Circulating Vitamin D Metabolites, Polymorphism in Vitamin D Receptor, and Colorectal Adenoma Risk

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Abstract

Objective: Vitamin D is a potential agent for the prevention of colorectal cancer possibly through mechanisms mediated by the vitamin D receptor (VDR). We investigated the association of circulating vitamin D metabolites and a genetic variant of the *VDR* gene with advanced colorectal adenoma, a precursor lesion of colorectal cancer. Methods: Cases with advanced adenoma of the distal large bowel and gender- and ethnicity-matched controls with a negative sigmoidoscopy were randomly selected from participants in the Prostate, Lung, Colorectal and Ovarian Cancer Screening trial. Genotype analysis of the *VDR* TaqI polymorphism was completed on 763 cases and 774 controls. Serum levels of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] were measured in a subset of 394 cases and 397 controls. Results: Serum levels of 25(OH)D were inversely associated with advanced adenoma risk in women but not in men. Comparing those in the highest quintile with those in the lowest quintile, the risk for advanced adenoma decreased by 73% in women [odds ratio (OR) = 0.27, 95% confidence interval (95% CI) = 0.11–0.69; *P* for trend = 0.0002], while the risk did not decrease in men (OR = 1.10, 95% CI = 0.60–2.05; *P* for trend = 0.85). In women, 25(OH)D levels were significantly higher in current users of hormone replacement therapy (HRT) than in former or never HRT users. Neither serum 1,25(OH)$_2$D nor *VDR* TaqI genotype was associated with advanced adenoma risk. Conclusion: Higher serum 25(OH)D levels were associated with decreased adenoma risk. Serum 1,25(OH)$_2$D and *VDR* TaqI genotype were not associated with adenoma risk. (Cancer Epidemiol Biomarkers Prev 2004;13(4):546–552)

Introduction

That U.S. colorectal cancer rates are greater in the northern United States, where sun exposure is lower, led to the hypothesis that sunlight-mediated dermal vitamin D synthesis provides protection from this disease. Several epidemiological studies support this hypothesis (1–6), showing decreased risk for colorectal tumors in relation to higher serum levels of vitamin D, an integrative measure of dermal vitamin D production and dietary and supplemental vitamin D intake; however, evidence from these studies is limited because of the small number of studies that have been done and the small sample sizes of half of these studies (34–146 cases; Refs. 4–6).

The cellular effects of vitamin D are primarily mediated through binding to the nuclear vitamin D receptor (VDR), which regulates the transcription of more than 60 genes, including genes involved in cellular differentiation and inhibition of proliferation (7, 8). A series of polymorphisms in the 3′ untranslated region (UTR) of *VDR* may affect mRNA stability (9, 10) possibly through linkage to other variants (11); however, functional studies have shown contradictory results (12, 13).

We assessed the association of serum vitamin D metabolite levels and a TaqI polymorphism in the 3′ UTR of *VDR* with risk of colorectal adenoma, a precursor lesion of colorectal cancer. We also evaluated the effects of calcium intake and hormone replacement therapy (HRT) in relation to vitamin D and adenoma risk because of their potential physiological correlates with vitamin D (14–21) and observed associations with colorectal tumor risk (22–24).

We conducted our analysis within a large colorectal cancer screening trial (25, 26) in which subjects received a standardized screening exam. The sample size allowed us to limit case to advanced adenomas, which have a higher potential for malignant transformation. Because of the standardized screening, we could randomly select for control subjects who had a negative sigmoidoscopy examination from the same study population.

Methods

The study was done as a nested case-control study within the Prostate, Lung, Colorectal and Ovarian Cancer Screening (PLCO) trial (25, 26). The trial recruited 154,952

participants, aged 55–74 years, at 10 U.S. study centers (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St. Louis, MO; and Washington, DC). Participants randomly assigned to the screening arm of the PLCO trial were offered a sigmoidoscopy examination at study entry and referred for colonoscopy follow-up examination if polyps or other suspect lesions were identified. Medical and pathological reports on follow-up were abstracted and coded by trained medical record abstractors. Written informed consent was obtained from participants and the trial received approval from the institutional review boards of the U.S. National Cancer Institute and the 10 study centers.

