Research Article

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Protective Effects of Low Calcium Intake and Low Calcium Absorption Vitamin D Receptor Genotype in the California Collaborative Prostate Cancer Study

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Abstract

Background: High calcium intake is consistently associated with increased prostate cancer risk in epidemiologic studies. We previously reported that the positive association between calcium intake and risk of aggressive prostate cancer was modified by the single-nucleotide polymorphism (SNP) in the CDX-2 binding site of the vitamin D receptor (VDR) gene, among African American men.

Methods: We expanded our previous study to include White men, a population with a higher calcium intake and a higher prevalence of the low absorption allele. We also examined VDR polymorphisms at other loci unrelated to calcium absorption. The study included 1,857 prostate cancer cases (1,140 with advanced stage at diagnosis, 717 with localized stage) and 1,096 controls. OR were estimated using conditional logistic regression.

Results: Among both Blacks and Whites, we observed a threshold for calcium intake (604 mg/d) below which prostate cancer risk declined sharply. Low calcium intake was most strongly associated with decreased risk among men with the VDR Cdx2 low calcium absorption genotype (P for interaction = 0.001 and $P = 0.06$ for Whites and African Americans, respectively). Among all men with this genotype, those in the lowest quartile of calcium intake ($\leq$604 mg/d) had a 50% reduction in risk as compared with those in the upper three quartiles [OR = 0.49; 95% confidence interval (CI), 0.36–0.67]. The association between calcium intake and prostate cancer risk was not modified by genotype at other VDR loci.

Conclusions: Our findings support the hypothesis that genetic determinants of calcium absorption influence prostate cancer risk.

Impact: The differences between African Americans and Whites in calcium absorption and dietary calcium intake may contribute to racial disparities in prostate cancer incidence and mortality rates. *Cancer Epidemiol Biomarkers Prev; 22(1); 16–24. ©2012 AACR.*

Introduction

Calcium intake has been consistently associated with risk of advanced or fatal prostate cancer in epidemiologic studies, although the mechanism(s) underlying this association remain(s) unclear (1–6). We recently reported that low calcium intake was associated with a decreased risk of advanced prostate cancer in African American men but only among those with a genotype of the vitamin D receptor (VDR) that is associated with poor intestinal absorption of calcium (7). To determine whether these associations also occur among Whites, we expanded our study to include White men from the California Collaborative Prostate Cancer Study. For comparison, we examined other VDR loci, unlinked to the single-nucleotide polymorphism (SNP) in the CDX-2 binding site of the *VDR* gene (Cdx2) polymorphism, that are not directly related to calcium absorption.

This study included 1,857 prostate cancer cases (500 African Americans and 1,357 Whites) and 1,096 controls (240 African Americans and 856 Whites). Among cases, 1,140 were diagnosed with advanced disease, making this one of the largest studies of advanced-stage prostate cancer in the epidemiologic literature.

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Materials and Methods

Study population

Study subjects were participants in the California Collaborative Prostate Cancer Study, a population-based multiethnic case–control study conducted between 1997 and 2005, enriched for aggressive prostate cancer cases, which has been described in detail previously (8, 9). Briefly, cases were identified from the Los Angeles County (LAC) Cancer Surveillance Program and the LAC and the Greater Bay Area Cancer Registries. A total of 2,008

cases completed the interview, including 1,232 from LAC and 776 from the San Francisco Bay Area (SFBA). Cases without a definitive stage were excluded, leaving 1,960 cases, including 542 African Americans and 1,418 Whites, 333 of whom were Hispanic and 1,085 non-Hispanic Whites. Advanced prostate cancer was defined according to Surveillance Epidemiology and End Results (SEER) 1995 pathologic and clinical extent of disease codes 41 to 85. Of the 1,960 cases, 1,199 were diagnosed with advanced stage and 761 were diagnosed with localized disease.

Controls were frequency-matched to the expected distribution of cases on race/ethnicity and 5-year age group. In LAC, controls were ascertained by a standard neighborhood walk algorithm that specifies an obligatory sequence of residences to be surveyed for eligible control subjects (10). A participating control was found within 40 residences in more than 90% of case neighborhoods surveyed. In SFBA, controls were identified through random-digit dialing and random selections from among beneficiaries of the Health Care Financing Administration. A total of 1,139 controls (594 from LAC, 545 from SFBA) completed the interview, including 253 African Americans and 886 Whites (122 Hispanic and 764 non-Hispanic).

