Research Article

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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

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Abstract

Epidemiologic evidence supports a role for vitamin D in colorectal cancer (CRC) risk. Variants in vitamin D–related genes might modify the association between vitamin D levels and CRC risk. In this analysis, we did a comprehensive evaluation of common variants in the vitamin D receptor (*VDR*) and the vitamin D–binding protein (*GC*; group-specific component) genes using a population-based case–unaffected sibling control design that included 1,750 sibships recruited into the Colon Cancer Family Registry. We also evaluated whether any associations differed by calcium supplement use, family history of CRC, or tumor characteristics. Heterogeneity by calcium and vitamin D intake was evaluated for a subset of 585 cases and 837 sibling controls who completed a detailed food frequency questionnaire. Age- and sex-adjusted associations were estimated using conditional logistic regression. Overall, we did not find evidence for an association between any single-nucleotide polymorphism (SNP) in *VDR* or *GC* and risk for CRC (range of unadjusted *P* values 0.01-0.98 for *VDR* and 0.07-0.95 for *GC*). None of these associations was significant after adjustment for multiple comparisons. We also found no evidence that calcium or vitamin D intake (food and supplement) from the food frequency questionnaire modified the association estimates between *VDR* and *GC* SNPs and CRC. We did observe associations between SNPs in *GC* and microsatellite unstable CRC, although these results should be confirmed in additional studies. Overall, our results do not provide evidence for a role of common genetic variants in *VDR* or *GC* in susceptibility to CRC. *Cancer Epidemiol Biomarkers Prev; 19(2); 525–36. ©2010 AACR.*

Introduction

Sunlight and vitamin D were first hypothesized to reduce risk for colorectal cancer (CRC) in 1980 (1). The epidemiologic evidence has supported this relationship because both cohort (2-5) and case-control studies (6-8) have reported an inverse relationship between vitamin D intake and CRC, although the association did not reach statistical significance in all studies. An inverse association between serum levels of 25-hydroxyvitamin D, a biomarker of vitamin D status, and CRC risk has been also shown in epidemiologic studies (5, 9-15). In addition, high plasma 25-hydroxyvitamin D was associated with decreased proliferation in colonic tissue from subjects at risk for colorectal neoplasms because they had a personal history of adenomas (16).

The vitamin D receptor (VDR) is a member of the steroid superfamily of nuclear receptors and plays a key role in regulating the transcriptional activity of the vitamin D metabolite, 1,25-dihydroxyvitamin D_3 (17). Polymorphisms in *VDR* have been studied extensively in epidemiologic studies on CRC (18-26). These studies have mostly focused on a few selected variants, including the *Fok*I (rs2228570, previously rs10735810), *Taq*I (rs731236), *Bsm*I (rs1544410), and *Apa*I (rs7975232) restriction sites; a polymorphism in the *CDX2*-binding element in *VDR* (rs11568820); and a poly-A microsatellite. Overall, the results have been mixed for studies on individual variants in CRC (18-22, 24-28). Several studies have suggested that haplotypes of these selected variants

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are associated with risk for CRC (22, 23, 29) and that the association with these variants may be modified by dietary factors (18, 20, 21, 30, 31), physical activity (32), and BMI (32).

The gene for the vitamin D–binding protein (*GC*) is also a logical candidate in the vitamin D pathway because its major function is to transport vitamin D metabolites in the blood. Variants in this gene have been shown to alter plasma concentrations of 25-hydroxyvitamin D (33, 34). Selected variants in *GC* have been evaluated in studies on breast (35, 36) and prostate cancers (37); however, to date, no studies have been reported with colorectal neoplasia.

Despite the large number of studies that have evaluated the association between selected genetic variants in *VDR* and CRC, a comprehensive analysis of *VDR* and *GC* has not yet been published. In this analysis, we used data from a multisite family-based case-control study conducted by the Colon Cancer Family Registry (Colon CFR) to evaluate associations between common single-nucleotide polymorphisms (SNP) in *VDR* and *GC* and CRC. In addition, we evaluated heterogeneity by calcium supplement use, dietary vitamin D and calcium intake, family history of CRC, tumor subsite in the colon or rectum, and presence/absence of tumor microsatellite instability (MSI).

Materials and Methods

Study Population

Participants were recruited for the Colon CFR from six registry centers: the University of Hawaii (Honolulu, HI), Fred Hutchinson Cancer Research Center (Seattle, WA), Mayo Clinic (Rochester, MN), University of Southern California Consortium (Los Angeles, CA), Cancer Care Ontario (Toronto, Ontario, Canada), and the University of Melbourne (Victoria, Australia). Families were ascertained through population-based cancer registries (population based) and high-risk clinics (clinic based). Some centers recruited all incident cases of CRC, whereas others oversampled cases with a family history of CRC or young age at diagnosis of CRC. Standardized procedures were used to collect epidemiologic data and blood samples. Tumor blocks and pathology reports from cases were obtained from the Colon CFR Jeremy Jass Memorial Pathology Bank. Detailed information about the Colon CFR can be found online[12] and is summarized in Newcomb et al. (38).

