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Associations between BMI, energy intake, energy expenditure, *VDR* genotype and colon and rectal cancers (United States)

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Abbreviations: VDR, vitamin D receptor; RFLP, restriction fragment length polymorphisms; 3′ UTR I′, 3′ untranslated region.

Abstract

Components of energy balance are important elements associated with colorectal cancer risk. In this study we examine the association between *VDR* genotypes, BMI, physical activity, and energy intake and risk of colorectal cancer. Data from a population-based case–control study of colon (1174 cases and 1174 controls) and rectal (785 cases and 1000 controls) cancer was used to evaluate the associations. The *Bsm*1, polyA, and *Fok*1 *VDR* polymorphisms were evaluated. For colon cancer, those who are obese were at greater risk of colon cancer if they had the SS or BB (OR = 3.50; 95% CI = 1.75–7.03; *p* interaction 0.03) or *ff* (OR = 2.62; 95% CI = 1.15–5.99; *p* interaction 0.12/) *VDR* genotypes. On the other hand, those who were least physically active were at greater risk of colon cancer if they had the *ff VDR* genotype (OR = 3.46; 95% CI = 1.58–7.58; *p* interaction 0.05. The association between energy intake and colon cancer appears to be driven more by energy intake than *Bsm*1 or polyA *VDR* genotypes, although there was a significant interaction between the *Fok*1 *VDR* polymorphism and energy intake and risk of both colon and rectal cancer (*p* interaction 0.01 for colon and 0.04 for rectal). These data suggest a relationship between *VDR* genotype and factors related to energy balance in modifying colorectal cancer risk.

Energy balance, or the ability to maintain body weight by balancing energy intake with energy expenditure, has been shown to be an important factor in the etiology of colon cancer [1]. Several studies have detected an increased risk of colon cancer associated with increasing body mass index (BMI) primarily among men [2–5] and post-menopausal women who take HRT or are estrogen positive [6, 7]; physical activity has been shown consistently to reduce risk of colon cancer [8–10]. Associations between energy intake and colon cancer are less consistent, with case–control studies showing increased risk while cohort studies generally reporting no association [11–13]. Animal studies show that restricting energy intake reduces tumor development [14, 15]. Data on the effect of energy balance on rectal cancer are less clear, although most studies do not show obesity as being a risk factor for rectal cancer [16, 17].

The vitamin D receptor (VDR)[4] is a nuclear receptor involved in the regulation of many physiological processes, including cell growth and differentiation and metabolic homeostasis [18]. Some studies suggest that VDR also may be involved in insulin and insulin-like growth factor mediated disease pathways [19–21]. Polymorphisms of the *VDR* gene most frequently studied include two restriction fragment length polymorphisms (RFLP's)[4] in intron 8 (*Bsm*I and *Apa*I) and one in exon 9 (*Taq*I). These are in linkage

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disequilibrium with each other and with several 3′ untranslated region (3′ UTR) polymorphisms, including a poly A repeat [22, 23]. The presence of the B, A, and t RFLP alleles (capital letters denote absence of restriction site; small letters presence of restriction site for *Bsm*I, *Apa*I, and *Taq*I RFLP's respectively) and the relatively short poly-A alleles are highly correlated. These alleles, either alone or in combination, have been associated with increased mRNA expression of the *VDR* gene, increased serum levels of 1,25-dihydroxy vitamin D, and increased levels of osteocalcin [24, 25]. *VDR* polymorphisms have been examined in conjunction with colorectal adenomas and cancer and *SS* and *BB* variants have been shown to reduce adenoma/cancer risk [23, 26–29].

At the start site of the gene, a polymorphism detected with a *Fok*I digest also has been studied and has been shown to not be in linkage disequilibrium with the other variants [23]. The polymorphism associated with lack of *Fok*1 digestion (F) changes the start site from the first STG to one three codons downstream; thus the F genotype is associated with a protein that is three amino acids shorter than that associated with the f genotype. The *ff* genotype of the *Fok*I polymorphism has been reported in one study to increase risk of colorectal cancer [28].

Given the involvement of the *VDR* in metabolic homeostasis and insulin-related mechanisms, it is possible that polymorphisms of the *VDR* gene are related to components of energy balance. Studies examining *VDR* polymorphisms with energy balance and cancers of the colon and rectum have not been reported, thus support for examination of the possible association must come from other studies. Studies of bone mineral density and *VDR* gene have shown that those with the bb *VDR* genotype had a greater response to brisk walking than those with the *BB VDR* genotype [30]. Other studies, focusing primarily on bone density, suggest that physical activity may interact with *VDR* genotype to alter fasting glucose levels [31]. Studies also have shown that people with early onset diabetes with the b allele of the *VDR* gene were more likely to be obese than people with the B allele [32]. Studies examining *VDR* genotype and diet have not examined associations with energy intake but one study suggested that dietary fat, a major energy contributing nutrient, may interact with the *Fok*1 polymorphism [28].