Blood or mouthwash samples were obtained for 1,164 advanced cases, 553 localized cases, and 1,119 controls. Biospecimens were not collected from localized cases in SFBA.

All study participants provided written informed consent. The protocol was approved by the Institutional Review Boards of the University of Southern California (Los Angeles, CA) and the Cancer Prevention Institute of California (Fremont, CA).

Data collection

Trained professional interviewers conducted home visits and administered a structured questionnaire on demographic background, medical history, body size, lifestyle factors (including physical activity, alcohol consumption, smoking, etc.), and family history of prostate cancer. Three measurements of standing height and 2 measurements of weight were taken and averaged. 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) was assessed using a 74-item food frequency questionnaire (FFQ) adapted from Block's 1995 Health Habits and History Questionnaire, which has been validated in middle-aged and older men (11–14). The FFQ assessed for each food item the frequency of consumption and portion size, using food models and utensils. Daily intake of specific nutrients, including calcium, was estimated using the DIETSYS software. Intake of supplemental calcium during the reference year was assessed by questions on use (number of tablets per week) of multivitamins, calcium pills with or without vitamin D, and calcium-based antacids (e.g., Tums, Rolaids).

Exposure variables

Calcium intake was estimated from the FFQ and from the use of supplements (i.e., multivitamin pills, single calcium tablets, and calcium-based antacids). Total calcium intake included calcium from foods, beverages, and supplements. Cutoff points were selected on the basis of quartiles of intake among controls. Subjects with dietary intake considered unreliable (<600 or >6,000 kcal/d) were excluded from analyses (103 cases and 43 controls), leaving 1,857 cases (500 African Americans and 1,357 Whites) and 1,096 controls (240 African Americans and 856 Whites) in the dietary analyses.

In addition to the Cdx2 polymorphism, we examined the FokI translation start site polymorphism in exon 2 that influences receptor transactivation ability and the synonymous TaqI polymorphism in exon 9 that is linked to variation in the 3′ untranslated region (3′UTR), which is important for mRNA stability.

Genotyping

SNPs in 3 regions of the *VDR* gene (gene encoding the vitamin D3 receptor) were examined: a missense SNP in a caudal type homeo box transcription factor 2 protein (CDX-2) protein binding site lying between exons 1D and 1G [rs11568820] (15), a missense SNP in the first of 2 potential start codons in exon 2 [rs10735810] (referred to as FokI), and a synonymous SNP in exon 9 (3′UTR) [rs731236] (referred to as TaqI; ref. 8).

The Cdx2, FokI, and TaqI SNPs were genotyped with TaqMan assays using the TaqMan Core Reagent Kit (Applied Biosystems) as previously described (7, 8). PCR reactions were conducted using conditions recommended by the manufacturer. Fluorescent signals were measured using an ABI 7900HT Detection System. Water blanks were included in all PCR batches. Ten percent of samples were blindly replicated. There were no discrepancies among replicated samples. Call rates were more than 97%. Laboratory technicians were blinded to case–control status. Genotype data were obtained for 1,857 cases and 1,096 controls.

Statistical analyses

Allele frequencies were estimated by gene counting. χ^2 Tests were used to test for departures of genotype frequencies from Hardy–Weinberg equilibrium among controls. To control for differences in race/ethnicity, socioeconomic status (SES) and case–control ratio across study sites, we created a variable that classified subjects according to study site, SES quintile, and race/ethnicity, as described previously (8) and fit conditional logistic regression models to estimate OR and 95% confidence intervals (CI). Models were adjusted for age (continuous variable) and first-degree family history of prostate cancer (yes, no) as potential confounders. We also checked for potential confounding by prostate-specific antigen (PSA) screening during the 5 years before the reference year. Reliable screening data were available only for SFBA cases and controls, and there was no evidence of confounding by PSA screening.

Dose–response trends were assessed by including quartiles as an ordinal value in the conditional logistic regression models. Cross-product terms were included and a 1 degree of freedom likelihood ratio test was used to evaluate effect modification (interaction). Separate analyses were conducted for cases with localized and advanced stage disease. Because the number of Hispanic Whites was too small for separate analyses, Hispanic and non-Hispanic Whites were combined.