Study Population. Subjects for this study were selected from 42,037 participants in the screening group who underwent a successful sigmoidoscopic examination (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified) between September 1993 and September 1999. Subjects provided information on risk factors and donated a blood sample for use in etiological studies. After exclusion of 4834 subjects with a self-reported history of cancer (except basal cell skin cancer), ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps, or Gardner's syndrome, we randomly selected 772 cases for study from among 1234 cases with advanced distal adenoma (adenoma ≥1 cm or containing high-grade dysplasia or villous elements). An equal number of gender- and ethnicity-matched participants ($n = 777$) with a negative screening sigmoidoscopy (*i.e.*, no polyp or other suspect lesion; $n = 26,651$) were selected as controls. Genotype analysis was attempted on samples from all selected cases and controls; genotypes were successfully obtained from 763 case samples and 774 control samples. Analyses of vitamin D metabolite levels were conducted in samples of 400 cases and 400 controls selected at random. Stored blood samples collected at study entry were unavailable for six cases and three controls. Therefore, the total number was 394 for cases and 397 for controls for the vitamin D metabolite analysis.

Vitamin D Metabolite Analysis. Levels of the two vitamin D metabolites, 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], were determined by RIA using radioiodinated tracer in serum derived from blood collected at study entry (27). Laboratory personnel were blinded to case-control status. Multiple blinded quality control samples from two different individuals were included in all batches (total $n = 96$). The coefficient of variation was 12.5% for 1,25(OH)$_2$D and 16.3% for 25(OH)D.

Genotyping. The TaqI polymorphism, a C-to-T base substitution (dbSNP rs731236) in exon 9 of the *VDR* gene, was detected by PCR amplification followed by restriction enzyme digestion (for details, see Appendix A. Alleles were scored as TT, Tt, or tt; lowercase letters indicate the presence of restriction site. Laboratory personnel were blinded to disease status of the participants. The P value of the Hardy-Weinberg equilibrium (28) was 0.31 in non-Hispanic white controls and 0.33 in all controls.

Assessment of Questionnaire-Based Factors. At the initial screening, all participants were asked to complete a questionnaire about sociodemographic factors, medical history including current and former use of HRT, and risk factors for cancer. Usual dietary intake over the 12 months before enrollment was assessed with a 137-item food frequency questionnaire including an additional 14 questions about intake of vitamin and mineral supplements (29). Daily dietary nutrient intake was calculated by multiplying the daily frequency of each consumed food item by the nutrient value of the gender-specific portion size (30) using the nutrient database from the U.S. Department of Agriculture (31). Total calcium and vitamin D intake was calculated by adding dietary and supplemental intakes.

Statistical Analysis. To account for seasonal differences and demographic factors, we adjusted all mean values of 25(OH)D and 1,25(OH)$_2$D for month of blood draw, gender, ethnic origin, study center, and age. Adjusted means (least squares means) and differences between adjusted means were calculated by general linear models. Using unconditional logistic regression analysis, we calculated prevalence odds ratios (ORs) for the relation between *VDR* and serum vitamin D metabolite levels and advanced distal colorectal adenoma with vitamin D metabolites entered continuously or in quintiles with cut points based on the distribution among controls. Additionally, we used season-specific cut points for quintiles (December to May and June to November). We also estimated the ORs comparing serum levels of 25(OH)D and 1,25(OH)$_2$D above and below clinically normal levels (cut points: 15 ng/ml and 26 pg/ml, respectively; Refs. 32, 33).

We frequency matched on ethnic origin to allow stratified genotype analysis. Because of a strong imbalance between male and female cases and controls, we further frequency matched on gender. To avoid small cell numbers, we did not match on other risk factors but adjusted for other potential risk factors in the statistical analysis. We adjusted all ORs for the matching factors, gender, and ethnic origin as well as for age at randomization, study center, and month of blood draw. In addition to this basic model, we included potential confounders based on *a priori* hypotheses for colorectal neoplasia risk factors if they changed the risk estimate (OR) by more than 10%. None of the factors (educational attainment, smoking, alcohol use, aspirin use, ibuprofen use, physical activity, body mass index, HRT use, dietary fiber, total calcium, total folate, and red meat) was included in the analyses because the factors, either separately or together, did not change the risk estimates of 25(OH)D, 1,25(OH)$_2$D, or the *VDR* TaqI polymorphism by more than 10%.

To explore effect modification, we performed stratified analyses and evaluated multiplicative interaction by creating product terms. We investigated the statistical significance of multiplicative interaction terms by comparing the log likelihood statistics of the main effect model with the joint effects model. Continuous variables were used to calculate the P value for trend. All P values are two-sided.

