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

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Abstract

Background—Experimental evidence has demonstrated an anti-neoplastic 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 (CRC). Genome-wide association studies have identified loci associated with levels of circulating 25(OH)D. The identified SNPs from four gene regions, collectively explain approximately 5% of the variance in circulating 25(OH)D.

Methods—We investigated whether six polymorphisms in *GC, CYP2R1, CYP24A1* and *DHCR7/NADSYN1*, genes previously shown to be associated with circulating 25(OH)D levels, were associated with CRC 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 performed 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 CRC marginally, conditionally, or as a GRS, or for colon or rectal cancer separately or combined.

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

Impact—There was no association observed between genetic markers of circulating 25(OH)D and CRC. These genetic markers account for a small proportion of the variance in 25(OH)D.

Introduction

Colorectal cancer (CRC) is the second leading cause of cancer death in men and women in the United States. It is estimated that in 2012, a total of 143,460 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 30-50% of the familial risk(4); much of the remaining familial aggregation is unexplained. Genome-wide association studies (GWAS) of sporadic CRC have identified at least 20 independent loci statistically significantly associated with risk (5-12). However, these variants cumulatively explain only a very small fraction of CRC risk (13).

Beyond inherited risk, there is a large body of evidence supporting the role of non-genetic factors, including vitamin D status, in the etiology of CRC. The first human evidence to suggest an association between vitamin D and CRC 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 ultraviolet B (UVB) radiation exposure in the U.S. (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 CRC 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 demonstrated reductions in colorectal cancer incidence. However, these trials have generally tested low doses of vitamin D and each included less than seven years of follow-up, which is likely insufficient to shown 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 SNPs significantly associated with lower circulating 25(OH)D levels in four gene regions that appear 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) (33, 34). Both the *GC* SNP rs2282679 and the *DHCR7/NADSYN1* SNPs are located in intronic regions, with the *GC* SNP demonstrating the largest magnitude of association with 25(OH)D <75 nmol/L (OR 1.63 (1.53–1.73)(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 CRC 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 NHS, HPFS and PHS with measured pre-diagnostic

plasma 25(OH)D levels, we also examined the joint effects of plasma 25(OH)D and 25(OH)D-associated SNPs on CRC 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 Supplemental Note and Supplemental Table 1. In brief, each study defined CRC 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); Health Professionals Follow-up Study (HPFS); Multiethnic Cohort (MEC); Nurses' Health Study (NHS); Ontario Familial Colorectal Cancer Registry (OFCCR); Physician's Health Study (PHS); Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial (PLCO); VITamins And Lifestyle (VITAL); and the Women's Health Initiative (WHI). Phase-one 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 done using Illumina HumanHap 550K, 610K, or combined Illumina 300K and 240K, and has been described previously (12). A total of 650 CRC cases and 522 controls from OFCCR are included in GECCO from previous genotyping using Affymetrix platforms (35). A total of 5,540 CRC 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 CRC 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 USA, 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 (CCFR), the Ontario Institute for Cancer Research (OFCCR), the University of Washington (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 (QA/QC) and statistical analysis. Details on the QA/QC can be found in Supplemental Table 2. In brief, samples were excluded based on 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 $\ge 10^{-4}$), and minor allele frequency (MAF $\ge 1\%$) 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 non-steroidal anti-inflammatory drugs (NSAIDs) was 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, and hormone replacement therapy in post-menopausal 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 analytical approach. For each study, we estimated the association between each SNP and risk for CRC by calculating betas, odds ratios (ORs), standard errors, 95% confidence intervals (CIs), and p-values using log-additive genetic models relating the genotype dose (0, 1 or 2 copies of the allele) to risk of CRC. 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), comprised of four SNPs from four distinct gene regions to ensure no single gene was over represented 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 0-8 alleles to derive estimates of allelic OR.

Minimally adjusted models included covariates for age, sex (when appropriate), center (when appropriate), smoking status, 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 CRC, BMI, NSAID use, 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 anatomical site (colon and rectum).

