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Association of *vitamin D receptor* gene variants, adiposity and colon cancer

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Vitamin D receptor (*VDR*) gene variants have been variably associated with risk of colon cancer in epidemiologic studies. We sought to further clarify the relationship between colon cancer and three single-nucleotide polymorphisms (SNPs) in the *VDR* gene (*Cdx-2*, *FokI* and *TaqI*) in a population-based case–control study of 250 incident cases and 246 controls. Colon cancer cases were more frequently homozygous for the *Cdx-2* A allele (9.2 versus 4.1%, $P = 0.06$). *Cdx-2* AA homozygotes were at increased risk with an unadjusted odds ratio (OR) of 2.47 [95% confidence interval (CI): 1.13–5.37, $P = 0.022$]; adjustment for age, sex, body mass index (BMI), non-steroidal anti-inflammatory use and family history of colorectal cancer yielded an OR of 2.27 (CI: 0.95–5.41, $P = 0.065$). Carriers of the *FokI* TT genotype were also at increased risk with an adjusted OR of 1.87 (CI: 1.03–3.38, $P = 0.038$). Haplotype analyses showed significant increased colon cancer risk for carriers of the *Cdx-2–FokI* A-T haplotype and the *FokI-TaqI* T-G haplotype. The three-SNP *Cdx-2–FokI–TaqI* (A-T-G) haplotype showed a similar association with an adjusted OR of 3.63 (CI: 1.01–13.07). A strong positive association was observed for the *Cdx-2* variant among individuals with low BMI or low waist circumference. Our results suggest that genetic variation at the *VDR* locus, in particular *Cdx-2* and *FokI* SNPs, may influence colon cancer risk and these associations may be modified by adiposity.

Introduction

The observation of an ecologic correlation between solar radiation exposure and colon cancer risk (1,2) has spawned a number of epidemiologic studies of the associations of dietary vitamin D intake, serum levels of vitamin D metabolites and more recently vitamin D receptor (*VDR*) gene variants and colon cancer. While a non-significant inverse association of dietary vitamin D intake and colon cancer risk was found in a number of prospective studies (3,4), analyses of plasma 25-hydroxyvitamin D (25(OH)D) concentrations, a biomarker for vitamin D, generally support an inverse association (1,5–8). In animal models, the active form of vitamin D, 1,25-dihydroxyvitamin D3, reduces cellular proliferation and promotes differentiation (9–11), providing biological evidence for the involvement of vitamin D in carcinogenesis.

The *VDR* gene, located on chromosome 12, plays an important role in a number of different pathways including calcium absorption, bone metabolism, immune cell differentiation and proliferation, as well as cellular processes of carcinogenesis, including differentiation, proliferation and apoptosis (12–15). Underlying variability at the *VDR* gene locus may therefore influence proliferation of cancer cells and differentiation directly, and also influence the downstream action of the nuclear *VDR* complex acting as a transcription factor on vitamin D-responsive genes.

Several epidemiologic studies have examined the association of various *VDR* genetic variants and colon cancer, and the results are inconsistent (16–30). The first study to date that analyzed the *VDR Cdx-2* single-nucleotide polymorphism (SNP) and colon cancer, which is functional and affects *VDR* transcription (31), reported increased risk for carriers of the *Cdx-2*-bearing haplotype, but no significant individual SNP association in 1574 cases and 1970 controls (29). Interestingly, the reported association for the *Cdx-2*-bearing haplotype was in the opposite hypothesized direction based on the functional effect of this SNP on *VDR* transcription. We sought to further clarify the association of the *Cdx-2* SNP as well as two other *VDR* SNPs (*FokI* and *TaqI*) with colon cancer in a population-based incident case–control study. We also explored potential effect modification by body mass index (BMI) and waist circumference since obesity is related to decreased serum 25(OH)D levels (32,33) and increased risk of colon cancer (34,35).

Abbreviations: BMI, body mass index; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; MAF, minor allele frequency; (25(OH)D), 25-hydroxyvitamin D; OR, odds ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; *VDR*, vitamin D receptor.