Results

Adenoma cases were slightly older than controls (average 62.9 and 62.3 years, respectively; Table 1). The

Table 1. Characteristics of the study population

Characteristics	Included in serum vitamin D		Included in *VDR* genotype	
	Cases	Controls	Cases	Controls
n	394	397	763	774
Age (yr), *n* (%)				
55–59	136 (34.5)	163 (41.1)	253 (33.2)	362 (46.8)
60–64	127 (32.2)	117 (29.5)	240 (31.5)	200 (25.8)
65–69	87 (22.1)	80 (20.2)	172 (22.5)	139 (18.0)
70–74	44 (11.2)	37 (9.3)	96 (12.8)	73 (9.4)
Female, *n* (%)	123 (31.2)	124 (31.2)	232 (30.4)	239 (30.9)
Ethnic origin, *n* (%)				
Non-Hispanic black	7 (1.8)	8 (2.0)	22 (2.9)	23 (3.0)
Non-Hispanic white	372 (94.4)	375 (94.5)	716 (93.8)	727 (93.9)
Others	15 (3.8)	14 (3.5)	25 (3.3)	24 (3.1)
Education, *n* (%)				
<12 yr	44 (11.2)	26 (6.6)	72 (9.4)	50 (6.5)
12 yr/high school equivalent	98 (24.9)	89 (22.4)	187 (24.5)	176 (22.8)
Some college	138 (35.0)	131 (33.0)	274 (35.9)	246 (31.8)
College and above	114 (28.9)	151 (38.0)	230 (30.1)	301 (38.9)

study population included about 31% female and 94% non-Hispanic white participants. Because we matched on ethnic origin and gender, the case and control distributions were very similar for these two variables. Controls attained higher education levels than did cases.

The average serum level of 25(OH)D in adenoma cases was lower than in controls ($P = 0.06$; Table 2). In contrast to 25(OH)D levels, mean serum levels of $1,25(OH)_2D$ were not different for cases and controls ($P = 0.66$). Within the control group, the average levels of 25(OH)D and $1,25(OH)_2D$ were higher in women than in men ($P = 0.15$ and 0.01, respectively). Non-Hispanic black controls had significantly lower 25(OH)D levels (16.6 ng/ml) than did non-Hispanic white controls (28.6 ng/ml; $P = 0.0007$), whereas $1,25(OH)_2D$ levels were similar (35.9 and 36.6 pg/ml; $P = 0.85$ respectively).

Overall, risk reduction $[(OR - 1) \times 100]$ of advanced distal adenoma was 13% for each 10 ng/ml increase in serum 25(OH)D level (Table 2). The overall inverse association between adenoma risk and serum 25(OH)D level was limited to women, showing a 41% reduction in risk with each 10 ng/ml increment in 25(OH)D and a highly significant trend ($P = 0.0002$; Table 2). The P value for the multiplicative interaction between gender and 25(OH)D was 0.02. Similar to the continuous analysis, the categorical analysis of serum 25(OH)D showed no association between 25(OH)D and adenoma risk in men but a strong inverse association in women (Table 3). Comparing women in the highest (5th) quintile with women in the lowest (1st) quintile of 25(OH)D, adenoma risk decreased by 73%. Further, a 70% decrease in risk was evident when women with normal serum 25(OH)D levels were compared with those with subnormal levels (<15 ng/ml). The association between 25(OH)D and advanced adenoma risk was stronger in older participants (Table 2). For non-Hispanic whites, adenoma risk decreased by 16% for each 10 ng/ml increment in 25(OH)D. Sample sizes of groups of other ethnic origins were too small to estimate ORs.

Table 2. Distribution of serum vitamin D metabolites and their association with advanced distal colorectal adenoma

Variable	Number of subjects		Mean ± SE		OR	95% CI	P_{trend}
	Cases	Controls	Cases	Controls			
Serum 25(OH) vitamin D (ng/ml)							
Overall	394	397	27.0 ± 0.49	28.3 ± 0.49	0.87	0.75–1.01	0.06
Female	123	124	25.1 ± 0.88	29.3 ± 0.87	0.59	0.45–0.78	0.0002
Male	271	273	27.9 ± 0.59	27.8 ± 0.59	1.02	0.84–1.23	0.85
Age, 55–64 yr	263	280	27.7 ± 0.69	28.6 ± 0.70	0.91	0.76–1.10	0.33
Age, 65–74 yr	131	117	25.4 ± 1.17	28.0 ± 1.19	0.72	0.54–0.95	0.02
Serum $1,25(OH)_2$ vitamin D (pg/ml)							
Overall	394	397	36.3 ± 0.50	36.0 ± 0.50	1.03	0.89–1.19	0.66
Female	123	124	37.1 ± 0.91	37.9 ± 0.90	0.92	0.72–1.16	0.46
Male	271	273	35.9 ± 0.61	35.1 ± 0.61	1.10	0.91–1.33	0.35
Age, 55–64 yr	263	280	36.4 ± 0.71	35.6 ± 0.72	1.08	0.91–1.28	0.39
Age, 65–74 yr	131	117	36.1 ± 1.21	36.9 ± 1.24	0.95	0.71–1.28	0.75