Thus, although evidence is limited, it is reasonable to determine if the associations between BMI, physical activity, and energy intake, components of energy balance, and colon and rectal cancer are altered by *VDR* genotype. We hypothesize that people with the L, b, or F alleles of the *VDR* gene will be at greater risk in the presence of obesity, physical inactivity, and high energy intake since these alleles are associated with diabetes. Using data from two large case–control studies of colon and rectal cancer, we determine these associations to obtain a better understanding of the interaction between energy balance and *VDR* genotype.

Methods

Study populations

Participants in the study were from the Kaiser Permanente Medical Care Program of Northern California (KPMCP) and the state of Utah. Two study populations are included in these analyses. The first population includes cases and controls from a population-based cases–control study of first primary colon cancer (ICD-O 2nd edition codes 18.0, 18.2–18.9) diagnosed between October 1, 1991 and September 30, 1994 conducted in both geographic areas. The second population consists of cases with a first primary tumor in the rectosigmoid junction or rectum identified between May 1997 and May 2001 in Utah and KPMC Case eligibility was determined by the Surveillance Epidemiology and End Results (SEER) Cancer Registries in Northern California and in Utah. In both studies, cases were identified using rapid-reporting systems. For both studies, eligibility included being between 30 and 79 years of age at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer [1], and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease.

Controls were matched to cases by sex and by five-year age groups. At the KPMCP, controls were randomly selected from membership lists and in Utah controls 65 years and older were randomly selected from HCFA lists and controls younger than 65 were randomly selected from random digit-dialing and driver's license lists.

Response rates, or the number interviewed over all persons identified, were 71.8% for colon cancer cases and 68.0% for controls selected for the colon cancer study, and 65.2% of cases and 65.3% of controls for the rectal cancer study. Of participants contacted, 80.8% of colon cancer study cases and 71.6% of colon cancer study controls participated. Corresponding participation rates for the rectal cancer study were 73.2% of cases and 68.8% of controls.

Data collection

Data were collected by trained and certified interviewers using laptop computers. Study participants were asked to recall the referent years of two years prior to the date

of selection (the date of diagnosis for cases or date of selection for controls). The interview took approximately 2 h. Data for both the colon and rectal studies were collected using the same questionnaire. Quality control methods used in the study were the same for both the colon and rectal studies and have been described previously in detail [33, 34].

Dietary data
Dietary intake was ascertained using an adaptation of the validated CARDIA diet history [34–36]. Participants were asked to recall foods eaten, the frequency at which they were eaten, serving size, and if fats were added in the preparation. Nutrient information was obtained by converting food intake data into nutrient data using the Minnesota Nutrition Coding Center (NCC) nutrient database.

Height and weight
Height and weight were measured at the time of interview and weight was reported for two and five years prior to referent date. The recalled weight from two years prior to referent date was used to calculate BMI; if weight from two years prior to referent date was missing weight reported for five years prior to referent date was used to calculate BMI. The BMI of weight/height^2 was calculated and used as an estimate of obesity.

Physical activity
Physical activity patterns were determined from a detailed questionnaire that asked about frequency and intensity of activities performed during the referent period and 10 and 20 years prior to the referent date [9]. An indicator of long-term vigorous activity was used to estimate physical activity level since this has been shown to be the most consistent predictor of risk for men and women for both colon and rectal cancer.

Other information
Information also was collected on smoking history, medical history, and regular use of aspirin and non-steroidal anti-inflammatory drugs (NSAIDs). Women also were asked a detailed reproductive history that included current use of hormone replacement therapy.

VDR

Intron 8 BsmI Polymorphism
Genomic DNA was amplified and digested as described previously [23]. Presence of the restriction site was scored as allele 'b', and absence of the restriction site was scored as allele 'B'.

3′UTR poly-A repeat
Genomic DNA was amplified and allele length determined as described previously [23]. Repeat length was classified as short (14–17 repeats) or S, or long (18–22 repeats) or L or as described by Ingles *et al*. [37].

