Vitamin D Receptor Polymorphism and the Risk of Colorectal Adenomas: Evidence of Interaction with Dietary Vitamin D and Calcium[1]

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Abstract

Laboratory studies and epidemiological investigations suggest that vitamin D plays a role in the etiology of colorectal adenomas, possibly through a mechanism mediated by the vitamin D receptor (VDR). We conducted a clinic-based case-control study to examine the association between VDR polymorphisms and colorectal adenomas. We selectively identified a random subset of 393 cases of colorectal adenomas and 406 colonoscopy-negative controls from a clinic-based case-control study conducted in the metropolitan Minneapolis/St. Paul area during 1991–1994. A self-administered questionnaire was used to collect data on dietary and supplement intake of vitamin D and calcium, as well as on demographics, physical activity, medical information, lifestyle factors, reproductive history, and anthropometry. DNA was extracted from whole blood and assayed for the *Bsm*I VDR polymorphism using an ABI 7700 TaqMan assay. Adjusted odds ratios (OR) and 95% confidence intervals (CIs) were evaluated using logistic regression. Compared with the *bb* genotype (33% of controls), neither the *Bb* (48.8% of controls) nor the *BB* (18.2% of controls) genotypes was strongly associated with risk of colorectal adenomas (OR = 0.86, CI = 0.63–1.19 and OR = 0.77, CI = 0.50–1.18, respectively). However, those with the lowest tertile of vitamin D intake and the *BB* genotype had a lower risk of colorectal adenoma (OR = 0.24, CI = 0.08–0.76) than those with the highest tertile of intake and the *bb* genotype. Similarly, those with the lowest tertile of calcium intake and the *BB* genotype had a reduced risk of colorectal adenoma (OR = 0.34, CI = 0.11–1.06). Although it has generally been shown that higher calcium and vitamin D intake are associated with a modestly reduced risk of colorectal neoplasia, our data suggest that those with the *BB Bsm*I VDR genotype may be at reduced risk of colorectal adenoma in the presence of lower calcium and vitamin D intake.

Introduction

Vitamin D is a hormone that has essential roles in endocrine functions and in regulating cell replication. It is critical in maintaining calcium homeostasis as well as in bone metabolism (1). The active metabolite of vitamin D [1,25$(OH)_2D_3$] has also been shown to regulate cell proliferation and differentiation in human colon cancer cell lines (2–8). Furthermore, the application of vitamin D analogues has inhibited colonic tumor formation *in vivo* and reversed malignant colon cancer cells *in vitro* to a normal morphological phenotype (9, 10).

Two mechanisms may explain the actions of 1,25$(OH)_2D_3$ on colorectal cancer cells. The first involves direct nongenomic actions of 1,25$(OH)_2D_3$ on calcium homeostasis. The second involves the genomic actions of 1,25$(OH)_2D_3$, mediated through the intracellular VDR.[3] Laboratory studies have shown that 1,25$(OH)_2D_3$ has antiproliferative actions on colorectal cancer cell growth and differentiation and that these actions only occur in those cell lines that express VDR (3, 7). However, a common polymorphism in the *VDR* gene may alter this relationship. Polymorphisms in the 3′-UTR region of the *VDR* gene alter transcriptional activity and mRNA stability in minigene reporter constructs (11). One of these polymorphisms, *Bsm*I, is located in intron 8 of the *VDR* gene (11). It is thought that the 3′-UTR region of the *VDR* gene is involved in the regulation of mRNA stability, and, therefore, polymorphisms in this region are involved in the degradation of the VDR mRNA and consequently reduce receptor density (12). There is also some speculation that these polymorphisms are in disequilibrium with other mutations that alter VDR function (13). In addition, the VDR also regulates vitamin D and calcium metabolism through a complex series of pathways and feedback loops. Bone mineral studies (11, 14) as well as studies on the association between prostate cancer risk and serum vitamin D levels (15) have demonstrated that *Bsm*I genotypes have distinct phenotypic characteristics in relation to calcium and vita-

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[3] The abbreviations used are: VDR, vitamin D receptor; UTR, untranslated region; TAMRA, 6-carboxytetramethylrhodamine; OR, odds ratio; CI, 95% confidence interval; BMI, body mass index; HRT, hormone replacement therapy; FFQ, food frequency questionnaire.

min D metabolism. This suggests a role for dietary calcium and vitamin D as effect modifiers of the association between VDR polymorphisms and colorectal neoplasia.