Note: Mean values are adjusted for age, gender, ethnic origin, study center, and months of blood draw. 95% CI, 95% confidence interval. OR for serum vitamin D levels per 10 units increase. ORs adjusted for age, gender, ethnic origin, study center, and month of blood draw.

Table 3. Association between categorical serum vitamin D metabolites and advanced distal colorectal adenomas

Categories	Range	Women				Men			
		Cases	Controls	OR	95% CI	Cases	Controls	OR	95% CI
Serum 25(OH) vitamin D (ng/ml)									
1st quintile	6.8–19.2	34	24	1.00	–	55	54	1.00	–
2nd quintile	>19.2–24.5	33	24	0.93	0.39–2.22	45	57	0.73	0.41–1.32
3rd quintile	>24.5–30.6	21	18	0.56	0.22–1.44	69	63	1.08	0.61–1.90
4th quintile	>30.6–36.6	14	26	0.28	0.11–0.75	49	52	0.89	0.49–1.64
5th quintile	>36.6–72.8	21	32	0.27	0.11–0.69	53	47	1.10	0.60–2.05
P_{trend}					0.0002				0.85
Low	<15	25	11	1.00	–	26	24	1.00	–
Normal-high	≥15	98	113	0.30	0.12–0.75	245	249	0.81	0.43–1.54
Serum 1,25(OH)$_2$ vitamin D (pg/ml)									
1st quintile	9.4–27.9	28	24	1.00		51	55	1.00	–
2nd quintile	>27.9–32.8	20	21	0.66	0.27–1.62	62	59	1.16	0.67–2.00
3rd quintile	>32.8–37.4	17	22	0.57	0.22–1.46	57	59	1.10	0.63–1.90
4th quintile	>37.4–44.1	28	24	1.10	0.47–2.61	53	54	1.07	0.60–1.90
5th quintile	>44.1–79.5	30	33	0.64	0.28–1.47	48	46	1.13	0.62–2.04
P_{trend}					0.46				0.35
Low	<26	19	17	1.00	–	26	40	1.00	–
Normal-high	≥26	104	107	0.77	0.36–1.62	245	233	1.40	0.85–2.31

Note: ORs adjusted for age, gender, ethnic origin, study center, and months of blood draw.

Serum levels of 1,25(OH)$_2$D (as continuous term or quintiles) were not associated with adenoma risk in either all participants or separately in men and women (Tables 2 and 3). Using season-specific cut points for the quintiles or stratifying on season of blood draw resulted in adenoma risk estimates for 25(OH)D and 1,25(OH)$_2$D (data not shown) very similar to those reported in Tables 2 and 3.

In women, mean serum 25(OH)D levels were significantly higher in current HRT users than in former or never HRT users (within cases, $P = 0.002$; within controls, $P = 0.006$; Table 4). However, the association between 25(OH)D and adenoma risk was similar in both groups and the P value for the multiplicative interaction between 25(OH)D and HRT use was 0.43. Current HRT users also had significantly higher mean levels of 1,25(OH)$_2$D than did former/never HRT users (within cases, $P = 0.003$; within controls, $P = 0.03$). 1,25(OH)$_2$D was not associated with adenoma risk in current HRT users or former/never HRT users (data not shown). Total intake of vitamin D and calcium was similar for current and former/never HRT users (mean vitamin D: 509 and 512 IU/day; mean calcium: 1319 and 1325 mg/day, respectively).

The *VDR* TaqI polymorphism was not associated with adenoma risk in all participants or in non-Hispanic whites (Table 5). The association between *VDR* TaqI genotype and adenoma risk was similar in non-Hispanic white women (OR for Tt *versus* TT and tt *versus* TT: 1.00, 1.10, and 1.03) and men (OR for Tt *versus* TT and tt *versus* TT: 1.00, 1.16, and 1.11).