*Fok*I initiation codon polymorphism: Genomic DNA was amplified and digested as described [38] with the following modifications. PCR amplification was performed on 20 ng of genomic DNA in the presence of 10% DMSO by an initial denaturation at 95 °C for two minutes followed by 35 cycles of 95 °C for 10 s, 60 °C for 30 seconds, and 72 °C for 30 seconds. A final five minutes extension at 72 °C was performed.

Statistical methods

SAS statistical package, version 8.2, CA, was used to conduct the analysis. Unconditional logistic regression models were used to estimate risk of rectal cancer from energy intake, energy expenditure, and BMI in combination with *VDR* genotype. Dietary variables were categorized based on the sex-specific control distribution. The B and b *Bsm*I alleles are highly associated with the short (S) and long (L) poly-A alleles, respectively. Since we did not have data on both polymorphisms for all cases, we combined the polymorphism results and report the genotypes as *BB* or *SS*, *bb* or *LL*, or other (most of which are *Bb*/*SL*). Separate analyses of *Bsm*I and poly-A polymorphisms showed no additional associations (data not shown). Among those that had data for both genotypes, concordance between the LL and bb alleles was 97%, between the SS and BB was 95%, and between the LS and Bb was 96%. Analysis also was done for the *Fok1 VDR* polymorphism, evaluating the *FF*, *Ff*, and *ff* genotypes.

Since the population was approximately 87–89% Caucasian, we did not evaluate race-specific associations. For tumor site-specific analyses, sites were defined as proximal (cecum through transverse colon), distal (splenic flexure, descending, and sigmoid colon), and rectal (rectosigmoid junction and rectum). Age-specific analysis was done assessing those diagnosed at age 65 or older and younger than age 65. Sex-specific analysis for men and women was done. Assessment of BMI was done stratifying data by gender as well as by estrogen status. Since results were similar for men and estrogen-positive women, data are presented for those groups combined.

In logistic models variables included as potentially confounding factors were: age at selection, sex, BMI, long-term vigorous physical activity, energy intake, dietary calcium, and dietary fiber. Adjustment for dietary fat and center did not alter observed

associations. All adjustment variables were treated as continuous variables in the model except for use of NSAIDS that was categorized as a dichotomous variable. Since approximately 87–89% of the population was Caucasian, we did not stratify by race. Interactions between dietary variables and *VDR* genotype were assessed both on multiplicative and additive scale. The relative excess risk from interaction (RERI) was assessed [39] to determine additive interaction; the cross-product of BMI, diet, and physical activity with VDR was used to estimate multiplicative interaction. The Wald χ^2 test of difference between slopes also was assessed to determine changes in ORs holding *VDR* genotype constant and varying dietary factors. A total of 1346 colon cancer cases and 1544 controls were interviewed between February 1991 and May 1994 and 952 rectal cancer cases and 1205 controls were interviewed between October 1997 and January 2002; we were able to obtain DNA. Of these, we were able to obtain VDR analysis for 1956 colorectal cases and 2174 controls. Slightly fewer individuals had the *Fok*1 *VDR* polymorphism assessed because of limited DNA available for analysis. Previous analyses of those with and without DNA have not shown any differences in demographic or study characteristics [40]. Numbers for some variables may vary because of missing values.

Results

In both the colon and rectal cancer studies, the majority of people were over 60 years of age at time of diagnosis, were mainly non-Hispanic white (89% of colon controls and 86% of rectal controls), and there was a slight excess of men (roughly 56%) (Table 1). The *SS* or *BB VDR* genotype was seen in roughly 19% of controls from the colon cancer study and 16% of controls from the rectal

