Cancer Causes Control (2014) 25:809–817
DOI 10.1007/s10552-014-0382-6

ORIGINAL PAPER

Associations between circulating 1,25(OH)$_2$D concentration and odds of metachronous colorectal adenoma

Elizabeth A. Hibler · Christine L. Sardo Molmenti · Peter Lance · Peter W. Jurutka · Elizabeth T. Jacobs

Received: 16 January 2014 / Accepted: 2 April 2014 / Published online: 16 April 2014

Abstract Cellular-level studies demonstrate that the availability of the secosteroid hormone 1α,25-dihydroxyvitamin D [1,25(OH)$_2$D] to colon cells promotes anti-carcinogenic activities. Although epidemiological data are relatively sparse, suggestive inverse trends have been reported between circulating 1,25(OH)$_2$D concentration and colorectal neoplasia. We therefore sought to evaluate the relationship between circulating 1,25(OH)$_2$D concentrations and odds for metachronous colorectal adenomas among 1,151 participants from a randomized trial of ursodeoxycholic acid for colorectal adenoma prevention. No relationship between 1,25(OH)$_2$D and overall odds for metachronous lesions was observed, with ORs (95 % CIs) of 0.80 (0.60–1.07) and 0.81 (0.60–1.10) for participants in the second and third tertiles, respectively, compared with those in the lowest (p-trend = 0.17). However, a statistically significant inverse association was observed between circulating 1,25(OH)$_2$D concentration and odds of proximal metachronous adenoma, with an OR (95 % CI) of 0.71 (0.52–0.98) for individuals in the highest tertile of 1,25(OH)$_2$D compared with those in the lowest (p-trend = 0.04). While there was no relationship overall between 1,25(OH)$_2$D and metachronous distal lesions, there was a significantly reduced odds for women, but not men, in the highest 1,25(OH)$_2$D tertile compared with the lowest (OR 0.53; 95 % CI 0.27–1.03; p-trend = 0.05; p-interaction = 0.08). The observed differences in associations with proximal and distal adenomas could indicate that delivery and activity of vitamin D metabolites in different anatomic sites in the colorectum varies, particularly by gender. These results identify novel associations between 1,25(OH)$_2$D and metachronous proximal and distal colorectal adenoma, and suggest that future studies are needed to ascertain potential mechanistic differences in 1,25(OH)$_2$D action in the colorectum.

Keywords Vitamin D · Colon cancer · 1,25(OH)$_2$D · Colorectal adenoma

E. A. Hibler (✉) · P. Lance · E. T. Jacobs
University of Arizona Cancer Center, 1515 N. Campbell Ave, Tucson, AZ 85724-5024, USA
e-mail: ehibler@email.arizona.edu

E. A. Hibler · C. L. Sardo Molmenti · E. T. Jacobs
Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, USA

C. L. Sardo Molmenti
Mailman School of Public Health, Columbia University, New York, NY, USA

P. W. Jurutka
School of Mathematical and Natural Sciences, Arizona State University, Phoenix, AZ, USA

Introduction

Colorectal cancer is the second leading cause of cancer-related deaths in the US [1, 2]. While colonoscopy is an effective means of detecting and removing colorectal lesions, screening rates currently fall well short of recommended coverage [3], and endoscopy appears to be more effective for lesions in the distal colorectum than for those the proximal region [4]. Therefore, an understanding of risk factors for this disease, particularly those with differential effects by colorectal sub-site, remains critical. Sporadic colorectal neoplasia has been associated with a variety of lifestyle factors, including vitamin D status [5–7]. Associations between low circulating 25(OH)D concentration and increased risk of colorectal adenomas and cancer are consistently observed [5, 8–11]; however, epidemiological

studies of the active vitamin D metabolite, 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D], are less common.