The association between *VDR* TaqI genotype and adenoma risk did not vary when stratified on total calcium intake (median or tertiles; data not shown). In addition, the association of 25(OH)D and 1,25(OH)$_2$D and adenoma risk did not vary after dichotomization on the median total calcium intake either in the whole study population or in each gender separately (data not shown). Gender-specific adenoma risk estimates of serum vitamin D level varied little by *VDR* TaqI genotype; only for women with the *VDR* TaqI genotype tt, the risk reduction for each 10 ng/ml increment in 25(OH)D was slightly stronger than that for women with genotypes TT or Tt (data not shown). None of the P values for multiplicative interaction between serum vitamin D level and *VDR* TaqI genotype or total calcium was below 0.2.

Discussion

Within our nested case-control study of participants in a colorectal cancer screening trial, we observed a 73%

Table 4. Serum 25(OH) vitamin D levels and their association with advanced distal colorectal adenoma in women based on use of HRT

Variable	Number of subjects		Mean ± SE (ng/ml)		OR	95% CI	P_{trend}
	Cases	Controls	Cases	Controls			
All women	123	124	25.1 ± 0.88	29.3 ± 0.87	0.59	0.45–0.78	0.0002
Current HRT users	58	60	27.9 ± 1.40	32.6 ± 1.37	0.62	0.44–0.88	0.007
Former or never HRT users	65	63	22.0 ± 1.34	27.3 ± 1.32	0.50	0.32–0.79	0.003

Note: Mean values are adjusted for age, ethnic origin, study center, and months of blood draw. OR for serum vitamin D levels per 10 units increase. ORs adjusted for age, ethnic origin, study center, and month of blood draw.

Table 5. Association between *VDR* TaqI polymorphism and advanced colorectal adenomas

VDR TaqI genotype	Cases	Controls	OR	95% CI
	n (%)	*n* (%)		
All				
TT (wild-type)	288 (37.7)	301 (38.9)	1.00	–
Tt	358 (46.9)	353 (45.6)	1.09	0.87–1.36
tt (variant)	117 (15.3)	120 (15.5)	1.04	0.76–1.42
Non-Hispanic whites				
TT (wild-type)	259 (36.2)	274 (37.7)	1.00	–
Tt	341 (47.6)	334 (45.9)	1.12	0.89–1.41
tt (variant)	116 (16.2)	119 (16.4)	1.06	0.78–1.45

Note: ORs adjusted for age, gender, ethnic origin, and study center.

reduction in advanced adenoma risk among women with serum 25(OH)D levels in the highest quintile compared with those in the lowest quintile. No association between serum 25(OH)D levels and adenoma risk was observed among men. Serum 1,25(OH)$_2$D levels and *VDR* TaqI genotype were not associated with adenoma risk in either men or women. The associations of adenoma risk with 25(OH)D, 1,25(OH)$_2$D, and *VDR* TaqI genotype were not appreciably modified by each other or by calcium intake or HRT use.

Two out of three case-control studies, with 236 cases (1) and 473 cases (2), reported inverse associations between 25(OH)D and adenoma risk. The third study (3) with 326 female cases found no clear association between 25(OH)D and risk of adenoma but did find an inverse association between 1,25(OH)$_2$D and adenoma. In addition to these adenoma studies, three nested case-control studies (4–6) of colorectal cancer with 34 (4), 57 (5), and 146 (6) cases were reported. All three studies showed an inverse association between serum 25(OH)D and colorectal cancer risk. Two of the studies (5, 6) also investigated 1,25(OH)$_2$D levels and found no association between 1,25(OH)$_2$D levels and colorectal cancer. Thus, although most observational studies, including the present study, suggest an inverse association between 25(OH)D and colorectal neoplasia, results concerning 1,25(OH)$_2$D have been less consistent.

A potential anticarcinogenic effect of 25(OH)D on colon tissue is supported by recent studies demonstrating that normal colon tissue expresses 1-α vitamin D hydroxylase and hence can locally produce the metabolically active form of vitamin D, 1,25(OH)$_2$D, from 25(OH)D (34–36). These findings suggest that 25(OH)D as well as 1,25(OH)$_2$D may have a localized effect in colon tissue to reduce cell proliferation and induce differentiation (37, 38). The potential significance of circulating 25(OH)D levels on colon neoplasia is further supported by the fact that serum levels of 1,25(OH)$_2$D are tightly regulated as part of their function to control calcium homeostasis, whereas circulating 25(OH)D levels fluctuate and reflect vitamin D status as determined by dietary intake and endogenous production (39–41). The half-life of 1,25(OH)$_2$D is only a few hours whereas the half-life of 25(OH)D is about 1 month (21, 42, 43). These data suggest that 25(OH)D levels, which are modifiable by behavioral changes (diet and sun exposure), may affect colorectal cancer development.