We investigated the relationships among VDR polymorphisms, vitamin D, and calcium in modulating risk of colorectal adenomas, the precursor lesions of colorectal cancer (16). This analysis is part of a clinic-based case-control study on the genetic epidemiology of colorectal neoplasia; the focus here is on the role of *Bsm*I VDR polymorphism, dietary vitamin D, and dietary calcium on colorectal adenoma risk.

Materials and Methods

Study Subjects. Subject recruitment for this case-control study has been described previously (17). Briefly, cases and controls were recruited through a large multiclinic private gastroenterology practice in the greater metropolitan Minneapolis/St. Paul area, Digestive Healthcare. All of the patients who were scheduled for colonoscopy at Digestive Healthcare clinics between April 1991 and April 1994 were screened for specific eligibility criteria and recruited for the study before colonoscopy. Eligibility criteria were: first diagnosis of incident colon or rectal adenomatous polyps; resident of Minneapolis/St. Paul metropolitan area; age 30–74 years; English-speaking; no known genetic syndrome associated with predisposition to colonic neoplasia; no personal history of cancer (except nonmelanoma skin cancer); and no history of inflammatory bowel disease.

If eligible, subjects were sent material describing the study and a self-administered questionnaire (including a FFQ) before their clinic visit. At colonoscopy, the signed consent form and completed questionnaires were collected, and blood was drawn. Both investigators and subjects were blind to the final diagnosis. The colonoscopy findings were recorded on standardized forms. Only participants with a complete colonoscopy reaching the cecum were eligible.

Data Collection. Information on physical activity, smoking habits, anthropometry, medical information, demographics, reproductive history (women), and family history of polyps and cancer was collected using the self-administered questionnaire. The dietary questionnaire was an adaptation of the Willett semiquantitative FFQ, which has been studied for validity and repeatability in a number of earlier studies (18–20). The questionnaire also contained queries about the brand and frequency of consumption of breakfast cereal and the brand and frequency of consumption of multivitamin and individual vitamin supplement use. Total daily nutrient intake incorporated multivitamin and individual supplement use in addition to dietary intake. Study staff followed up by phone when data were incomplete.

Pathology. On removal, any polyps detected were examined histologically by the study pathologist using diagnostic criteria established for the National Polyp Study (21). On the basis of pathology findings, participants were assigned to one of the following three groups: (*a*) adenomatous polyp group (defined as either adenomatous or mixed pathology; $n = 575$); (*b*) hyperplastic polyp-only group ($n = 219$); and (*c*) colonoscopy-negative group (controls, $n = 708$). For this analysis, participants with polyps showing invasive carcinoma were excluded, as were those in the hyperplastic group. The participation rate for all of the eligible, colonoscoped patients was 68%. In this study, a total of 394 cases were randomly drawn from the adenomatous polyp group, and 406 controls were randomly selected from the colonoscopy-negative group, for a total of 800 subjects.

Blood Collection and Processing. A venous blood sample was collected at the clinic on the day of the appointment before colonoscopy. White cells were stored in appropriate cell culture medium at −70°C for DNA extraction. DNA was extracted from buffy coats at the Core Laboratory of the Public Health Science Division of the Fred Hutchinson Cancer Research Center using the Pure Gene DNA isolation kit (Gentra Systems Inc., Minneapolis, MN). DNA was quantitated and examined for purity by UV absorption at 260 and 280 nm (22).