In this analysis, we used a case–unaffected sibling control design including only population-based families. Cases were defined as probands who were diagnosed with invasive CRC from 1997 to 2005 and affected relatives of probands with a diagnosis of invasive CRC. All cases were interviewed within 5 y of diagnosis (75% within 2 y). Controls were defined as siblings without a diagnosis of CRC. A total of 4,704 population-based cases (n = 1,815) and unaffected sibling controls (n = 2,889) were genotyped for this analysis. Most discordant sibships had only one case (96.3%), and the remaining sibships included one or more affected relatives. In addition, we also genotyped a random set of unrelated population-based controls (n = 447) from one of the Colon CFR sites (Fred Hutchinson Cancer Research Center).

We obtained informed consent from all participants. The study was approved by the Institutional Review Board(s) at each Colon CFR site.

Data Collection

A core risk-factor questionnaire, administered to all participants at the time of recruitment, was used to collect information on personal and family history of polyps, colorectal cancer, and other cancers, as well as other risk factors, including demographic information, medication use, reproductive history (females only), physical activity, alcohol consumption, tobacco use, and a brief section on diet. In addition, a well-validated food frequency questionnaire, developed at the Cancer Research Center of Hawaii (39), was administered to all participants at baseline for three of the Colon CFR sites (University of Southern California consortium, Ontario, and Hawaii), representing 32% of the overall study population. The food frequency questionnaire included questions on dietary and supplemental intake of calcium and vitamin D. Calcium and vitamin D intake from food and supplements were evaluated as nutrient densities (per 1,000 kcal/d). Individuals were asked to report exposures at a reference time of 2 y before diagnosis for cases and 2 y before recruitment for unaffected siblings.

Genotyping

TagSNPs were selected using Haploview Tagger (40) with the following criteria: minor allele frequency > 5%; pairwise r^2 > 0.95; and distance from closest SNP > 60 bp. Linkage disequilibrium blocks were determined using data from HapMap data release 16c.1, June 2005, on NCBI B34 assembly, dbSNP b124. For each gene, we extended examination of the 5′- and 3′-untranslated region to include the 5′- and 3′-most SNP within the linkage disequilibrium block (~10 kb upstream and 5 kb downstream). In regions with no or low linkage disequilibrium, SNPs (minor allele frequency > 5%) were selected from either HapMap or dbSNP at a density of ~1 per kb. Finally, all nonsynonymous SNPs and SNPs previously reported in the literature were included, regardless of minor allele frequency.

DNA was extracted from blood samples for genotyping (38). Genotyping was done using the Illumina GoldenGate genotyping assay (41). We implemented a series of quality control checks based on Illumina metrics, and SNPs were excluded from analysis based on the following standards: GenTrain Score < 0.4; 10% GC Score < 0.25; AB T Dev > 0.1239; call rate < 0.95; and >2 P-P-C errors. Interplate and intraplate replicates were included at a rate of ~5% on all plates, and SNPs

[12] http://epi.grants.cancer.gov/CFR/

were excluded from the analysis if there were greater than two errors on replicate genotypes. In addition, genotype data from 30 CEPH trios (Coriell Cell Repository) were used to confirm reliability and reproducibility of the genotyping. SNPs were excluded from the analysis if more than three discordant genotypes were discovered in comparison with genotypes from the International HapMap Project (42).

We did additional genotyping using Sequenom iPLEX Gold for tagSNPs that were not successfully genotyped on the Illumina platform and for additional SNPs selected to ensure adequate coverage based on updated HapMap data (v.21). These additional SNPs were selected using Haploview Snagger ($r^2 > 0.95$; minor allele frequency > 0.05; ref. 43). PCR and extension primers for these SNPs were designed using the MassARRAY Assay Design 3.0 software (Sequenom, Inc.) and are available upon request. PCR amplification and single base extension reactions were done according to the manufacturer's instructions. Extension product sizes were determined by mass spectrometry using Sequenom's Compact MALDI-TOF mass spectrometer. The resulting mass spectra were converted to genotype data using SpectroTYPER-RT software.

As a QC measure, the frequency of discordant genotypes was estimated: 2 of 281 (0.7%) blinded replicates were discordant; these samples were excluded from all analyses. In addition, three samples were excluded because their call rate was <0.85 for SNPs genotyped on the Illumina platform.

In this analysis, we report results for two genes in the vitamin D pathway: *VDR* and *GC*. We genotyped 50 tagSNPs in *VDR* and 26 tagSNPs in *GC*. Seven SNPs in *VDR* were excluded because of discordant genotypes compared with HapMap data (rs3847987) or poor quality by Illumina QC standards (rs1540339, rs4237855, rs739837, rs7975232, rs7136534, rs8179174). We excluded one SNP in *GC* from the analysis because of discordant genotypes compared with HapMap data for the 30 CEPH trios (rs6837549).

MSI Status

MSI was evaluated for all cases with available tumor tissue using a panel of 10 markers (BAT25, BAT26, BAT40, MYCL, D5S346, D17S250, ACTC, D18S55, D10S197, and BAT34C4), using standard techniques (44). Results were required for at least four markers to determine MSI status. Tumors were deemed MSI-H if instability was observed at ≥30% of markers, MSI-L if >0 and <30% of markers were unstable, and MSS if all markers were stable.

Tumor Location

Tumors were classified by location in the colon using International Classification of Diseases for Oncology, third edition (ICDO-3), codes (45). Tumors located in the cecum, ascending colon, hepatic flexure, transverse colon, and splenic flexure (ICDO-3 codes C180, C182, C183, C184, and C185) were classified as proximal colon. Tumors located in the descending colon and sigmoid colon (ICDO-3 codes C186 and C187) were classified as distal colon. Rectal tumors included those of the rectosigmoid junction and rectum (ICDO-3 codes C199 and C209).