The hormone 1,25$(OH)_2$D plays a central role in bone health as a component of the system regulating calcium homeostasis, and its concentration is maintained within a relatively narrow range due to this role [12, 13]. For this reason, 1,25$(OH)_2$D concentrations have rarely been studied in epidemiologic investigations, which have generally focused on 25(OH)D. However, a recent study by Skinner and Schwartz ascertained that serum calcium concentration, which also does not generally exhibit substantial variation within healthy individuals, is significantly associated with risk of fatal prostate cancer [14]. Furthermore, it was recently reported that circulating 1,25$(OH)_2$D levels are significantly inversely associated with serum fibroblast growth factor 23 (FGF23), suggesting that circulating 1,25$(OH)_2$D concentrations may be modifiable while remaining within a physiologically functional range [15]. These studies demonstrate that levels of homeostatically controlled metabolites vary enough in circulation to justify evaluation in epidemiological studies. Further, it is possible that there may be differential effects of 1,25$(OH)_2$D in the colorectum by factors such as gender, adenoma characteristics, or location in the colorectum.

Variation in risk of colorectal neoplasia by gender, anatomic location, or advanced histologic characteristics has been reported and may be differentially influenced by specific risk factors [6, 16, 17]. The hormone 1,25$(OH)_2$D is an example of one such risk factor, as it is not fully understood how circulating 1,25$(OH)_2$D levels or availability of 1,25$(OH)_2$D at the colonic epithelial cell level may vary by gender or differentially affect metachronous neoplasia by colorectal sub-site or by adenoma features. Therefore, the purpose of this study was to evaluate associations between circulating 1,25$(OH)_2$D concentration and metachronous colorectal adenomas, while accounting for participant and adenoma characteristics that could influence these relationships.

Methods

Study population

The present study was conducted among participants of a randomized, double blind, placebo-controlled phase III trial of ursodeoxycholic acid (UDCA) for adenoma prevention conducted at the University of Arizona Cancer Center, which has been described in detail previously [18]. Briefly, eligible participants were male and female Arizona residents ($n = 1{,}192$) ranging in age from 40 to 80 years, and who had one or more colorectal adenomas (>3 mm in diameter) removed during a colonoscopic evaluation within 6 months of entering the study. The primary study aim was to compare the effect of UDCA (8–10 mg/kg of body weight/day) versus placebo on the risk of metachronous colorectal adenoma [18]. There was no evidence that UDCA treatment decreased the overall rate of metachronous adenoma in the primary analyses [18]. Complete data for metachronous adenomas and circulating 1,25$(OH)_2$D concentration were available for 1,150 participants. The University of Arizona Human Subjects committee and local hospital committees approved the UDCA study, and written informed consent was obtained from each participant prior to study enrollment.

Dietary, sociodemographic, and medical history data were collected at baseline using self-administered questionnaires. Dietary data were obtained with the Arizona Food Frequency Questionnaire (AFFQ), a validated, 113-item, semi-quantitative, scannable instrument, with study participants asked to report their usual dietary intake during the prior year [19]. A scale of seven categories ranging from >3 times/day to rarely/never was employed for most items; for beverages and commonly consumed foods, the scale ranged from >6 times/day to rarely/never [19]. Portion sizes of small, medium, or large were also recorded for each food item, and total nutrients intake were calculated by multiplying the frequency of each item's consumption by the nutrient composition for each portion size [19].

Study endpoints

For this study, metachronous colorectal adenomas were defined as adenomas detected by colonoscopy at least 6 months after randomization to the parent trial. In the past, these lesions have been defined as "recurrences"; however, to account for the possibility that some of the lesions discovered at the follow-up colonoscopy may have been missed at baseline colonoscopy, the term "metachronous" adenomas will be used herein [20]. Detailed data regarding adenoma characteristics including number, size, location, and histology were obtained via medical records and pathology reports for each subject. Adenomas were classified as advanced if they had a diameter of 1 cm or more and/or tubulovillous/villous architecture (at least 25 % villous) and/or the presence of high-grade dysplasia and/or cancer. Additionally, adenocarcinomas ($n = 12$) were counted as advanced lesions. All other adenomas were considered non-advanced (<1 cm in size, tubular architecture, and no high-grade dysplasia). In subjects with more than one adenoma, size and characterization of the histologic type were based on the largest and/or most advanced adenoma.

