

Calcium Intake and Prostate Cancer Among African Americans: Effect Modification by Vitamin D Receptor Calcium Absorption Genotype

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ABSTRACT

High dietary intake of calcium has been classified as a probable cause of prostate cancer, although the mechanism underlying the association between dietary calcium and prostate cancer risk is unclear. The vitamin D receptor (VDR) is a key regulator of calcium absorption. In the small intestine, VDR expression is regulated by the CDX-2 transcription factor, which binds a polymorphic site in the VDR gene promoter. We examined VDR Cdx2 genotype and calcium intake, assessed by a food frequency questionnaire, in 533 African-American prostate cancer cases (256 with advanced stage at diagnosis, 277 with localized stage) and 250 African-American controls who participated in the California Collaborative Prostate Cancer Study. We examined the effects of genotype, calcium intake, and diet-gene interactions by conditional logistic regression. Compared with men in the lowest quartile of calcium intake, men in the highest quartile had an approximately twofold increased risk of localized and advanced prostate cancer (odds ratio [OR] = 2.20, 95% confidence interval [CI] = 1.40, 3.46), with a significant dose-response. Poor absorbers of calcium (VDR Cdx2 GG genotype) had a significantly lower risk of advanced prostate cancer (OR = 0.41, 95% CI = 0.19, 0.90). The gene-calcium interaction was statistically significant ($p = 0.03$). Among men with calcium intake below the median (680 mg/day), carriers of the G allele had an approximately 50% decreased risk compared with men with the AA genotype. These findings suggest a link between prostate cancer risk and high intestinal absorption of calcium. © 2012 American Society for Bone and Mineral Research.

KEY WORDS: VITAMIN D RECEPTOR; CALCIUM ABSORPTION; GENETIC POLYMORPHISM, PROSTATE CANCER; AFRICAN-AMERICAN

Introduction

The relationship between diets high in calcium and risk of prostate cancer has been the subject of numerous epidemiologic studies. In a recent review of this literature, the World Cancer Research Fund (WCFR) classified calcium as a probable cause of prostate cancer.⁽¹⁾ The Agency for Health Care Research and Quality (AHCQR) reached a similar conclusion.⁽²⁾ The assessment of the WCFR was based on examination of 9 cohort and 12 case-control studies. On meta-analysis, higher intake of calcium was associated with increased risk of prostate cancer, consistent with a dose-response relationship in the cohort studies. The AHCQR report considered that of the four cohort studies with the highest methodologic quality, three showed that diets high in calcium were associated with an increased risk of advanced or fatal disease. Recently, two

prospective studies showed that serum levels of calcium that are high but within the normal reference range (high normocalcemia) were associated with a two- to threefold increase in prostate cancer mortality.^(3,4) Because levels of ionized calcium in serum are increased after absorption of a moderate dose of calcium, we hypothesized that prostate cancer risk may be influenced by polymorphisms in genes that influence the efficiency of calcium absorption.

Many of the genes affecting calcium absorption are regulated by the vitamin D receptor (VDR).^(5,6) In the small intestine, VDR expression is regulated by the tissue-specific transcription factor, CDX-2⁽⁷⁾ which binds a site in the VDR upstream 1 e promoter.⁽⁸⁾ This CDX-2 binding site harbors a single nucleotide A/G polymorphism (denoted Cdx2). The CDX-2 protein binds more efficiently to the A than to the G allele, and the A allele has been shown to more efficiently drive transcription in in vitro reporter

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gene assays.⁽⁹⁾ The VDR Cdx2 low activity G allele has been associated consistently with lower bone mineral density in candidate gene studies,⁽¹⁰⁾ and data from genome-wide association studies (GWAS) support these findings. Although the association did not attain genome-wide significance ($p = 0.0007$), a single nucleotide polymorphism (SNP) (rs7132324) tightly linked to the Cdx2 SNP ($D' = 0.967$) was among the top 0.2% of hits for bone mineral density at the hip.⁽¹¹⁾ Although the effect of the Cdx2 SNP on intestinal calcium absorption remains to be experimentally verified, these data suggest that the differences observed between alleles in vitro influence intestinal calcium absorption in vivo.