Statistical Analysis

Statistical analyses were conducted in R (version 2.7.1; ref. 46). Minor allele frequency was estimated using genotype data collected on unrelated population based controls. We determined pairwise linkage disequilibrium between SNPs within a gene using r^2 between markers. We also evaluated Hardy-Weinberg equilibrium for each SNP.

We estimated associations between variants and risk for CRC using multivariable conditional logistic regression with sibship as the matching factor. All analyses were adjusted for age (continuous) and sex. We evaluated calcium supplement use (yes/no), multivitamin use (yes/no), and physical activity (average weekly metabolic equivalent (MET) hours of physical activity throughout adulthood) as potential confounders. Potential confounders that changed the odds ratio by >10% were included in the final model. We tested the associations between tagSNPs and CRC using a log additive model, in which the estimates of effect represent the risk due to one additional variant. Because we do not know which, if any, of these tagSNPs are causal variants, we used a robust variance estimator to prevent biased estimates from testing association in the presence of linkage (47). *P* values were adjusted for multiple testing, taking into account correlated tagSNPs using the p_{ACT} test of Conneely and Boehnke (48) with a modification to allow for inclusion of covariates.

We estimated stratum-specific odds ratios to evaluate heterogeneity by calcium supplement use, total vitamin D, and calcium intake from the food frequency questionnaire, and family history of CRC in a first-degree relative, as reported by the proband. We evaluated differences in the associations by MSI status and by tumor location by stratifying the matched sets on the tumor characteristics of the case. We assigned the unaffected sibling to the same MSI or tumor-site category as the case and included interaction terms in the conditional logistic regression models to estimate these stratum-specific odds ratios. A likelihood ratio test was used to assess heterogeneity by comparing models with the interaction terms to a model that included only the main effects of genotype. We used a Bonferroni correction to determine statistical significance of the stratum-specific odds ratios.

Results

Selected characteristics of the study population are illustrated in Table 1. Nineteen individuals (9 cases and 10 sibling controls) were removed from the analysis because of missing data on age or sex. The cases and unaffected siblings had similar age and sex distributions, and most

Table 1. Selected characteristics of the study population

Person characteristic	Cases (*n* = 1,806)	Sibling controls (*n* = 2,879)
Mean age ± SD	53.5 ± 10.9	54.0 ± 11.8
Sex, *n* (%)		
Male	927 (51.3)	1,278 (44.4)
Female	879 (48.7)	1,601 (55.6)
Race, *n* (%)		
Non-Hispanic White	1,580 (87.5)	2,512 (87.3)
Black	32 (1.8)	42 (1.5)
Asian	69 (3.8)	113 (3.9)
Other*	104 (5.8)	189 (6.6)
Unknown/missing	21 (1.2)	23 (0.8)
BMI, kg/m^{2}†		
15-18 (Underweight)	22 (1.2)	25 (0.9)
18-25 (Normal)	629 (34.8)	1,155 (40.1)
25-30 (Overweight)	670 (37.1)	1,036 (36.0)
30+ (Obese)	422 (23.4)	594 (20.6)
Unknown/missing	63 (3.5)	69 (2.4)
Physical activity, MET hours‡		
0-6 (Inactive)	438 (24.3)	669 (23.2)
6.01-20 (Less active)	491 (27.2)	777 (27.0)
20.1-44 (Active)	415 (23)	642 (22.3)
>44 (Very active)	378 (20.9)	631 (21.9)
Unknown/missing	84 (4.7)	160 (5.6)
Multivitamins§		
No	820 (45.4)	1,497 (52.0)
Yes	971 (53.8)	1,346 (46.8)
Unknown/missing	15 (0.8)	36 (1.3)
Calcium supplements§		
No	1,335 (73.9)	2,063 (71.7)
Yes	459 (25.4)	785 (27.3)
Unknown/missing	12 (0.7)	31 (1.1)
Dietary calcium, mg/d, mean ± SD‖	442.0 ± 141.2	449.1 ± 154.7
Total calcium, mg/d, mean ± SD‖	546.9 ± 277.3	596.1 ± 347.3
Dietary vitamin D, IU/d, mean ± SD‖	89.3 ± 45.9	89.4 ± 47.4
Total vitamin D, IU/d; mean ± SD‖	164.0 ± 120.6	182.3 ± 224.8
Tumor characteristics		
Site, *n* (%)		
Right colon	598 (33.1)	
Left colon	525 (29.1)	—
Rectum	593 (32.8)	
Unknown/missing	90 (5.0)	
MSI, *n* (%)		
MSS	855 (47.3)	—
MSI-L	151 (8.4)	
MSI-H	179 (9.9)	
Unknown/missing	621 (34.4)	

Abbreviations: BMI, body mass index; MSS, microsatellite stable; MSI-L, MSI-low; MSI-H, MSI-high.

*Includes individuals who self-identified themselves as Hispanic, Native Hawaiian/Pacific Islander, and mixed race.

†Self-reported weight and height 2 years before questionnaire completion date used to calculate body mass index.

‡Average weekly total lifetime MET hours.

§Ever use of supplements during lifetime regularly (twice a week for >1 month).

‖Calorie adjusted; calculated from food frequency questionnaire (*n* cases = 585; *n* sibling controls = 837).

individuals reported non-Hispanic White ethnicity (Table 1). MSI results were available for 66% of these population-based cases. Cases with missing MSI status were younger and less likely to have a family history of cancer (data not shown). Family history of CRC, defined as having at least one first-degree relative affected with CRC, was reported by 30% of the cases.