Table 1. Description of the population

	Colon			Rectal		
	Cases N(%)	Controls N(%)		Cases N(%)	Controls N(%)	
Age						
30–39	13 (1.1)	20 (1.7)		20 (2.6)	22 (2.2)	
40–49	77 (6.6)	87 (7.4)		98 (12.5)	107 (10.7)	
50–59	230 (19.6)	196 (16.7)		206 (26.2)	247 (24.7)	
60–69	404 (34.4)	409 (34.8)		266 (33.9)	346 (34.6)	
70–79	450 (38.3)	462 (39.4)		195 (24.8)	278 (27.8)	
Ethnicity						
White	1020 (87.0)	1047 (89.3)		644 (83.7)	851 (86.1)	
Hispanic	72 (6.1)	73 (6.2)		53 (6.8)	66 (6.7)	
African-American	80 (6.8)	52 (4.4)		30 (3.9)	40 (4.0)	
Asian	0	0		38 (4.9)	30 (3.0)	
Native American	0	0		10 (1.3)	2 (0.2)	
Gender						
Men	657 (56.0)	646 (55.0)		464 (59.1)	566 (56.6)	
Women	517 (44.0)	528 (45.0)		321 (40.9)	434 (43.4)	
Center						
Kaiser	857 (73.0)	807 (68.7)		499 (63.6)	613 (61.3)	
Utah	317 (27.0)	367 (31.3)		268 (36.4)	387 (38.7)	
VDR						
LL or *bb*	458 (39.0)	429 (36.5)		320 (40.8)	383 (38.3)	
Mostly *SL* and *Bb*	535 (45.6)	521 (44.4)		341 (43.4)	453 (45.3)	
SS or *BB*	181 (15.4)	224 (19.1)		124 (15.8)	164 (16.4)	
ff	115 (12.3)	148 (13.5)		101 (13.4)	126 (13.1)	
Ff	420 (44.8)	540 (49.3)		367 (48.8)	452 (47.1)	
FF	403 (43.0)	407 (37.2)		284 (37.8)	382 (39.8)	
	Mean (SD)	Mean (SD)	*p*-value[a]	Mean (SD)	Mean (SD)	*p*-value
Energy (kcal)	2502 (1190)	2370 (1147)	< 0.01	2750 (1446)	2591 (1231)	< 0.01
PAL (hours vigorous activity/Wk)	2.18 (3.77)	2.56 (4.10)	0.02	2.53 (3.67)	3.07 (4.49)	< 0.01
BMI (kg/m^2)	27.7 (5.3)	26.9 (5.0)	< 0.01	27.8 (5.6)	27.4 (4.8)	0.12

cancer study. Both the *SS* and *BB* genotypes were inversely associated with colon cancer relative to the *LL* and *bb* genotypes (OR = 0.79; 95% CI = 0.56–0.96 and OR = 0.84; 95% CI = 0.70–1.02 respectively). The *ff Fok*1 genotype was present in roughly 13% of cases in both the colon and rectal cancer studies. Those with the *FF* genotype were more likely to develop colon cancer than those with the *FF* genotype (OR = 1.28; 95% CI = 0.97–1.69). In both colon and rectal cancer studies, cases ate significantly more calories and performed less vigorous physical activity. BMI was significantly higher among colon cancer cases compared to age and gender matched controls.

There was a significant interaction between BMI and *Bsm*1/polyA *VDR* genotype for colon cancer but not rectal cancer (RERI *p*-value = 0.06; multiplicative *p*-value = 0.03; Wald χ^2 = 0.04) (Table 2). The *SS*/*BB VDR* genotypes (S and B alleles are in linkage disequilibrium; see Methods) were associated with a significant increase in colon cancer risk among obese individuals (OR = 3.50; 95% CI = 1.75–7.03). A similar interaction was not observed for BMI and *VDR* genotype for rectal cancer. The *Fok*1 *VDR* genotype did not interact significantly with BMI.

There was no significant interaction between the BSMI/polyA *VDR* genotypes and physical activity for colon cancer, although for rectal cancer there was a borderline significant interaction (*p* for multiplicative interaction = 0.08; Wald χ^2 for difference in slopes 0.02) (Table 3). Those at greatest risk of colon cancer from having no vigorous physical activity were those with the SS or BB genotypes. There was a significant multiplicative interaction between physical activity level and the *Fok*1 *VDR* genotype and colon cancer (*p* = 0.05; Wald χ^2 for difference in slopes = 0.08). Those who reported high levels of physical activity were at a two-fold increased risk of developing colon cancer if they had the *FF VDR* genotype, while those with the *ff VDR* genotype and no long-term vigorous physical activity were at over a three-fold increased risk of both colon and rectal cancer.

There was no significant interaction between the BSMI and polyA *VDR* genotypes and energy intake for either colon or rectal cancer (Table 4) while there was a significant multiplicative interaction for energy intake, *Fok*1 *VDR* genotype and colon cancer (*p* = 0.01) and a significant additive interaction between energy intake, *Fok*I genotype, and rectal cancer (*p* = 0.04). For colon

Table 2. Associations between BMI and *VDR* genotype and coloteral cancer in men and women