We hypothesized that the high activity A allele, which is common in populations of African origin, may contribute to the high prostate cancer incidence and mortality rates and younger ages at diagnosis observed in African-Americans compared with other racial/ethnic groups.^(12,13) We examined dietary calcium intake and VDR Cdx2 genotype among 533 African-American cases (256 with advanced stage at diagnosis; 277 with localized stage) and 250 African-American controls from the California Collaborative Prostate Cancer Study, a population-based multiethnic case-control study that had a high proportion of cases diagnosed with aggressive prostate cancer.

Materials and Methods

Study population

The study population from the San Francisco Bay area and Los Angeles County has been described in detail previously.⁽¹⁴⁾ Cases were identified by the Greater Bay Area Cancer Registry, the Los Angeles County Cancer Surveillance Program, and the Los Angeles County Cancer Registry. Five hundred fifty-nine African-American cases (including 377 from Southern California and 182 from Northern California) completed the interview. Twenty-six cases did not have definitive stage and were excluded, leaving 533 cases. Four hundred fifty-four cases (246 advanced and 208 localized) provided a biospecimen. Biospecimens were not collected from localized cases at the Northern California site.

In both studies, advanced prostate cancer was defined according to SEER (Surveillance Epidemiology and End Results) 1995 pathologic and clinical extent of disease codes. Of the 533 participating cases, 256 (116 from Northern California and 140 from Southern California) were diagnosed with advanced stage, 277 (66 from Northern California and 211 from Southern California) were diagnosed with localized disease.

Controls were identified through random-digit dialing and from random selections from the rosters of beneficiaries of the Health Care Financing Administration at the Northern California site and by a standard neighborhood walk algorithm⁽¹⁵⁾ at the Southern California site. Controls were frequency matched to cases on self-reported race/ethnicity and expected 5-year age distributions. Two hundred fifty controls (including 162 from Southern California and 88 from Northern California) completed the interview and 245 provided a biospecimen.

The study was approved by the institutional review boards of the Cancer Prevention Institute of California (formerly the

Northern California Cancer Center) and the University of Southern California. Written informed consent was obtained for all study participants.

Data collection

Trained interviewers conducted in-person interviews and administered a structured questionnaire that asked about demographic background, lifestyle factors (physical activity, alcohol consumption, smoking), body size, use of supplements containing calcium, family history of prostate cancer in first-degree relatives, medical history, and screening for prostate cancer. A 74-item food frequency questionnaire adapted from Block's Health History and Habits Questionnaire⁽¹⁶⁾ assessed usual dietary intake during the reference year, defined as the calendar year before diagnosis for cases and the year before selection into the study for controls. The interviewers also took three measurements of standing height and weight, which were averaged. Calcium intake was assessed from single calcium tablets, multivitamin pills, and calcium-based antacids, such as Tums, Roloids, Alka-Mints, or Chooz Antacid gum. Data were collected on age at first use, frequency of use, and duration of use.

Exposure variables

Dietary questionnaires from subjects reporting total energy intake greater than 6000 or less than 600 kilocalories per day (33 cases and 10 controls) were considered to be unreliable and were excluded from the analysis, leaving 500 cases and 240 controls with dietary information.

We derived two measures of calcium exposure, including total calcium from foods, beverages, and supplements, and dietary calcium from foods and beverages only. Cut points were selected based on the calcium intake of controls. Calcium supplementation was divided into dichotomous categories reflecting usual daily calcium contained in multivitamins (400 mg/day) versus less than 400 mg/day. Body mass index (BMI) was calculated as reported weight in the reference year (in kilograms) divided by measured height squared (in meters [m]) and dichotomized as obese ($BMI \geq 30$) and nonobese ($BMI < 30$).