There was no association between calcium supplement use and CRC (odds ratio, 1.07; 95% CI, 0.92-1.25; $P = 0.39$ after adjustment for age and sex). In the subset of individuals with food frequency questionnaire data, total calcium and vitamin D intake were similar in cases and controls [vitamin D odds ratio, 0.86 (95% CI, 0.63-1.18 for highest versus lowest quartile); calcium odds ratio, 0.79 (95% CI 0.56-1.11 for highest versus lowest quartile)].

After adjustment for multiple testing, we observed no statistically significant associations between SNPs in *VDR* or *GC* and CRC using log-additive models (Table 2). Models were adjusted for age and sex. Addition of other potential confounders [calcium supplement use (yes/no), multivitamin use (yes/no), and physical activity (average weekly MET hours of physical activity throughout adulthood)] did not appreciably modify the risk estimates (data not shown), and the more parsimonious models are presented. In the three *VDR* SNPs in our data set that have previously been evaluated in epidemiologic studies on CRC, we observed no evidence for an association [*Taq*I odds ratio, 1.04 (95% CI, 0.92-1.17); *Bsm*I odds ratio, 1.04 (95% CI, 0.92-1.18); *CDX*2 odds ratio, 1.07 (95% CI, 0.92-1.25)]. We repeated the analysis of SNP main effects in the subset of cases with MSI data, and the results did not differ substantially from those shown above (data not shown). We observed no indication that any of the associations with SNPs in *VDR* or *GC* were modified by calcium supplement use (Supplementary Table S1).

We evaluated associations between *VDR* and *GC* variants and risk for CRC after stratification by total vitamin D intake in the subset of sibships from Colon CFR centers that administered the food frequency questionnaire (N = 585 cases and 837 unaffected siblings from 563 sibships). We found an inverse relationship between CRC and *VDR* rs2239186 in the stratum with vitamin D intake above the median (odds ratio, 0.72; 95% CI, 0.53-0.97) and saw odds ratios > 1 for rs11574143, rs11168267, rs2107301, and rs2239180 in the stratum with vitamin D intake below the median (Table 3). None of these associations was statistically significant after Bonferroni correction for multiple testing. We observed no evidence for modification by total calcium intake measured by the food frequency questionnaire (supplemental or dietary intake; data not shown).

There was no evidence for heterogeneity by tumor location or family history of CRC for either *VDR* or *GC* (data not shown). There was also no evidence for heterogeneity by MSI status for SNPs in *VDR* (data not shown). However, we observed inverse associations that were statistically significant at the 0.05 level for 15 SNPs in *GC* (rs13117483, rs1491709, rs222014, rs222016, rs222017, rs16847015, rs1352843, rs222029, rs1352844, rs3733359, rs6817912, rs16847039, rs705125, rs16847047, rs843007) and risk for MSI-H tumors (Table 4). These are unlikely to be independent associations because each of these SNPs was highly correlated ($r^2 > 0.80$) with at least one other SNP within this list.

Discussion

In this family-based case-control study on CRC, we did not find evidence to suggest that variation in *VDR* and *GC* is associated with risk for CRC overall. Our data also suggested that associations between SNPs in these genes are not modified by calcium supplement use, family history of CRC, or total calcium intake estimated from an food frequency questionnaire. We found only weak suggestions that dietary vitamin D could modify the association between SNPs in *VDR* and risk for CRC (Table 3), although these associations were not statistically significant after correction for multiple testing. We observed statistically significant associations between several SNPs in *GC* and MSI-H CRC; however, these results should be interpreted with caution because of the many comparisons we have made.

There are several mechanisms that may explain the inhibitory action of vitamin D on carcinogenesis, including reduced cellular proliferation (16, 49), induction of apoptosis (49-53), inhibition of inflammation (54) and angiogenesis (55), and protection of colonic epithelium from the damaging effects of bile acids (56). Given that VDR is expressed in a wide variety of tissues, including some with no known role in calcium metabolism (17), it is logical to hypothesize that variation in *VDR* may influence these potential anticarcinogenic effects of vitamin D through altered transcriptional control of 1,25-dihydroxyvitamin D_3. In addition, two SNPs in *GC* (rs4588 and rs7041) have been shown to influence 25-hydroxyvitamin D levels (33, 34), which suggests that variation in this gene could also influence the actions of vitamin D. We would also expect that the effect of variants in these genes may be most relevant in the context of vitamin D levels.

Whereas most epidemiologic studies on *VDR* variants and risk for CRC have found associations with the disease, the results for specific variants have not been entirely consistent. Inconsistency has been a common problem in genetic epidemiology studies (57, 58) and is not limited to studies on the *VDR* gene. Potential explanations for heterogeneity in genetic association studies include inadequate power, bias, and population differences.