	VDR genotype					
	SS/*BB*	Mostly *SL*/*Bb* N (cases/controls)	*LL*/*bb*	*SS*/*BB* OR (95% CI)[b]	Mostly *SL*/*Bb* OR (95% CI)	*LL*/*bb* OR (95% CI)
Colon cancer[a]						
BMI						
< 25	42/61	112/155	99/116	1.00	1.05 (0.66–1.68)	1.27 (0.78–2.05)
25–29	53/86	166/155	144/143	0.87 (0.51–1.47)	1.55 (0.98–2.44)	1.39 (0.88–2.21)
≥ 30	42/17	109/83	81/61	3.50 (1.75–7.03)	1.80 (1.10–2.95)	1.74 (1.03–2.93)
P interaction (REEI, multiplicative, Wald χ^2): 0.06, 0.03, 0.04						
	ff	*fF*	*FF*	*ff*	*fF*	*FF*
< 25	22/43	92/158	85/110	1.00	1.09 (0.61–1.94	1.40 (0.78–2.54)
25–29	29/54	136/174	124/124	0.95 (0.47–1.89)	1.41 (0.81–2.50)	1.71 (0.96–3.05)
≥ 30	24/16	81/63	90/76	2.62 (1.15–5.99)	2.26 (1.22–4.19)	1.94 (1.06–3.56)
P interaction (RERI, multiplicative, Wald χ^2): 0.39, 0.12, 0.11						
Rectal cancer						
BMI	*SS*/*BB*	Mostly *SL*/*Bb*	*LL*/*bb*	*SS*/*BB*	Mostly *SL*/*Bb*	*LL*/*bb*
< 25	38/39	105/158	116/131	1.00	0.72 (0.43–1.21)	0.90 (0.54–1.51)
25–29	52/79	140/172	121/175	0.68 (0.38–1.20)	0.87 (0.52–1.44)	0.69 (0.41–1.15)
≥ 30	34/46	96/123	83/77	0.75 (0.40–1.42)	0.80 (0.47–1.35)	1.02 (0.58–1.76
P interaction (RERI, multiplicative, Wald χ^2): 0.07, 0.54, 0.50						
	ff	*ff*	*FF*	*ff*	*ff*	*FF*
< 25	29/40	121/149	98/131	1.00	1.03 (0.60–1.78)	0.96 (0.55–1.66)
25–29	40/47	154/203	110/160	1.09 (0.57—2.08)	0.97 (0.57–1.65)	0.87 (0.50–1.49)
≥ 30	32/39	92/100	76/91	1.05 (0.54—2.07)	1.11 (0.63–1.95)	1.01 (0.56–1.79)
P interaction (RERI, multiplicative, Wald χ^2): 0.99, 0.78, 0.88						

[a] Colon cancer associations include only men and estrogen positive women no associations were made detected for estrogen negative women.
[b] Adjusted for age, sex, physical activity, energy intake, dietary fiber and calcium.

Table 3. Associations between Long-term Vigorous activity, *VDR* genotype, and colorectal cancer

	VDR genotype					
	SS/BB	Mostly *SL/Bb*	*LL/bb*	*SS/BB*	Mostly *SL/Bb*	*LL/bb*
		N (cases/controls)		OR (95% CI)[a]	OR (95% CI)	OR (95% CI)
Colon cancer						
Physical activity						
High	35/51	102/129	85/103	1.00	1.12 (0.67–1.87)	1.19 (0.70–2.01)
Intermediate	103/125	274/279	245/238	1.16 (0.70–1.94)	1.40 (0.87–2.23)	1.40 (0.87–2.26)
None	42/48	156/112	127/87	1.20 (0.65–2.20)	1.94 (1.17–3.21)	1.98 (1.18–3.33)
p interaction (RERI, multiplicative, Wald χ^2): 0.53, 0.83, 0.67						
	ff	*fF*	*FF*	*ff*	*fF*	*FF*
High	15/39	82/135	87/98	1.00	1.64 (0.84–3.18)	2.30 (1.18–4.49)
Intermediate	15/39	82/135	87/98	2.06 (1.03-4.11)	2.01 (1.07–3.78)	2.44 (1.29–4.59)
None	36/28	119/113	105/92	3.46 (1.58–7.58)	2.72 (1.40–5.26)	2.82 (1.44–5.51)
p interaction (RERI, multiplicative, Wald χ^2): 0.70, 0.05, 0.08						
Rectal cancer	*SS/BB*	Mostly *SL/Bb*	*LL/bb*	*SS/BB*	Mostly *SL/Bb*	*LL/bb*
Physical activity						
High	27/55	94/160	83/93	1.00	1.25 (0.73–2.12)	1.85 (1.06–3.21)
Intermediate	69/78	169/233	158/225	1.84 (1.04–3.26)	1.52 (0.92–2.53)	1.40 (0.85–2.34)
None	28/30	74/58	78/63	1.91 (0.95–3.85)	2.72 (1.40–5.26)	2.82 (1.44–5.51)
p interaction (RERI, multiplicative, Wald χ2): 0.32, 0.08, 0.02						
	ff	*fF*	*FF*	*ff*	*fF*	*FF*
High	20/38	101/132	72/127	1.00	1.45 (0.79–2.65)	1.05 (0.57–1.94)
Intermediate	53/71	176/254	152/189	1.44 (0.75–2.77)	1.25 (0.70–2.23)	1.48 (0.82–2.66)
None	27/17	86/63	60/64	3.10 (1.37–7.05)	2.58 (1.37–4.90)	1.72 (0.89–3.30))
p interaction (RERI, multiplicative, Wald χ^2): 0.33, 0.87, 0.15						