Genotyping

The CDX-2 protein binding site SNP⁽¹⁰⁾ (rs11568820) was genotyped on the TaqMan 7900HT Sequence Detection System using the TaqMan Core Reagent Kit (Applied Biosystems, Foster City, CA, USA). Polymerase chain reactions (PCRs) were carried out as recommended by the manufacturer. The following primer and minor groove binder probe sequences were used: forward primer 5'-CATTGTAGAACATCTTTTGTATCAGGAAC-3', reverse primer 5'-GGTCTCCCAGGACAGTATTTTCA-3', G allele FAM-AGGTCACAGTAAAAAC-3', and A allele VIC-AGGTCACAATAAAC-3'. Ten percent of samples were blindly replicated and samples with known genotype were included as controls on each run. Clusters were manually called without knowledge of case-control status. There were no discrepancies among replicate samples. Genotypes were called for 447 cases and 233 controls, giving a call rate of 97%.

Statistical analysis

Allele frequencies were estimated by gene counting. Tests for departures from Hardy-Weinberg equilibrium among controls were conducted by comparing observed and expected genotype frequencies using a chi-square test.

A matching variable for conditional logistic regression was constructed by creating study site/socioeconomic status (SES) bins, as previously described.⁽¹⁴⁾ Odds ratios (OR) and 95% confidence intervals (CI) were estimated by fitting conditional logistic regression models, using study site/SES as the matching variable and adjusting for age (continuous variable) and family history of prostate cancer in first-degree relatives (yes or no). Calcium was categorized according to quartiles among the controls. Dose-response trends were assessed by including quartile as an ordinal variable in logistic regression models. Tests of interaction were conducted by including crossproduct terms in the conditional logistic models and conducting a 1 degree of freedom likelihood ratio test.

Results

Characteristics of participants are shown in Table 1. The median age at diagnosis was 64 years. On average, advanced cases were 3 years younger at diagnosis than localized cases. Cases and controls were similar with respect to education, SES, and BMI. Cases were more likely to report a family history of prostate cancer and consumed more calcium than did controls.

The majority of participants (82% of cases and 76% of controls) consumed less than the recommended intake of 1200 mg of calcium per day.⁽¹⁷⁾ The predominant sources of dietary calcium were similar for cases and controls: dairy products (45%), followed by vegetables (predominantly spinach, other greens, and broccoli) (25%), and grains (including corn tortillas) (15%). Dietary calcium and total calcium showed similar patterns of increased risk of both advanced and localized disease associated with increasing intake (Table 2). Men in the highest quartile of total calcium intake (>1059 mg/day) had a more than twofold increased risk of prostate cancer (advanced or localized) versus

Table 1. Characteristics of Prostate Cancer Cases and Controls

	Controls <i>N</i> = 250		Cases <i>N</i> = 533	
	<i>N</i> (250) <i>N</i> (%)		Advanced (<i>N</i> = 256) <i>N</i> (%)	Localized (<i>N</i> = 277) <i>N</i> (%)
Age (years)				
≤ 49	17 (7%)		15 (6%)	13 (5%)
50–59	63 (25%)		79 (31%)	56 (20%)
60–69	105 (42%)		106 (41%)	114 (41%)
70–79	60 (24%)		53 (21%)	78 (28%)
≥ 80	5 (2%)		3 (1%)	16 (6%)
mean (SD)	63.5 (9.15)		62.7 (8.46)	65.8 (8.85)
Education				
High school or less	99 (40%)		120 (47%)	120 (43%)
College degree/some college	95 (38%)		79 (31%)	111 (40%)
Postgraduate	49 (20%)		57 (22%)	45 (16%)
Unknown	7 (2%)		0 (0%)	1 (<1%)
SES (census tract-based)				
1 = Low	51 (20%)		69 (27%)	96 (35%)
2	71 (28%)		54 (21%)	70 (25%)
3	57 (23%)		63 (25%)	58 (21%)
4	48 (19%)		45 (18%)	33 (12%)
5 = High	23 (9%)		25 (10%)	20 (7%)
Family history of prostate cancer				
No	217 (87%)		195 (76%)	214 (77%)
Yes	28 (11%)		61 (24%)	63 (23%)
Unknown	5 (2%)		0 (0%)	0 (0%)
	Mean (SD)		Mean (SD)	Mean (SD)
BMI (kg/m ²)	28.8 (5.5)		28.3 (5.5)	28.5 (4.8)
Calcium intake				
Total calcium (mg/day)	818 (474)		979 (577)	945 (510)
Dietary calcium (mg/day)	755 (444)		890 (517)	869 (497)
Supplemental calcium (mg/day)	64 (155)		89 (217)	75 (130)