Analysis of vitamin D metabolite levels

Vitamin D metabolite concentrations at baseline were measured at Heartland Assays (Ames, IA). The lab utilized an ^{125}I-based radioimmunoassay, which is an established practice and has been described in detail previously [21]. Quality assurance and control measures, including pooled sample analysis with batches of study samples for measuring analytical precision and possible laboratory shifts over time as well as duplicate testing in batches, were utilized by the laboratory. The coefficient of variation was 11.5 % for 1,25(OH)$_2$D analysis [22] and the analyses were conducted in a blinded fashion.

Statistical analysis

Participant characteristics were compared by tertile of 1,25(OH)$_2$D concentration with means and standard deviations calculated for the continuous variables, and frequencies and percentages for the categorical variables. Circulating 1,25(OH)$_2$D concentrations were evaluated as overall and gender-specific tertiles. Unconditional logistic regression modeling was used to evaluate the associations between 1,25(OH)$_2$D concentrations and metachronous colorectal adenoma characteristics. Potential confounding was assessed for age, body mass index (BMI), gender, race, family history of colorectal cancer, current smoking, history of previous polyps, physical activity, and aspirin use. Dietary intake of several nutrients was also evaluated for confounding and included measurements of vitamin D, dietary fat, fiber, folate, magnesium, calcium supplement use, and total energy intake; in addition, we considered intake of red meat as a potential confounder. A confounding variable was included in the final analysis if it changed the point estimate by 10 % or greater [23], and likelihood ratio tests comparing models with and without interaction terms were used to evaluate interactions by gender. We also assessed the possibility of heterogeneity of effect by treatment arm of the UDCA trial. We conducted stratified analyses by treatment arm of the association between 1,25(OH)$_2$D and adenoma recurrence and found no differences in the point estimate between intervention groups. We further assessed heterogeneity of effect between the two treatment groups by employing an interaction term for treatment arm and tertile of 1,25(OH)$_2$D and evaluating with a likelihood ratio test; no heterogeneity was observed. The STATA statistical software package (version 9.0, Stata Corporation, College Station, TX) was used for data management and statistical analysis.

Results

Participant characteristics by tertile of 1,25(OH)$_2$D concentration are presented in Table 1. Overall, UDCA trial participants had a mean age of 66.1 ± 8.5 years, 94.6 % reported White race, and 67.3 % were male. Dietary intake included a mean energy intake of 1,986.9 ± 819.9 kcal/day, calcium intake of 1,007.0 ± 492.9 mg/day, and vitamin D intake of 136.2 ± 98.6 IU/day. On average, 71.9 % of all participants reported regular use of supplements. Lower age and BMI, as well as fewer participants reporting a history of polyps or aspirin use, were observed in individuals in the highest tertile of 1,25(OH)$_2$D; however, only BMI, age, and gender were identified as confounding factors for inclusion on the logistic regression models.

There were no statistically significant associations between 1,25(OH)$_2$D and overall odds for metachronous adenoma (Table 2), with ORs (95 % CIs) of 0.80 (0.60–1.07) and 0.81 (0.60–1.10) for participants in the second and third tertiles, respectively, compared with those in the lowest (p-trend = 0.17). In addition, no statistically significant relationships were observed for multiplicity, large size, or villous histology. Heterogeneity of effect by gender was evaluated and no significant differences were observed. We also conducted exploratory analyses of associations between 1,25(OH)$_2$D concentrations and features of baseline adenomas. No statistically significant associations were identified (data not shown).