[a] Adjusted for age, sex, BMI, energy intake, dietary fiber and calcium.

cancer the greatest risk was associated with high levels of energy intake that was slightly greater for those with the *ff VDR* genotype. For rectal cancer, low energy intake was associated with reduced risk of rectal cancer among those with the FF genotype compared to those with the ff genotype (RERI p value = 0.04).

Further assessment of the colorectal cancer risk associated with the *VDR* genotype was done by level of activity, BMI, and energy intake. There were few clear differences in *VDR* genotype by level of physical activity and BMI, however it appears that rectal cancer risk was reduced among those with the *SS/BB VDR* genotypes who reported low energy intake (Table 5). Both the *SS/BB* and *ff VDR* genotypes reduced risk of proximal tumors among those with high energy intake; the *ff* genotype also was associated with reduced risk of distal tumors among those with high energy consumption.

Discussion

These data provide some support for an interaction between the *VDR* genotype and components of energy balance. For colon cancer, those who are obese were at greater risk of colon cancer if they had the *SS, BB, or ff VDR* genotypes. On the other hand, those who were least physically active were at greater risk of rectal cancer if they had the *SL*, *Bb*, *LL*, *bb, or ff VDR* genotypes. For rectal cancer it appeared that those with the *ff VDR* enotype were at greatest risk if they were sedentary. The association between energy intake and colon cancer appears to be driven more by energy intake than *VDR* genotype, although there was a significant interaction between the *Fok*1 *VDR* polymorphism and both colon and rectal cancer, again with the greatest risk associated with the *ff* genotype.

Studies in animals suggest that low levels of energy intake reduce tumor incidence and development [41]. Epidemiological studies further suggest that energy balance, or the maintaining equilibrium between energy intake and expenditure, is important in the development of many cancers including colon cancer [42, 43]. However, the relevant physiological mechanisms associated with energy balance and cancer risk are less clear. Possibilities include that energy balance results in reduced endogenous free radical formation and oxidative damage, enhanced DNA repair, enhanced immune response, alterations in the activity of carcinogen-metabolizing enzymes, or alterations in endogenous hormone metabolism [43]. Of these hypothesized mech-

Table 4. Associations between energy intake, *VDR* genotype, and colorectal cancer

	VDR genotype					
	SS/*BB*	Mostly *SL*/*Bb*	*LL*/*bb*	*SS*/*BB*	Mostly *SL*/*Bb*	*LL*/*bb*
	N (cases/controls)			OR (95% CI)[a]		
Colon cancer						
Energy intake						
Low	55/76	170/185	130/144	1.00	1.29 (0.86–1.93)	1.28 (0.84–1.96)
Intermediate	65/79	179/174	142/151	1.32 (0.81–2.15)	1.63 (1.08–2.46)	1.46 (0.96–2.24)
High	60/69	183/161	185/133	1.71 (1.01—2.90)	2.19 (1.40–3.43)	2.69 (1.71–4.23)
p interaction (RERI, multiplicative, Wald χ^2): 0.54, 0.52, 0.38						
	ff	*fF*	*FF*	*Ef*	*fF*	*FF*
Low	37/58	112/190	124/132	1.00	0.95 (0.59–1.53)	1.51 (0.93–2.44)
Intermediate	35/53	147/181	138/142	1.17 (0.64–2.13)	1.45 (0.90–2.34)	1.69 (1.05–2.74)
High	42/37	160/167	140/133	2.48 (1.30–4.71)	2.07 (1.24–3.45)	2.23 (1.33–3.74)
p interaction (RERI, multiplicative, Wald χ^2): 0.63, 0.01, 0.22						
Rectal cancer						
Energy intake	*SS*/*BB*	Mostly *SL*/*Bb*	*LL*/*bb*	*SS*/*BB*	Mostly *SL*/*Bb*	*LL*/*bb*
Low	21/45	100/128	75/119	1.00	1.78 (0.99–3.19)	1.77 (0.99–3.19)
Intermediate	40/50	102/136	90/117	1.77 (0.90–3.40)	1.71 (0.95–3.08)	1.72 (0.95–3.11)
High	63/68	136/188	133/146	2.19 (1.14–4.20)	1.70 (0.93–3.12)	2.04 (1.11–3.74)
p interaction (RERI, multiplicative, Wald χ2): 0.59, 0.24, 0.43						
	Ff	*fF*	*FF*	*ff*	*fF*	*FF*
Low	37/34	100/128	75/119	1.00	0.73 (0.43–1.25)	0.58 (0.33–1.01)
Intermediate	25/34	109/147	87/108	0.70 (0.34–1.41)	0.69 (0.40–1.18)	0.75 (0.43–1.31)
High	38/58	154/174	122/153	0.62 (0.32–1.20)	0.84 (0.48–1.47)	0.77 (0.43–1.35)
p interaction (RERI, multiplicative, Wald χ2): 0.04, 0.71, 0.14						

