minor allele homozygotes, with no effect estimates presented in the table.

Table 3. Associations of SNPs with CRC risk overall and by tumor location (colon vs. rectum), the EPIC study, 1992–2003.

| Gene/SNP | Genotype | Colorectal Cancer | | | | | Colon Cancer | | | | Rectal Cancer | | | | |
|------------|-----------|-------------------|----------|--------------------------|-------|-----------------|--------------|--------------------------|-------|-----------------|---------------|--------------------------|-------|------------------------------|--|
| | | Cases | Controls | OR (95% CI) ^a | P | P _{BH} | Cases | OR (95% CI) ^a | P | P _{BH} | Cases | OR (95% CI) ^a | P | P _{BH} ^b | |
| CUBN | | | | | | | | | | | | | | | |
| rs12243895 | GG | 702 | 767 | 1.00 (ref) | 0.009 | 0.569 | 435 | 1.00 (ref) | 0.006 | 0.509 | 267 | 1.00 (ref) | 0.261 | 0.969 | |
| | GA | 551 | 513 | 1.18 (1.00, 1.38) | | | 354 | 1.20 (1.00, 1.44) | | | 197 | 1.12 (0.90, 1.40) | | | |
| | AA | 140 | 104 | 1.48 (1.12, 1.96) | | | 94 | 1.59 (1.17, 2.16) | | | 46 | 1.34 (0.91, 1.97) | | | |
| | Additive | 1393 | 1384 | 1.20 (1.07, 1.35) | 0.002 | 0.274 | 883 | 1.24 (1.08, 1.41) | 0.002 | 0.254 | 510 | 1.14 (0.97, 1.34) | 0.106 | 0.824 | |
| | Dominant | 1393 | 1384 | 1.22 (1.05, 1.43) | 0.009 | 0.561 | 883 | 1.27 (1.07, 1.50) | 0.007 | 0.571 | 510 | 1.16 (0.94, 1.43) | 0.169 | 0.896 | |
| rs1801224 | Recessive | 1393 | 1384 | 1.38 (1.05, 1.80) | 0.020 | 0.898 | 883 | 1.46 (1.08, 1.97) | 0.013 | 0.827 | 510 | 1.27 (0.88, 1.85) | 0.204 | 0.939 | |
| | AA | 601 | 669 | 1.00 (ref) | 0.015 | 0.677 | 359 | 1.00 (ref) | 0.004 | 0.473 | 242 | 1.00 (ref) | 0.517 | 0.998 | |
| | AC | 614 | 582 | 1.18 (1.00, 1.38) | | | 399 | 1.26 (1.05, 1.51) | | | 215 | 1.04 (0.84, 1.30) | | | |
| | CC | 180 | 144 | 1.40 (1.09, 1.80) | | | 120 | 1.52 (1.15, 2.01) | | | 60 | 1.22 (0.87, 1.73) | | | |
| | Additive | 1395 | 1395 | 1.18 (1.06, 1.32) | 0.004 | 0.374 | 878 | 1.24 (1.09, 1.41) | 0.001 | 0.169 | 517 | 1.08 (0.93, 1.27) | 0.308 | 0.926 | |
| rs7096079 | Dominant | 1395 | 1395 | 1.22 (1.05, 1.42) | 0.010 | 0.561 | 878 | 1.31 (1.10, 1.56) | 0.002 | 0.377 | 517 | 1.07 (0.87, 1.32) | 0.494 | 0.965 | |
| | Recessive | 1395 | 1395 | 1.29 (1.02, 1.63) | 0.035 | 0.898 | 878 | 1.35 (1.04, 1.76) | 0.026 | 0.875 | 517 | 1.20 (0.86, 1.67) | 0.276 | 0.986 | |
| | CC | 275 | 338 | 1.00 (ref) | 0.023 | 0.677 | 159 | 1.00 (ref) | 0.007 | 0.509 | 116 | 1.00 (ref) | 0.587 | 0.998 | |
| | CA | 654 | 620 | 1.31 (1.08, 1.60) | | | 417 | 1.43 (1.14, 1.80) | | | 237 | 1.13 (0.87, 1.48) | | | |
| | AA | 301 | 305 | 1.22 (0.97, 1.53) | | | 194 | 1.36 (1.04, 1.77) | | | 107 | 1.03 (0.75, 1.40) | | | |
| | Additive | 1230 | 1263 | 1.10 (0.99, 1.24) | 0.084 | 0.894 | 770 | 1.16 (1.02, 1.32) | 0.025 | 0.712 | 460 | 1.02 (0.87, 1.18) | 0.841 | 0.996 | |
| | Dominant | 1230 | 1263 | 1.28 (1.07, 1.54) | 0.008 | 0.561 | 770 | 1.41 (1.13, 1.75) | 0.002 | 0.377 | 460 | 1.10 (0.86, 1.41) | 0.461 | 0.965 | |
| | Recessive | 1230 | 1263 | 1.02 (0.85, 1.22) | 0.861 | 1.000 | 770 | 1.06 (0.86, 1.31) | 0.578 | 0.991 | 460 | 0.95 (0.73, 1.22) | 0.668 | 0.997 | |
| VDR | | | | | | | | | | | | | | | |
| rs886441 | AA | 885 | 926 | 1.00 (ref) | 0.024 | 0.677 | 563 | 1.00 (ref) | 0.028 | 0.729 | 322 | 1.00 (ref) | 0.179 | 0.963 | |
| | AG | 444 | 404 | 1.16 (0.98, 1.36) | | | 273 | 1.12 (0.93, 1.35) | | | 171 | 1.21 (0.97, 1.52) | | | |
| | GG | 57 | 36 | 1.66 (1.08, 2.56) | | | 40 | 1.83 (1.15, 2.93) | | | 17 | 1.32 (0.73, 2.41) | | | |
| | Additive | 1386 | 1366 | 1.20 (1.05, 1.38) | 0.009 | 0.508 | 876 | 1.20 (1.03, 1.40) | 0.020 | 0.670 | 510 | 1.19 (0.99, 1.44) | 0.067 | 0.690 | |
| | Dominant | 1386 | 1366 | 1.20 (1.02, 1.40) | 0.027 | 0.693 | 876 | 1.18 (0.98, 1.41) | 0.078 | 0.896 | 510 | 1.22 (0.99, 1.52) | 0.067 | 0.750 | |
| | Recessive | 1386 | 1366 | 1.59 (1.04, 2.43) | 0.034 | 0.898 | 876 | 1.77 (1.11, 2.81) | 0.016 | 0.827 | 510 | 1.24 (0.68, 2.25) | 0.481 | 0.997 | |
| NCOA2 | | | | | | | | | | | | | | | |
| rs10087049 | AA | 393 | 472 | 1.00 (ref) | 0.007 | 0.569 | 240 | 1.00 (ref) | 0.003 | 0.448 | 153 | 1.00 (ref) | 0.180 | 0.963 | |
| | AG | 724 | 665 | 1.32 (1.11, 1.56) | | | 464 | 1.40 (1.15, 1.71) | | | 260 | 1.17 (0.92, 1.48) | | | |
| | GG | 173 | 182 | 1.14 (0.89, 1.46) | | | 120 | 1.30 (0.98, 1.72) | | | 53 | 0.88 (0.61, 1.26) | | | |
| | Additive | 1290 | 1319 | 1.12 (1.00, 1.26) | 0.054 | 0.848 | 824 | 1.19 (1.04, 1.36) | 0.010 | 0.596 | 466 | 1.00 (0.85, 1.17) | 0.959 | 0.996 | |
| | Dominant | 1290 | 1319 | 1.28 (1.08, 1.51) | 0.004 | 0.469 | 824 | 1.38 (1.14, 1.67) | 0.001 | 0.297 | 466 | 1.11 (0.88, 1.39) | 0.385 | 0.963 | |

Table 3. Cont.