BMI = body mass interval; SES = socioeconomic status.

Table 2. Total, Dietary and Supplemental Calcium Intake and Prostate Cancer Risk

	All Cases versus Controls		Advanced Cases versus Controls		Localized Cases versus Controls		
	Controls N = 240	N = 500	N = 235	N = 265	N = 265	N = 265	
Total calcium^a	N (%)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
<488 mg/day	61 (25%)	84 (17%)	1.0 (ref)	41 (17%)	1.00 (ref)	43 (16%)	1.00 (ref)
488–680 mg/day	59 (25%)	75 (15%)	0.91 (0.56, 1.47)	34 (14%)	0.84 (0.47, 1.51)	41 (15%)	0.96 (0.54, 1.71)
681–1059 mg/day	61 (25%)	162 (32%)	1.89 (1.21, 2.97)	77 (33%)	1.87 (1.10, 3.19)	85 (32%)	1.89 (1.11, 3.21)
>1059 mg/day	59 (25%)	179 (36%)	2.20 (1.40, 3.46)	83 (35%)	2.08 (1.22, 3.53)	96 (36%)	2.14 (1.26, 3.62)
<i>p</i> for Trend			<i>p</i> < 0.001		<i>p</i> = 0.001		<i>p</i> = 0.001
Dietary calcium	N (%)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
<449 mg/day	60 (25%)	77 (15%)	1.0 (ref)	34 (14%)	1.00 (ref)	43 (16%)	1.00 (ref)
449–633 mg/day	60 (25%)	94 (19%)	1.19 (0.74, 1.92)	44 (19%)	1.30 (0.72, 2.33)	50 (19%)	1.09 (0.62, 1.90)
634–973 mg/day	60 (25%)	149 (30%)	1.87 (1.18, 2.97)	71 (30%)	2.11 (1.21, 3.68)	78 (29%)	1.60 (0.94, 2.74)
>973 mg/day	60 (25%)	180 (36%)	2.26 (1.43, 3.57)	86 (37%)	2.48 (1.43, 4.28)	94 (35%)	1.94 (1.14, 3.30)
<i>p</i> for Trend			<i>p</i> = 0.001		<i>p</i> = 0.001		<i>p</i> = 0.005
Supplements	N (%)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
< 400 mg/day	235 (98%)	479 (96%)	1.0 (ref)	222 (94%)	1.00 (ref)	257 (97%)	1.00 (ref)
≥ 400 mg/day	5 (2%)	21 (4%)	2.50 (0.92, 6.79)	13 (6%)	3.15 (1.09, 9.15)	8 (3%)	1.86 (0.58, 5.98)

^aTotal calcium is dietary calcium plus calcium supplements. Models adjusted for age and family history of prostate cancer. CI = confidence interval; OR = odds ratio.

men in the lowest quartile (<488 mg/day) (OR = 2.20, 95% CI = 1.40, 3.46, *p* for trend = 0.01). Intake of calcium from supplements was low, with fewer than 5% of men consuming at least 400 mg/day of supplemental calcium. However, consumers of 400 mg/day or more had a significantly increased risk for advanced prostate cancer (OR = 3.15, 95% CI = 1.09, 9.15) (Table 2).