Materials and methods

Study population

The study design of this population-based case–control study has been described elsewhere (36). Briefly, eligible cases were identified through the population-based Surveillance, Epidemiology and End Results Kentucky Cancer Registry covering all residents living in the State of Kentucky at the time of diagnosis. We queried the cancer registry database every 3 months and identified all histopathologically confirmed incident primary colon cancer cases reported within 6 months of diagnosis preceding the recruitment. Cancers of the rectosigmoid were classified as colon cancer and included in the study, whereas rectal cancers were excluded.

We first sent an introductory letter explaining the study to potentially eligible cases. After 3 weeks or when subject initiated contact, we phoned each case subject for a screening interview to determine eligibility and their willingness to participate in the study. We collected information on demographics, family history of colorectal cancer and personal history of cancer at the recruitment phone call. We then used a two-step approach to collect blood samples and lifestyle questionnaire data. First, we sent a prepacked phlebotomy kit with detailed written instructions for blood sample collection and written consent forms to each case subject. Participants were instructed to go to their physician offices or adjacent medical facilities for blood draw after overnight fasting. The samples were collected in purple-top (K3EDTA) blood collection tubes and shipped overnight on frozen ice pack. Upon receipt, the blood tubes were spun for 15 min at 600*g* and aliquots of plasma and concentrated buffy coat were prepared and frozen at −80°C. We then sent each participant a self-administered lifestyle risk factor questionnaire developed by the National Cancer Institute Colon Cancer Familial Cancer Registry (http://epi.grants.cancer.gov/documents/CFR/center_questionnaires/Colon/LA/ColonRiskFactor_USC.pdf) to collect detailed information on family history of colorectal cancer, lifestyle and behavioral risk factors. Height, weight and waist and hip circumference were also collected via self-measurement.

We used random digital dialing to recruit population controls following a protocol similar to that described above for the cases. As a proxy for frequency match of the residential locations between the cases and the controls, the area codes and exchanges of the phone numbers of potential cases were used, along with randomly generated four digit numbers, to produce the list of phone numbers for control recruitment. Recruitment was not conducted when a business was reached. We originally recruited controls who were ≥30 years, but later changed the study protocol to recruit controls age ≥40 years, in efforts to recruit a control

sample that was more similar in age to the study cases. Controls were free of personal history of cancer other than skin cancer. We excluded those with known inflammatory bowel diseases, family history of familial adenomatous polyposis and hereditary non-polyposis colorectal cancer.

As with case subjects, each control first donated a sample of blood after fasting overnight and then was asked to complete the self-administered risk factor questionnaire.

The participation rates were 72.2% for the cases and 62.5% for eligible controls. All participants provided written informed consent. The study was approved by the Institutional Review Boards of the University of Kentucky, Lexington and Case Western Reserve University/University Hospitals of Cleveland.

Genotyping

Genomic DNA was extracted from buffy coats/blood using the EZ1 DNA Blood kit (QIAGEN, Valencia, CA). All purified DNA samples were diluted to a constant DNA concentration of 5 ng/μl in 10 mM Tris and 5 mM ethylenediaminetetraacetic acid buffer (pH 8). Three *VDR* variants were examined as follows: the *Cdx-2* SNP in the 5′ promoter region of the *VDR* gene (rs11568820), the *FokI* polymorphism in exon 2 (rs10735810) and *TaqI* in exon 9 (rs731236).

Two of the *VDR* variants, *FokI* and *TaqI*, were detected by using the Amplifluor® SNPs HT Genotyping System (Chemicon International, Temecula, CA). Amplifluor® AssayArchitect™ software was used to design polymerase chain reaction (PCR) primers. The PCRs were performed in a 384-well format with the following primers: *TaqI* reverse primer 5′-TATCCCCGTGCCCACAGAT-3′; A allele forward specific primer 5′-GAAGGTGACCAAGTTCATGCTTCGGTCCTGGATGGCCTCAA-3′; G allele forward specific primer 5′-GAAGGTCGGAGTCAACGGATTCGGTCCTGGATGGCCTCG-3′ and *FokI* reverse primer 5′-AAGTGCTGGCCGCCATT-3′; T allele forward specific primer 5′-GAAGGTGACCAAGTTCATGCTGCTTGCTGTTCTTACAGGGAT-3′; C allele forward specific primer 5′-GAAGGTCGGAGTCAACGGATTTGCTTGCTGTTCTTACAGGGAC-3′. PCRs were performed in a total reaction volume of 5 μl using Titanium™ Taq polymerase (BD Clontech®, Mountain View, CA) and 5 ng of genomic DNA as recommended by the manufacturer, followed by an end-point read and genotype analysis using the ABI Prism 7900HT and SDS 2.1 software (Applied Biosystems, Foster City, CA).