Results

The demographic characteristics of study participants are shown in Table 1. The mean age at diagnosis was 64 years. On average, advanced cases were diagnosed 3 years earlier than localized cases among African Americans, and 5 years earlier among Whites. Cases and controls were similar in education, SES, and body mass index (BMI). Cases consumed more calcium than controls and were more likely to report a first-degree family history of prostate cancer. African Americans consumed less calcium than Whites (mean of 818 vs. 1,078 mg/d for African American vs. White controls). In both racial groups, advanced cases consumed more calcium than localized cases (1,173 vs. 1,119 mg/d among Whites; 979 vs. 945 mg/d among African Americans for advanced vs. localized disease).

Table 1. Characteristics of prostate cancer cases and controls

	Controls		Cases			
	N = 1,096		*N* = 1,857			
			Localized		Advanced	
			N = 717		*N* = 1,140	
	N (%)		*N* (%)		*N* (%)	
Categories	**African American**	**White**	**African American**	**White**	**African American**	**White**
Race	***N* = 240**	***N* = 856**	***N* = 265**	***N* = 452**	***N* = 235**	***N* = 905**
Age, y						
≤49	14 (6%)	40 (5%)	11 (4%)	7 (2%)	13 (6%)	27 (3%)
50–59	61 (25%)	248 (29%)	52 (20%)	63 (14%)	69 (29%)	247 (27%)
60–69	104 (43%)	337 (39%)	108 (41%)	160 (35%)	99 (42%)	382 (42%)
70–79	57 (24%)	202 (24%)	78 (29%)	181 (40%)	52 (22%)	223 (25%)
≥80	4 (2%)	29 (3%)	16 (6%)	41 (9%)	2 (<1%)	26 (3%)
Mean (SD)	64 (8.9)	64 (9.1)	66 (8.8)	69 (8.5)	63 (8.5)	64 (8.4)
Education						
High school or less	98 (41%)	186 (22%)	116 (44%)	160 (30%)	109 (46%)	270 (30%)
College degree/some college	92 (38%)	235 (27%)	104 (39%)	115 (25%)	71 (30%)	236 (26%)
Postgraduate	49 (20%)	432 (50%)	44 (17%)	175 (39%)	55 (23%)	396 (44%)
Unknown	1 (<1%)	3 (<1%)	1 (<1%)	2 (<1%)	0 (0%)	3 (<1%)
Socioeconomic Status (census tract-based)						
1 = Low	48 (20%)	54 (6%)	92 (35%)	54 (12%)	64 (27%)	79 (8%)
2	68 (28%)	68 (8%)	68 (26%)	61 (14%)	51 (22%)	89 (10%)
3	56 (23%)	145 (17%)	55 (21%)	65 (14%)	56 (24%)	151 (17%)
4	45 (19%)	229 (27%)	30 (11%)	103 (23%)	41 (17%)	186 (21%)
5 = High	23 (10%)	360 (42%)	20 (8%)	169 (37%)	23 (10%)	400 (44%)
Family history of prostate cancer						
No	212 (88%)	745 (87%)	204 (77%)	363 (80%)	178 (76%)	742 (82%)
Yes	28 (12%)	111 (13%)	61 (23%)	89 (20%)	57 (24%)	163 (18%)
BMI						
<25	51 (21%)	235 (28%)	60 (23%)	137 (30%)	66 (28%)	220 (24%)
25.0–29.9	110 (46%)	387 (45%)	123 (47%)	232 (51%)	104 (44%)	451 (50%)
≥30	78 (33%)	232 (27%)	81 (31%)	82 (18%)	64 (27%)	234 (26%)
Calcium intake	Mean (SD)		Mean (SD)		Mean (SD)	
Total calcium, mg/d	818 (474)	1,078 (613)	945 (510)	1,119 (649)	979 (577)	1,173 (671)
Dietary calcium, mg/d	755 (444)	970 (559)	869 (497)	1,001 (542)	890 (517)	1,071 (614)
Supplemental calcium, mg/d	64 (155)	108 (249)	75 (130)	119 (316)	89 (217)	102 (266)