The VDR Cdx2 minor (G) allele frequency was 28%. Genotype frequencies among the controls were in Hardy-Weinberg equilibrium. The VDR Cdx2 genotype was significantly associated with advanced, but not localized, prostate cancer (Table 3). Risk decreased with increasing number of G alleles (*p* trend = 0.02). Compared with men with genotype AA, those with the GG genotype were 59% less likely to have been diagnosed with advanced prostate cancer. When combining all cases (advanced plus localized), the reduction in risk for genotype GG versus AA was slightly attenuated (42%) and was of borderline statistical significance (*p* = 0.09).

The relationship between Cdx2 genotype and risk of advanced prostate cancer was modified by calcium intake (*p* for interaction = 0.03) (Table 3). Among men with calcium intake below the median, carriers of the G allele had an approximately 50% decreased risk compared with those with genotype AA. Among men with calcium intake above the median, Cdx2 G alleles were not associated with reduced risk of advanced prostate cancer. There was no association between Cdx2 genotype and risk of localized prostate cancer, regardless of the level of calcium intake. High calcium intake was a risk factor for advanced prostate cancer among men of all genotypes (Table 4), although the association was of borderline statistical significance among men with genotype AA (*p* = 0.08).

The relationship between dietary calcium and prostate cancer risk was modified by obesity (Table 5). High calcium intake was a

risk factor among both obese and nonobese men, although the association appeared to be stronger among the obese (*p* for interaction = 0.06). Data were too sparse to determine whether the relationship between VDR Cdx2 genotype and prostate cancer risk was modified by obesity, because genotype was a risk factor only for advanced disease.

Discussion

Our finding of an association between dietary calcium and prostate cancer risk is consistent with a sizable literature on dietary calcium and prostate cancer; see reviews, refs. (18,19). However, few studies have examined genotypes related to calcium absorption. To our knowledge, the VDR Cdx2 polymorphism has been examined in four prostate cancer studies, with inconsistent findings. Two studies examined the Cdx2 polymorphism with respect to sun exposure and prostate cancer risk. A UK study in an exclusively Caucasian population found a twofold increased risk among carriers of the A allele, consistent with our findings, but that finding was limited to men with high sunlight exposure.⁽²⁰⁾ Conversely, in a US study of non-Hispanic White men, we found no significant association between VDR Cdx2 genotype and advanced prostate cancer risk, regardless of sun exposure.⁽²¹⁾ Cdx2 genotype was examined in conjunction with serum vitamin D levels in the Physician's Health study.⁽²²⁾ Although there was a significant interaction between VDR Cdx2 genotype and vitamin D status, genotype was not significantly related to prostate cancer risk within strata defined by serum 25(OH)D levels (deficient/sufficient). Finally, in a study that did not examine vitamin D status or sunlight exposure, Torkko et al.⁽²³⁾ found a borderline significant association between the A