Analysis of the *Cdx-2* variant was performed by Custom TaqMan® SNP Genotyping assay (Applied Biosystems). PCRs were performed according to the manufacturer's instructions in a 384-well format in a total reaction volume of 5 μl using 10 ng of genomic DNA and the following primer sequences; forward 5′-ACATCTTTTGTATCAGGAACTTATATATATTCCTGAGTA-3′; reverse 5′-CAGTATTTTTCAAAATTTTAACTGCAACCCAT-3′; FAM probe 5′-CTAGGTCACAATAAAA-3′ and VIC probe 5′-AACTAGGTCACAGTAAAA-3′. An ABI Prism 7900HT instrument was used to perform plate reading. Automated allele calling was performed by allelic discrimination plots using the SDS version 2.1 software from Applied Biosystems.

The genotyping failure rate was <0.1%. Two percent replicate samples were sequenced for quality control and had a concordance rate of 100%. Laboratory personnel were blinded to case–control status.

Statistical analysis

The analyses presented here are based on a sample of 266 incident colon cancer cases and 267 cancer-free controls from an ongoing case–control study. We excluded a small number of participants of other self-reported ethnic groups (16 cases and 21 controls) to minimize the possibility of population stratification bias—spurious associations due to differences in genotype frequencies and disease incidences in individuals of different ancestries. All analyses herein include the Caucasian sample of 250 cases and 246 controls.

We examined differences in demographic and lifestyle risk factors using chi-square tests and Student's *t*-tests. We estimated allele and genotype frequencies and assessed deviations from Hardy–Weinberg equilibrium (HWE) in cases and controls separately using chi-square tests with one degree of freedom. The difference in disequilibrium between cases and controls and the overall population disequilibrium assuming a disease prevalence ranging from 1 to 5% were also assessed using chi-square tests with one degree of freedom.

Calculation of the extent of linkage disequilibrium (LD) between SNPs was performed using Haploview (37). We computed genotype-specific odds ratios (ORs) using unconditional logistic regression models under additive, dominant and recessive genetic models for each of the three SNPs. We used individuals homozygous for the common allele as the referents for additive and dominant models. For the recessive model, we combined those with one or two copies of the common allele and used this as the reference category. We estimated the crude ORs and multivariate ORs adjusted for age, sex, non-steroidal anti-inflammatory drug use, BMI and family history of colorectal cancer. We dichotomized BMI and waist circumference based on the median values in control subjects (26.78 kg/m^2 and 93.98 cm, respectively) when examining effect modification of the association of *VDR* genotype and colon cancer risk by adiposity. In addition, we examined effect modification by age and sex, judging a *P*-value <0.10 as evidence for statistical interaction. We used the Statistical Package for Social Sciences software unless otherwise noted (38).

We estimated haplotype frequencies of 2- and 3-SNP haplotypes using the expectation maximization algorithm within the UNPHASED software and estimated risk associated with each haplotype with the most common haplotype as the reference category using unconditional logistic regression (39,40).

Results

Table I shows the descriptive characteristics of the study sample and allele frequencies of *VDR*. Cases were more likely to be older and male, with cases and controls ranging from 21 to 90 years and 29 to 87 years, respectively. Cases had higher BMI and waist circumference, but the differences were not statistically significant. There were no significant differences according to smoking status; however, we found a higher proportion of current smokers in controls. In our study population, there was no significant population deviation from HWE. The *Cdx-2* allele frequency was consistent with HWE among the controls ($P = 0.95$), but was out of Hardy–Weinberg proportions in colon cancer cases ($P = 0.03$), with increased homozygosity. Allele distributions of *FokI* and *TaqI* were consistent with HWE for both cases and controls. Also, the estimated disequilibrium coefficients for cases and controls were in opposite directions for all the SNPs, with no significant deviation from HWE in the population as a whole assuming a disease prevalence between 1% ($P = 0.57$) and 5% ($P = 0.17$). Overall, we observed weak LD between *TaqI* and *FokI* and between *FokI* and *Cdx-2*, with D' values of 0.05 and 0.01, respectively. We observed moderate LD between the *TaqI* and *Cdx-2* SNPs, with a D' estimate of 0.20 in the cases and 0.30 in the controls.