| Gene/SNP | Genotype | Colorectal Cancer | | | | | Colon Cancer | | | | Rectal Cancer | | | |
|---------------------|-----------|-------------------|----------|--------------------------|----------|-----------------------|--------------|--------------------------|----------|-----------------------|---------------|--------------------------|----------|------------------------------------|
| | | Cases | Controls | OR (95% CI) ^a | <i>P</i> | <i>P_{BH}</i> | Cases | OR (95% CI) ^a | <i>P</i> | <i>P_{BH}</i> | Cases | OR (95% CI) ^a | <i>P</i> | <i>P_{BH}</i> ^b |
| NCOA7 rs10223441 | CC | 648 | 709 | 1.00 (ref) | 0.007 | 0.569 | 399 | 1.00 (ref) | 0.009 | 0.531 | 249 | 1.00 (ref) | 0.153 | 0.939 |
| | CG | 640 | 561 | 1.25 (1.07, 1.47) | | | 413 | 1.30 (1.09, 1.55) | | | 227 | 1.17 (0.95, 1.45) | | |
| | GG | 128 | 149 | 0.93 (0.72, 1.21) | | | 84 | 0.98 (0.72, 1.31) | | | 44 | 0.85 (0.59, 1.23) | | |
| | Additive | 1416 | 1419 | 1.06 (0.95, 1.19) | 0.277 | 0.921 | 896 | 1.09 (0.96, 1.24) | 0.183 | 0.920 | 520 | 1.01 (0.87, 1.18) | 0.882 | 0.996 |
| | Dominant | 1416 | 1419 | 1.19 (1.02, 1.38) | 0.025 | 0.669 | 896 | 1.23 (1.04, 1.46) | 0.017 | 0.685 | 520 | 1.10 (0.90, 1.35) | 0.338 | 0.944 |
| | Recessive | 1416 | 1419 | 0.84 (0.65, 1.08) | 0.169 | 0.948 | 896 | 0.86 (0.65, 1.14) | 0.295 | 0.991 | 520 | 0.79 (0.55, 1.13) | 0.202 | 0.939 |
| | GG | 594 | 639 | 1.00 (ref) | 0.004 | 0.569 | 375 | 1.00 (ref) | 0.019 | 0.659 | 219 | 1.00 (ref) | 0.031 | 0.882 |
| | GA | 657 | 575 | 1.23 (1.05, 1.44) | | | 416 | 1.21 (1.01, 1.46) | | | 241 | 1.26 (1.01, 1.57) | | |
| | AA | 148 | 185 | 0.86 (0.67, 1.10) | | | 97 | 0.86 (0.65, 1.13) | | | 51 | 0.85 (0.60, 1.21) | | |
| rs17292488 | Additive | 1399 | 1399 | 1.01 (0.90, 1.13) | 0.841 | 0.998 | 888 | 1.00 (0.89, 1.14) | 0.942 | 0.994 | 511 | 1.02 (0.88, 1.19) | 0.802 | 0.996 |
| | Dominant | 1399 | 1399 | 1.14 (0.98, 1.33) | 0.087 | 0.876 | 888 | 1.13 (0.95, 1.34) | 0.174 | 0.944 | 511 | 1.16 (0.94, 1.43) | 0.155 | 0.896 |
| | Recessive | 1399 | 1399 | 0.77 (0.61, 0.97) | 0.028 | 0.898 | 888 | 0.77 (0.59, 1.01) | 0.059 | 0.973 | 511 | 0.76 (0.54, 1.06) | 0.102 | 0.939 |
| NCOR2 rs10846670 | AA | 288 | 360 | 1.00 (ref) | 0.021 | 0.677 | 169 | 1.00 (ref) | 0.007 | 0.509 | 119 | 1.00 (ref) | 0.676 | 0.998 |
| | AG | 688 | 669 | 1.29 (1.07, 1.56) | | | 441 | 1.39 (1.12, 1.74) | | | 247 | 1.12 (0.86, 1.44) | | |
| | GG | 274 | 265 | 1.30 (1.03, 1.64) | | | 177 | 1.42 (1.09, 1.86) | | | 97 | 1.12 (0.81, 1.53) | | |
| | Additive | 1250 | 1294 | 1.15 (1.02, 1.29) | 0.020 | 0.663 | 787 | 1.20 (1.05, 1.37) | 0.007 | 0.564 | 463 | 1.06 (0.91, 1.24) | 0.461 | 0.927 |
| | Dominant | 1250 | 1294 | 1.29 (1.08, 1.55) | 0.005 | 0.528 | 787 | 1.40 (1.13, 1.73) | 0.002 | 0.377 | 463 | 1.12 (0.87, 1.43) | 0.377 | 0.962 |
| | Recessive | 1250 | 1294 | 1.09 (0.90, 1.32) | 0.356 | 0.987 | 787 | 1.13 (0.91, 1.41) | 0.256 | 0.991 | 463 | 1.04 (0.80, 1.36) | 0.777 | 0.997 |
| | GG | 1032 | 1082 | 1.00 (ref) | 0.010 | 0.569 | 666 | 1.00 (ref) | 0.005 | 0.496 | 366 | 1.00 (ref) | 0.025 | 0.827 |
| | GA | 359 | 298 | 1.26 (1.06, 1.51) | | | 220 | 1.20 (0.98, 1.47) | | | 139 | 1.37 (1.08, 1.74) | | |
| | AA | 20 | 32 | 0.66 (0.38, 1.17) | | | 6 | 0.30 (0.13, 0.74) | | | 14 | 1.36 (0.71, 2.62) | | |
| rs906304 | Additive | 1411 | 1412 | 1.12 (0.96, 1.31) | 0.141 | 0.894 | 892 | 1.02 (0.85, 1.22) | 0.822 | 0.990 | 519 | 1.30 (1.07, 1.59) | 0.010 | 0.514 |
| | Dominant | 1411 | 1412 | 1.21 (1.02, 1.43) | 0.032 | 0.693 | 892 | 1.12 (0.92, 1.36) | 0.268 | 0.954 | 519 | 1.37 (1.09, 1.73) | 0.007 | 0.416 |
| | Recessive | 1411 | 1412 | 0.63 (0.35, 1.11) | 0.106 | 0.931 | 892 | 0.29 (0.12, 0.70) | 0.006 | 0.714 | 519 | 1.26 (0.66, 2.40) | 0.490 | 0.997 |
| CHAF1A rs243352 | CC | 410 | 369 | 1.00 (ref) | 0.014 | 0.677 | 250 | 1.00 (ref) | 0.240 | 0.977 | 160 | 1.00 (ref) | 0.003 | 0.453 |
| | CA | 695 | 673 | 0.93 (0.78, 1.11) | | | 438 | 0.97 (0.79, 1.19) | | | 257 | 0.85 (0.67, 1.08) | | |
| | AA | 285 | 346 | 0.74 (0.60, 0.91) | | | 190 | 0.82 (0.65, 1.05) | | | 95 | 0.60 (0.44, 0.80) | | |
| | Additive | 1390 | 1388 | 0.86 (0.78, 0.96) | 0.006 | 0.417 | 878 | 0.91 (0.81, 1.03) | 0.128 | 0.920 | 512 | 0.78 (0.67, 0.90) | 0.001 | 0.132 |
| | Dominant | 1390 | 1388 | 0.86 (0.73, 1.02) | 0.087 | 0.876 | 878 | 0.92 (0.76, 1.11) | 0.392 | 0.956 | 512 | 0.76 (0.61, 0.95) | 0.018 | 0.629 |
| | Recessive | 1390 | 1388 | 0.77 (0.65, 0.92) | 0.005 | 0.648 | 878 | 0.84 (0.69, 1.03) | 0.097 | 0.981 | 512 | 0.66 (0.51, 0.86) | 0.002 | 0.729 |
| | AA | 461 | 417 | 1.00 (ref) | 0.023 | 0.677 | 277 | 1.00 (ref) | 0.315 | 0.995 | 184 | 1.00 (ref) | 0.003 | 0.453 |
| | AG | 648 | 681 | 0.86 (0.72, 1.02) | | | 413 | 0.92 (0.76, 1.13) | | | 235 | 0.75 (0.60, 0.95) | | |
| | GG | 254 | 307 | 0.74 (0.60, 0.92) | | | 166 | 0.83 (0.65, 1.06) | | | 88 | 0.61 (0.46, 0.83) | | |
| rs9352 | Additive | 1363 | 1405 | 0.86 (0.78, 0.96) | 0.006 | 0.417 | 856 | 0.91 (0.81, 1.03) | 0.131 | 0.920 | 507 | 0.78 (0.67, 0.90) | 0.001 | 0.132 |
| | Dominant | 1363 | 1405 | 0.82 (0.70, 0.97) | 0.018 | 0.597 | 856 | 0.89 (0.74, 1.08) | 0.238 | 0.954 | 507 | 0.71 (0.57, 0.88) | 0.002 | 0.243 |
| | Recessive | 1363 | 1405 | 0.81 (0.68, 0.98) | 0.032 | 0.898 | 856 | 0.87 (0.70, 1.07) | 0.192 | 0.991 | 507 | 0.73 (0.56, 0.95) | 0.018 | 0.939 |

Table 3. Cont.

| Gene/SNP | Genotype | Colorectal Cancer | | | | | Colon Cancer | | | | Rectal Cancer | | | |
|-----------------------------|-----------|-------------------|----------|--------------------------|----------|-----------------------|--------------|--------------------------|----------|-----------------------|---------------|--------------------------|----------|------------------------------------|
| | | Cases | Controls | OR (95% CI) ^a | <i>P</i> | <i>P_{BH}</i> | Cases | OR (95% CI) ^a | <i>P</i> | <i>P_{BH}</i> | Cases | OR (95% CI) ^a | <i>P</i> | <i>P_{BH}</i> ^b |
| <i>HDAC9</i> rs2520361 | AA | 881 | 841 | 1.00 (ref) | 0.021 | 0.677 | 556 | 1.00 (ref) | 0.161 | 0.960 | 325 | 1.00 (ref) | 0.033 | 0.882 |
| | AG | 385 | 395 | 0.92 (0.78, 1.09) | | | 240 | 0.93 (0.76, 1.13) | | | 145 | 0.91 (0.72, 1.15) | | |
| | GG | 50 | 79 | 0.60 (0.41, 0.87) | | | 36 | 0.68 (0.45, 1.02) | | | 14 | 0.46 (0.26, 0.83) | | |
| | Additive | 1316 | 1315 | 0.85 (0.75, 0.97) | 0.018 | 0.641 | 832 | 0.88 (0.75, 1.02) | 0.086 | 0.920 | 484 | 0.81 (0.67, 0.98) | 0.027 | 0.664 |
| | Dominant | 1316 | 1315 | 0.87 (0.74, 1.02) | 0.089 | 0.876 | 832 | 0.89 (0.74, 1.07) | 0.197 | 0.946 | 484 | 0.84 (0.67, 1.05) | 0.122 | 0.853 |
| | Recessive | 1316 | 1315 | 0.61 (0.43, 0.88) | 0.009 | 0.898 | 832 | 0.69 (0.46, 1.04) | 0.079 | 0.973 | 484 | 0.48 (0.27, 0.85) | 0.013 | 0.939 |
| | AA | 1028 | 1072 | 1.00 (ref) | 0.007 | 0.569 | 645 | 1.00 (ref) | 0.006 | 0.509 | 383 | 1.00 (ref) | 0.146 | 0.921 |
| | AG | 366 | 304 | 1.26 (1.06, 1.50) | | | 238 | 1.30 (1.07, 1.58) | | | 128 | 1.23 (0.97, 1.57) | | |
| | GG | 18 | 31 | 0.60 (0.33, 1.09) | | | 10 | 0.54 (0.26, 1.12) | | | 8 | 0.71 (0.32, 1.56) | | |
| | Additive | 1412 | 1407 | 1.11 (0.95, 1.30) | 0.176 | 0.916 | 893 | 1.13 (0.95, 1.35) | 0.166 | 0.920 | 519 | 1.10 (0.90, 1.36) | 0.346 | 0.926 |
| <i>SMARCC1</i> rs3755637 | Dominant | 1412 | 1407 | 1.20 (1.01, 1.42) | 0.039 | 0.752 | 893 | 1.23 (1.01, 1.49) | 0.035 | 0.806 | 519 | 1.18 (0.93, 1.49) | 0.164 | 0.896 |
| | Recessive | 1412 | 1407 | 0.57 (0.32, 1.03) | 0.062 | 0.916 | 893 | 0.51 (0.25, 1.05) | 0.067 | 0.973 | 519 | 0.67 (0.30, 1.49) | 0.328 | 0.986 |
| | GG | 661 | 605 | 1.00 (ref) | 0.015 | 0.677 | 412 | 1.00 (ref) | 0.026 | 0.729 | 249 | 1.00 (ref) | 0.073 | 0.882 |
| | GA | 520 | 601 | 0.79 (0.67, 0.93) | | | 322 | 0.78 (0.64, 0.93) | | | 198 | 0.81 (0.65, 1.02) | | |
| | AA | 132 | 141 | 0.86 (0.66, 1.12) | | | 90 | 0.95 (0.70, 1.27) | | | 42 | 0.70 (0.48, 1.03) | | |
| | Additive | 1313 | 1347 | 0.87 (0.78, 0.98) | 0.023 | 0.712 | 824 | 0.90 (0.79, 1.03) | 0.111 | 0.920 | 489 | 0.83 (0.70, 0.97) | 0.023 | 0.650 |
| | Dominant | 1313 | 1347 | 0.80 (0.69, 0.93) | 0.005 | 0.517 | 824 | 0.81 (0.68, 0.96) | 0.017 | 0.685 | 489 | 0.79 (0.64, 0.98) | 0.030 | 0.679 |
| | Recessive | 1313 | 1347 | 0.96 (0.75, 1.24) | 0.758 | 1.000 | 824 | 1.07 (0.80, 1.41) | 0.659 | 0.991 | 489 | 0.78 (0.54, 1.12) | 0.174 | 0.939 |
| | AA | 948 | 884 | 1.00 (ref) | 0.022 | 0.677 | 612 | 1.00 (ref) | 0.011 | 0.531 | 336 | 1.00 (ref) | 0.460 | 0.993 |
| | AG | 353 | 415 | 0.79 (0.66, 0.93) | | | 220 | 0.74 (0.61, 0.90) | | | 133 | 0.87 (0.68, 1.10) | | |
| <i>TOP2B</i> rs1001647 | GG | 57 | 55 | 0.94 (0.64, 1.39) | | | 37 | 0.91 (0.59, 1.41) | | | 20 | 1.06 (0.61, 1.81) | | |
| | Additive | 1358 | 1354 | 0.86 (0.75, 0.99) | 0.032 | 0.792 | 869 | 0.83 (0.71, 0.97) | 0.017 | 0.650 | 489 | 0.93 (0.77, 1.13) | 0.454 | 0.926 |
| | Dominant | 1358 | 1354 | 0.80 (0.68, 0.95) | 0.009 | 0.561 | 869 | 0.76 (0.63, 0.92) | 0.004 | 0.442 | 489 | 0.89 (0.71, 1.11) | 0.298 | 0.943 |
| | Recessive | 1358 | 1354 | 1.02 (0.70, 1.49) | 0.922 | 1.000 | 869 | 1.00 (0.65, 1.55) | 0.982 | 0.999 | 489 | 1.11 (0.65, 1.90) | 0.702 | 0.997 |