It is puzzling that we found an association between adenoma risk and levels of 25(OH)D in women but not in men. The mechanism by which vitamin D mediates an anticarcinogenic effect does not appear to be gender specific. Most case-control studies have not stratified their analysis of vitamin D and colorectal neoplasia on gender (1, 2, 4, 5). However, one study that only included men showed an inverse association between 25(OH)D and colorectal cancer (6), and one study that only included women found no consistent association between 25(OH)D and adenoma risk (3). We reanalyzed an earlier published study (1) and found that the inverse association between 25(OH)D levels and adenoma risk was not substantially stronger in women than in men (quintiles 1–5, women: 1.0, 0.5, 0.4, 0.2, and 0.3; men: 1.0, 0.4, 0.8, 0.6, and 0.5). Based on the results of these previous studies, our finding of a gender-specific inverse association between vitamin D and colorectal adenoma was unexpected and might be due to chance. Therefore, we believe that the potential anticarcinogenic effect of 25(OH)D is unlikely to be gender specific.

Current HRT users had significantly higher serum 25(OH)D and 1,25(OH)$_2$D levels than did former/never users and the difference was not accounted for by differences in vitamin D intake. This finding is consistent with previous reports of higher 25(OH)D and 1,25(OH)$_2$D levels in women with increased levels of endogenous estrogens and in women taking exogenous estrogen formulations (14–20). The estrogen-related increase in 25(OH)D and 1,25(OH)$_2$D levels is possibly due to stimulation of renal 1-α hydroxylase and inhibition of 24-vitamin D hydroxylase, resulting in a reduced proportion of 25(OH)D being metabolized to 24,25(OH)$_2$D (14, 15, 19). Alternatively, estrogen may alter the relative proportions of free and protein-bound vitamin D: some but not all studies have shown that estrogen increases levels of vitamin D binding protein or that estrogen increases levels of free vitamin D (44, 45). The decreased risk of colorectal cancer associated with HRT observed in other studies (22, 23) may be related in part to the HRT effect on vitamin D levels. However, HRT and estrogen likely affect colorectal cancer risk through multiple pathways, such as interaction with insulin and insulin-like growth factor axis (23) or reduction in CpG methylation in the *VDR* gene, which results in increased *VDR* expression (46).

We did not observe an association between *VDR* TaqI polymorphism and colorectal adenoma. Two case-control studies reported a decreased risk of colorectal neoplasia in participants with the TaqI tt genotype compared with those with Tt or TT genotype either overall (11) or in conjunction with low calcium or vitamin D intake (9). In contrast, in our study and two case-control studies (47, 48), TaqI genotype was not associated with colorectal adenoma risk and neither vitamin D nor calcium modified this association. Results concerning the association between TaqI polymorphism in the 3′ UTR of *VDR* and risk of colorectal neoplasia remain inconsistent.

A theoretical limitation of our study is that serum nutrient levels may be influenced by disease status at the time of blood draw. However, we studied screen-detected adenomas so it is not likely that disease-related behavioral modifications such as dietary changes or exposure to sun affected vitamin D levels.

Our design had the advantage of ensuring that cases and controls came from the same source population and were screened with a standardized procedure (*i.e.*, cases

were not screened based on symptoms). The large study population allowed us to confine the analysis to cases with advanced adenoma, which have a higher potential for malignant transformation and are a particularly meaningful intermediate outcome for studying factors related to colorectal cancer. However, because we restricted our study to advanced adenomas, we could not examine whether vitamin D metabolites and the TaqI polymorphism prevented any adenoma formation but only whether they prevented the formation and/or subsequent persistence of adenomas with increased malignant potential.

In summary, results from our study suggest an inverse association between serum 25(OH)D level and risk of developing colorectal neoplasia. Our study does not support an association between adenoma and TaqI polymorphism in the 3′ UTR of *VDR* or between adenoma and serum 1,25(OH)$_2$D level.