Odds of colorectal adenoma by anatomic location in the colon, proximal versus distal, were also evaluated (Table 3). There was an overall statistically significant association between odds of proximal metachronous adenoma and tertile of circulating 1,25(OH)$_2$D, with ORs (95 % CIs of 0.76 (0.56–1.04) for the second tertile and 0.71 (95 % CI 0.52–0.98) for the third tertile, compared with the lowest (p-trend = 0.04). No heterogeneity of effect by gender was observed for proximal adenomas. In contrast, a statistically significant interaction by gender (p = 0.08) was found for distal adenomas. While there was no association observed between 1,25(OH)D and distal metachronous lesions among men, for women, a statistically significant inverse trend (p = 0.05) for reduced odds of distal metachronous adenoma was observed, with ORs (95 % CIs) of 0.61 (0.33–1.15) and 0.53 (0.27–1.03)for the second and third tertiles compared with those in the lowest tertile. Overall, these results provide evidence for associations between circulating 1,25(OH)$_2$D concentration and odds of colorectal neoplasia that vary by gender and anatomic location in the colorectum.

Discussion

The results of the present study demonstrated that higher circulating 1,25(OH)$_2$D concentrations were not associated with odds for metachronous adenoma overall, nor with advanced features of adenomas. However, 1,25(OH)$_2$D

Table 1 Baseline characteristics of participants, by tertile of $1,25(OH)_2D$ concentration

Baseline characteristics	Tertile of $1,25(OH)_2D$ concentration (pg/ml) (mean ± SD)			
	All $n = 1,151$	22.7 ± 4.5 $n = 390$	33.4 ± 2.8 $n = 379$	47.0 ± 8.2 $n = 382$
Age at baseline (mean ± SD)	66.1 ± 8.5	67.6 ± 7.9	66.1 ± 8.8	64.7 ± 8.5
BMI (kg/m^2, mean ± SD)	28.2 ± 4.8	29.0 ± 5.1	28.2 ± 4.6	27.3 ± 4.6
Male, *n* (%)	744 (67.3)	239 (61.3)	273 (72.0)	262 (68.6)
White, *n* (%)	1,070 (94.6)	362 (94.0)	355 (95.2)	353 (94.6)
Family History CRC, *n* (%)[a]	315 (27.4)	100 (25.6)	120 (31.7)	95 (24.9)
Current Smoker (yes), *n* (%)	134 (11.6)	42 (10.8)	47 (12.4)	45 (11.8)
Previous Polyps (yes), *n* (%)[b,c]	515 (44.7)	185 (47.4)	183 (48.3)	147 (38.5)
Aspirin Use (yes), *n* (%)[d]	319 (27.7)	109 (28.0)	113 (29.8)	97 (25.4)
Dietary intake				
Energy (kcal/day)	1,986.9 ± 819.9	1,937.9 ± 818.8	2,019.3 ± 829.8	2,004.6 ± 810.9
Fat (g/day)	62.2 ± 32.0	60.8 ± 30.7	62.2 ± 32.7	63.7 ± 32.5
Fiber (g/day)	22.2 ± 10.7	22.2 ± 10.5	22.7 ± 10.9	21.8 ± 10.8
Vitamin D (IU/day)	136.2 ± 98.6	130.7 ± 96.8	144.1 ± 102.9	134.0 ± 96.0
Calcium (mg/day)	1,007.0 ± 492.9	981.4 ± 472.7	1,025.9 ± 482.8	1,014.5 ± 522.2
Folate (mcg/day)	472.5 ± 235.2	459.7 ± 218.5	483.5 ± 237.0	474.7 ± 249.4
Magnesium (mg/day)	338.6 ± 136.3	332.4 ± 133.1	344.9 ± 138.0	338.5 ± 138.0
Supplement use (yes)	827 (71.9)	296 (75.9)	269 (71.0)	262 (68.6)

[a] Family history of colorectal cancer in one or more first degree relatives

[b] History of polyps prior to baseline

[c] Number do not add up to 100 % due to missing data

[d] Aspirin use in the last month

was statistically significantly associated with reduced risk of proximal metachronous colorectal adenoma in our study population. We also found that the relationship between $1,25(OH)_2D$ and distal adenoma varied by gender, with significantly reduced odds among women with higher serum concentration of $1,25(OH)_2D$, but not among men. The differences in associations between vitamin D metabolites and colorectal adenoma by anatomic location and gender have been suggested by previous work, as discussed below, though the epidemiological literature overall is relatively sparse for $1,25(OH)_2D$.