^a Unconditional logistic regression adjusted for age at blood collection, sex, and study center. ^b *P* of false discovery rate (BH; Benjamini–Hochberg) method.

Table 4. P-values of pathway- and gene-level associations with CRC risk overall and by tumor location (colon vs. rectal) and of interactions with 25(OH)D concentrations (per 24.96 nmol/L), the EPIC study, 1992–2003.

| Pathway/Gene | No. of SNPs | No. of SNPs Retained After Pruning | Colorectal Cancer | | | | Colon Cancer | | | | Rectal Cancer | | | |
|--|-------------|------------------------------------|----------------------|------------------------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|
| | | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | |
| | | | <i>P</i> | <i>P_{BH}</i> ^a | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> |
| Vitamin D metabolism | 276 | 245 | 0.580 | | 0.159 | | 0.550 | | 0.160 | | 0.418 | | 0.116 | |
| Identified in GWAS of 25(OH)D ^b | 2 | 2 | 0.235 | 0.759 | 0.867 | 0.999 | 0.167 | 0.657 | 0.923 | 0.990 | 0.561 | 0.944 | 0.499 | 0.991 |
| CUBN | 116 | 106 | 0.173 | 0.741 | 0.764 | 0.999 | 0.130 | 0.657 | 0.896 | 0.990 | 0.083 | 0.470 | 0.490 | 0.991 |
| CYP24A1 | 25 | 23 | 0.443 | 0.777 | 0.358 | 0.999 | 0.083 | 0.647 | 0.666 | 0.990 | 0.622 | 0.944 | 0.007 | 0.595 |
| CYP27A1 | 5 | 5 | 0.500 | 0.777 | 0.299 | 0.999 | 0.488 | 0.819 | 0.086 | 0.522 | 0.968 | 0.968 | 0.256 | 0.991 |
| CYP27B1 | 6 | 5 | 0.448 | 0.777 | 0.037 | 0.446 | 0.585 | 0.829 | 0.041 | 0.448 | 0.514 | 0.944 | 0.154 | 0.991 |
| CYP2R1 | 12 | 9 | 0.115 | 0.741 | 0.811 | 0.999 | 0.368 | 0.815 | 0.921 | 0.990 | 0.044 | 0.459 | 0.727 | 0.991 |
| CYP3A4 | 7 | 5 | 0.241 | 0.759 | 0.730 | 0.999 | 0.461 | 0.815 | 0.533 | 0.990 | 0.262 | 0.747 | 0.392 | 0.991 |
| DHCR7 | 12 | 6 | 0.997 | 0.997 | 0.549 | 0.999 | 0.800 | 0.911 | 0.614 | 0.990 | 0.434 | 0.944 | 0.716 | 0.991 |
| GC | 24 | 20 | 0.484 | 0.777 | 0.018 | 0.406 | 0.912 | 0.954 | 0.026 | 0.442 | 0.241 | 0.747 | 0.316 | 0.991 |
| LRP2 | 67 | 64 | 0.804 | 0.926 | 0.377 | 0.999 | 0.508 | 0.819 | 0.677 | 0.990 | 0.904 | 0.967 | 0.487 | 0.991 |
| Mineral homeostasis | 58 | 40 | 0.834 | | 0.313 | | 0.912 | | 0.431 | | 0.537 | | 0.782 | |
| CASR | 31 | 23 | 0.580 | 0.784 | 0.736 | 0.999 | 0.536 | 0.819 | 0.957 | 0.990 | 0.643 | 0.944 | 0.565 | 0.991 |
| PTH | 6 | 5 | 0.931 | 0.982 | 0.671 | 0.999 | 0.773 | 0.911 | 0.739 | 0.990 | 0.539 | 0.944 | 0.736 | 0.991 |
| CALB1 | 2 | 2 | 0.489 | 0.777 | 0.741 | 0.999 | 0.400 | 0.815 | 0.847 | 0.990 | 0.882 | 0.967 | 0.819 | 0.991 |
| TRPV5 | 9 | 7 | 0.657 | 0.846 | 0.054 | 0.456 | 0.920 | 0.954 | 0.081 | 0.522 | 0.337 | 0.818 | 0.225 | 0.991 |
| TRPV6 | 10 | 3 | 0.263 | 0.777 | 0.880 | 0.999 | 0.520 | 0.819 | 0.954 | 0.990 | 0.112 | 0.595 | 0.713 | 0.991 |
| VDR complex/Transcriptional Co-regulators and Co-activators | 538 | 490 | 0.634 | | 0.041 | | 0.874 | | 0.105 | | 0.180 | | 0.727 | |
| ACTL6A | 3 | 3 | 0.239 | 0.759 | 0.395 | 0.999 | 0.262 | 0.815 | 0.497 | 0.990 | 0.506 | 0.944 | 0.613 | 0.991 |
| ARID1A | 8 | 7 | 0.408 | 0.777 | 0.032 | 0.446 | 0.306 | 0.815 | 0.048 | 0.448 | 0.133 | 0.628 | 0.068 | 0.924 |
| BAZ1B | 14 | 9 | 0.478 | 0.777 | 0.955 | 0.999 | 0.360 | 0.815 | 0.867 | 0.990 | 0.385 | 0.909 | 0.935 | 0.993 |
| CARM1 | 4 | 4 | 0.641 | 0.839 | 0.006 | 0.406 | 0.831 | 0.929 | 0.022 | 0.442 | 0.290 | 0.747 | 0.120 | 0.991 |
| CHAF1A | 5 | 4 | 0.035 | 0.511 | 0.013 | 0.406 | 0.307 | 0.815 | 0.047 | 0.448 | 0.007 | 0.187 | 0.098 | 0.926 |
| CREBBP | 15 | 12 | 0.388 | 0.777 | 0.285 | 0.999 | 0.800 | 0.911 | 0.215 | 0.865 | 0.011 | 0.187 | 0.793 | 0.991 |
| EP300 | 6 | 5 | 0.771 | 0.926 | 0.791 | 0.999 | 0.434 | 0.815 | 0.835 | 0.990 | 0.917 | 0.967 | 0.342 | 0.991 |
| HDAC9 | 149 | 141 | 0.559 | 0.784 | 0.873 | 0.999 | 0.524 | 0.819 | 0.578 | 0.990 | 0.553 | 0.944 | 0.970 | 0.993 |

Table 4. Cont.