The results for the main effects of *VDR* genotype and risk of colon cancer are given in Table II. We observed increased risk for carriers of the *Cdx-2* AA genotype in both the additive and recessive models. Adjustment for covariates slightly reduced the OR estimates to only borderline significance ($P = 0.065$ and 0.069, respectively). Carriers of homozygous *FokI* T allele were also at increased risk of colon cancer in both additive and recessive models. There was no statistically significant association for *TaqI*.

Table III shows the results for haplotype analyses. In the adjusted models, individuals carrying the *Cdx-2–FokI* A-T haplotype and the *FokI-TaqI* T-G haplotype were both at increased risk. Consistent with these results, those carrying a haplotype comprised of the three minor *Cdx-2–FokI–TaqI* alleles (A-T-G) had >3-fold increase of risk in covariate-adjusted models.

We found no evidence for effect modification of the association of *VDR* variants and colon cancer by age or sex. However, when we stratified the analyses by sex, we found a statistically significant association of *FokI* with colon cancer in men, with adjusted ORs and 95% confidence intervals (CIs) of 2.79 (1.03–7.58) and 2.03 (1.05–3.89) for recessive (TT) and dominant (CT or TT) models, respectively (data not shown). The minor allele frequency (MAF) for *FokI* in men was 0.44 and 0.36 in cases and controls, respectively, whereas for women the estimates were 0.40 for both cases and controls.

Table IV summarizes results from analyses stratified by BMI. We observed considerable differences in OR estimates for each of the three SNPs in stratified analyses, although tests for interactions were not statistically significant. Most notably, for *Cdx-2* AA genotype, we found an >3-fold increase in risk for the low-BMI group (<26.8 kg/m^2), and almost a 5-fold increase in risk for the low-waist circumference group (<94 cm) (OR = 5.04, 95% CI: 1.4–18.7) (data not shown), whereas the corresponding OR estimates were essentially at unity for those with higher BMI or waist circumference. In addition, for the high categories of BMI and waist circumference, individuals who carry two *TaqI* variant alleles were at increased risk in unadjusted models; however, these associations became non-significant in covariate-adjusted models. We observed a relatively high degree of correlation between BMI and waist circumference; Spearman correlation coefficients of 0.70 and 0.73 for cases and controls, respectively.

Approximately 61% of colon cancers were proximal and 39% were distal. When we analyzed whether *VDR* variants are more strongly associated with either proximal or distal tumors, we found stronger associations for proximal tumors (data not shown). The *Cdx-2* AA genotype was associated with increased risk of proximal tumors, OR = 2.84, 95% CI: 1.21–6.68, but this association became non-significant when adjusted for covariates. For *TaqI*, there was also increased risk for the rare (GG) genotype, with significant crude and adjusted OR estimates of 1.98, 95% CI: 1.10–3.56 and 2.22, 95% CI: 1.17–4.23, respectively. There were no significant associations for distal cancers in our study.

Table I. Kentucky colon cancer study demographics and allele frequencies

Variables	Cases (n = 250)	Controls (n = 246)	P-value[a]
Age, mean (SD)	62.76 (10.21)	58.47 (12.11)	0.001
Sex, *n* (%)			
Male	120 (48%)	81 (33%)	0.001
Female	130 (52%)	165 (67%)	
Smoking status, *n* (%)			
Never smoker	100 (46.5%)	99 (46.9%)	
Former smoker	90 (41.9%)	74 (35.1%)	0.12
Current smoker	25 (11.6%)	38 (18.0%)	
Regular NSAID use[b], *n* (%)	134 (60.1%)	163 (71.5%)	0.01
Family history of colorectal cancer, *n* (%)	57 (24.4%)	38 (16.1%)	0.03
BMI (kg/m^2), mean (SD)	28.9 (6.4)	27.8 (5.8)	0.07
Waist circumference (cm), mean (SD)	96.8 (15.3)	95.7 (16.4)	0.47
Allele frequencies			
Cdx-2 (rs11568820)			
G	0.74	0.80	0.048
A	0.26	0.20	
FokI (rs10735810)			
C	0.58	0.61	0.301
T	0.42	0.39	
TaqI (rs731236)			
A	0.58	0.63	0.107
G	0.42	0.37	