***Bsm*I Genotyping.** Determination of the VDR *Bsm*I polymorphism was conducted by the Molecular Biomarkers Laboratory in the Center for Ecogenetics and Environmental Health at the University of Washington, Seattle, WA. This *Bsm*I polymorphism is a C to T base substitution (nucleotide 47; GenBank accession no. S82984) and was determined using a new 5′ nuclease assay that uses specific fluorogenic TaqMan probes. A 400-bp region surrounding this variant site was imported into PrimerExpress software (PE/Applied Biosystems, Foster City, CA), and specific PCR primers as well as the corresponding allelic probes were determined using this software package. The PCR primers were: (sense) 5′-GAGCCCAGTTCACGCAAGAG-3′; and (antisense) 5′-GGGGGGATTCTGAGGAACTAGATA-3′. Both fluorogenic wild-type (*b* allele) and variant (*B* allele) allele- specific probes were complementary to their corresponding antisense strands and were both 3′ labeled with the TAMRA quencher dye. In addition, the specific wild type and variant probes were 5′ labeled with the 6-FAM reporter dye (6-FAM-5′-ACAGGCCTGCGCATTCCCATT-3′-TAMRA) and the VIC reporter dye (VIC-5′-ACAGGCCTGCACATTCCCATT-3′-TAMRA), respectively. This fluorescent 5′ nuclease assay was performed on an ABI PRISM 7700 Sequence Detection System (PE/Applied Biosystems). Both the sense and antisense PCR primers were used at a final concentration of 0.2 μM in a 3 mM $MgCl_2$ PCR solution. The wild-type and variant fluorogenic probes were used at a final concentration of 0.05 mM and detected alleles from 30 ng of genomic DNA template. Thermocycling parameters started with an initial denaturation step of 94°C for 10 min followed by 40 cycles of 94°C for 20 s and 62°C for 20 s.

Laboratory personnel were blinded to disease status of the participants. Random blinded repeats of 10% of the 799 samples genotyped ($n = 80$) yielded a reproducibility of 100%. DNA quality was insufficient for *Bsm*I genotyping in 1 case; therefore, the final study population consisted of 393 cases and 406 controls.

Statistical Data Analysis. Unconditional logistic regression was used to estimate the OR and 95% CI for the *Bsm*I VDR genotype (*bb* genotype, *Bb* genotype, and *BB* genotype) and incidence of colorectal adenomas (23). The following potential confounding factors were evaluated: age (continuous), sex, HRT (ever/never), regular use of aspirin (yes/no, at least once/week), regular use of nonsteroidal anti-inflammatory drugs (yes/no, at least once/week), metabolic rate hours of physical activity (continuous), smoking (never/ever/current), BMI (kg/m^2), use of oral contraceptives (ever/never), alcohol consumption (never/ever/current), total caloric intake (continuous), total dietary fiber intake (continuous), total folate intake (continuous), and percentage calories from fat (continuous). The choice of variables for the final multivariate logistic model was based on a 10% change in the OR and included the following covariates: age, sex, BMI, smoking, HRT, and total caloric intake.

The association between the *Bsm*I VDR genotype and incidence of colorectal adenomas was evaluated first in the entire population and then in subpopulations stratified by sex, location of largest adenoma, number of polyps, age, and family

Table 1 Selected characteristics of the study population by cases and controls

	Cases (n = 393)	Controls (n = 406)	P
Location of largest adenoma[a]			
Proximal	83 (21.3%)		
Distal	245 (63.0%)		
Rectum	61 (15.7%)		
Number of polyps[a]			
1	186 (47.3%)		
≥2	207 (52.7%)		
*Bsm*I polymorphism genotype[a]			
bb	144 (36.6%)	134 (33.0%)	
Bb	189 (48.1%)	198 (48.8%)	
BB	60 (15.3%)	74 (18.2%)	0.402
Sex[a]			
Men	215 (54.7%)	208 (51.2%)	
Women	178 (45.3%)	198 (48.8%)	0.325
Age (yr)			
Mean	57.98 ± 9.71	53.04 ± 10.96	
Range	(31–74)	(30–74)	0.000
Family history of colon cancer[a]			
Yes	60 (15.3%)	130 (32.0%)	
No	333 (84.7%)	276 (68.0%)	0.001
Smoking history[a]			
Current	79 (20.1%)	64 (15.8%)	
Ex	179 (45.5%)	160 (39.4%)	
Never	135 (34.4%)	182 (44.8%)	0.009
HRT[a]			
Yes	68 (38.4%)	101 (51.0%)	
No	103 (58.5%)	92 (46.5%)	0.055
Regular nonsteroidal anti-inflammatory drug use[a]			
Yes	42 (10.7%)	68 (16.7%)	
No	350 (89.3%)	338 (83.3%)	0.013
Regular aspirin use[a]			
Yes	106 (27.0%)	130 (32.0%)	
No	287 (73.0%)	276 (68.0%)	0.118
BMI			
Mean	27.15 ± 4.65	26.94 ± 4.60	
Range	(14.40–44.90)	(17.14–41.54)	0.525
Total vitamin D intake (IU)			
Mean	321.21 ± 258.04	337.59 ± 241.56	
Range	(10.71–1826.87)	(16.18–1265.28)	0.359
Total calcium intake (mg)			
Mean	966.18 ± 546.51	992.95 ± 536.64	
Range	(148.29–2695.29)	(211.62–3575.87)	0.490