[a] Adjusted for age, sex, BMI, physical activity, dietary fiber and calcium.

anisms, those related to hormone metabolism, both sex steroid metabolism and insulin and insulin-like growth factors (IGF), have received the most attention, especially in conjunction with colon cancer.

Data have shown that estrogen and hormone replacement therapy (HRT) are involved in colon cancer etiology [44]. Studies have shown that estrogen and HRT also may modify the effect of BMI on colon cancer risk in women [6, 7]. Other studies suggest the importance of insulin and IGF in colon cancer etiology [45–47] and the association of obesity with insulin levels [48, 49]. Components of energy balance, mainly obesity, physical activity, and energy intake, may be further regulated by genetic factors thought to influence endogenous hormone metabolism. The *VDR* gene, given its role in metabolic regulation and its association with insulin, is therefore one gene that may modify the effects of obesity, physical activity, and energy intake on risk of colorectal cancer [19, 20, 50].

Some studies suggest that obesity may be associated with *VDR* genotype. Among people with early age onset of type 2 diabetes, those with the bb *VDR* alleles had more obesity [32]. Another study did not show a relationship between *VDR* genotype and body size [51]. Our data suggest that those with the *SS* or *BB* *VDR* alleles who are obese are at an increased risk of colon cancer.

Studies of the associations between *VDR* genotype and physical activity also are limited, but have shown that people with the *bb VDR* genotype respond to physical activity in relationship to bone density more than those with the *BB VDR* genotype [52]. Another study showed that men who were inactive and had the *BB VDR* genotype were more likely to have higher fasting glucose levels than men with other *VDR* genotypes [31]. Our data suggest the importance of physical activity on reducing risk of colorectal cancer, but also suggest risk associated with lack of physical activity was offset by those with the *SS* or *BB VDR* genotypes. On the other hand, the *ff* genotype was protective against colon cancer amongst those with high levels of physical activity, but was deleterious in the sedentary. The *ff* genotype also was associated with the highest risk of rectal cancer in the sedentary.

There is some indication of site-specific associations, especially at different levels of energy intake. In these data those at low levels of energy intake have reduced risk of rectal cancer if they also have the *SS* or *BB VDR* genotype; those with high energy intake and the *SS*, *BB*, or *ff VDR* genotypes were at reduced risk of proximal

Table 5. Colorectal cancer risk associated with *VDR* genotype by BMI, PAL, and KCAL