Table 2. Total calcium intake and prostate cancer risk

All men	Controls $N = 1{,}096$	All cases vs. controls $N = 1{,}857$		Localized cases vs. controls $N = 717$		Advanced cases vs. controls $N = 1{,}140$	
Total calcium	*N* (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)
>1,258 mg/d	275 (25%)	356 (18%)	1.0 (ref.)	185 (26%)	1.00 (ref.)	351 (31%)	1.00 (ref.)
895–1,258 mg/d	273 (25%)	479 (26%)	0.95 (0.77–1.17)	201 (28%)	1.04 (0.78–1.38)	306 (27%)	0.89 (0.71–1.13)
604–894 mg/d	274 (25%)	507 (27%)	0.90 (0.72–1.11)	189 (26%)	0.92 (0.69–1.22)	290 (25%)	0.88 (0.70–1.11)
<604 mg/d	274 (25%)	536 (29%)	0.62 (0.49–0.77)	142 (20%)	0.67 (0.50–0.91)	193 (17%)	0.59 (0.46–0.76)
P for trend			$P = 0.001$		$P = 0.008$		$P = 0.001$
	$N = 240$	$N = 500$		$N = 235$		$N = 265$	
African Americans	*N* (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)
>1,258 mg/d	38 (16%)	100 (20%)	1.0 (ref.)	49 (18%)	1.00 (ref.)	51 (22%)	1.00 (ref.)
895–1,258 mg/d	46 (19%)	140 (86%)	1.10 (0.66–1.82)	81 (31%)	1.36 (0.76–2.42)	59 (25%)	0.91 (0.51–1.64)
604–894 mg/d	58 (24%)	130 (26%)	0.79 (0.48–1.29)	67 (25%)	0.88 (0.49–1.55)	63 (27%)	0.77 (0.44–1.35)
<604 mg/d	98 (41%)	130 (26%)	0.49 (0.31–0.78)	68 (26%)	0.58 (0.33–1.00)	62 (26%)	0.46 (0.27–0.79)
P for trend			$P = 0.001$		$P = 0.004$		$P = 0.002$
	$N = 856$	$N = 1{,}357$		$N = 452$		$N = 905$	
Whites	*N* (%)	*N* (%)	OR (95% CI)	N (%)	OR (95% CI)	*N* (%)	OR (95% CI)
>1,258 mg/d	237 (28%)	436 (32%)	1.0 (ref.)	136 (30%)	1.00 (ref.)	300 (33%)	1.00 (ref.)
895–1,258 mg/d	227 (27%)	367 (27%)	0.91 (0.72–1.15)	120 (27%)	0.94 (0.67–1.31)	247 (27%)	0.89 (0.69–1.14)
604–894 mg/d	216 (25%)	349 (26%)	0.92 (0.72–1.17)	122 (27%)	0.93 (0.67–1.29)	227 (25%)	0.90 (0.69–1.17)
<604 mg/d	176 (21%)	205 (15%)	0.69 (0.53–0.89)	74 (16%)	0.78 (0.53–1.13)	131 (14%)	0.65 (0.49–0.87)
P for Trend			$P = 0.01$		$P = 0.22$		$P = 0.01$

NOTE: Total calcium from diet and supplements.
All models adjusted for age, study site, SES, and family history of prostate cancer.

Table 2 shows that low calcium intake is associated with lower prostate cancer risk among both African Americans and Whites. The association was seen for both advanced and localized disease. Men in the lowest quartile of total calcium intake (<604 mg/d) had an approximately 40% lower risk of prostate cancer (advanced or localized) than men in the highest quartile (>1,258 mg/d; OR = 0.62; 95% CI, = 0.49–0.77; *P* for trend: 0.001). The top 3 quartiles, however, did not differ significantly with respect to prostate cancer risk. The associations were somewhat stronger among African Americans, with a 54% decrease in advanced disease risk (quartile 4 vs. 1) compared with 35% in White men.

Table 3 shows associations between risk and the genotypes for the 3 SNPs, Cdx2, FokI, and TaqI, in the *VDR* gene. All genotypes were in Hardy–Weinberg equilibrium among African American and White controls. The Cdx2 genotype was associated with prostate cancer risk only among African Americans; specifically, the GG genotype was associated with reduced risk of advanced disease. The FokI genotype was associated with risk only among Whites and only for advanced disease. No significant associations were observed for TaqI. For all pairwise combinations of SNPs, there was no linkage disequilibrium detected among either African Americans or Whites.

Table 4 shows the association with genotype, stratified by total calcium intake (lowest quartile vs. top 3 quartiles). Effect modification was present only for the Cdx2 genotype. Among both African Americans and Whites, the Cdx2 G allele was associated with reduced risk, but only among those with low (lowest quartile) calcium intake (*P* for interaction = 0.001 in Whites; 0.06 in African Americans). Among men with low calcium intake (<604 mg/d), each additional G allele was associated with a 39% to 46% decrease in risk.

Table 5 shows the association with calcium consumption, stratified by Cdx2 genotype. Low calcium intake (<604 mg/d vs. ≥604 mg/d) was significantly associated with decreased risk only among men with the protective genotype (GG; OR = 0.49; 95% CI, = 0.36–0.67). A similar pattern was seen in African Americans and Whites (data not shown). Both races were combined because of sparse numbers of men with both the GG genotype and low calcium intake.