NSAID, non-steroidal anti-inflammatory drug.
[a]Student's *t*-test or chi-square for differences, complete data on BMI, smoking, NSAID use and family history were available for 216 cases and 214 controls.
[b]Use at least twice per week ≥6 months.

Discussion

In this population-based case–control study, we found a statistically significant association between *VDR Cdx-2* variant and colon cancer risk. Those carrying two copies of *Cdx-2* A allele had a >2-fold increase of risk. We also found evidence for increased risks for the *FokI* variant (T allele) in recessive and additive models. Haplotype analyses further showed that the (*Cdx-2–FokI–TaqI* A-T-G) haplotype is associated with >3-fold increase of colon cancer risk. These results add evidence for a potential role of inherited *VDR* variants in the development of colon cancer.

The *Cdx-2* SNP (G/A polymorphism), located in the promoter at the 5′ end of the *VDR* gene, is a known binding site for the *Cdx-2* intestinal-specific transcription factor (31,41). The *Cdx-2* polymorphism is believed to be important in the regulation of calcium absorption; the G allele substitution eliminates the *Cdx-2* transcription factor-binding site and therefore reduces *VDR* transcription (31). Since the *Cdx-2* A allele is hypothesized to increase *VDR* transcription and consequently increased intestinal calcium absorption, we hypothesized that the A allele is protective for colon cancer. However, we found that the *Cdx-2* AA (rare) genotype increases risk for colon cancer in our study. Our results are in line with a previous study by Slattery *et al.* (29) showing increased risk of colon cancer for carriers of a haplotype that includes the *Cdx-2* A allele (n = 1574 cases, 1970 controls). The *Cdx-2* MAFs in our control sample and that reported by Slattery *et al.* were very similar (0.19–0.20), whereas another recent study that found no significant association of *Cdx-2* and colorectal cancer had a MAF of 0.34 in a Russian sample of controls (30). Flugge *et al.* (30) found no differences in the *Cdx-2* MAF between cases and controls, which may partly explain the lack of significant association with colon cancer; however, these are different populations and these differences may influence the association of *VDR* and colon cancer.

There are four published studies that have investigated *Cdx-2* and prostate cancer and the results are not in agreement (42–45). Two of

Table II. Association of *VDR* SNP genotypes and colon cancer risk

VDR SNPs	Cases/controls	Crude	Adjusted[a]
		OR (95% CI), P-value	OR (95% CI), P-value
Cdx-2 (rs11568820)			
GG (reference)	145/156	1.0 (reference)	1.0 (reference)
GA[b]	82/80	1.10 (0.75–1.62), 0.62	1.07 (0.70–1.63), 0.75
AA[b]	23/10	2.47 (1.13–5.37), 0.02	2.27 (0.95–5.41), 0.07
GA or AA[c]	105/90	1.26 (0.88–1.80), 0.22	1.19 (0.80–1.79), 0.38
AA versus others[d]	23/10	2.39 (1.11–5.14), 0.03	2.21 (0.94–5.21), 0.07
FokI (rs10735810)			
CC (reference)	89/89	1.0 (reference)	1.0 (reference)
CT[b]	113/124	0.91 (0.62–1.35), 0.64	1.00 (0.65–1.54), 0.99
TT[b]	48/33	1.46 (0.86–2.48), 0.17	1.87 (1.03–3.38), 0.04
CT or TT[c]	161/157	1.03 (0.71–1.48), 0.89	1.17 (0.78–1.76), 0.46
TT versus others[d]	48/33	1.53 (0.95–2.49), 0.08	1.87 (1.09–3.19), 0.02
TaqI (rs731236)			
AA (reference)	89/97	1.0 (reference)	1.0 (reference)
AG[b]	111/115	1.05 (0.71–1.55), 0.80	1.10 (0.71–1.69), 0.67
GG[b]	50/34	1.60 (0.95–2.70), 0.08	1.66 (0.92–2.97), 0.09
AG or GG[c]	161/149	1.18 (0.82–1.70), 0.38	1.22 (0.82–1.83), 0.33
GG versus others[d]	50/34	1.56 (0.97–2.51), 0.07	1.57 (0.92–2.68), 0.10