VDR genotype	Low KCAL			High KCAL		
	LL/*bb*	Mostly *SL*/*Bb*	*SS*/*BB*	*LL*/*bb*	Mostly *SL*/*Bb*	*SS*/*BB*
Proximal						
N (cases/controls)	99/387	141/475	45/189	115/425	113/499	38/199
OR (95% CI)	1.00	1.14 (0.86–1.53)	0.92 (0.62–1.36)	1.00	0.84 (0.63–1.12)	0.69 (0.46–1.03)
Distal						
N (cases/controls)	93/387	131/475	42/189	136/425	131/499	54/199
OR (95% CI)	1.00	1.15 (0.86–1.55)	0.93 (0.62–1.39)	1.00	0.82 (0.63–1.08)	0.83 (0.58–1.19)
Rectal						
N (cases/controls)	142/387	149/475	41/189	178/425	192/499	83/199
OR (95% CI)	1.00	0.87 (0.66–1.13)	0.60 (0.41–0.89)	1.00	0.92 (0.72–1.18)	1.04 (0.76–1.42)
	FF	*Ff*	*ff*	*FF*	*Ff*	*ff*
Proximal						
N (cases/controls)	99/374	90/487	35/130	88/415	109/505	23/144
OR (95% CI)	1.00	0.69 (0.51–0.95)	1.02 (0.66–1.58)	1.00	1.03 (0.76–1.41)	0.74 (0.45–1.22)
Distal						
N (cases/controls	88/374	97/487	27/130	117/415	109/505	27/144
OR (95% CI)	1.00	0.85 (0.62–1.17)	0.89 (0.55–1.42)	1.00	0.78 (0.58–1.04)	0.65 (0.41–1.04)
Rectal						
N (cases/controls)	117/374	153/487	52/130	167/415	214/505	49/144
OR (95% CI)	1.00	1.03 (0.78–1.36)	1.30 (0.89–1.91)	1.00	1.05 (0.83–1.34)	0.87 (0.60–1.26)

[a] Adjusted for age, sex, and calcium intake.

and distal (*ff* genotype only) tumors. Wong and colleagues [28] have reported similar associations for dietary fat in conjunction with the *Fok*I *VDR* polymorphism; those with the *ff* genotype were found to be at greater risk of colorectal cancer when dietary fat intake was low, but not at risk in the presence of high dietary fat. Adjustment for total fat did not alter associations in the current study.

An intriguing pattern appears to be developing with respect to *VDR* genotypes and colorectal cancer risk. Overall there is a weak protective effect associated with the *BB*/*SS* and *ff* genotypes. Also, in a previous study we detected an interaction between the *Bsm*I/polyA *VDR* genotype and dietary calcium and vitamin D, but any decreased risk of cancer was always associated with the *BB*/*SS* genotype [53]. In the current study there are also many contexts (such as decreased BMI or high physical activity) in which the *SS*/*BB* or *ff* genotypes are protective, but there are other contexts (obesity, sedentary life style, high energy intake, either alone or in combination) in which these genotypes are associated with the highest risk of colonic or rectal cancer. The fact that the in some contexts these genotypes are protective and in other contexts they increase risk suggests the possibility that VDR may be acting through more than one pathway to influence carcinogenesis. The precise pathway and the mechanism by which the polymorphisms exert effects is difficult to be sure of at this time, as very little is known about all of the activities of *VDR* and how the polymorphisms affect these activities. *VDR* is known to influence calcium metabolism, however, and recent studies have suggested that some of its anticarcinogenic activity may be related to binding to bile acids. One could speculate, then, that under conditions of relatively good energy balance the *BB*/*SS* and/or *ff* genotypes affect *VDR*'s actions in calcium/vitamin D related pathways to decrease the risk of cancer, while under conditions of poor energy balance these genotypes affect *VDR*'s impact on bile acids or insulin-related pathways. Differences in association by polymorphism examined may provide clues to functionality of the relevant polymorphisms.

This study has several strengths, including the quality of the data collected and the large sample size available for analysis. Because of the sample size, we have been able to assess both site-specific and sex-specific associations. However, even with our large sample size, we have limited power to look at both sex- and site-specific associations simultaneously or with combinations of BMI, level of activity, and energy intake. Dietary data and physical activity data were collected using detailed and validated questionnaires as described in the methods. This is one of the first reports to look at multiple polymorphisms of the *VDR* gene and components of energy balance. Our data suggest that not all polymorphisms work in the same manner. The *Fok*I polymorphism is not in linkage disequilibrium with the *Bsm*I and polyA polymorphisms and may operate differently than *Bsm*I and polyA polymorphisms. With respect to cancer risk, the

*Bsm*I and polyA polymorphisms appeared to be more associated with BMI, while the *Fok*I appeared to be more associated with physical activity; both polymorphisms appeared to be associated with energy intake. The reasons for these differences are not well understood, as at the present time the impact of the various polymorphisms on the perhaps multiple activities of *VDR* is not completely understood.

In summary, these data provide some support for an interaction between obesity and *VDR* genotype in risk associated with colon cancer, suggesting some possible genetic regulation of energy balance as it relates to colon cancer. However, it appears that physical activity and energy intake may be less influenced by *VDR* genotype as they relate to colorectal cancer risk. Energy intake, on the other hand, may modify the effects of *VDR* genotype of rectal cancer risk

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