We conducted inverse-variance weighted, fixed-effects meta-analysis to combine beta estimates and standard errors 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 comprised 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 pre-diagnostic levels of 25(OH)D and also had genotype data (21, 44). We calculated ORs and 95% CI for CRC comparing extreme quartiles of 25(OH)D defined according to cohort-specific cutpoints determined by the distribution in controls (44). We compared the GRS-associated risk for CRC across categories of high versus low vitamin D levels and quartiles of vitamin D, as well as tested for multiplicative interactions between GRS and a 1ng/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, Cary, NC) to conduct the statistical analyses. We estimated our power to detect an association between a GRS and CRC using the method of Tosteson et al. (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 CRC cases and 12,768 controls. Overall 53% were female, and the mean age at CRC diagnosis was 64.0 years (+/- 9.6 SD), 55.0% past/current smokers and with risk allele frequencies ranging from 16 – 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 demonstrate a statistically significant association with CRC risk (Table 2). In analyses stratified by anatomic site each of the four SNP associations remained non-significant (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 CRC. However, an analysis of a GRS comprised of the risk alleles from the four SNPs associated with plasma 25(OH)D and CRC risk did not demonstrate any significant association (Table 4). The figure 1 forest plot depicts the ORs and 95% CIs of the GRS and CRC association of the individual GECCO and CCFR studies showing a balanced distribution of study-specific odds ratios around the null value of 1.0.

Among the subset of NHS, HPFS and PHS subjects with measured prediagnostic plasma 25(OH)D levels (N=672 cases and N=909 controls), we observed that a 1 allele change in our GRS was associated with a mean 1.5ng/mL decrease in 25(OH)D (p-value <0.0001). Compared with the lowest quartile of 25(OH)D, the highest quartile of 25(OH)D was associated with an OR of 0.66 (95% CI 0.47, 0.92) for CRC. We did not observe a differential association of GRS with risk of CRC according to strata of quartile levels of plasma 25(OH)D or according to high or low levels of 25(OH)D (p-heterogeneity>0.05).

Discussion

Consistent evidence from epidemiologic studies supports an inverse association between circulating 25(OH)D, the best integrated biomarker of vitamin D status, and risk of CRC. However, contrary to expectation, we did not observe a statistically significant association between SNPs associated with circulating 25(OH)D and CRC, marginally or in an additive GRS.

A number of epidemiologic studies have reported inverse associations between 25(OH)D and CRC. A meta-analysis of five nested case–control studies reported a pooled odds ratio of 0.49 (95% CI; 0.35– 0.68) for CRC 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 pooled RRs for CRC of 0.67 (95% CI, 0.54 to 0.80) comparing extreme quintiles

of 25(OH)D (22). Overall, the estimated OR of CRC for a 10ng/mL increase in circulating 25(OH)D was 0.74 (95% CI, 0.63 to 0.89)with the relationship appearing approximately linear (22). Several mechanisms could explain an anti-cancer 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 D_3 every four months over five years was not associated with CRC incidence (RR=1.02, 95% CI 0.60-1.74)(25). Similarly, among 36,000 women in the WHI calcium-vitamin D trial, a combination of calcium (1000 mg/day) plus low-dose vitamin D_3 (400 IU/d) for a mean of 7 years did not reduce CRC 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 CRC 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 D_3 (1100 IU/d) observed a significantly lower cancer incidence among those supplemented with calcium and vitamin D over just four years of treatment (61). However, follow-up of total cancers was a secondary outcome and there were only a small number of CRC cases, limiting the interpretation of these results.

Over 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), 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 CRC, 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 CRC. A prior DALS multicenter population-based case-control study of 1,600 CRC 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 CRC, we did not consider total genetic variation in CYP24A1 with CRC risk. It is possible that alternative CYP24A1 SNPs may be associated with CRC 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 the risk of CRC, 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 *Fok1* and *BsmI*, have yielded inconsistent results (68-71). Notably, among over 10,000 men with prostate cancer, a greater number of low 25(OH)D SNPs were associated with a decreased risk of aggressive prostate cancer (OR 0.66; 95% CI, 0.44-0.98 for 6–8 vs. 0–1 alleles,; Ptrend = 0.003)(72).