| Pathway/Gene | No. of SNPs | No. of SNPs Retained After Pruning | Colorectal Cancer | | | | Colon Cancer | | | | Rectal Cancer | | | |
|---------------------------|-------------|------------------------------------|----------------------|------------------------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|
| | | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | |
| | | | <i>P</i> | <i>P_{BH}</i> ^a | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> |
| MED1 | 5 | 5 | 0.507 | 0.777 | 0.561 | 0.999 | 0.719 | 0.899 | 0.694 | 0.990 | 0.131 | 0.628 | 0.617 | 0.991 |
| NCOA1 | 18 | 14 | 0.581 | 0.784 | 0.065 | 0.504 | 0.804 | 0.911 | 0.201 | 0.854 | 0.239 | 0.747 | 0.355 | 0.991 |
| NCOA2 | 19 | 16 | 0.542 | 0.781 | 0.579 | 0.999 | 0.145 | 0.657 | 0.456 | 0.990 | 0.938 | 0.967 | 0.505 | 0.991 |
| NCOA3 | 11 | 9 | 0.067 | 0.636 | 0.051 | 0.456 | 0.056 | 0.647 | 0.069 | 0.489 | 0.522 | 0.944 | 0.350 | 0.991 |
| NCOA7 | 31 | 31 | 0.518 | 0.777 | 0.142 | 0.755 | 0.646 | 0.872 | 0.056 | 0.448 | 0.223 | 0.747 | 0.482 | 0.991 |
| NCOR1 | 7 | 3 | 0.312 | 0.777 | 0.802 | 0.999 | 0.377 | 0.815 | 0.759 | 0.990 | 0.577 | 0.944 | 0.489 | 0.991 |
| PCAF/KAT2B | 31 | 31 | 0.801 | 0.926 | 0.891 | 0.999 | 0.470 | 0.815 | 0.865 | 0.990 | 0.938 | 0.967 | 0.797 | 0.991 |
| PRMT1 | 4 | 4 | 0.346 | 0.777 | 0.351 | 0.999 | 0.407 | 0.815 | 0.184 | 0.823 | 0.618 | 0.944 | 0.624 | 0.991 |
| RXRA | 30 | 27 | 0.683 | 0.866 | 0.763 | 0.999 | 0.091 | 0.647 | 0.732 | 0.990 | 0.662 | 0.944 | 0.726 | 0.991 |
| RXRB | 7 | 3 | 0.824 | 0.926 | 0.074 | 0.526 | 0.445 | 0.815 | 0.058 | 0.448 | 0.617 | 0.944 | 0.356 | 0.991 |
| RXRG | 24 | 24 | 0.853 | 0.929 | 0.716 | 0.999 | 0.558 | 0.819 | 0.923 | 0.990 | 0.875 | 0.967 | 0.087 | 0.924 |
| SMARCA2 | 1 | 1 | 0.506 | 0.777 | 0.019 | 0.406 | 0.381 | 0.815 | 0.012 | 0.442 | 0.944 | 0.967 | 0.307 | 0.991 |
| SMARCA4 | 12 | 9 | 0.474 | 0.777 | 0.794 | 0.999 | 0.559 | 0.819 | 0.975 | 0.990 | 0.557 | 0.944 | 0.923 | 0.993 |
| SMARCC1 | 4 | 4 | 0.103 | 0.741 | 0.893 | 0.999 | 0.344 | 0.815 | 0.824 | 0.990 | 0.082 | 0.470 | 0.164 | 0.991 |
| SMARCD1 | 3 | 3 | 0.312 | 0.777 | 0.703 | 0.999 | 0.356 | 0.815 | 0.550 | 0.990 | 0.519 | 0.944 | 0.998 | 0.998 |
| SMARCE1 | 4 | 4 | 0.048 | 0.582 | 0.197 | 0.881 | 0.191 | 0.706 | 0.390 | 0.990 | 0.083 | 0.470 | 0.059 | 0.924 |
| SNW1 | 10 | 10 | 0.615 | 0.816 | 0.863 | 0.999 | 0.740 | 0.911 | 0.947 | 0.990 | 0.813 | 0.967 | 0.807 | 0.991 |
| SUPT16H | 7 | 6 | 0.809 | 0.926 | 0.990 | 0.999 | 0.696 | 0.899 | 0.960 | 0.990 | 0.663 | 0.944 | 0.841 | 0.991 |
| TOP2B | 6 | 5 | 0.192 | 0.741 | 0.965 | 0.999 | 0.097 | 0.647 | 0.327 | 0.990 | 0.281 | 0.747 | 0.400 | 0.991 |
| NCOR2 | 62 | 61 | 0.701 | 0.877 | 0.960 | 0.999 | 0.770 | 0.911 | 0.609 | 0.990 | 0.459 | 0.944 | 0.935 | 0.993 |
| VDR | 30 | 28 | 0.270 | 0.777 | 0.652 | 0.999 | 0.372 | 0.815 | 0.707 | 0.990 | 0.477 | 0.944 | 0.706 | 0.991 |
| TSC2 | 8 | 7 | 0.135 | 0.741 | 0.885 | 0.999 | 0.354 | 0.815 | 0.811 | 0.990 | 0.284 | 0.747 | 0.761 | 0.991 |
| TGF-beta signaling | 110 | 98 | 0.0001 | | 0.616 | | 0.0001 | | 0.729 | | 0.0001 | | 0.342 | |
| RHPN2 | 25 | 23 | 0.452 | 0.777 | 0.722 | 0.999 | 0.144 | 0.657 | 0.871 | 0.990 | 0.956 | 0.967 | 0.701 | 0.991 |
| SMAD7 | 23 | 18 | 0.0001 | 0.008 | 0.927 | 0.999 | 0.001 | 0.043 | 0.947 | 0.990 | 0.0005 | 0.021 | 0.627 | 0.991 |
| SMAD3 | 39 | 38 | 0.0002 | 0.008 | 0.367 | 0.999 | 0.0001 | 0.009 | 0.514 | 0.990 | 0.0003 | 0.021 | 0.085 | 0.924 |
| BMP2 | 5 | 5 | 0.016 | 0.346 | 0.081 | 0.527 | 0.014 | 0.298 | 0.224 | 0.865 | 0.072 | 0.470 | 0.049 | 0.924 |
| BMP4 | 1 | 1 | 0.201 | 0.742 | 0.431 | 0.999 | 0.334 | 0.815 | 0.615 | 0.990 | 0.261 | 0.747 | 0.276 | 0.991 |
| TGFB1 | 11 | 8 | 0.159 | 0.741 | 0.293 | 0.999 | 0.469 | 0.815 | 0.112 | 0.595 | 0.068 | 0.470 | 0.306 | 0.991 |
| TGFBR1 | 4 | 3 | 0.950 | 0.982 | 0.741 | 0.999 | 0.306 | 0.815 | 0.838 | 0.990 | 0.446 | 0.944 | 0.851 | 0.991 |
| SCG5/GREM1 | 2 | 2 | 0.141 | 0.741 | 0.412 | 0.999 | 0.170 | 0.657 | 0.371 | 0.990 | 0.323 | 0.808 | 0.656 | 0.991 |

Table 4. Cont.

| Pathway/Gene | No. of SNPs | No. of SNPs Retained After Pruning | Colorectal Cancer | | | | Colon Cancer | | | | Rectal Cancer | | | |
|---|-------------|------------------------------------|----------------------|------------------------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|
| | | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | |
| | | | <i>P</i> | <i>P_{BH}</i> ^a | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> |
| Inflammation | 133 | 97 | 0.888 | | 0.479 | | 0.620 | | 0.156 | | 0.200 | | 0.784 | |
| ALOX5 | 22 | 11 | 0.839 | 0.926 | 0.264 | 0.999 | 0.801 | 0.911 | 0.593 | 0.990 | 0.782 | 0.967 | 0.165 | 0.991 |
| IL10 | 13 | 8 | 0.462 | 0.777 | 0.030 | 0.446 | 0.854 | 0.931 | 0.006 | 0.442 | 0.009 | 0.187 | 0.419 | 0.991 |
| IL10R | 9 | 7 | 0.734 | 0.904 | 0.552 | 0.999 | 0.913 | 0.954 | 0.751 | 0.990 | 0.663 | 0.944 | 0.591 | 0.991 |
| IL2/IL21 | 6 | 5 | 0.441 | 0.777 | 0.933 | 0.999 | 0.538 | 0.819 | 0.978 | 0.990 | 0.666 | 0.944 | 0.607 | 0.991 |
| IL6 | 13 | 10 | 0.983 | 0.995 | 0.807 | 0.999 | 0.982 | 0.988 | 0.557 | 0.990 | 0.696 | 0.967 | 0.843 | 0.991 |
| IL12B | 18 | 17 | 0.128 | 0.741 | 0.870 | 0.999 | 0.079 | 0.647 | 0.796 | 0.990 | 0.791 | 0.967 | 0.761 | 0.991 |
| IFNG | 7 | 4 | 0.300 | 0.777 | 0.686 | 0.999 | 0.453 | 0.815 | 0.608 | 0.990 | 0.287 | 0.747 | 0.497 | 0.991 |
| TNF | 4 | 4 | 0.959 | 0.982 | 0.218 | 0.926 | 0.988 | 0.988 | 0.375 | 0.990 | 0.790 | 0.967 | 0.076 | 0.924 |
| NFKB1 | 22 | 15 | 0.816 | 0.926 | 0.644 | 0.999 | 0.873 | 0.939 | 0.736 | 0.990 | 0.856 | 0.967 | 0.794 | 0.991 |
| IL12A | 1 | 1 | 0.523 | 0.777 | 0.168 | 0.795 | 0.452 | 0.815 | 0.159 | 0.770 | 0.814 | 0.967 | 0.706 | 0.991 |
| IL18 | 4 | 4 | 0.348 | 0.777 | 0.514 | 0.999 | 0.613 | 0.840 | 0.766 | 0.990 | 0.182 | 0.737 | 0.290 | 0.991 |
| IL1A/IL1B | 11 | 8 | 0.951 | 0.982 | 0.124 | 0.701 | 0.846 | 0.931 | 0.054 | 0.448 | 0.761 | 0.967 | 0.711 | 0.991 |
| IL8 | 1 | 1 | 0.153 | 0.741 | 0.978 | 0.999 | 0.060 | 0.647 | 0.593 | 0.990 | 0.811 | 0.967 | 0.669 | 0.991 |
| RELA (p65) | 2 | 2 | 0.921 | 0.982 | 0.835 | 0.999 | 0.938 | 0.961 | 0.962 | 0.990 | 0.922 | 0.967 | 0.690 | 0.991 |
| Oxidative Stress | 51 | 37 | 0.726 | | 0.471 | | 0.598 | | 0.460 | | 0.913 | | 0.747 | |
| GSR | 9 | 7 | 0.530 | 0.777 | 0.116 | 0.701 | 0.712 | 0.899 | 0.110 | 0.595 | 0.656 | 0.944 | 0.376 | 0.991 |
| GPx2 | 15 | 8 | 0.834 | 0.926 | 0.772 | 0.999 | 0.713 | 0.899 | 0.748 | 0.990 | 0.412 | 0.944 | 0.915 | 0.993 |
| TXNRD1 (TR1) | 17 | 14 | 0.232 | 0.759 | 0.859 | 0.999 | 0.168 | 0.657 | 0.875 | 0.990 | 0.757 | 0.967 | 0.946 | 0.993 |
| SOD2 | 10 | 8 | 0.530 | 0.777 | 0.374 | 0.999 | 0.546 | 0.819 | 0.599 | 0.990 | 0.896 | 0.967 | 0.281 | 0.991 |
| Insulin growth factor (IGF) signaling | 61 | 52 | 0.105 | | 0.320 | | 0.550 | | 0.135 | | 0.007 | | 0.346 | |
| Associated with IGF levels in GWAS ^c | 4 | 4 | 0.414 | 0.777 | 0.999 | 0.999 | 0.579 | 0.829 | 0.901 | 0.990 | 0.036 | 0.437 | 0.879 | 0.993 |
| IGF1 | 17 | 15 | 0.131 | 0.741 | 0.452 | 0.999 | 0.113 | 0.657 | 0.668 | 0.990 | 0.225 | 0.747 | 0.655 | 0.991 |
| IGF2BP2 | 3 | 1 | 0.303 | 0.777 | 0.923 | 0.999 | 0.596 | 0.830 | 0.580 | 0.990 | 0.156 | 0.698 | 0.658 | 0.991 |
| IGFBP2/IGFBP5 | 24 | 21 | 0.187 | 0.741 | 0.052 | 0.456 | 0.692 | 0.899 | 0.018 | 0.442 | 0.029 | 0.411 | 0.607 | 0.991 |
| IGFBP3 | 13 | 11 | 0.057 | 0.601 | 0.882 | 0.999 | 0.273 | 0.815 | 0.997 | 0.997 | 0.050 | 0.459 | 0.058 | 0.924 |