The *Fok*I restriction site polymorphism (rs2228570) is the most frequently reported *VDR* variant in epidemiologic studies on CRC (18-20, 22, 24-26) and adenoma (31, 59, 60). In studies on CRC, three of the seven studies that evaluated this variant found evidence for an association with risk. In contrast, this variant was not associated with adenoma risk in three studies (31, 59, 60),

Table 2. Associations between SNPs in VDR and GC and CRC in population-based sibships

Gene	Variant	Minor allele frequency*	*n* (sibships)	Multivariable-adjusted OR† (95% CI)	Unadjusted *P*	p_{ACT}‡
VDR	rs12721364	0.16	1,750	0.91 (0.77-1.08)	0.29	0.99
	rs10783215	0.50	1,748	0.88 (0.78-0.99)	0.03	0.59
	rs11574143	0.09	1,746	1.29 (1.05-1.57)	0.01	0.32
	rs11574139	0.03	1,750	1.25 (0.91-1.73)	0.17	0.96
	rs731236 (Taq1)	0.39	1,743	1.04 (0.92-1.17)	0.57	1.00
	rs1544410 (BsmI)	0.40	1,750	1.04 (0.92-1.18)	0.49	1.00
	rs2525044	0.44	1,750	0.86 (0.76-0.97)	0.01	0.30
	rs11168267	0.08	1,750	1.21 (0.97-1.51)	0.08	0.84
	rs11574077	0.05	1,749	0.89 (0.68-1.17)	0.40	1.00
	rs2248098	0.50	1,750	0.89 (0.79-1.00)	0.06	0.74
	rs987849	0.46	1,742	0.88 (0.78-0.99)	0.03	0.56
	rs2283343	0.49	1,749	1.12 (0.99-1.26)	0.07	0.81
	rs2239182	0.50	1,750	1.06 (0.93-1.20)	0.38	1.00
	rs2107301	0.29	1,748	1.07 (0.94-1.23)	0.32	0.99
	rs2283342	0.17	1,736	0.91 (0.77-1.07)	0.26	0.99
	rs2239180	0.11	1,750	1.20 (0.99-1.46)	0.07	0.79
	rs2239179	0.42	1,750	1.05 (0.93-1.19)	0.41	1.00
	rs886441	0.19	1,750	0.98 (0.84-1.14)	0.80	1.00
	rs2189480	0.38	1,739	1.00 (0.88-1.13)	0.98	1.00
	rs3819545	0.40	1,750	0.99 (0.88-1.12)	0.89	1.00
	rs3782905	0.31	1,746	1.01 (0.89-1.15)	0.90	1.00
	rs2239186	0.22	1,749	0.87 (0.75-1.01)	0.06	0.77
	rs2254210	0.38	1,722	0.92 (0.81-1.05)	0.21	0.97
	rs2238136	0.25	1,750	0.91 (0.79-1.05)	0.21	0.97
	rs1989969	0.41	1,749	0.91 (0.80-1.03)	0.14	0.93
	rs2238135	0.24	1,750	0.94 (0.82-1.09)	0.44	1.00
	rs4760648	0.42	1,750	1.13 (1.00-1.27)	0.05	0.70
	rs2853559	0.41	1,645	0.92 (0.81-1.04)	0.18	0.96
	rs11168287	0.49	1,750	1.05 (0.93-1.18)	0.46	1.00
	rs4328262	0.42	1,745	0.99 (0.88-1.12)	0.93	1.00
	rs11574026	0.11	1,748	1.11 (0.91-1.35)	0.32	0.99
	rs10875695	0.26	1,750	1.06 (0.92-1.22)	0.42	1.00
	rs11168293	0.33	1,748	0.94 (0.83-1.08)	0.40	1.00
	rs4760655	0.36	1,750	0.98 (0.86-1.11)	0.74	1.00
	rs12581281	0.02	1,750	1.39 (0.88-2.17)	0.18	0.96
	rs7299460	0.30	1,750	1.09 (0.95-1.25)	0.20	0.97
	rs4760658	0.34	1,750	0.94 (0.82-1.07)	0.33	0.99
	rs4516035	0.41	1,749	0.96 (0.84-1.08)	0.48	1.00
	rs11568820(CDX2)	0.21	1,750	1.07 (0.92-1.25)	0.36	1.00
	rs7310552	0.41	1,747	0.96 (0.85-1.09)	0.54	1.00
	rs7970314	0.23	1,739	1.11 (0.96-1.29)	0.16	0.96
	rs3923693	0.13	1,672	1.16 (0.96-1.39)	0.12	0.92
	rs4237856	0.24	1,750	1.00 (0.87-1.15)	0.98	1.00
GC	rs16846876	0.32	1,746	1.00 (0.89-1.14)	0.95	1.00
	rs16846880	0.07	1,750	1.11 (0.88-1.41)	0.37	0.98
	rs13117483	0.27	1,748	0.99 (0.86-1.14)	0.91	1.00
	rs12512631	0.34	1,750	0.99 (0.87-1.12)	0.86	1.00
	rs17383291	0.06	1,748	0.83 (0.64-1.07)	0.16	0.83
	rs17467825	0.28	1,749	1.01 (0.88-1.15)	0.93	1.00
	rs705117	0.18	1,749	1.16 (0.99-1.37)	0.07	0.56

(*Continued on the following page*)

Table 2. Associations between SNPs in VDR and GC and CRC in population-based sibships (Cont'd)