[a]Adjusted for age, sex, non-steroidal inflammatory drug use, BMI and family history of colorectal cancer.
[b]Additive genetic model.
[c]Dominant genetic model.
[d]Recessive genetic model.

these studies that measured ultraviolet exposure are conflicting, as carriers of the *Cdx-2* A allele with high ultraviolet exposure at increased risk in one study (42) and reduced risk in the other (44). Two other studies found no significant association of *Cdx-2* and prostate cancer risk (43,45). A recent study of squamous cell lung cancer with similar *Cdx-2* allele frequencies reported enhanced survival for carriers of the GA or AA *Cdx-2* genotypes (46), whereas two other studies of skin and ovarian cancer were non-significant for *Cdx-2* (47,48).

A notable finding is the deviation of the *Cdx-2* SNP from Hardy–Weinberg proportions in our case subjects, for an excess of the rare (A) allele. We examined whether age difference between the cases and controls could have explained this deviation, but found no such evidence for an influence by age (data not shown). A departure from Hardy–Weinberg proportions may be explained by population stratification, genotyping error, chance or a true association when the departure is observed with opposite sign in cases and controls. Since our sample is solely Caucasian and our genotype data are of high quality, we believe that population stratification and genotyping error are unlikely explanations. This is further supported by the fact that the other two SNPs examined were consistent with Hardy–Weinberg proportions in both cases and controls in our study population and that for all three SNPs there was no significant population deviation from HWE. The most probable explanation is that our observation represents a real association with *Cdx-2* conferring increased risk for colon cancer in an additive or recessive manner or being in LD with an unknown causal variants (49–51).

The *FokI* SNP (C/T polymorphism) in exon 2 is also of particular interest, owing to its functional significance. The C allele (also referred to as the 'F' allele) results in a *VDR* protein that is shorter by three amino acids than the T allele (f) (49). The more active, shorter protein is thought to result in enhanced *VDR* transcription activity. Consistent with this functional study, our results show that the *FokI* T allele (the minor allele in Caucasians) is associated with increased risk of colon cancer. Others who have analyzed *FokI* genotype variants and risk of adenomas (239 cases and 228 controls) (23) and colon cancer (1174 cases and 1174 controls) (25) found no significant association. We did not find evidence for a statistical interaction of sex and *FokI*, but we did find a strong association of *FokI* and colon cancer in men in stratified analyses. Like adiposity, sex is related to serum (25(OH)D) levels, with women having lower concentrations than men (50). Further replication is needed, as well as future investigation regarding how these functional *VDR* variants correlate with *VDR* expression and

Table III. Association of *VDR* haplotypes and colon cancer risk[a]

	Frequency in cases/controls	Crude OR (95% CI)	Adjusted OR (95% CI)
Cdx-2–FokI			
G-C	0.43/0.49	1.0 (reference)	1.0 (reference)
G-T	0.31/0.31	1.16 (0.84–1.60)	1.16 (0.82–1.64)
A-C	0.15/0.13	1.38 (0.88–2.17)	1.07 (0.64–1.78)
A-T	0.10/0.08	1.51 (0.90–2.54)	1.97 (1.06–3.67)
Cdx-2–TaqI			
G-A	0.45/0.54	1.0 (reference)	1.0 (reference)
G-G	0.28/0.26	1.30 (0.95–1.78)	1.43 (0.99–2.05)
A-A	0.12/0.09	1.58 (0.96–2.60)	1.69 (0.98–2.89)
A-G	0.14/0.11	1.40 (0.94–2.11)	1.27 (0.81–2.00)
FokI–TaqI			
C-A	0.33/0.37	1.0 (reference)	1.0 (reference)
C-G	0.25/0.24	1.12 (0.75–1.67)	1.19 (0.73–1.93)
T-A	0.24/0.26	1.04 (0.72–1.51)	1.21 (0.78–1.88)
T-G	0.17/0.13	1.51 (0.99–2.30)	1.68 (1.04–2.71)
Cdx-2–FokI–TaqI			
G-C-A	0.26/0.31	1.0 (reference)	1.0 (reference)
G-C-G	0.17/0.18	1.15 (0.70–1.88)	1.67 (0.91–3.07)
G-T-A	0.20/0.23	1.06 (0.67–1.67)	1.27 (0.76–2.14)
G-T-G	0.11/0.08	1.73 (0.97–3.08)	1.50 (0.81–2.75)
A-C-A	0.07/0.06	1.60 (0.74–3.44)	1.84 (0.77–4.43)
A-C-G	0.08/0.07	1.40 (0.67–2.91)	0.85 (0.39–1.83)
A-T-A	0.05/0.03	1.61 (0.69–3.76)	1.96 (0.71–5.41)
A-T-G	0.06/0.04	1.51 (0.66–3.47)	3.63 (1.01–13.07)