The evidence to date for a relationship between serum $1,25(OH)_2D$ and risk of adenoma is equivocal. There are numerous studies that demonstrate a statistically significantly increased risk of colorectal neoplasia, including both adenomas and cancer, with lower circulating 25(OH)D concentration [5, 7, 24, 25]. A prior analysis of UDCA trial participants by Jacobs et al. [8] revealed a suggestive but not statistically significant association between circulating 25(OH)D concentration and metachronous adenoma, with a stronger association in women compared with men. An expanded analysis of a pooled population including UDCA and Wheat Bran Fiber trial participants identified statistically significant associations between 25(OH)D levels and specific adenoma characteristics at baseline, but not with odds of metachronous adenoma [26]. In the present study, we observed no associations between $1,25(OH)_2D$ and baseline adenomas (data not shown), but we did detect significant associations between this metabolite and metachronous proximal lesions, as well as distal metachronous adenoma in women. We can only speculate as to why 25(OH)D concentrations were associated with only baseline adenoma characteristics in this study population [26], while $1,25(OH)_2D$ levels were related only to some types of metachronous adenomas. One possibility is that the different vitamin D metabolites have actions in various stages of adenoma formation, such that the active hormonal form can prevent new growth of lesions, but does not inhibit the progression of lesions that have already been initiated. Another consideration regarding the study population is the potential role of the intervention agent, UDCA. Although the primary findings of the trial were null [18], Thompson et al. [27] conducted a further investigation of the role of gender on treatment effect in the UDCA trial. This study demonstrated that UDCA treatment was significantly associated with reduced odds of metachronous adenomas in men, but not women [28]. However, no heterogeneity of effect for the association between $1,25(OH)_2D$ and adenoma recurrence was observed by treatment arm in the present study, indicating that this was not a factor in the overall or gender-specific findings.

As with calcium, circulating $1,25(OH)_2D$ concentrations are maintained within a tight homeostatic range due to their importance to multiple physiologic functions [13, 29, 30]. However, recent work by Skinner and Schwartz [14] identified a statistically significant association between variation in serum calcium concentration and risk of fatal prostate cancer among the National Health and Nutrition

Table 2 Adjusted odds ratios[a] (95 % confidence intervals) for the association between $1,25(OH)_2D$ concentration and metachronous colorectal adenoma