Gene	Variant	Minor allele frequency*	*n* (sibships)	Multivariable-adjusted OR† (95% CI)	Unadjusted *P*	p_{ACT}‡
	rs842999	0.46	1,744	1.11 (0.98-1.25)	0.08	0.62
	rs1491709	0.08	1,748	1.05 (0.85-1.31)	0.63	1.00
	rs7041 (Asp432Glu)	0.46	1,748	1.08 (0.96-1.22)	0.21	0.88
	rs222035	0.46	1,739	1.08 (0.96-1.22)	0.21	0.88
	rs222014	0.10	1,750	0.98 (0.81-1.20)	0.88	1.00
	rs222016	0.17	1,742	1.00 (0.86-1.17)	0.95	0.95
	rs222017	0.10	1,749	0.99 (0.81-1.20)	0.90	1.00
	rs16847015	0.06	1,745	1.08 (0.84-1.37)	0.57	1.00
	rs1352843	0.10	1,749	0.99 (0.81-1.21)	0.93	1.00
	rs1155563	0.29	1,747	0.99 (0.86-1.13)	0.83	1.00
	rs222029	0.17	1,749	0.99 (0.84-1.15)	0.86	1.00
	rs1352844	0.12	1,750	0.97 (0.80-1.18)	0.78	1.00
	rs3733359	0.07	1,748	0.94 (0.74-1.18)	0.57	1.00
	rs6817912	0.07	1,749	0.92 (0.73-1.16)	0.47	1.00
	rs16847039	0.18	1,748	0.96 (0.82-1.11)	0.56	1.00
	rs705125	0.14	1,744	1.01 (0.87-1.18)	0.89	1.00
	rs16847047	0.11	1,750	0.99 (0.82-1.21)	0.93	1.00
	rs843007	0.20	1,749	0.96 (0.82-1.12)	0.59	1.00

Abbreviations: OR, odds ratio.

*Minor allele frequencies were estimated using a sample of 447 unrelated population-based controls from the Seattle Cancer Family Registry.

†Odds ratios estimated using a log-additive model. Adjusted for age and sex.

‡*P* values were adjusted for multiple comparisons using a modification of p_{ACT} for correlated tests developed by Conneely and Boehnke (48).

although a statistically significant association for risk for large adenomas was observed in one of these (31). In a recent meta-analysis, the summary odds ratio for this variant was not associated with CRC, although there was significant heterogeneity among studies (27). Unfortunately, we were unable to evaluate associations between the *Fok*I variant and CRC risk in our study because this SNP did not pass quality control checks.

In three studies, the *Bsm*I variant (rs1544410) was not associated with risk for colorectal adenoma (30, 31, 61); however, data from two of the studies suggested that the variant may modify the relationship between calcium and/or vitamin D intake and risk for adenoma (30, 61). In studies on CRC, this variant was associated with risk for CRC overall in three studies (19, 21, 26), with evidence for modification by dietary intake of calcium and vitamin D (21). However, two additional studies showed no association with CRC (18, 25). In a recent meta-analysis, the summary odds ratio was borderline protective for this variant after one study contributing to heterogeneity in risk estimates was excluded (27). In our analysis, we observed no overall association between the *Bsm*I variant and CRC (Table 2). In addition, we observed no evidence of modification by calcium supplement use or by dietary calcium and vitamin D intake in the subset of individuals with food frequency questionnaire data.

The *Taq*I variant (rs731236) was not associated with risk for colorectal adenoma or cancer in three studies (25, 59, 60) but was associated with a borderline statistically significant risk for CRC in one (19). We observed no overall association between this variant and risk for CRC (Table 2). Three previous studies have evaluated associations between the *VDR CDX2* variant (rs11568820) and CRC risk, with two studies reporting no association between this variant and risk (18, 23), whereas the other study reported a statistically significant increased risk in individuals with the AA genotype (24). We observed no association between this SNP and CRC in our study population (Table 2).

To our knowledge, this is the first epidemiologic study to evaluate the association between variation in *GC* and risk for CRC. Our data suggest that this gene is unlikely to play a major role in genetic susceptibility to this malignancy. We observed limited evidence that variants in *GC* may be associated with a reduced risk for MSI-H CRC, although these results should be interpreted with caution because of the many comparisons that we have made, as well as the lack (at least to date) of a biological rationale for a role of this gene only in MSI-H CRC.

This study has several strengths, including the comprehensive evaluation of *VDR* and *GC*, the availability of systematically collected data on tumor characteristics, and the case–unaffected sibling study design that

Table 3. Associations between *VDR* and *GC* variants and CRC by total vitamin D intake