Table 3. VDR Cdx2 Polymorphism and Prostate Cancer Risk

VDR-Cdx2	Controls	All Cases versus Controls		Advanced Cases versus Controls		Localized Cases versus Controls	
	N = 223	N = 414		N = 222		N = 192	
	N (%)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
AA	123 (55%)	252 (61%)	1.0 (ref)	140 (63%)	1.0 (ref)	112 (58%)	1.0 (ref)
AG	78 (35%)	137 (33%)	0.88 (0.61, 1.27)	71 (32%)	0.78 (0.51, 1.18)	66 (34%)	1.06 (0.67, 1.66)
GG	22 (10%)	25 (6%)	0.58 (0.31, 1.09)	11 (5%)	0.41 (0.19, 0.90)	14 (7%)	0.74 (0.35, 1.59)
<i>p</i> for trend			<i>p</i> = 0.12		<i>p</i> = 0.02		<i>p</i> = 0.67
Stratified by Total Calcium Intake							
Low calcium ≤ 680 (mg/day)	Controls	All Cases versus Controls		Advanced Cases versus Controls		Localized Cases versus Controls	
	N = 111	N = 126		N = 71		N = 55	
VDR-Cdx2	N (%)	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
AA	59 (53%)	84 (67%)	1.0 (ref)	51 (72%)	1.0 (ref)	33 (60%)	1.0 (ref)
AG	37 (33%)	38 (30%)	0.69 (0.38, 1.26)	19 (27%)	0.54 (0.27, 1.12)	19 (35%)	1.08 (0.50, 2.30)
GG	15 (14%)	4 (3%)	0.18 (0.05, 0.60)	1 (1%)	0.08 (0.01, 0.62)	3 (6%)	0.40 (0.10, 1.63)
<i>p</i> for trend			<i>p</i> = 0.005		<i>p</i> = 0.002		<i>p</i> = 0.38
High calcium > 680 (mg/day)	Controls	All Cases versus Controls		Advanced Cases versus Controls		Localized Cases versus Controls	
	N = 112	N = 288		N = 151		N = 137	
VDR-Cdx2	N %	N (%)	OR (95% CI)	N (%)	OR (95% CI)	N (%)	OR (95% CI)
AA	64 (57%)	168 (58%)	1.0 (ref)	89 (59%)	1.0 (ref)	79 (58%)	1.0 (ref)
AG	41 (37%)	99 (34%)	1.00 (0.62, 1.61)	52 (34%)	0.93 (0.55, 1.58)	47 (34%)	1.05 (0.59, 1.86)
GG	7 (6%)	21 (7%)	1.18 (0.47, 2.96)	10 (7%)	0.92 (0.33, 2.58)	11 (8%)	1.15 (0.41, 3.23)
<i>p</i> for trend			<i>p</i> = 0.81		<i>p</i> = 0.79		<i>p</i> = 0.49
<i>p</i> for interaction			<i>p</i> = 0.03		<i>p</i> = 0.03		<i>p</i> = 0.78

Models adjusted for age and family history of prostate cancer.

CI = confidence interval; OR = odds ratio; VDR = vitamin D receptor.

allele and decreased prostate cancer risk, among Hispanic but not among non-Hispanic White men.

Our finding that the high transcription A allele is associated with increased prostate cancer risk is not explained by the well-documented antiproliferative, pro-differentiating effects of the VDR and its ligands on prostate epithelial cells.⁽²⁴⁾ Increased expression of VDR in the prostate should decrease, not increase, risk. Furthermore, VDR Cdx2 genotype should not affect prostatic VDR expression in the absence of the CDX-2 transcription factor, which is generally believed to be restricted to the intestine. However, it is noteworthy that CDX-2 expression has been

reported in some other organs, including the prostate. In 70 radical prostatectomy specimens, Herawi et al.⁽²⁵⁾ observed CDX-2 staining in 5.7% of the specimens. No staining was observed in any of 185 metastatic prostate tumors. The role of this protein in prostate tissue is presently unclear. However, the relative rarity of its expression suggests that it is unlikely to significantly influence the results of this study.

We believe that our findings are intelligible on the hypothesis that high calcium absorption genotypes and/or diets high in calcium increase serum calcium levels and the increase in serum calcium affects prostate cancer cells. Prostate cells, including

Table 4. Advanced Prostate Cancer and VDR Cdx2 Polymorphism Stratified by Total Calcium Intake

Genotype	Total Calcium		<i>p</i> Value
	Low Calcium ≤ 680 mg/day	High Calcium >680 mg/day	
VDR-Cdx2	OR (95% CI)		
AA	1.00 (ref)	1.58 (0.95, 2.63)	<i>p</i> = 0.08
AG/GG	0.43 (0.22, 0.82)	1.44 (0.83, 2.49)	<i>p</i> = 0.01
<i>p</i> for trend	<i>p</i> = 0.03	<i>p</i> = 0.70	

Model adjusted for age and family history of prostate cancer.