The lack of association that we observed between genetic markers associated with circulating 25(OH)D and CRC is consistent with prior clinical trials of vitamin D and CRC and would, at least initially, seem to argue against a causal association between vitamin D and CRC(73). However, prior work has demonstrated that these 4 SNPs, though correlated with circulating 25(OH)D, explain only a small fraction (5%) of the variance in circulating 25(OH)(74). Recently, a Scottish case-control study observed a significant association between direct plasma measurements of 25(OH)D and CRC risk, yet failed to replicate the association using an instrumental-variable method of mendelian randomization (MR) with the same four genome-wide significant risk loci examined in our analysis (75). 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 CRC 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 CRC(22), our power to detect a 1-allele change in our GRS is 96% (significance level of 0.05). However, if the true magnitude of association with a 10ng/mL increase in 25(OH)D is in fact an OR of 0.85 for CRC, we would have only had 56% power to detect a 1-allele change in our GRS.

We are not certain of the precise pathway or biological mediators by which 25(OH)D influences CRC risk. Our GRS assumes that each included SNP would be associated with increased CRC 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 CRC. 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 1 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 CRC, this could confound our results(73). Third, despite our large sample size, we had limited power to detect associations between individual SNPs and risk of CRC.

In conclusion, our findings do not support an association between SNPs associated with circulating 25(OH)D and risk of CRC. 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 CRC 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 CRC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Study	OR	95%CI	P
ASTERISK	1.03	(0.95-1.12)	5.08e-01
COLO23	1.11	(0.89-1.37)	3.58e-01
CCFR	1.02	(0.95-1.09)	5.72e-01
DACHS	0.99	(0.94-1.05)	8.16e-01
DALS	1.03	(0.96-1.10)	4.14e-01
HPFS	1.02	(0.92-1.15)	6.69e-01
MEC	0.92	(0.82-1.04)	2.04e-01
NHS	0.93	(0.85-1.01)	8.83e-02
OFCCR	1.04	(0.94-1.14)	4.65e-01
PHS	0.89	(0.79-1.00)	4.85e-02
PLCO	0.97	(0.91-1.03)	3.68e-01
VITAL	1.02	(0.89-1.17)	7.66e-01
WHI	1.05	(1.00-1.11)	5.63e-02
Summary	**1.00**	**(0.98-1.03)**	**7.25e-01**