Gene	Variant	Total vitamin D intake above median,* OR adjusted† (95% CI)	Total vitamin D intake below median,* OR adjusted† (95% CI)	P‡
VDR	rs12721364	0.82 (0.56-1.18)	1.19 (0.85-1.67)	0.10
	rs10783215	0.83 (0.66-1.04)	0.99 (0.79-1.24)	0.11
	rs11574143	1.30 (0.86-1.97)	2.26 (1.45-3.51)	0.004
	rs11574139	0.75 (0.37-1.52)	1.69 (0.83-3.45)	0.16
	rs731236 (Taq1)	0.97 (0.76-1.25)	0.88 (0.69-1.13)	0.57
	rs1544410 (Bsml)	0.99 (0.77-1.26)	0.88 (0.69-1.11)	0.47
	rs2525044	0.84 (0.66-1.07)	0.95 (0.76-1.20)	0.32
	rs11168267	1.40 (0.88-2.22)	1.88 (1.13-3.11)	0.07
	rs11574077	1.43 (0.66-3.08)	0.94 (0.50-1.76)	0.51
	rs2248098	0.85 (0.67-1.07)	0.97 (0.78-1.21)	0.27
	rs987849	0.84 (0.67-1.07)	0.96 (0.77-1.20)	0.28
	rs2283343	1.10 (0.87-1.39)	1.06 (0.84-1.33)	0.73
	rs2239182	1.17 (0.93-1.48)	1.02 (0.82-1.27)	0.26
	rs2107301	0.92 (0.70-1.21)	1.37 (1.06-1.77)	0.005
	rs2283342	0.81 (0.58-1.15)	1.25 (0.90-1.72)	0.05
	rs2239180	1.08 (0.71-1.64)	1.54 (1.05-2.26)	0.11
	rs2239179	1.19 (0.94-1.50)	1.04 (0.83-1.31)	0.31
	rs886441	0.91 (0.66-1.25)	0.81 (0.59-1.10)	0.43
	rs2189480	0.99 (0.77-1.26)	1.27 (0.98-1.65)	0.06
	rs3819545	0.94 (0.74-1.21)	1.25 (0.99-1.59)	0.02
	rs3782905	1.06 (0.81-1.40)	0.99 (0.76-1.28)	0.85
	rs2239186	0.72 (0.53-0.97)	1.10 (0.82-1.47)	0.02
	rs2254210	0.94 (0.73-1.21)	0.96 (0.75-1.24)	0.89
	rs2238136	0.83 (0.62-1.12)	0.92 (0.70-1.22)	0.49
	rs1989969	0.91 (0.71-1.16)	0.91 (0.72-1.16)	0.69
	rs2238135	0.87 (0.65-1.16)	0.93 (0.71-1.22)	0.67
	rs4760648	1.06 (0.83-1.35)	1.22 (0.97-1.53)	0.21
	rs2853559	0.94 (0.74-1.20)	0.92 (0.73-1.17)	0.81
	rs11168287	1.05 (0.83-1.34)	1.16 (0.92-1.45)	0.42
	rs4328262	0.89 (0.70-1.13)	1.02 (0.81-1.27)	0.42
	rs11574026	1.59 (1.02-2.48)	1.41 (0.94-2.11)	0.09
	rs10875695	1.09 (0.82-1.46)	1.19 (0.89-1.59)	0.53
	rs11168293	0.93 (0.71-1.21)	0.88 (0.68-1.15)	0.67
	rs4760655	0.90 (0.69-1.16)	1.04 (0.81-1.34)	0.40
	rs12581281	1.31 (0.49-3.47)	0.94 (0.45-1.95)	0.82
	rs7299460	1.17 (0.89-1.54)	1.21 (0.92-1.58)	0.39
	rs4760658	0.92 (0.71-1.20)	0.88 (0.67-1.14)	0.64
	rs4516035	0.87 (0.67-1.11)	0.90 (0.70-1.15)	0.53
	rs11568820(CDX2)	1.29 (0.93-1.78)	1.18 (0.86-1.63)	0.29
	rs7310552	0.87 (0.68-1.12)	0.91 (0.71-1.17)	0.56
	rs7970314	1.33 (0.98-1.82)	1.20 (0.89-1.61)	0.19
	rs3923693	1.39 (0.92-2.09)	1.52 (1.03-2.23)	0.10
	rs4237856	0.82 (0.61-1.10)	0.82 (0.63-1.08)	0.33
GC	rs16846876	1.05 (0.80-1.36)	1.06 (0.82-1.37)	0.90
	rs16846880	0.58 (0.34-0.98)	0.57 (0.31-1.04)	0.06
	rs13117483	1.03 (0.78-1.35)	1.01 (0.75-1.35)	0.98
	rs12512631	0.93 (0.73-1.20)	1.26 (0.98-1.61)	0.05
	rs17383291	0.90 (0.50-1.61)	0.59 (0.34-1.04)	0.24
	rs17467825	1.20 (0.91-1.56)	1.07 (0.83-1.39)	0.43
	rs705117	0.85 (0.60-1.19)	0.79 (0.57-1.09)	0.36

(*Continued on the following page*)

Table 3. Associations between VDR and GC variants and CRC by total vitamin D intake (Cont'd)

Gene	Variant	Total vitamin D intake above median,* OR adjusted† (95% CI)	Total vitamin D intake below median,* OR adjusted† (95% CI)	*P*‡
	rs842999	1.07 (0.85-1.34)	0.96 (0.77-1.19)	0.53
	rs1491709	0.96 (0.62-1.50)	0.92 (0.58-1.46)	0.94
	rs7041 (Asp432Glu)	1.03 (0.81-1.31)	0.96 (0.77-1.21)	0.77
	rs222035	1.03 (0.81-1.30)	0.95 (0.76-1.20)	0.75
	rs222014	0.75 (0.49-1.14)	0.96 (0.61-1.50)	0.46
	rs222016	0.91 (0.66-1.26)	0.99 (0.71-1.39)	0.86
	rs222017	0.76 (0.50-1.16)	1.00 (0.64-1.58)	0.47
	rs16847015	1.17 (0.67-2.05)	1.15 (0.67-1.98)	0.82
	rs1352843	0.75 (0.49-1.15)	0.97 (0.61-1.53)	0.47
	rs1155563	1.12 (0.85-1.49)	1.14 (0.87-1.50)	0.59
	rs222029	0.86 (0.61-1.20)	0.95 (0.67-1.35)	0.69
	rs1352844	0.85 (0.56-1.29)	0.81 (0.49-1.33)	0.60
	rs3733359	1.05 (0.63-1.74)	1.07 (0.65-1.77)	0.96
	rs6817912	1.02 (0.62-1.68)	1.04 (0.62-1.74)	0.99
	rs16847039	0.90 (0.65-1.25)	0.92 (0.64-1.31)	0.80
	rs705125	0.92 (0.66-1.28)	1.01 (0.71-1.43)	0.88
	rs16847047	0.89 (0.58-1.35)	0.92 (0.55-1.53)	0.85
	rs843007	0.93 (0.68-1.29)	0.93 (0.66-1.32)	0.89