CI = confidence interval; OR = odds ratio; VDR = vitamin D receptor.

Table 5. Total Calcium Intake and Prostate Cancer Risk, Stratified by Obesity

	Controls	All Cases versus Controls	
<30 BMI (Nonobese)	N = 161	N = 353	OR (95% CI)
Total calcium ^a	N (%)	N (%)	
≤ 680 mg/day	81 (50%)	128 (36%)	1.0 (ref)
> 680 mg/day	80 (50%)	225 (64%)	1.80 (1.23, 2.65)
<i>p</i> for trend			<i>p</i> = 0.003
	Controls	All Cases versus Controls	
≥ 30 BMI (Obese)	N = 78	N = 152	OR (95% CI)
Total calcium ^a	N (%)	N (%)	
≤ 680 mg/day	38 (49%)	29 (21%)	1.0 (ref)
> 680 mg/day	40 (56%)	116 (79%)	3.70 (1.99, 6.90)
<i>p</i> for trend			<i>p</i> < 0.01
<i>p</i> for Interaction			<i>p</i> = 0.06

^aTotal calcium = dietary calcium and supplements. Models adjusted for age and family history of prostate cancer. BMI = body mass index; CI = confidence interval; OR = odds ratio.

prostate cancer cells, possess both the calcium-sensing receptor⁽²⁶⁾ and calcium-dependent voltage-gated channels,⁽²⁷⁾ which respond to an increase in calcium with an increase in proliferation and a decrease in apoptosis. Levels of total serum calcium are generally very stable, and are little influenced by dietary intakes over a wide range of intake.⁽²⁸⁾ However, the results of carefully conducted metabolic studies indicate that serum levels of ionized calcium, the biologically active fraction of total serum calcium, increase significantly for several hours after calcium intake.^(29,30) High normal levels of serum and ionized serum calcium have been associated with increased risk of fatal prostate cancer in two prospective epidemiologic studies.^(3,4) Because serum calcium is presumed to promote prevalent (existing) cancer, rather than having an effect on the initiation of cancer, this interpretation is consistent with the observation that VDR Cdx2 genotype was associated with advanced but not localized disease.

For advanced disease, we observed a statistical interaction between dietary calcium intake and genotype. The high absorption variant conferred less risk among men consuming higher levels of calcium. Although vitamin D aids calcium absorption, on a typical diet containing 1000 mg of calcium, the majority of calcium absorption is passive (vitamin D independent).⁽³¹⁾ The passive absorption of calcium may explain why men who consume greater amounts of calcium are at increased prostate cancer risk, regardless of genotype.

We also observed a borderline significant interaction between calcium intake and obesity, with calcium being a stronger risk factor among the obese than the nonobese. This finding contrasts with a report from the Singapore Health Study, which found calcium to be a risk factor only among thin men (BMI < 22.9 kg/m²).⁽³²⁾ However, the interaction between calcium and BMI in that study was not statistically significant.

Although numerous studies indicate that calcium is associated with an increased risk of prostate cancer, particularly of advanced and or fatal disease, other studies have suggested that adequate (versus deficient) levels of 25OHD are associated with a decreased risk of subsequent prostate cancer. As the