Tertile of $1,25(OH)_2D$ concentration (pg/ml)	Metachronous Adenoma OR (95 % CI)[a]							
	Any metachronous adenoma		Multiplicity (≥3 adenoma)		Large size (≥1 cm)		Villous histology[b]	
	n (%)	OR (95 % CI)	*n* (%)	OR (95 % CI)	*n* (%)	OR (95 % CI)	*n* (%)	OR (95 % CI)
Pooled population								
T1 (22.7 ± 4.5)	181 (46.4)	1.00	42 (10.8)	1.00	40 (10.3)	1.00	36 (9.3)	1.00
T2 (33.4 ± 2.8)	154 (40.6)	0.80 (0.60–1.07)	29 (7.7)	0.74 (0.44–1.23)	39 (10.3)	1.00 (0.62–1.61)	25 (6.6)	0.74 (0.43–1.27)
T3 (47.0 ± 8.2)	150 (39.3)	0.81 (0.60–1.10)	28 (7.3)	0.80 (0.47–1.34)	29 (7.6)	0.70 (0.41–1.18)	29 (7.6)	0.90 (0.53–1.53)
p-trend		0.17		0.37		0.20		0.66
Men								
T1 (23.6 ± 4.5)	126 (47.9)	1.00	32 (12.2)	1.00	30 (11.4)	1.00	22 (8.4)	1.00
T2 (33.9 ± 2.6)	111 (43.9)	0.91 (0.64–1.29)	22 (8.7)	0.81 (0.45–1.46)	28 (11.1)	1.00 (0.58–1.74)	21 (8.3)	1.14 (0.60–2.16)
T3 (47.0 ± 7.6)	109 (42.3)	0.90 (0.63–1.30)	24 (9.3)	0.97 (0.54–1.75)	19 (7.4)	0.63 (0.33–1.17)	20 (7.8)	1.09 (0.56–2.12)
p-trend		0.58		0.90		0.16		0.79
Women								
T1 (21.1 ± 4.3)	54 (42.5)	1.00	10 (7.9)	1.00	11 (8.7)	1.00	12 (9.5)	1.00
T2 (32.2 ± 3.2)	43 (33.9)	0.68 (0.41–1.15)	7 (5.5)	0.67 (0.24–1.82)	9 (7.1)	0.79 (0.31–1.98)	5 (4.0)	0.38 (0.13–1.10)
T3 (47.2 ± 9.4)	42 (34.2)	0.76 (0.45–1.29)	4 (3.3)	0.46 (0.14–1.53)	11 (8.9)	1.00 (0.40–2.49)	10 (8.2)	0.91 (0.37–2.23)
p-trend		0.30		0.19		0.99		0.77
p-interaction[c]		0.66		0.50		0.47		0.17

[a] Odds ratios adjusted for body mass index, gender (except for stratified analysis), and age

[b] Villous histology was present if adenoma exhibited tubulovillous or villous histology (at least 25 % villous)

[c] *p*-interaction for gender and vitamin D status calculated using a likelihood ratio test

Examination Survey (NHANES) participants. Furthermore, recent studies have shown that circulating $1,25(OH)_2D$ concentrations are influenced by serum fibroblast growth factor 23 (FGF23) [15, 31–33]. These studies indicate that circulating concentrations of homeostatically controlled metabolites involved in the vitamin D endocrine system may exhibit enough variation to warrant study in an epidemiological setting. Prior epidemiological studies of $1,25(OH)_2D$ and colorectal neoplasia have shown suggestive results.

In a population from the Nurses' Health Study (NHS), Platz et al. found that in 326 matched case and control pairs, participants with circulating $1,25(OH)_2D$ in the lowest quartile demonstrated increased odds of distal colorectal adenoma (OR 1.58, 95 % CI 1.03–2.40). There was also a non-significant trend of increased odds for women in the lower two quartiles of plasma $1,25(OH)_2D$ (p-trend = 0.20) [17]. These results are similar to those of the current study, as we observed statistically significantly decreased odds of distal adenoma for women with higher circulating $1,25(OH)_2D$ concentration (OR 0.53, 95 % CI (0.27–1.03); p-trend = 0.05). In contrast to these results, Lee et al. [6] evaluated data from The Physician's Health Study and reported no significant association between $1,25(OH)_2D$ concentration and colorectal cancer among men. An additional study by Feskanich et al. [16] also of colorectal cancer identified no statistically significant associations among NHS participants; however, they did observe that women in the highest quintile of $1,25(OH)_2D$ concentration demonstrated increased odds of overall colorectal cancer (OR 1.77 95 % CI 0.93–3.36). The results for colorectal adenoma and cancer are conflicting, but indicate that any role of circulating $1,25(OH)_2D$ in colorectal neoplasia may vary stage of carcinogenesis, gender, or colorectal sub-site. This concept is supported by the results of the present study, which identified statistically significant variations in the relationship between circulating $1,25(OH)_2D$ and odds of metachronous adenomas by gender as well as by anatomic location. These findings support the hypothesis that colorectal neoplasia in specific anatomic areas of the GI tract may represent sub-types of the disease.