Table 4. Cont.

| Pathway/Gene | No. of SNPs | No. of SNPs Retained After Pruning | Colorectal Cancer | | | | Colon Cancer | | | | Rectal Cancer | | | |
|--------------------------------|-------------|------------------------------------|----------------------|------------------------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|----------------------|-----------------------|--------------------------------------|-----------------------|
| | | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | | Gene or Pathway Only | | Gene- or Pathway-25(OH)D Interaction | |
| | | | <i>P</i> | <i>P_{BH}</i> ^a | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> | <i>P</i> | <i>P_{BH}</i> |
| Cell Cycle | 60 | 56 | 0.120 | | 0.852 | | 0.030 | | 0.845 | | 0.482 | | 0.916 | |
| KRAS | 13 | 13 | 0.511 | 0.777 | 0.498 | 0.999 | 0.262 | 0.815 | 0.436 | 0.990 | 0.182 | 0.737 | 0.925 | 0.993 |
| FOS (c-fos) | 10 | 9 | 0.389 | 0.777 | 0.425 | 0.999 | 0.230 | 0.815 | 0.518 | 0.990 | 0.764 | 0.967 | 0.574 | 0.991 |
| JUN | 7 | 7 | 0.569 | 0.784 | 0.407 | 0.999 | 0.411 | 0.815 | 0.163 | 0.770 | 0.917 | 0.967 | 0.811 | 0.991 |
| C-MYC region ^d | 13 | 13 | 0.010 | 0.295 | 0.843 | 0.999 | 0.051 | 0.647 | 0.857 | 0.990 | 0.054 | 0.459 | 0.959 | 0.993 |
| CCND1 | 4 | 3 | 0.177 | 0.741 | 0.893 | 0.999 | 0.155 | 0.657 | 0.565 | 0.990 | 0.549 | 0.944 | 0.763 | 0.991 |
| BCL2A1 | 2 | 2 | 0.118 | 0.741 | 0.587 | 0.999 | 0.083 | 0.647 | 0.574 | 0.990 | 0.250 | 0.747 | 0.915 | 0.993 |
| BAX | 6 | 5 | 0.308 | 0.777 | 0.892 | 0.999 | 0.155 | 0.657 | 0.748 | 0.990 | 0.922 | 0.967 | 0.988 | 0.998 |
| CDKN1A | 5 | 4 | 0.521 | 0.777 | 0.168 | 0.795 | 0.099 | 0.647 | 0.459 | 0.990 | 0.867 | 0.967 | 0.216 | 0.991 |
| VDR binding sites | 19 | 18 | 0.036 | | 0.530 | | 0.008 | | 0.410 | | 0.256 | | 0.798 | |
| VDR binding sites ^e | 19 | 18 | 0.036 | 0.511 | 0.530 | 0.999 | 0.008 | 0.227 | 0.410 | 0.990 | 0.256 | 0.747 | 0.798 | 0.991 |

^a *P* of false discovery rate (BH; Benjamini–Hochberg or BH) method. ^b rs10485165 and rs10507577 (Bejamin et al. 2007) [27]. ^c rs1245541, rs4234798, rs700752, and rs780094. ^d Chromosome 8q24 region. ^e SNPs located in the VDR binding sites relating to colorectal cancer and Crohn’s disease risk as previously published (Ramagopalan et al. 2010) [19].

Table 5. Associations of season-adjusted 25(OH)D concentrations (per 24.96 nmol/L) with CRC risk by genotypes, the EPIC study, 1992–2003.

| Gene/SNP | Major Allele Homozygotes | | Heterozygotes | | Minor Allele Homozygotes | | <i>P</i> _{interaction} ^b |
|---|--------------------------|-------------------------|----------------|-------------------------|--------------------------|-------------------------|--|
| | Cases/Controls | OR (95%CI) ^a | Cases/Controls | OR (95%CI) ^a | Cases/Controls | OR (95%CI) ^a | |
| Vitamin D metabolism | | | | | | | |
| CYP27B1 rs10877013 | 557/383 | 1.00 (0.86,1.17) | 510/311 | 0.84 (0.73,0.97) | 108/70 | 0.61 (0.40,0.93) | 0.024 |
| CYP27B1 rs4646536 | 551/381 | 1.00 (0.86,1.17) | 485/300 | 0.85 (0.74,0.99) | 110/70 | 0.62 (0.41,0.95) | 0.034 |
| GC rs1352846 | 500/319 | 0.96 (0.81,1.13) | 406/286 | 0.79 (0.67,0.93) | 104/68 | 0.58 (0.35,0.97) | 0.049 |
| GC rs16846876 | 530/331 | 1.05 (0.90,1.23) | 477/337 | 0.75 (0.65,0.88) | 144/81 | 0.88 (0.63,1.21) | 0.017 |
| GC rs2298850 | 584/384 | 1.00 (0.86,1.15) | 445/293 | 0.80 (0.68,0.94) | 86/61 | 0.56 (0.33,0.97) | 0.038 |
| GC rs3755967 | 569/359 | 0.99 (0.85,1.15) | 436/297 | 0.78 (0.66,0.91) | 101/64 | 0.66 (0.40,1.09) | 0.034 |
| GC rs842873 | 261/192 | 0.73 (0.58,0.91) | 588/354 | 0.84 (0.73,0.97) | 240/196 | 1.22 (0.97,1.52) | 0.002 |
| VDR complex/Transcriptional Co-regulators and Co-activators | | | | | | | |
| ARID1A rs11247596 | 720/474 | 0.96 (0.85,1.09) | 400/256 | 0.76 (0.63,0.92) | 53/34 | 0.42 (0.20,0.86) | 0.051 |
| ARID1A rs12737946 | 998/640 | 0.83 (0.74,0.93) | 168/116 | 1.25 (0.95,1.66) | 9/8 | - ^c | 0.016 |
| ARID1A rs12752833 | 998/641 | 0.83 (0.74,0.93) | 165/114 | 1.22 (0.92,1.62) | 9/8 | - | 0.025 |
| CARM1 rs7254708 | 764/515 | 0.82 (0.73,0.93) | 208/144 | 1.04 (0.81,1.32) | 9/8 | - | 0.0001 |
| CHAF1A rs243341 | 596/403 | 0.78 (0.68,0.90) | 468/276 | 0.95 (0.80,1.13) | 102/78 | 1.42 (0.97,2.07) | 0.020 |
| CHAF1A rs243365 | 607/434 | 0.80 (0.70,0.91) | 408/257 | 1.02 (0.86,1.22) | 50/44 | 1.64 (0.89,3.02) | 0.027 |
| SMARCA2 rs2296212 | 920/607 | 0.81 (0.72,0.91) | 229/142 | 1.15 (0.89,1.48) | 14/7 | 4.09 (0.29,58.01) | 0.035 |
| Inflammation | | | | | | | |
| IL10 rs3024509 | 1015/657 | 0.92 (0.83,1.03) | 132/100 | 0.62 (0.45,0.85) | 3/2 | - | 0.024 |
| IL10 rs6686931 | 747/476 | 0.80 (0.70,0.91) | 373/243 | 1.02 (0.86,1.20) | 49/38 | 1.42 (0.59,3.39) | 0.029 |

^a Adjusted for age at blood collection, sex, and center. ^b Interactions between SNPs and 25(OH)D stratifying by categories of 25(OH)D concentrations and assuming a log-additive model for genetic markers. ^c Not estimatable due to small sample size.

4. Discussion

In this large European prospective case-control study nested within the EPIC cohort, we investigated whether genetic variation in the genes and pathways related to vitamin D metabolism and vitamin D genomic effects is associated with CRC risk, and whether these associations are modified by 25(OH)D concentrations.

We identified several genes related to vitamin D metabolism, the VDR complex, and VDR transcriptional activity associated with 25(OH)D concentrations, with an unadjusted $P < 0.01$ before BH correction among controls. We confirmed two genes related to vitamin D metabolism, *CYP27B1* and *GC*, and one in the *VDR*, which were identified in previous GWAS studies [28–32]. We also identified other genes in our study including 1) two genes that encode the transcription-related factors *HDAC9* and *NCOA7*, involved in vitamin D transcriptional activity and VDR complex formation, and 2) two genes that encode the vitamin D-related transporters *LRP2* and *CUBN* [33,34]. *LRP2*, commonly known as megalin, is responsible for the endocytosis of the 25(OH)D vitamin D binding protein complex [35]. *CUBN* is an important co-receptor in the megalin-mediated endocytic pathway and patients without functioning *CUBN* were found to have abnormal 25(OH)D metabolism [33].

The genes associated with circulating 25(OH)D concentrations were also associated with CRC risk at unadjusted $P < 0.01$ before BH correction. *HDAC9* is located in a region on chromosome 7p21 [36] in which chromosomal gains were observed in primary CRC [37]. Furthermore, *HDAC9* has been observed via chromatin immunoprecipitation (ChIP) assay in human osteosarcoma tissues to suppress p53 transcription and, thereby, promote cell proliferation [38]. An association of *CUBN* with CRC was previously reported in a meta-analysis of six GWAS studies [39], while no studies have investigated a possible association of *LRP2* with CRC. *LRP2* is expressed in multiple epithelial cell lines, including colon [35,40] and is often co-expressed with *CUBN* [34]. Additionally, there are no previous GWAS regarding CRC and *NCOA7*, although Higginbotham et al., found statistically significant associations of *NCOA7* gene variants with reduced breast cancer risk across three different study cohorts [41]. The *NCOA7* SNPs identified in our study, however, differed from those identified by Higginbotham suggesting a possible novel CRC susceptibility locus.

Three *VDR* binding site SNPs were associated with CRC risk in our study population, but the associations were not statistically significant after BH correction. We a priori selected these *VDR* binding sites for genotyping based on the results of a previous study that used ChIP followed by DNA sequencing to identify 2776 *VDR* binding sites in lymphoblastoid cell lines treated with calcitriol, an active form of vitamin D [19]. The study found a statistically significant 4-fold increase in the enrichment of *VDR* binding sites located in genes associated with CRC, and a 3.5-fold increase in enrichment located in genes associated with Crohn's disease [19]. Our findings suggest that genetic variation in these *VDR* binding sites, upregulated in response to treatment with vitamin D and relevant to colorectal carcinogenesis and inflammatory bowel diseases, may be associated with CRC risk.