“classic” role of vitamin D is to increase the efficiency of calcium uptake, the literature for serum vitamin D and for calcium appears conflicting. However, this apparent conflict can be understood by considering the “nonclassical” (autocrine/paracrine) role of vitamin D in the prostate. Prostate cells possess 1-alpha hydroxylase and convert 25OHD into 1,25(OH)₂D, which exerts prodifferentiating and antiproliferative effects on prostate cancer cells.⁽³³⁾ Thus, the classical (calcium-mediated) and nonclassical roles of vitamin D operate “against” each other to influence prostate cancer risk, probably through different mechanisms. Although it had been speculated that the increased risk for prostate cancer that is associated with higher levels of dietary calcium was because of a reduction in the hepatic conversion of 25OHD into 1,25(OH)₂D by calcium,⁽³⁴⁾ this hypothesis has not been supported by subsequent investigations.⁽³⁵⁾

It is important to note that although some studies have reported an increased risk of prostate cancer with low levels of vitamin D,^(21,36) these have not been confirmed by other investigations. Many subsequent studies of serum 25-OHD and prostate cancer risk have been null, and some have reported an increased risk of prostate cancer with both low and high levels of 25OHD.^(37,38) A positive effect of serum calcium on prostate cancer risk may confound the relationship between 25(OH)D and prostate cancer risk in some studies, which may account for some discrepant results in the literature.⁽³⁹⁾

Our study has several limitations, including its retrospective design, which could have introduced recall bias in the reporting of calcium intake. However, our findings for calcium intake are consistent with those of several prospective studies, where recall bias obviously is not a contributor. Similarly, although we measured calcium intake, we did not measure serum calcium. Future studies would benefit from including measurements of serum total and ionized calcium.

Strengths of the study include its population-based design and the oversampling of cases with advanced-stage disease, which allowed us to distinguish stage-specific genotype–diet interactions that would have been difficult or impossible to detect in a case series that consisted mainly of early-

stage disease. Finally, few epidemiologic studies of diet and prostate cancer have included large numbers of African-American cases.

Population stratification is a potential confounding issue in our study. The VDR Cdx2 A allele is associated with both increased prostate cancer risk and with African ancestry. It could be that the A allele is simply marking those men with increased African ancestry and therefore at increased risk because of some other factor that is associated with African ancestry. In fact, among the 518 men for whom genetic ancestry estimates were available, adjusting for European ancestry did not alter the results. The ancestry-adjusted odds ratio comparing genotype GG to AG/AA for advanced cases versus controls was 0.37 after ancestry adjustment (versus an unadjusted OR of 0.38).

The more active A allele is most prevalent in populations of African origin (98% in Yorubans in Ibadan, 89% in Luhya in Kenya, 71% in African-Americans of the US Southwest; in contrast to 45% in Japanese in Tokyo, Japan, and 20% in Utah residents with ancestry from northern and western Europe).⁽⁴⁰⁾ Prostate cancer incidence rates in African-Americans are 36% higher than those of non-Hispanic Whites, and African-Americans are diagnosed at younger ages and are twice as likely to die of the disease (SEER 2003–2007).⁽¹³⁾ Therefore, by promoting efficient calcium absorption even on a relatively low calcium diet, the ancestral African VDR Cdx2 allele may contribute to racial/ethnic disparities in prostate cancer incidence and mortality.

In conclusion, our data support the hypothesis, substantiated in many epidemiologic studies, that dietary calcium is causally related to prostate cancer risk.^(18,19) Our finding that prostate cancer risk is increased among high absorbers of calcium adds to the biological plausibility of the calcium hypothesis, for which the underlying mechanism has been a subject of considerable debate.^(34,35,41,42) Because calcium is essential for bone health and appears to protect against colorectal cancer (and possibly other diseases),⁽¹⁾ we must be cautious about making public health recommendations to limit calcium intake. Our data indicate that, although calcium intake increases prostate cancer risk in African-American men as a group, it is associated with a significantly greater risk among high absorbers of calcium (men with the AA genotype). If confirmed by other dietary-seroepidemiologic studies, African-American men with the AA genotype may be advised to restrict their calcium intake in order to reduce their risk of developing prostate cancer.

Disclosures

All authors state that they have no conflicts of interest.

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