[a] No. of cases.

history. Effect modification of the relationship between VDR genotype and colorectal adenomas by vitamin D, calcium intake, and other factors was then evaluated by stratification on the tertiles of the variable of interest; ORs within each stratum were compared.

Effect modification of the relationship between VDR genotype and colorectal adenomas by vitamin D and calcium intake was evaluated by testing whether including the interaction term in the multivariate logistic model (with vitamin D and calcium intake modeled as continuous variables) significantly changed the log likelihood of the model. All of the tests for statistical significance were two-sided. Stata version 6 for Microsoft Windows 95/98 (Stata Corporation, College Station, Texas) was used for analysis.

Results

Adenoma cases and controls in this study were similar with respect to sex, BMI, dietary vitamin D intake, and dietary calcium intake (Table 1). Cases were generally older than controls, were less likely to have a family history of colon cancer, and more likely to be a current or ex-smoker. Among women, cases were less likely to have used postmenopausal hormones. The control population was in Hardy-Weinberg equilibrium ($\chi^2 = 0.003$, $P > 0.95$).

Most of the subjects were heterozygous for the *Bsm*I polymorphism, with similar proportions of cases (48.1%) and controls (48.8%). A slightly higher proportion of cases (36.6%) than controls (33.0%) had the *bb* genotype, and a slightly higher proportion of controls (18.2%) than cases (15.3%) had the *BB* genotype. Table 2 describes the association between colorectal adenoma risk and VDR genotype. Among all of the subjects, after adjusting for age and sex, having the heterozygous *Bb* genotype or the *BB* genotype was slightly inversely associated with the risk for colorectal adenomas when compared with the *bb* genotype. Multivariate adjustment for age, sex, BMI, smoking, hormone replacement therapy, and total caloric intake did not change the estimates appreciably.

The risk of colorectal adenoma and the *Bsm*I VDR genotype was evaluated by individual characteristics; stratification by sex, age, location of largest adenoma, number of

Table 2 Relative risks of adenomas by *Bsm*I genotype and selected characteristics

	Genotype					
	bb (144/134)		*Bb* (189/198)		*BB* (60/74)	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
All subjects (age- and sex-adjusted)	1.00	(ref)[b]	0.86	(0.63–1.19)	0.77	(0.50–1.18)
All subjects (multivariate adjusted)[a]	1.00	(ref)	0.86	(0.62–1.20)	0.71	(0.46–1.11)
By gender[a]						
Men	1.00	(ref)	0.92	(0.58–1.45)	0.70	(0.38–1.29)
Women	1.00	(ref)	0.71	(0.43–1.18)	0.71	(0.37–1.36)
By age[a]						
<60 yr	1.00	(ref)	0.76	(0.44–1.32)	0.78	(0.39–1.54)
≥60 yr	1.00	(ref)	0.75	(0.40–1.42)	0.67	(0.28–1.63)
By location of largest adenoma[a]						
Proximal	1.00	(ref)	0.60	(0.35–1.04)	0.52	(0.24–1.10)
Distal	1.00	(ref)	0.97	(0.66–1.43)	0.88	(0.53–1.46)
Rectum	1.00	(ref)	0.94	(0.51–1.75)	0.53	(0.20–1.41)
By no. of polyps[a]						
One	1.00	(ref)	1.03	(0.69–1.55)	0.72	(0.41–1.27)
Two or more	1.00	(ref)	0.72	(0.48–1.08)	0.73	(0.42–1.25)

[a] Adjusted for age, gender, HRT, total caloric intake, BMI, and smoking.
[b] Reference category.