TGF β has an important role in the regulation of cell proliferation, differentiation, migration and apoptosis [42], and may be modulated by vitamin D [43]. *SMAD7* and *SMAD3*, in the TGF β signaling pathway, were statistically significantly associated with CRC risk after BH correction for multiple testing. *SMAD7* SNPs were previously identified to be associated with CRC risk in several different populations [44–46] as well as in a meta-analysis of 2906 cases and 3416 controls from four previous GWAS studies [47]. *SMAD7* is transcriptionally induced by cytokines from the TGF β family and regulates the TGF β signaling pathway via a negative feedback loop [42]; therefore, the overexpression of *SMAD7* inhibits the pathway and its associated anti-neoplastic effects [42]. The active form of vitamin D was shown to inhibit *SMAD7* in experimental models [48]. The role of *SMAD3* in the development of CRC is less understood and somatic tumor mutations in this gene have been observed in only 4.3% of CRC cases [49]. *SMAD3* has been identified to interact with *VDR* and mediate a cross-talk between TGF β and vitamin D signaling pathways [50]. An animal model found that *SMAD3* may also play an important role in the TGF β response to inflammation and bacteria-induced colon carcinogenesis [51]. Inflammation is further associated with CRC risk in our study as indicated by the interaction between

circulating 25(OH)D concentrations, which has anti-inflammatory properties [52,53], and genetic variation in the *IL10* gene encoding anti-inflammatory cytokine interleukin (IL)-10 involved in immune response to pathogens [54]. The induction of IL-10 is mediated by 1,25(OH)₂D and is repressed with SMAD3 inhibition [48].

Chromosome 8q24 polymorphisms in the cell cycle pathway were previously identified to be strongly associated with CRC risk [47,55,56]. However, they were not statistically significant after BH correction. Although the 8q24 region is described as a gene desert, it is closely located to the region encoding *c-MYC* oncogene [57]. *C-MYC* controls processes related to cell growth regulation, metabolism and proliferation and is not only activated by numerous oncogenic pathways but also stimulates metabolic changes which can lead to malignant transformation [58]. Multiple studies have identified long-range physical interaction of the 8q24 region with *c-MYC* via enhancer elements and chromatin loops [57,59,60]. In an experimental study, 1,25(OH)₂D and the *VDR* were shown to affect the *c-MYC/MXD1* pathway leading to inhibition of *c-MYC* protein expression [61]. Using 8q24 SNPs as a proxy, our results confirm an association between *c-MYC* and CRC risk, but do not indicate a potential modification by 25(OH)D despite a previously reported possible interaction for fatal prostate cancer risk [62].

The IGF signaling pathway plays a key role in cell growth [63]. In our study, IGF-related genetic variation was associated with rectal cancer risk at the pathway level as well as for several individual genes before BH correction. Contrary to our results, IGF genetic variants [64,65] in addition to high circulating IGF peptides [66] have been previously associated more strongly with increased colon versus rectal cancer risk.

The strengths of our study include its prospective design and high follow-up rate. The hypothesis-driven selection of pathways, genes, and SNPs, and relatively large samples size within a large cohort study allowed an extensive investigation of vitamin D-related and -responsive genetic variation and the effect modification by established biomarker of vitamin D status with CRC risk. We used the detailed data from EPIC to address potential confounding by body size and other factors; with our careful analyses suggesting no or little confounding. However, we cannot altogether discount the possibility of residual confounding nor changes in lifestyle habits between enrolment into the cohort and the eventual cancer diagnosis. Although our study was large, most interaction and stratified analyses had limited power, especially by sex and tumor location. Our power analyses (Supplementary Table S10) showed that we have sufficient power (80%) to detect the effect associations in the range of 1.17 to 1.27 for relatively common SNPs with MAF between 40 and 10%, respectively, using our full data set ($n = 1419$ matched case-control sets). In addition, most of our results were not statistically significant after BH correction for multiple testing. As to the selection of genes and pathways, we were limited by published literature on vitamin D at the time of genotyping, so we may have not included all vitamin D-responsive genes. Additional experimental studies are needed to understand the biological mechanisms of the identified associations.

5. Conclusions

This large and comprehensive study has confirmed genetic variations in several previously identified vitamin D-related pathways associated with CRC risk in European populations, and has suggested potential new pathways related to vitamin D genomic effects and colorectal carcinogenesis.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2072-6643/11/8/1954/s1>, Table S1: Characteristics of SNPs used in the study, Table S2: Vitamin D metabolism and transcriptional activity-related SNPs associated (unadjusted P -value < 0.05) with season-adjusted 25(OH)D concentrations among controls, the EPIC study, 1992–2003, Table S3A: Associations between SNPs in the genes involved in the vitamin D metabolism, mineral homeostasis/endocrine regulation of 1,25(OH)₂D synthesis, and vitamin D transcriptional activity with season-adjusted 25(OH)D concentrations among controls, Table S3B: Associations between SNPs in the genes involved in the vitamin D metabolism, mineral homeostasis/endocrine regulation of 1,25(OH)₂D synthesis, and vitamin D transcriptional activity with season-adjusted 25(OH)D concentrations among cases and controls combined, Table S4: Associations of SNPs with CRC risk overall and by tumor location (colon vs. rectum) using unconditional logistic regression with adjustment for age at recruitment, study center and sex, the EPIC

study, 1992–2003, Table S5 Associations of SNPs with CRC risk overall and by tumor location (colon vs. rectum) among men and women using unconditional logistic regression with adjustment for age at recruitment, study center and sex, the EPIC study, 1992–2003, Table S6: Associations of SNPs in vitamin D-responsive genes with CRC risk overall and by tumor location (colon vs. rectum) using unconditional logistic regression with adjustment for age at recruitment, study center and sex, the EPIC study, 1992–2003, Table S7: Statistically significant associations (unadjusted $p < 0.01$) of SNPs in vitamin D-responsive genes with CRC risk overall and by tumor location (colon vs. rectum) using unconditional logistic regression with adjustment for age at recruitment, study center and sex, the EPIC study, 1992–2003, Table S8: Associations of SNPs in vitamin D-responsive genes with CRC risk overall and by tumor location (colon vs. rectum) among men and women using unconditional logistic regression with adjustment for age at recruitment, study center and sex, the EPIC study, 1992–2003, Table S9: Associations of season-adjusted 25(OH)D concentrations (per 24.96 nmol/L) with CRC risk by genotypes, the EPIC study, 1992–2003, Table S10: SNP-Only Minimal Detectable Effect Associations by Minor Allele Frequency for 80% Power and $N = 1419$ matched cases and controls, the EPIC study, 1992–2003.

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Abbreviations

CRC—colorectal cancer; MR—Mendelian randomization; RCT—randomized control trial; VDR—vitamin D receptor; 1,25(OH)₂D—1,25-dihydroxyvitamin D; 25(OH)D—25-hydroxyvitamin D; SNP—single nucleotide polymorphism; EPIC—European Prospective Investigation into Cancer and Nutrition; IGF—insulin growth factor; TGFβ—tumor growth factor β; MAF—minor allele frequency; tagSNPs—tagging SNPs; BMI—body mass index; ARTP—Adaptive Rank Truncated Product; BH—Benjamini–Hochberg; ChIP—chromatin immunoprecipitation.

References

1. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* **2015**, *136*, E359–E386. [[CrossRef](#)] [[PubMed](#)]
2. Touvier, M.; Chan, D.S.; Lau, R.; Aune, D.; Vieira, R.; Greenwood, D.C.; Kampman, E.; Riboli, E.; Hercberg, S.; Norat, T. Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. *Cancer Epidemiol. Biomark. Prev.* **2011**, *20*, 1003–1016. [[CrossRef](#)] [[PubMed](#)]
3. McCullough, M.L.; Zoltick, E.S.; Weinstein, S.J.; Fedirko, V.; Wang, M.; Cook, N.R.; Eliassen, A.H.; Zeleniuch-Jacquotte, A.; Agnoli, C.; Albanes, D.; et al. Circulating Vitamin D and Colorectal Cancer Risk: An International Pooling Project of 17 Cohorts. *J. Natl. Cancer Inst.* **2018**, *111*, 158–169. [[CrossRef](#)] [[PubMed](#)]