[a]Adjusted for age, sex, non-steroidal inflammatory drug use, BMI and family history of colorectal cancer.

Table IV. Association of *VDR* SNPs genotypes and colon cancer risk according to BMI category

VDR SNPs under different genetic models	Low BMI (kg/m^2)		High BMI (kg/m^2)	
	Crude OR (95% CI), *P*-value	Adjusted OR[a] (95% CI), *P*-value	Crude OR (95% CI), *P*-value	Adjusted OR[a] (95% CI), *P*-value
Cdx-2 (rs11568820)				
GG (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
GA[b]	1.23 (0.68–2.23), 0.506	1.11 (0.59–2.10), 0.75	0.95 (0.56–1.63), 0.86	0.98 (0.56–1.74), 0.95
AA[b]	3.61 (1.22–10.73), 0.02	3.22 (1.03–10.05), 0.04	1.59 (0.45–5.66), 0.48	1.25 (0.33–4.83), 0.74
GA or AA[c]	1.52 (0.88–2.64), 0.14	1.38 (0.77–2.48), 0.28	1.52 (0.88–2.64), 0.14	1.00 (0.58–1.75), 0.98
AA versus others[d]	3.36 (1.15–9.78), 0.03	3.11 (1.02–9.50), 0.05	1.62 (0.46–5.68), 0.45	1.26 (0.33–4.78), 0.73
P for interaction, *Cdx-2* × BMI (continuous variable) = 0.27				
FokI (rs10735810)				
CC (reference)	1.0 (reference)	1.0 (reference)	0.81 (reference), 0.50	1.0 (reference)
CT[b]	0.58 (0.32–1.06), 0.08	0.61 (0.32–1.15), 0.13	1.14 (0.81–2.46), 0.22	1.46 (0.80–2.66), 0.22
TT[b]	1.30 (0.59–2.87), 0.51	1.33 (0.58–3.03), 0.50	1.80 (0.83–3.91), 0.14	2.54 (1.07–6.00), 0.03
CT or TT[c]	0.72 (0.41–1.27), 0.26	0.76 (0.42–1.38), 0.36	1.50 (0.89–2.54), 0.13	1.65 (0.93–2.92), 0.09
TT versus others[d]	1.79 (0.88–3.63), 0.11	1.76 (0.84–3.70), 0.14	1.48 (0.73–2.99), 0.28	2.02 (0.93–4.43), 0.08
P for interaction, *FokI* × BMI (continuous variable) = 0.25				
TaqI (rs731236)				
AA (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
AG[b]	0.84 (0.47–1.52), 0.57	0.89 (0.48–1.66), 0.71	1.28 (0.73–2.23), 0.39	1.31 (0.72–2.39), 0.37
GG[b]	1.26 (0.58–2.76), 0.56	1.26 (0.55–2.90), 0.58	2.33 (1.09–5.00), 0.03	2.13 (0.92–4.92), 0.08
AG or GG[c]	0.94 (0.51–1.63), 0.83	0.98 (0.55–1.75), 0.95	1.50 (0.89–2.52), 0.13	1.48 (0.84–2.60), 0.17
GG versus others[d]	1.38 (0.68–2.84), 0.38	1.34 (0.63–2.87), 0.45	2.04 (1.02–4.10), 0.045	1.82 (0.85–3.92), 0.12
P for interaction, *TaqI* × BMI (continuous variable) = 0.25				

[a]Adjusted for age, sex, non-steroidal inflammatory drug use and family history of colorectal cancer.
[b]Additive genetic model.
[c]Dominant genetic model.
[d]Recessive genetic model.

how the variants are therefore related to circulating (25(OH)D) levels in men and women.