Table 3 Association between *Bsm*I genotype and adenomatous polyps stratified by vitamin D and calcium intake[a]

	Genotype						
	bb (144/134)		*Bb* (189/198)		*BB* (60/74)		
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	
Vitamin D intake (IU)							
Highest tertile	1.00	(ref)[b]	0.81	(0.45–1.44)	1.24	(0.53–2.31)	
Medium tertile	1.16	(0.62–2.17)	0.94	(0.42–2.13)	0.63	(0.22–1.80)	
Lowest tertile	1.40	(0.73–2.70)	1.26	(0.56–2.86)	0.24	(0.08–0.76)	
P for interaction							0.094
Calcium intake (mg)							
Highest tertile	1.00	(ref)	0.67	(0.38–1.19)	0.91	(0.44–1.89)	
Medium tertile	0.76	(0.40–1.46)	1.50	(0.66–3.40)	1.18	(0.41–3.40)	
Lowest tertile	1.22	(0.63–2.37)	1.48	(0.66–3.32)	0.34	(0.11–1.06)	
P for interaction							0.255

[a] Adjusted for age, gender, HRT, total caloric intake, BMI, and smoking.
[b] Reference category.

polyps, yielded results similar to those for the entire population (Table 2).

The association between VDR genotype and colorectal adenomas varied by dietary vitamin D and calcium intake (Table 3). Using those with the *bb* genotype with the highest tertile of vitamin D intake as the reference group, there was a statistically significant inverse association for those with the *BB* variant and the lowest tertile of vitamin D intake (OR = 0.24, CI = 0.08–0.76). Stratification by dietary calcium intake also suggested an interaction between calcium intake and VDR genotype variant in determining colorectal adenoma risk. Again, using those with the *bb* genotype and the highest tertile of dietary calcium intake as referent, there was an inverse association with the *BB* genotype with the lowest tertile of dietary calcium intake (OR = 0.34, CI = 0.11–1.06).

Discussion

In this clinic-based case-control study, we found evidence to suggest that the variant allele *B* of the *Bsm*I VDR polymorphism was inversely associated with colorectal adenoma risk. In addition, the data suggest that dietary vitamin D intake modifies the association between *Bsm*I VDR genotype and colorectal adenoma risk. There was a statistically significant decrease in risk in those with the *BB* variant and the lowest tertile of vitamin D intake. There was also some evidence to suggest that dietary calcium may also modify the association between *Bsm*I VDR genotype and colorectal adenoma risk.

To our knowledge, no epidemiological studies on the relationship between VDR polymorphism, vitamin D and calcium exposure, and colorectal adenoma risk have been reported previously. However, VDR polymorphisms have been associated with the risk of other types of cancer, including breast and prostate cancer (15, 24). This study design had several strengths that enhance our confidence in the validity of our findings. Because the study population is clinic-based and only those who were screened using colonoscopy were eligible for this study we are assured that all of the cases have colorectal adenomas, and more importantly, all of the controls are free of adenomas. Furthermore, because the clinic-based study design assessed past exposures before the subjects knew their disease status, the possibility of differential recall bias is reduced. Lastly, a

relatively large sample size allowed us to investigate gene-environment interactions, even with the *Bsm*I VDR polymorphism, which has a *BB* genotype prevalence rate of only 15%.

Some limitations in the study should be considered in interpreting our results. The lower risk for colorectal adenoma for those with the *BB* genotype and low vitamin D intake may be an artifact caused by the high level of correlation between dietary vitamin D intake and dietary calcium intake. In this study, the Spearman correlation coefficient between total dietary vitamin D intake (including supplement intake) and total dietary calcium intake (including supplement intake) is 0.62 ($P < 0.0001$). Furthermore, dietary vitamin D intake as measured by the FFQ does not take into account vitamin D photolysed by exposure to UV radiation, nor obviously does it take into account the longer history of dietary vitamin D intake. Among young people, sunlight exposure is a very important source of total vitamin D (25). Therefore, dietary vitamin D intake may not accurately reflect the true exposure to vitamin D; rather, it may be a surrogate for calcium intake, because the major sources of dietary vitamin D are also major sources of calcium.