4. Jiang, X.; O'Reilly, P.F.; Aschard, H.; Hsu, Y.-H.; Richards, J.B.; Dupuis, J.; Ingelsson, E.; Karasik, D.; Pilz, S.; Berry, D.; et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat. Commun.* **2018**, *9*, 260. [[CrossRef](#)] [[PubMed](#)]
5. Dimitrakopoulou, V.I.; Tsilidis, K.K.; Haycock, P.C.; Dimou, N.L.; Al-Dabhani, K.; Martin, R.M.; Lewis, S.J.; Gunter, M.J.; Mondul, A.; Shui, I.M.; et al. Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study. *BMJ* **2017**, *359*, j4761. [[CrossRef](#)] [[PubMed](#)]
6. He, Y.; Timofeeva, M.; Farrington, S.M.; Vaughan-Shaw, P.; Svinti, V.; Walker, M.; Zgaga, L.; Meng, X.; Li, X.; Spiliopoulou, A.; et al. Exploring causality in the association between circulating 25-hydroxyvitamin D and colorectal cancer risk: A large Mendelian randomisation study. *BMC Med.* **2018**, *16*, 142. [[CrossRef](#)]
7. Wactawski-Wende, J.; Kotchen, J.M.; Anderson, G.L.; Assaf, A.R.; Brunner, R.L.; O'Sullivan, M.J.; Margolis, K.L.; Ockene, J.K.; Phillips, L.; Pottern, L.; et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N. Engl. J. Med.* **2006**, *354*, 684–696. [[CrossRef](#)]
8. Baron, J.A.; Barry, E.L.; Mott, L.A.; Rees, J.R.; Sandler, R.S.; Snover, D.C.; Bostick, R.M.; Ivanova, A.; Cole, B.F.; Ahnen, D.J.; et al. A Trial of Calcium and Vitamin D for the Prevention of Colorectal Adenomas. *N. Engl. J. Med.* **2015**, *373*, 1519–1530. [[CrossRef](#)]
9. Manson, J.E.; Cook, N.R.; Lee, I.M.; Christen, W.; Bassuk, S.S.; Mora, S.; Gibson, H.; Gordon, D.; Copeland, T.; D'Agostino, D.; et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. *N. Engl. J. Med.* **2018**, *380*, 33–44. [[CrossRef](#)]
10. Barry, E.L.; Rees, J.R.; Peacock, J.L.; Mott, L.A.; Amos, C.I.; Bostick, R.M.; Figueiredo, J.C.; Ahnen, D.J.; Bresalier, R.S.; Burke, C.A.; et al. Genetic Variants in CYP2R1, CYP24A1, and VDR Modify the Efficacy of Vitamin D3 Supplementation for Increasing Serum 25-Hydroxyvitamin D Levels in a Randomized Controlled Trial. *J. Clin. Endocrinol. Metab.* **2014**, *99*, E2133–E2137. [[CrossRef](#)]
11. Matusiak, D.; Murillo, G.; Carroll, R.E.; Mehta, R.G.; Benya, R.V. Expression of vitamin D receptor and 25-hydroxyvitamin D3-1 α -hydroxylase in normal and malignant human colon. *Cancer Epidemiol. Biomark. Prev.* **2005**, *14*, 2370–2376. [[CrossRef](#)] [[PubMed](#)]
12. Tangpricha, V.; Flanagan, J.N.; Whitlatch, L.W.; Tseng, C.C.; Chen, T.C.; Holt, P.R.; Lipkin, M.S.; Holick, M.F. 25-hydroxyvitamin D-1 α -hydroxylase in normal and malignant colon tissue. *Lancet* **2001**, *357*, 1673–1674. [[CrossRef](#)]
13. Matusiak, D.; Benya, R.V. CYP27A1 and CYP24 expression as a function of malignant transformation in the colon. *J. Histochem. Cytochem.* **2007**, *55*, 1257–1264. [[CrossRef](#)] [[PubMed](#)]
14. Ball, G.F.M. *Vitamins: Their Role in the Human Body*; Blackwell Science: Ames, IA, USA, 2004.
15. Feldman, D.; Krishnan, A.V.; Swami, S.; Giovannucci, E.; Feldman, B.J. The role of vitamin D in reducing cancer risk and progression. *Nat. Rev. Cancer* **2014**, *14*, 342–357. [[CrossRef](#)] [[PubMed](#)]
16. Slatter, M.L.; Yakumo, K.; Hoffman, M.; Neuhausen, S. Variants of the VDR gene and risk of colon cancer (United States). *Cancer Causes Control* **2001**, *12*, 359–364. [[CrossRef](#)] [[PubMed](#)]
17. Poynter, J.N.; Jacobs, E.T.; Figueiredo, J.C.; Lee, W.H.; Conti, D.V.; Campbell, P.T.; Levine, A.J.; Limburg, P.; Le Marchand, L.; Cotterchio, M.; et al. Genetic variation in the vitamin D receptor (VDR) and the vitamin D-binding protein (GC) and risk for colorectal cancer: Results from the Colon Cancer Family Registry. *Cancer Epidemiol. Biomark. Prev.* **2010**, *19*, 525–536. [[CrossRef](#)] [[PubMed](#)]
18. Gibbs, D.C.; Fedirko, V.; Um, C.; Gross, M.D.; Thyagarajan, B.; Bostick, R.M. Associations of Circulating 25-Hydroxyvitamin D3 Concentrations With Incident, Sporadic Colorectal Adenoma Risk According to Common Vitamin D-Binding Protein Isoforms. *Am. J. Epidemiol.* **2018**, *187*, 1923–1930. [[CrossRef](#)]
19. Ramagopalan, S.V.; Heger, A.; Berlanga, A.J.; Maugeri, N.J.; Lincoln, M.R.; Burrell, A.; Handunnetthi, L.; Handel, A.E.; Disanto, G.; Orton, S.M.; et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: Associations with disease and evolution. *Genome Res.* **2010**, *20*, 1352–1360. [[CrossRef](#)]
20. Gil, A.; Plaza-Diaz, J.; Mesa, M.D. Vitamin D: Classic and Novel Actions. *Ann. Nutr. Metab.* **2018**, *72*, 87–95. [[CrossRef](#)]
21. Riboli, E.; Hunt, K.J.; Slimani, N.; Ferrari, P.; Norat, T.; Fahey, M.; Charrondiere, U.R.; Hemon, B.; Casagrande, C.; Vignat, J.; et al. European Prospective Investigation into Cancer and Nutrition (EPIC): Study populations and data collection. *Public Health Nutr.* **2002**, *5*, 1113–1124. [[CrossRef](#)]
22. Bingham, S.; Riboli, E. Diet and cancer—the European Prospective Investigation into Cancer and Nutrition. *Nat. Rev. Cancer* **2004**, *4*, 206–215. [[CrossRef](#)] [[PubMed](#)]

23. Jenab, M.; Bueno-de-Mesquita, H.B.; Ferrari, P.; van Duijnhoven, F.J.; Norat, T.; Pischon, T.; Jansen, E.H.; Slimani, N.; Byrnes, G.; Rinaldi, S.; et al. Association between pre-diagnostic circulating vitamin D concentration and risk of colorectal cancer in European populations: a nested case-control study. *BMJ* **2010**, *340*, b5500. [[CrossRef](#)] [[PubMed](#)]
24. Gail, M.H.; Wu, J.; Wang, M.; Yaun, S.S.; Cook, N.R.; Eliassen, A.H.; McCullough, M.L.; Yu, K.; Zeleniuch-Jacquotte, A.; Smith-Warner, S.A.; et al. Calibration and seasonal adjustment for matched case-control studies of vitamin D and cancer. *Stat. Med.* **2016**, *35*, 2133–2148. [[CrossRef](#)] [[PubMed](#)]
25. Yu, K.; Li, Q.; Bergen, A.W.; Pfeiffer, R.M.; Rosenberg, P.S.; Caporaso, N.; Kraft, P.; Chatterjee, N. Pathway analysis by adaptive combination of *P*-values. *Genet. Epidemiol.* **2009**, *33*, 700–709. [[CrossRef](#)] [[PubMed](#)]
26. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate—A Practical and Powerful Approach to Multiple Testing. *J. Roy. Stat. Soc. B Methodol.* **1995**, *57*, 289–300. [[CrossRef](#)]
27. Benjamin, E.J.; Dupuis, J.; Larson, M.G.; Lunetta, K.L.; Booth, S.L.; Govindaraju, D.R.; Kathiresan, S.; Keaney, J.F., Jr.; Keyes, M.J.; Lin, J.P.; et al. Genome-wide association with select biomarker traits in the Framingham Heart Study. *BMC Med. Genet.* **2007**, *8* (Suppl. 1), S11. [[CrossRef](#)] [[PubMed](#)]
28. Hibler, E.A.; Klimentidis, Y.C.; Jurutka, P.W.; Kohler, L.N.; Lance, P.; Roe, D.J.; Thompson, P.A.; Jacobs, E.T. CYP24A1 and CYP27B1 Polymorphisms, Concentrations of Vitamin D Metabolites, and Odds of Colorectal Adenoma Recurrence. *Nutr. Cancer* **2015**, *67*, 1131–1141. [[CrossRef](#)]
29. Ahn, J.; Albanes, D.; Berndt, S.I.; Peters, U.; Chatterjee, N.; Freedman, N.D.; Abnet, C.C.; Huang, W.-Y.; Kibel, A.S.; Crawford, E.D.; et al. Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk. *Carcinogenesis* **2009**, *30*, 769–776. [[CrossRef](#)]
30. Wang, T.J.; Zhang, F.; Richards, J.B.; Kestenbaum, B.; van Meurs, J.B.; Berry, D.; Kiel, D.; Streeten, E.A.; Ohlsson, C.; Koller, D.L.; et al. Common genetic determinants of vitamin D insufficiency: A genome-wide association study. *Lancet* **2010**, *376*, 180–188. [[CrossRef](#)]
31. Bu, F.X.; Armas, L.; Lappe, J.; Zhou, Y.; Gao, G.; Wang, H.W.; Recker, R.; Zhao, L.J. Comprehensive association analysis of nine candidate genes with serum 25-hydroxy vitamin D levels among healthy Caucasian subjects. *Hum. Genet.* **2010**, *128*, 549–556. [[CrossRef](#)]
32. Mokry, L.E.; Ross, S.; Ahmad, O.S.; Forgetta, V.; Smith, G.D.; Goltzman, D.; Leong, A.; Greenwood, C.M.; Thanassoulis, G.; Richards, J.B. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. *PLoS Med.* **2015**, *12*, e1001866. [[CrossRef](#)] [[PubMed](#)]
33. Nykjaer, A.; Fyfe, J.C.; Kozyraki, R.; Leheste, J.-R.; Jacobsen, C.; Nielsen, M.S.; Verroust, P.J.; Aminoff, M.; de la Chapelle, A.; Moestrup, S.K.; et al. Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D3. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13895–13900. [[CrossRef](#)] [[PubMed](#)]
34. Kaseda, R.; Hosojima, M.; Sato, H.; Saito, A. Role of megalin and cubilin in the metabolism of vitamin D(3). *Ther. Apher. Dial.* **2011**, *15* (Suppl. 1), 14–17. [[CrossRef](#)] [[PubMed](#)]
35. Rowling, M.J.; Kemmis, C.M.; Taffany, D.A.; Welsh, J. Megalin-Mediated Endocytosis of Vitamin D Binding Protein Correlates with 25-Hydroxycholecalciferol Actions in Human Mammary Cells. *J. Nutr.* **2006**, *136*, 2754–2759. [[CrossRef](#)] [[PubMed](#)]
36. Mählknecht, U.; Schnittger, S.; Will, J.; Cicek, N.; Hoelzer, D. Chromosomal organization and localization of the human histone deacetylase 9 gene (HDAC9). *Biochem. Biophys. Res. Commun.* **2002**, *293*, 182–191. [[CrossRef](#)]
37. Aragane, H.; Sakakura, C.; Nakanishi, M.; Yasuoka, R.; Fujita, Y.; Taniguchi, H.; Hagiwara, A.; Yamaguchi, T.; Abe, T.; Inazawa, J.; et al. Chromosomal aberrations in colorectal cancers and liver metastases analyzed by comparative genomic hybridization. *Int. J. Cancer* **2001**, *94*, 623–629. [[CrossRef](#)] [[PubMed](#)]
38. Zhao, Y.-X.; Wang, Y.-S.; Cai, Q.-Q.; Wang, J.-Q.; Yao, W.-T. Up-regulation of HDAC9 promotes cell proliferation through suppressing p53 transcription in osteosarcoma. *Int. J. Clin. Exp. Med.* **2015**, *8*, 11818–11823. [[PubMed](#)]
39. Al-Tassan, N.A.; Whiffin, N.; Hosking, F.J.; Palles, C.; Farrington, S.M.; Dobbins, S.E.; Harris, R.; Gorman, M.; Tenesa, A.; Meyer, B.F.; et al. A new GWAS and meta-analysis with 1000Genomes imputation identifies novel risk variants for colorectal cancer. *Sci. Rep.* **2015**, *5*, 10442. [[CrossRef](#)] [[PubMed](#)]
40. Ternes, S.B.; Rowling, M.J. Vitamin D Transport Proteins Megalin and Disabled-2 Are Expressed in Prostate and Colon Epithelial Cells and Are Induced and Activated by All-Trans-Retinoic Acid. *Nutr. Cancer* **2013**, *65*, 900–907. [[CrossRef](#)]