We observed a significantly increased risk for carriers of the *Cdx-2–FokI* A-T, the *FokI–TaqI* T-G and the *Cdx-2–FokI–TaqI* A-T-G haplotypes, which is consistent with the identified individual SNP genotypic risks. It is important to note that it is unlikely that the departure from Hardy–Weinberg proportions for *Cdx-2* introduced bias during estimation of haplotype frequencies because the excess is toward homozygosity (51). Our haplotype results are not in agreement with those published by Sweeney *et al.* (27), where common haplotypes carrying the *FokI* common C allele (also referred to as F) were associated with increased colon cancer risk. These differences are not easily explained. The *FokI* allele frequencies in our study were exactly the same as reported by Sweeney *et al.* (27) and their study was much larger (1811 cases and 1451 controls). The authors used two different SNPs when constructing their haplotypes (*FokI*, *BsmI* and *polyA* repeat), whereas our haplotypes were constructed using *Cdx-2*, *TaqI* and *FokI*.

The *TaqI* SNP is located at the 3′ end of the gene, and is reported to be in high LD with neighboring polymorphisms including *BsmI* and *ApaI* (52,53). Also nearby is the *polyA* repeat polymorphism, which is also in LD with the four aforementioned SNPs (24,52,54,55). In our study, we did not observe significant associations for the *TaqI* SNP in the overall analysis, but did so in analyses stratified by adiposity. Many studies have analyzed the association of *TaqI* and colon cancer, and also other variants in the 3′ region including *BsmI*, *ApaI* and the *polyA* repeat. Slattery *et al.* (24) was the first group to report that purported low-risk genotypes that included 3′ *VDR* variants were associated with decreased cancer risk in a study of 250 cases and 364 controls. The same group reported an interaction of low-risk *VDR* genotype (*BsmI* and *polyA* variants) with BMI (25), calcium and vitamin D intake (26).

Obese individuals may have lower levels of circulating 25(OH)D than non-obese individuals (56,57), possibly due to sequestration by adipose tissue (58), resulting in a vitamin D-deficient state; therefore, adiposity may modify the relation between *VDR* and colon cancer. Indeed, we found evidence for significant interactions of *Cdx-2* and *TaqI* with BMI and waist circumference, with the highest colon cancer risk associated with *Cdx-2* in the low-adiposity category and increased risk associated with *TaqI* in the high-adiposity category. It is important to note, however, that due to the limited power in our study, these analyses of interactions shall be considered as exploratory only.

In spite of the weak epidemiologic evidence for differences in proximal and distal colon cancers, there is some evidence that these sites are biologically different (59,60). In our study, we found evidence for stronger associations of *VDR* variants with proximal colon cancers, but this may be due to low power to detect associations in the distal group; therefore, further studies are necessary in larger samples.

Weaknesses of our study include potential information bias from the case–control study design and use of self-reported height, weight and waist circumference. However, correlations between self-reported and measured weight are generally quite high (61) and self-reported weight has been shown to be accurately recalled up to 28 years prior in elderly subjects (62). Although we performed several statistical tests, we did not adjust for multiple comparisons here when we stratified our analyses by adiposity because our intent was to generate hypotheses about potential effect modification by adiposity on the association of *VDR* genetic polymorphisms with colon cancer risk and inducing such statistical penalties would inhibit discovery (63). The relatively modest sample size also limited the statistical power to test for interaction; thus, we could not completely rule out the possibility of chance findings for the observed differential associations. Confirmation and replication of our results in larger studies are warranted.

In summary, in this population-based case–control study, we found that *VDR* gene variation, especially the *Cdx-2* and *FokI* SNPs, may influence risk of colon cancer in Caucasians. More research is necessary to confirm these findings and functionally characterize the *VDR* variants. Also, the interaction of *VDR* variants with adiposity is an interesting avenue for further research.

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