Another potential limitation of this analysis is that the clinic-based study population may not be representative of the entire population. Because only those who were screened using colonoscopy were eligible for this study and because worry about family history may induce a higher likelihood of seeking colonoscopic examination, controls were actually more likely to have a positive family history of colorectal cancer than the general population. If familial risk is linked to VDR genotype, these data may be providing an inappropriate estimate of risk.

Both epidemiological and laboratory evidence support a role for vitamin D in colorectal cancer etiology, and this may be mediated by the VDR. Ecological and population-based studies have shown some evidence that vitamin D exposure, as measured through sunlight exposure, diet, and serum levels, is associated with a lower risk of colorectal cancer (26–36). Laboratory evidence demonstrates that $1,25(OH)_2D_3$, the biologically active form of vitamin D, has a role in the regulation of cell proliferation and differentiation and in calcium metabolism (reviewed in Ref. 37). *In vitro* studies on colon cancer cell lines have shown that the presence of $1,25(OH)_2D_3$ and its synthetic analogues slows tumor cell proliferation and enhances differentiation (3, 8). However, inhibition of tumor cell growth and enhancement of differentiation occurred only in cell lines with high VDR expression (3). It has also been shown that normal and malignant colonic tissue express VDR. Furthermore, the effects of calcium on colorectal cancer risk may also be mediated by the VDR. Epidemiological and laboratory data show that calcium may reduce the risk of colorectal adenomas as well as cancer (33–36, 38–41). Laboratory evidence points to several possible pathways involving calcium that are mediated by $1,25(OH)_2D_3$ and indirectly by the VDR (42–44).

Given this biology, a VDR-mediated mechanism of growth inhibition involving vitamin D and calcium exists. Additional polymorphic variation in VDR expression may influence the way in which $1,25(OH)_2D_3$ and calcium control cellular growth and differentiation. *Bsm*I is located in intron 8 and is closely linked to a second polymorphism, *Taq*I, located in exon 9. Another polymorphism, a poly(A) microsatellite, is located in the 3′-UTR (15). The mechanism through which these polymorphisms affect the VDR is still unclear, as the polymorphisms do not alter the amino acid sequence of the VDR protein (13). However, it is thought that the 3′-UTR region of the *VDR* gene is involved in the regulation of mRNA stability, and, therefore, may alter the rate of degradation of the VDR mRNA (12). There is also some speculation that these polymorphisms are in disequilibrium with other mutations that alter VDR function (13). Although the mechanism is still unknown, studies have shown that VDR *Bsm*I genotypes are associated with certain phenotypes. The *Bsm*I *BB* genotype has been associated with lower calcium absorption and lower bone density in several studies (14), as well as significantly elevated levels of circulating serum $1,25(OH)_2D_3$ (15).

The results of this study are unusual in light of the fact that higher calcium and vitamin D intake are generally associated with a modestly reduced risk of colorectal neoplasia. However, these data suggest that those with the *BB Bsm*I VDR genotype may be at reduced risk in the presence of lower calcium and vitamin D intake. These results are similar to those reported by Ma *et al.* (15), who reported an inverse association between the *Bsm*I *BB* genotype and prostate cancer risk among those with serum $25(OH)D_3$ levels lower than the median in a study on VDR polymorphisms, prostate cancer risk, and serum vitamin D levels.

The results of this case-control study suggest that the *BB* genotype is inversely associated with colorectal adenoma risk in the presence of lower vitamin D and calcium intake. These findings are novel in light of the fact that higher calcium and vitamin D intake are generally associated with a modestly reduced risk of colorectal neoplasia. These findings should be replicated to establish whether this VDR polymorphism does have a role in the etiology of colorectal neoplasia and that the relationship is modified by vitamin D and calcium exposure.

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