41. Higginbotham, K.S.P.; Breyer, J.P.; Bradley, K.M.; Schuyler, P.A.; Plummer, W.D.; Freudenthal, M.E.; Trentham-Dietz, A.; Newcomb, P.A.; Sanders, M.E.; Page, D.L.; et al. A multi-stage association study identifies a breast cancer genetic locus at NCOA7. *Cancer Res.* **2011**, *71*, 3881–3888. [[CrossRef](#)]
42. Yan, X.; Liu, Z.; Chen, Y. Regulation of TGF-beta signaling by Smad7. *Acta Biochim. Biophys. Sin.* **2009**, *41*, 263–272. [[CrossRef](#)] [[PubMed](#)]
43. Tu, H.; Flanders, W.D.; Ahearn, T.U.; Daniel, C.R.; Gonzalez-Feliciano, A.G.; Long, Q.; Rutherford, R.E.; Bostick, R.M. Effects of Calcium and Vitamin D(3) on Transforming Growth Factors in Rectal Mucosa of Sporadic Colorectal Adenoma Patients: A Randomized Controlled Trial. *Mol. Carcinog.* **2015**, *54*, 270–280. [[CrossRef](#)] [[PubMed](#)]
44. Jiang, X.; Castelao, J.E.; Vandenberg, D.; Carracedo, A.; Redondo, C.M.; Conti, D.V.; Paredes Cotore, J.P.; Potter, J.D.; Newcomb, P.A.; Passarelli, M.N.; et al. Genetic variations in SMAD7 are associated with colorectal cancer risk in the colon cancer family registry. *PLoS ONE* **2013**, *8*, e60464. [[CrossRef](#)] [[PubMed](#)]
45. Zhang, B.; Jia, W.H.; Matsuo, K.; Shin, A.; Xiang, Y.B.; Matsuda, K.; Jee, S.H.; Kim, D.H.; Cheah, P.Y.; Ren, Z.; et al. Genome-wide association study identifies a new SMAD7 risk variant associated with colorectal cancer risk in East Asians. *Int. J. Cancer* **2014**, *135*, 948–955. [[CrossRef](#)] [[PubMed](#)]
46. Kirac, I.; Matosevic, P.; Augustin, G.; Simunovic, I.; Hostic, V.; Zupancic, S.; Hayward, C.; Antoljak, N.; Rudan, I.; Campbell, H.; et al. SMAD7 variant rs4939827 is associated with colorectal cancer risk in Croatian population. *PLoS ONE* **2013**, *8*, e74042. [[CrossRef](#)] [[PubMed](#)]
47. Peters, U.; Hutter, C.M.; Hsu, L.; Schumacher, F.R.; Conti, D.V.; Carlson, C.S.; Edlund, C.K.; Haile, R.W.; Gallinger, S.; Zanke, B.W.; et al. Meta-analysis of New Genome-wide Association Studies of Colorectal Cancer Risk. *Hum. Genet.* **2012**, *131*, 217–234. [[CrossRef](#)] [[PubMed](#)]
48. Nanduri, R.; Mahajan, S.; Bhagyaraj, E.; Sethi, K.; Kalra, R.; Chandra, V.; Gupta, P. The Active Form of Vitamin D Transcriptionally Represses Smad7 Signaling and Activates Extracellular Signal-regulated Kinase (ERK) to Inhibit the Differentiation of a Inflammatory T Helper Cell Subset and Suppress Experimental Autoimmune Encephalomyelitis. *J. Biol. Chem.* **2015**, *290*, 12222–12236. [[CrossRef](#)] [[PubMed](#)]
49. Fleming, N.I.; Jorissen, R.N.; Mouradov, D.; Christie, M.; Sakthianandeswaren, A.; Palmieri, M.; Day, F.; Li, S.; Tsui, C.; Lipton, L.; et al. SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer. *Cancer Res.* **2013**, *73*, 725–735. [[CrossRef](#)] [[PubMed](#)]
50. Yanagisawa, J.; Yanagi, Y.; Masuhiro, Y.; Suzawa, M.; Watanabe, M.; Kashiwagi, K.; Toriyabe, T.; Kawabata, M.; Miyazono, K.; Kato, S. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* **1999**, *283*, 1317–1321. [[CrossRef](#)]
51. Maggio-Price, L.; Treuting, P.; Zeng, W.; Tsang, M.; Bielefeldt-Ohmann, H.; Iritani, B.M. Helicobacter infection is required for inflammation and colon cancer in SMAD3-deficient mice. *Cancer Res.* **2006**, *66*, 828–838. [[CrossRef](#)]
52. Lamprecht, S.A.; Lipkin, M. Chemoprevention of colon cancer by calcium, vitamin D and folate: Molecular mechanisms. *Nat. Rev. Cancer* **2003**, *3*, 601–614. [[CrossRef](#)] [[PubMed](#)]
53. Bostick, R.M.; Goodman, M.; Sidelnikov, E. Calcium and vitamin D. In *Genetics of Colorectal Cancer*; Potter, J.D., Lindor, N.M., Eds.; Springer Science + Business Media, LLC: New York, NY, USA, 2009; pp. 277–296.
54. Iyer, S.S.; Cheng, G. Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. *Crit. Rev. Immunol.* **2012**, *32*, 23–63. [[CrossRef](#)] [[PubMed](#)]
55. Hutter, C.M.; Slattery, M.L.; Duggan, D.J.; Muehling, J.; Curtin, K.; Hsu, L.; Beresford, S.A.; Rajkovic, A.; Sarto, G.E.; Marshall, J.R.; et al. Characterization of the association between 8q24 and colon cancer: Gene-environment exploration and meta-analysis. *BMC Cancer* **2010**, *10*, 670. [[CrossRef](#)] [[PubMed](#)]
56. Wang, Y.P.; Zhang, J.; Zhu, H.Y.; Qian, C.L.; Liu, H.; Ji, F.; Shen, Z.Y. Common variation rs6983267 at 8q24.1 and risk of colorectal adenoma and cancer: Evidence based on 31 studies. *Tumour Biol.* **2014**, *35*, 4067–4075. [[CrossRef](#)] [[PubMed](#)]
57. Pomerantz, M.M.; Ahmadiyeh, N.; Jia, L.; Herman, P.; Verzi, M.P.; Doddapaneni, H.; Beckwith, C.A.; Chan, J.A.; Hills, A.; Davis, M.; et al. The 8q24 cancer risk variant rs6983267 demonstrates long-range interaction with MYC in colorectal cancer. *Nat. Genet.* **2009**, *41*, 882–884. [[CrossRef](#)] [[PubMed](#)]
58. Miller, D.M.; Thomas, S.D.; Islam, A.; Muench, D.; Sedoris, K. c-Myc and Cancer Metabolism. *Clin. Cancer Res.* **2012**, *18*, 5546–5553. [[CrossRef](#)] [[PubMed](#)]

59. Ahmadiyeh, N.; Pomerantz, M.M.; Grisanzio, C.; Herman, P.; Jia, L.; Almendro, V.; He, H.H.; Brown, M.; Liu, X.S.; Davis, M.; et al. 8q24 prostate, breast, and colon cancer risk loci show tissue-specific long-range interaction with MYC. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 9742–9746. [[CrossRef](#)] [[PubMed](#)]
60. Wright, J.B.; Brown, S.J.; Cole, M.D. Upregulation of c-MYC in cis through a Large Chromatin Loop Linked to a Cancer Risk-Associated Single-Nucleotide Polymorphism in Colorectal Cancer Cells. *Mol. Cell. Biol.* **2010**, *30*, 1411–1420. [[CrossRef](#)]
61. Salehi-Tabar, R.; Nguyen-Yamamoto, L.; Tavera-Mendoza, L.E.; Quail, T.; Dimitrov, V.; An, B.-S.; Glass, L.; Goltzman, D.; White, J.H. Vitamin D receptor as a master regulator of the c-MYC/MXD1 network. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 18827–18832. [[CrossRef](#)]
62. Dimitrakopoulou, V.I.; Travis, R.C.; Shui, I.M.; Mondul, A.; Albanes, D.; Virtamo, J.; Agudo, A.; Boeing, H.; Bueno-de-Mesquita, H.B.; Gunter, M.J.; et al. Interactions Between Genome-Wide Significant Genetic Variants and Circulating Concentrations of 25-Hydroxyvitamin D in Relation to Prostate Cancer Risk in the National Cancer Institute BPC3. *Am. J. Epidemiol.* **2017**, *185*, 452–464. [[CrossRef](#)]
63. Vigneri, P.G.; Tirrò, E.; Pennisi, M.S.; Massimino, M.; Stella, S.; Romano, C.; Manzella, L. The Insulin/IGF System in Colorectal Cancer Development and Resistance to Therapy. *Front. Oncol.* **2015**, *5*, 230. [[CrossRef](#)] [[PubMed](#)]
64. Wong, H.L.; Delellis, K.; Probst-Hensch, N.; Koh, W.P.; Van Den Berg, D.; Lee, H.P.; Yu, M.C.; Ingles, S.A. A new single nucleotide polymorphism in the insulin-like growth factor I regulatory region associates with colorectal cancer risk in singapore chinese. *Cancer Epidemiol. Biomark. Prev.* **2005**, *14*, 144–151.
65. Simons, C.C.J.M.; Schouten, L.J.; Godschalk, R.W.L.; van Engeland, M.; van den Brandt, P.A.; van Schooten, F.J.; Weijenberg, M.P. Genetic Variants in the Insulin-like Growth Factor Pathway and Colorectal Cancer Risk in the Netherlands Cohort Study. *Sci. Rep.* **2015**, *5*, 14126. [[CrossRef](#)] [[PubMed](#)]
66. Chi, F.; Wu, R.; Zeng, Y.C.; Xing, R.; Liu, Y. Circulation insulin-like growth factor peptides and colorectal cancer risk: An updated systematic review and meta-analysis. *Mol. Biol. Rep.* **2013**, *40*, 3583–3590. [[CrossRef](#)] [[PubMed](#)]



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