The proximal colon includes the transverse colon, hepatic flexure, ascending colon, and cecum; which are the areas proximal to the splenic flexure [34, 35], while the distal colon includes the areas distal to the splenic flexure with the descending colon, sigmoid colon, and rectum [34]. These sub-sites of the colorectum have different embryonic

Table 3 Adjusted odds ratios[a] (95 % confidence intervals) for the association between $1,25(OH)_2D$ concentration and metachronous adenoma, by colorectal sub-site and gender

Tertile of $1,25(OH)_2D$ concentration	Colorectal sub-site OR (95 % CI)[a]			
	Distal adenoma		Proximal adenoma	
	n (%)	OR (95 % CI)	*n* (%)	OR (95 % CI)
Pooled population				
T1 (22.7 ± 4.5)	96 (24.7)	1.00	142 (36.5)	1.00
T2 (33.4 ± 2.8)	77 (20.4)	0.80 (0.56–1.13)	113 (30.0)	0.76 (0.56–1.04)
T3 (47.0 ± 8.2)	87 (22.8)	0.96 (0.68–1.36)	101 (26.5)	0.71 (0.52–0.98)
p-trend		0.82		0.04
Men				
T1 (23.6 ± 4.5)	64 (24.43)	1.00	104 (39.7)	1.00
T2 (33.9 ± 2.6)	57 (22.7)	1.00 (0.66–1.52)	79 (31.5)	0.76 (0.52–1.11)
T3 (47.0 ± 7.6)	69 (26.9)	1.32 (0.87–2.00)	73 (28.4)	0.72 (0.49–1.05)
p-trend		0.19		0.09
Women				
T1 (21.1 ± 4.3)	31 (24.4)	1.00	37 (29.1)	1.00
T2 (32.2 ± 3.2)	21 (16.5)	0.61 (0.33–1.15)	34 (26.8)	0.87 (0.49–1.52)
T3 (47.2 ± 9.4)	18 (14.6)	0.53 (0.27–1.03)	29 (23.6)	0.84 (0.47–1.51)
p-trend		0.05		0.56
p-interaction[b]		0.08		0.27

[a] Odds ratios adjusted for body mass index, gender (except for stratified analysis), and age

[b] *p*-interaction for gender and vitamin D status calculated using a likelihood ratio test

origins, with the proximal colon originating from the midgut and the distal colorectum from the hindgut [34, 36]. There are functional differences by sub-site that range from water and electrolyte absorption to storage of feces, which may lead to unique carcinogenic exposures for cells in each region [35]. This is supported by additional evidence that indicates differential carcinogenic pathways for distal versus proximal colorectal neoplasia [37]. For example, microsatellite instability (MSI) is more frequently observed in proximal lesions compared with chromosomal instability in distal colon neoplasia [38, 39], while epigenetic methylation patterns also differ by region [40]. Studies by Slattery et al. have found associations between tumors with MSI and vitamin D receptor (*VDR*) polymorphisms, such as FOKI, as well as evidence that the *VDR* polymorphisms are associated with proximal colorectal cancer [41, 42]. Overall, it is possible that exposure of colonic epithelial cells to circulating $1,25(OH)_2D$ may have unique effects in the proximal colon versus the distal colon, particularly among men, due to differential factors promoting carcinogenesis, though further investigation is warranted.