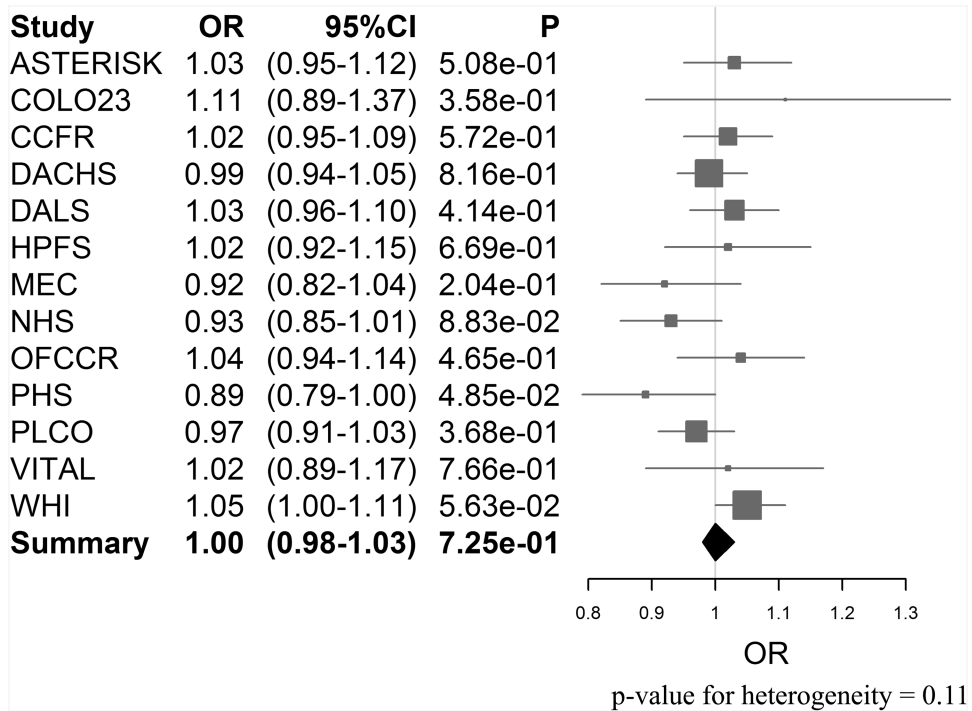0.8   0.9   1   1.1   1.2   1.3

OR

p-value for heterogeneity = 0.11

Figure 1. Forest plot of the genetic risk score and CRC for individual studies and meta-analysis of all studies (allelic OR, 95% CI)

Table 1

Characteristics of colorectal cancer patients in included study populations

Study*	Abbreviation	Number of Cases	Number of Controls	% Female	Mean age diagnosis	% CRC family history
Ontario Familial Colorectal Cancer Registry	OFCCR	650	522	52	62.0	20.1
Colon Cancer Family Registry	CCFR	1,171	983	50	54.2	16.9
Diet, Activity, and Lifestyle Study	DALS	1,116	1174	45	63.9	13.3
A case-control study from the University of Hawai'i	COLO 2&3	87	125	45	65.2	11.8
Multiethnic Cohort	MEC	328	346	46	63.0	12.2
Darmkrebs: Chancen der Verhütung durch Screening	DACHS	1,710	1,708	41	68.6	12.5
Prostate, Lung, Colorectal, and Ovarian	PLCO	1,019	2,391	31	64.0	11.4
Women's Health Initiative	WHI	1,476	2,538	100	65.8	17.6
Association Study Evaluation RISK for sporadic colorectal cancer	ASTERISK	892	947	41	65.2	NA
VITamins And Lifestyle Study	VITAL	285	288	48	66.5	13.6
Health Professionals Follow-up Study	HPFS	403	402	0	65.2	17.3
Nurses' Health Study	NHS	549	955	100	59.8	15.2
Physicians' Health Study	PHS	375	389	0	58.9	NA
TOTAL		**10,061**	**12,768**	**53**	**64.0**	**13.0**

*
DALS Set 2, Colo2&3, DACHS, MEC, PLCO Set 2, WHI Set 2, ASTERISK, and VITAL were genotyped using Illumina 550K and 550K duo platforms; PLCO Set 1 was genotyped using Illumina 550K and 610K platforms; DALS set 1 was genotyped using Illumina 610K and 550K platforms; OFCCR was genotyped using Affymetrix GeneChip Human Mapping 100K and 500K Array Set and a 10K non-synonymous SNP chip; CCFR was genotyped using Illumina 1M, 1MDuo and 1M-Omni platforms; and HPFS, NHS, and PHS were genotyped on the OmniExpress platform.

Table 2

Association between five 25(OH)D associated SNPs and CRC among 10,061cases and 12,768 controls.

SNP	Position (Nearest Gene)	Major/Minor Allele	Minor Allele Frequency Mean	Age, Sex and PCA-Adjusted OR (95% CI)**	p-value	Multivariable-Adjusted OR (95% CI)**	p-value	N studies genotyped[†]	Mean R^2	p-value for Heterogeneity[‡]
rs2282679	GC	T/G*	0.29	1.03 (0.99, 1.08)	0.14	1.02 (0.77, 1.09)	0.14	11	1.00	0.55
rs10741657	CYP2R1	G*/A	0.39	0.98 (0.94, 1.02)	0.22	0.97 (0.93, 1.02)	0.23	0	0.99	0.73
rs12785878	DHCR7/NADSYN1	T/G*	0.26	1.03 (0.99, 1.08)	0.15	1.04 (0.99, 1.10)	0.10	0	0.99	0.19
rs11234027	DHCR7/NADSYN1	G/A*	0.16	1.01 (0.96, 1.07)	0.63	1.03 (0.97, 1.10)	0.29	9	0.99	0.18
rs6013897	CYP24A1	T/A*	0.21	0.97 (0.92, 1.02)	0.30	0.98 (0.92, 1.04)	0.46	0	0.88	0.74