Appendix A. Description of Genotyping for TaqI Polymorphism in the *VDR* Gene

The TaqI polymorphism was detected by PCR amplification followed by restriction enzyme digestion. Genomic DNA was amplified by PCR using the following primers: 5′-CAG AGC ATG GAC AGG GAG CAA-3′ (sense) and 5′-GCA ACT CCT CAT GGC TGA GGT CTC-3′ (antisense). The amplified product is a 740-bp fragment containing the variant site. PCR was started with a denaturation step of 94°C for 3 min followed by 30 cycles at 94°C for 45 s, 58°C for 60 s, and 72°C for 90 s. The concentrations of the sense and antisense primers were 250 nM in an amplification reaction. PCR products were digested by the TaqI restriction enzyme (1 unit/20 μl reaction) and electrophoresed through a 3% agarose gel at 125 V for 30 min. The gels were stained with ethidium bromide and photographed under UV light. Alleles were scored as TT, Tt, or tt; lowercase letters indicate the presence of restriction site. In each batch, we included samples of the homozygote wild-type (TT) and heterozygote (Tt) genotypes as positive controls and blank restriction reactions as negative controls.

References

1. Peters U, McGlynn KA, Chatterjee N, et al. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. Cancer Epidemiol Biomarkers & Prev, 2001;10:1267–74.
2. Levine AJ, Harper JM, Ervin CM, et al. Serum 25-hydroxyvitamin D, dietary calcium intake, and distal colorectal adenoma risk. Nutr Cancer, 2001;39:35–41.
3. Platz EA, Hankinson SE, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. Cancer Epidemiol Biomarkers & Prev, 2000;9:1059–65.
4. Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet, 1989;2:1176–8.
5. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Colon cancer and serum vitamin D metabolite levels 10–17 years prior to diagnosis. Am J Epidemiol, 1995;142:608–11.
6. Tangrea J, Helzlsouer K, Pietinen P, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes & Control, 1997;8:615–25.
7. Marcinkowska E. A run for a membrane vitamin D receptor. Biol Signals Recept, 2001;10:341–9.
8. Norman AW, Bishop JE, Bula CM, et al. Molecular tools for study of genomic and rapid signal transduction responses initiated by 1 α,25(OH)(2)-vitamin D(3). Steroids, 2002;67:457–66.
9. Kim HS, Newcomb PA, Ulrich CM, et al. Vitamin D receptor polymorphism and the risk of colorectal adenomas: evidence of interaction with dietary vitamin D and calcium. Cancer Epidemiol Biomarkers & Prev, 2001;10:869–74.
10. Beelman CA, Parker R. Degradation of mRNA in eukaryotes. Cell, 1995;81:179–83.
11. Slattery ML, Yakumo K, Hoffman M, Neuhausen S. Variants of the VDR gene and risk of colon cancer (United States). Cancer Causes & Control, 2001;12:359–64.
12. Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. Nature, 1994;367:284–7.
13. Durrin LK, Haile RW, Ingles SA, Coetzee GA. Vitamin D receptor 3′-untranslated region polymorphisms: lack of effect on mRNA stability. Biochim Biophys Acta, 1999;1453:311–20.
14. Buchanan JR, Santen R, Cauffman S, Cavaliere A, Greer RB, Demers LM. The effect of endogenous estrogen fluctuation on metabolism of 25-hydroxyvitamin D. Calcif Tissue Int, 1986;39:139–44.
15. Aarskog D, Aksnes L, Markestad T, Rodland O. Effect of estrogen on vitamin D metabolism in tall girls. J Clin Endocrinol Metab, 1983;57:1155–8.
16. Harris SS, Dawson-Hughes B. The association of oral contraceptive use with plasma 25-hydroxyvitamin D levels. J Am Coll Nutr, 1998;17:282–4.
17. Landin-Wilhelmsen K, Wilhelmsen L, Wilske J, et al. Sunlight increases serum 25(OH) vitamin D concentration whereas 1,25(OH)2D3 is unaffected. Results from a general population study in Goteborg, Sweden (The WHO MONICA Project). Eur J Clin Nutr, 1995;49:400–7.
18. Sowers MR, Wallace RB, Hollis BW, Lemke JH. Parameters related to 25-OH-D levels in a population-based study of women. Am J Clin Nutr, 1986;43:621–8.
19. Gallagher JC, Riggs BL, DeLuca HF. Effect of estrogen on calcium absorption and serum vitamin D metabolites in postmenopausal osteoporosis. J Clin Endocrinol Metab, 1980;51:1359–64.
20. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr, 2002;76:187–92.
21. Heaney RP, Barger-Lux MJ, Dowell MS, Chen TC, Holick MF. Calcium absorptive effects of vitamin D and its major metabolites. J Clin Endocrinol Metab, 1997;82:4111–6.
22. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA, 2002;288:321–33.
23. Slattery ML, Ballard-Barbash R, Edwards S, Caan BJ, Potter JD. Body mass index and colon cancer: an evaluation of the modifying effects of estrogen (United States). Cancer Causes & Control, 2003;14:75–84.
24. Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. N Engl J Med, 1999;340:101–7.
25. Gohagan JK, Prorok PC, Hayes RB, Kramer BS. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial of the National Cancer Institute: history, organization, and status. Control Clin Trials, 2000;21:251S–72S.
26. Hayes RB, Reding D, Kopp W, et al. Etiologic and early marker studies in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. Control Clin Trials, 2000;21:349S–55S.
27. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. Methods Enzymol, 1997;282:174–86.
28. Hernández JL, Weir BS. A disequilibrium coefficient approach to Hardy-Weinberg testing. Biometrics, 1989;45:53–70.
29. Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Accessed May 2003. Available from: http://www3.cancer.gov/prevention/plco/DQX.pdf.
30. Subar AF, Midthune D, Kulldorff M, et al. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. Am J Epidemiol, 2000;152:279–86.
31. Tippett KS, Cypel YS. Design and operation: the continuing survey of food intakes by individuals and the diet and health knowledge survey, 1994-96. In: Continuing survey of food intakes by individuals 1994–96. Nationwide Food Surveys Rep. No. 96-1. U.S. Department of Agriculture, Agricultural Research Service; 1997.
32. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr, 1999;69:842–56.
33. Thomas CL. Taber's cyclopedic medical dictionary. Philadelphia: F.A. Davis Co; 1989.