*Includes a subset of individuals who completed food frequency questionnaire (585 cases and 837 unaffected siblings from 563 sibships).
†Adjusted for age and sex.
‡*P* value for heterogeneity from the 2 degrees of freedom likelihood ratio test; not adjusted for multiple comparisons.

controls for any potential confounding by ethnicity. This family-based design is more powerful than studies that include unrelated controls for detecting gene-environment interactions (62).

Several limitations must also be considered. Although the case–unaffected sibling design is more powerful for detecting gene-environment interactions, this design may have lower power for detecting genetic main effects because the study sample is often overmatched on genotype (62). Because overmatching is an issue of power rather than bias, evaluating the width of the confidence intervals can provide information about the overall power of the study. For the main effects of the genotypes (Table 2), the confidence intervals are quite narrow, especially for the more common variants. This would indicate that the lack of association in our study is not simply an issue of inadequate power. Detailed food frequency questionnaire data were collected only for a subset of the participants in this study (32%), so we have limited power to detect heterogeneity by calcium and vitamin D intake. In addition, we did not collect information on sun exposure, so we are unable to account for endogenously synthesized vitamin D levels. Cases were interviewed up to 5 years after diagnosis, which introduces the potential for possible survival bias and increases the potential for exposure misclassification; however, when we restricted our analyses to subjects interviewed within the first 2 years after diagnosis, we observed very similar results. In addition, we have no strong a priori hypothesis that any of these SNPs would be strongly associated with prognosis.

In conclusion, we did not observe any statistically significant associations between variants in *VDR* and *GC* and CRC overall in this family based analysis. After correction for multiple comparisons, we observed no indication that these associations were modified by calcium supplement use and dietary and total intake of calcium and vitamin D. We did observe associations between SNPs in *GC* and MSI-H CRC, although these results should be confirmed in additional studies.

Disclosure of Potential Conflicts of Interest

Dr. Limburg has a consulting agreement with Genomic Health, Inc.

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Table 4. Association between SNPs in *GC* and CRC by MSI status

Gene	Variant	MSI-H,* OR adjusted† (95% CI)	MSI-L/MSS,* OR adjusted† (95% CI)	*P* heterogeneity‡
GC	rs16846876	1.04 (0.66-1.63)	1.05 (0.89-1.24)	0.83
	rs16846880	1.26 (0.62-2.52)	1.07 (0.79-1.46)	0.74
	rs13117483	0.60 (0.40-0.89)	1.11 (0.93-1.33)	0.04
	rs12512631	1.30 (0.88-1.92)	0.89 (0.75-1.05)	0.15
	rs17383291	1.15 (0.43-3.09)	0.84 (0.61-1.14)	0.54
	rs17467825	1.34 (0.87-2.06)	1.05 (0.88-1.24)	0.35
	rs705117	0.74 (0.46-1.20)	1.27 (1.03-1.57)	0.05
	rs842999	1.11 (0.76-1.60)	1.20 (1.03-1.40)	0.05
	rs1491709	0.52 (0.27-0.98)	1.26 (0.95-1.68)	0.04
	rs7041 (Asp432Glu)	1.07 (0.73-1.57)	1.17 (1.00-1.37)	0.12
	rs222035	1.09 (0.74-1.62)	1.18 (1.00-1.38)	0.13
	rs222014	0.38 (0.18-0.78)	1.14 (0.88-1.47)	0.02
	rs222016	0.39 (0.23-0.66)	1.16 (0.95-1.42)	0.0006
	rs222017	0.41 (0.20-0.82)	1.14 (0.88-1.48)	0.02
	rs16847015	0.37 (0.16-0.86)	1.23 (0.88-1.70)	0.03
	rs1352843	0.38 (0.18-0.78)	1.15 (0.89-1.49)	0.01
	rs1155563	1.76 (1.13-2.76)	1.01 (0.85-1.20)	0.05
	rs222029	0.34 (0.19-0.60)	1.16 (0.94-1.42)	0.0002
	rs1352844	0.47 (0.26-0.87)	1.04 (0.80-1.34)	0.05
	rs3733359	0.46 (0.21-0.99)	1.12 (0.82-1.54)	0.09
	rs6817912	0.49 (0.24-1.00)	1.09 (0.79-1.49)	0.14
	rs16847039	0.43 (0.27-0.69)	1.09 (0.89-1.35)	0.002
	rs705125	0.53 (0.33-0.86)	1.13 (0.92-1.39)	0.02
	rs16847047	0.44 (0.24-0.79)	1.11 (0.85-1.44)	0.02
	rs843007	0.47 (0.30-0.75)	1.07 (0.87-1.32)	0.005

*Includes 1,200 cases (182 MSI-H and 1,018 MSS/MSI-L) and 1,880 unaffected siblings from 1,163 sibships wherein the case had MSI testing results.
†Adjusted for age and sex.
‡*P* heterogeneity from the 2 degrees of freedom likelihood ratio test; not adjusted for multiple comparisons.

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