The results of the current work also demonstrate reduced risk of both proximal and distal metachronous colorectal adenoma for women, though only the association with distal metachronous adenoma achieved statistical significance. Recent studies have demonstrated that men may be at increased risk for colorectal adenoma and recurrence [4, 43, 44]; however, the evidence is more consistent for colorectal cancer [45, 46]. An interaction between estrogen and vitamin D has been proposed to explain the observed reduced risk of colorectal neoplasia in women [47, 48]. Estrogen and vitamin D are both in the steroid hormone family and studies have proposed differences in estrogen receptor expression by colonic region [45, 49]. Furthermore, Protiva et al. [48] demonstrated that estrogen replacement therapy in postmenopausal women increased expression of the vitamin D receptor as well as genes related to the vitamin D endocrine system, including CYP24A1 and E-cadherin. In turn, increased expression of E-cadherin inhibits the activity of the Wnt/β-catenin pathway, which acts to promote cell adhesion and differentiation [50, 51]. Therefore, estrogen and vitamin D may work synergistically to reduce activity of this cancer promotion pathway, which may help explain a more consistent overall association between $1,25(OH)_2D$ concentrations and adenoma recurrence among women in the present study.

The biologic activity of vitamin D is initiated when $1,25(OH)_2D$ binds to the vitamin D receptor [30]. At the cellular level, autocrine processing of 25(OH)D to $1,25(OH)_2D$ by intracellular 1α-hydroxylase (CYP27B1) is demonstrated in several tissues [52]; however, there are many factors influencing this process. For example, variation in *CYP27B1* has been demonstrated to affect conversion of 25(OH)D to $1,25(OH)_2D$ [53]. Furthermore, polymorphic variation in the Gc-globulin (GC), also known as the vitamin D binding protein, has been associated with circulating 25(OH)D and GC levels as well as

concentrations of "free" versus GC-bound vitamin D metabolites [54, 55]. Our own work has also demonstrated that Gc-globulin isotypes are associated with 25(OH)D and $1,25(OH)_2D$ availability to colon cancer cells [56]. Therefore, it is of interest to determine which vitamin D metabolite is most reflective of tissue-level activity. While circulating 25(OH)D concentration is the most common measure of vitamin D status, it is known to be affected by a variety of factors including sun exposure, dietary intake, gender, physical activity levels, body size, and genetic variation [57, 58]. However, recent work has suggested that $1,25(OH)_2D$ may also be influenced by factors such as body size and season as well [59]. Recent literature suggests that 25(OH)D alone may not be a sufficient measure of vitamin D status [60]; however, it is not clear if $1,25(OH)_2D$ would be a better marker or a potential biomarker to consider in combination with 25(OH)D and GC concentrations. The present work supports substantial future investigation of the potential for more complex assessment of vitamin D status.

The strengths of the present study include a large sample size, with over 1,000 participants, and prospective nature of the data, including measurements of metachronous adenoma among study participants. Furthermore, the data available for participants included comprehensive pathology of adenomas, circulating vitamin D metabolite concentrations, and complete demographic as well as dietary information. Limitations include that a single baseline measure of circulating $1,25(OH)_2D$ concentrations was available for each participant, and the population was largely White with an average age of approximately 65 years. Future studies in large, diverse populations with longitudinal measures of vitamin D metabolite concentrations will improve our understanding of the role of circulating $1,25(OH)_2D$ concentration in risk of colorectal neoplasia.

Conclusions

The results of the current study identify statistically significant associations between circulating $1,25(OH)_2D$ concentration and odds of colorectal metachronous adenoma that varies by anatomic location in the colon, as well as by gender. These findings add to a growing literature which suggests that variation in metabolites with homeostatically controlled concentrations, such as $1,25(OH)_2D$ and calcium, may have a wide enough range to observe changes in risk of disease in some populations. It will be important to continue evaluating the role of $1,25(OH)_2D$ concentration in risk of colorectal adenoma and cancer in additional diverse populations in order to identify individuals at risk for disease and implement cancer prevention strategies.

Acknowledgments This work was supported by the R25T training fellowship (R25CA078447) awarded to EH as well as a grant from the National Cancer Institute (R01CA140285) to EJ and PJ. EH was responsible for conducting the epidemiologic analysis and writing the manuscript. EJ, CS, PL, and PJ were responsible for assisting with manuscript preparation and editing as well as evaluating the analysis. No authors have any financial conflicts of interest to disclose.

Conflict of interest The authors have no conflicts to disclose.

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