34. Dusso A, Brown A, Slatopolsky E. Extrarenal production of calcitriol. Semin Nephrol, 1994;14:144–55.

35. Tangpricha V, Flanagan JN, Whitlatch LW, et al. 25-Hydroxyvitamin D-1α-hydroxylase in normal and malignant colon tissue. Lancet, 2001;357:1673–4.

36. Zehnder D, Bland R, Williams MC, et al. Extrarenal expression of 25-hydroxyvitamin d(3)-1 α-hydroxylase. J Clin Endocrinol Metab, 2001;86:888–94.

37. Holt PR, Arber N, Halmos B, et al. Colonic epithelial cell proliferation decreases with increasing levels of serum 25-hydroxy vitamin D. Cancer Epidemiol Biomarkers & Prev, 2002;11:113–9.

38. Cross HS, Bareis P, Hofer H, et al. 25-Hydroxyvitamin D(3)-1α-hydroxylase and vitamin D receptor gene expression in human colonic mucosa is elevated during early cancerogenesis. Steroids, 2001;66:287–92.

39. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an ^{125}I-labeled tracer. Clin Chem, 1993;39:529–33.

40. Fraser DR. Regulation of the metabolism of vitamin D. Physiol Rev, 1980;60:551–613.

41. Stern PH, Taylor AB, Bell NH, Epstein S. Demonstration that circulating 1 α, 25-dihydroxyvitamin D is loosely regulated in normal children. J Clin Invest, 1981;68:1374–7.

42. Holick MF. The use and interpretation of assays for vitamin D and its metabolites. J Nutr, 1990;120 Suppl 11:1464–9.

43. Hollis BW. Assessment of vitamin D nutritional and hormonal status: what to measure and how to do it. Calcif Tissue Int, 1996;58:4–5.

44. ten Bolscher M, de Valk-de Roo GW, Barto R, van der Vijgh WJ, Neugut AI. Oestrogen has no short-term effect on intestinal strontium absorption in healthy postmenopausal women. Clin Endocrinol (Oxf), 2003;50:387–92.

45. van Hoof HJ, de Sevaux RG, van Baelen H, et al. Relationship between free and total 1,25-dihydroxyvitamin D in conditions of modified binding. Eur J Endocrinol, 2001;144:391–6.

46. Smirnoff P, Liel Y, Gnainsky J, Shany S, Schwartz B. The protective effect of estrogen against chemically induced murine colon carcinogenesis is associated with decreased CpG island methylation and increased mRNA and protein expression of the colonic vitamin D receptor. Oncol Res, 1999;11:255–64.

47. Boyapati SM, Bostick RM, McGlynn KA. Vitamin D intake, vitamin D receptor *Bsm*I polymorphisms, and risk for incident sporadic colorectal adenoma [abstract]. 92nd AACR Annual Meeting; March 24–28, 2001.

48. Ingles SA, Wang J, Coetzee GA, Lee ER, Frankl HD, Haile RW. Vitamin D receptor polymorphisms and risk of colorectal adenomas (United States). Cancer Causes & Control, 2001;12:607–14.

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