Abbreviations: CRC=colorectal cancer, SNP=single-nucleotide polymorphism, OR=odds ratio, CI=confidence interval, N studies genotyped=number of studies with directly genotyped in all the studies, Mean r^2=average imputation R2 value across all the studies which the SNP was imputed

* Allele associated with decreased circulating 25(OH)D in prior GWAS (Ahn et al. HMG 2010; Wang et al. Lancet 2010.).

** Odds ratio 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 CRC, BMI, NSAID use, alcohol use, dietary calcium, folate and red meat intake, sedentary status, and hormone replacement therapy.

[†] Number of studies directly genotyping and imputing SNPs varies for each SNP.

[‡] p-value for heterogeneity across study in the multivariable-adjusted models.

Table 3

Association between five 25(OH)D associated SNPs and CRC stratified by site

SNP	Position (Nearest Gene)	Colon Cancer Cases Only			Rectal Cancer Cases Only			
		Age, Sex and PCA-Adjusted OR (95% CI)[**]	p-value	N Studies[*]	p-value for heterogeneity	Age, Sex and PCA-Adjusted OR (95% CI)[**]	p-value	N studies[*]	p-value for Heterogeneity
rs2282679	GC	1.03 (0.98, 1.08)	0.23	12	0.29	1.05 (0.98, 1.13)	0.19	11	0.90
rs10741657	CYP2R1	0.98 (0.94, 1.02)	0.35	12	0.57	0.97 (0.90, 1.04)	0.34	11	0.97
rs12785878	DHCR7/NADSYN1	1.05 (1.00, 1.10)	0.06	12	0.21	0.97 (0.90, 1.05)	0.49	11	0.009
rs11234027	DHCR7/NADSYN1	1.02 (0.97, 1.08)	0.30	12	0.39	0.94 (0.85, 1.03)	0.19	11	0.19
rs6013897	CYP24A1	0.96 (0.91, 1.02)	0.21	12	0.75	1.01 (0.92, 1.11)	0.79	11	0.72

Abbreviations: CRC=colorectal cancer, SNP=single-nucleotide polymorphism, OR=odds ratio, CI=confidence interval

[*]
A total of 7220 colon cancer and 2,308 rectal cancer cases were included in each site-specific analysis with 12,768 controls.

[**]
Odds ratio calculated in reference to the allele associated with decreased 25(OH)D.

Table 4

Association between 4 mutually adjusted 25(OH)D associated SNPs, a composite SNP additive genetic risk score and CRC

SNP	Position (Nearest Gene)	N Cases/N Controls	Major/Minor Allele	Age, Sex and PCA-Adjusted OR (95% CI)[**]	p-value	N studies	p-value for Heterogeneity
rs2282679	*GC*	10,128/12,768	T/G[*]	1.03 (0.99, 1.08)	0.13	13	0.20
rs10741657	*CYP2R1*	10,128/12,768	G[*]/A	0.98 (0.94, 1.02)	0.22	13	0.77
rs12785878	*DHCR7/NADSYN1*	10,128/12,768	T/G[*]	1.03 (0.99, 1.08)	0.16	13	0.10
rs6013897	*CYP24A1*	10,128/12,768	T/A[*]	0.97 (0.93, 1.03)	0.33	13	0.89
Score	-	10,128/12,768	-	1.00 (0.98, 1.03)	0.72	13	0.11

Abbreviations: CRC=colorectal cancer, SNP=single-nucleotide polymorphism, OR=odds ratio, CI=confidence interval

[*]
Allele associated with decreased circulating 25(OH)D in prior GWAS (Ahn et al. HMG 2010; Wang et al. Lancet 2010.).

[**]
Odds ratio calculated in reference to the allele associated with decreased 25(OH)D.