Discussion

In this multiethnic study of 1,857 prostate cancer cases and 1,096 controls, we observed a positive association between calcium intake and prostate cancer risk, consistent with a large epidemiologic literature (1–6). Moreover, among both African Americans and Whites, the association was modified by VDR calcium absorption GG genotype. Specifically, low calcium intake was most strongly associated with decreased prostate cancer risk among

Table 3. VDR polymorphism and prostate cancer risk

Cdx-2	Controls	All cases vs. controls		Localized cases vs. controls		Advanced cases vs. controls	
African Americans	$N = 223$	$N = 414$		$N = 192$		$N = 222$	
	N (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)
AA	123 (55%)	252 (61%)	1.0 (ref.)	112 (58%)	1.00 (ref.)	140 (63%)	1.00 (ref.)
AG	78 (35%)	137 (33%)	0.88 (0.61–1.27)	66 (34%)	1.06 (0.67–1.66)	71 (32%)	0.78 (0.51–1.18)
GG	22 (10%)	25 (6%)	0.58 (0.31–1.09)	14 (7%)	0.74 (0.35–1.59)	11 (5%)	0.41 (0.19–0.90)
P for trend		$P = 0.12$		$P = 0.06$		$P = 0.02$	
White Americans	$N = 795$	$N = 1,117$		$N = 285$		$N = 832$	
	N (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI)
AA	43 (5%)	64 (6%)	1.0 (ref.)	23 (8%)	1.00 (ref.)	4 (5%)	1.00 (ref.)
AG	271 (34%)	347 (31%)	0.89 (0.58–1.36)	90 (32%)	0.58 (0.32–1.07)	257 (31%)	0.95 (0.60–1.52)
GG	481 (61%)	706 (63%)	0.98 (0.64–1.48)	172 (60%)	0.67 (0.37–1.19)	534 (64%)	1.06 (0.68–1.67)
P for trend		$P = 0.58$		$P = 0.66$		$P = 0.38$	
Fok1							
African Americans	$N = 234$	$N = 421$		$N = 196$		$N = 225$	
	N (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI	*N* (%)	OR (95% CI)
CC	134 (57%)	236 (56%)	1.0 (ref.)	107 (55%)	1.00 (ref.)	129 (57%)	1.00 (ref.)
CT	90 (38%)	161 (38%)	1.02 (0.72–1.43)	75 (38%)	1.09 (0.71–1.67)	86 (38%)	1.01 (0.68–1.50)
TT	10 (4%)	24 (6%)	1.14 (0.51–2.51)	14 (7%)	1.56 (0.63–3.83)	10 (4%)	1.00 (0.39–2.51)
P for trend		$P = 0.80$		$P = 0.39$		$P = 0.97$	
White Americans	$N = 836$	$N = 1,197$		$N = 316$		$N = 881$	
	N (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI	*N* (%)	OR (95% CI)
CC	311 (37%)	406 (34%)	1.0 (ref.)	111 (35%)	1.0 (ref.)	295 (33%)	1.0 (ref.)
CT	413 (49%)	598 (50%)	1.06 (0.87–1.30)	164 (52%)	1.04 (0.77–1.41)	434 (49%)	1.10 (0.89–1.35)
TT	112 (13%)	193 (16%)	1.32 (0.99–1.76)	41 (13%)	0.98 (0.62–1.54)	152 (17%)	1.42 (1.06–1.91)
			$P = 0.08$		$P = 0.99$		$P = 0.03$
Taq1							
African Americans	$N = 235$	$N = 421$		$N = 196$		$N = 225$	
	N (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI	*N* (%)	OR (95% CI)
TT	118 (50%)	211 (50%)	1.0 (ref.)	90 (46%)	1.00 (ref.)	121 (54%)	1.00 (ref.)
TC	95 (40%)	183 (43%)	1.07 (0.76–1.52)	92 (47%)	1.20 (0.79–1.83)	91 (40%)	0.94 (0.63–1.39)
CC	22 (9%)	27 (6%)	0.71 (0.38–1.32)	14 (7%)	0.79 (0.37–1.72)	13 (6%)	0.52 (0.25–1.11)
P for trend		$P = 0.62$		$P = 0.93$		$P = 0.18$	
White Americans	$N = 837$	$N = 1,196$		$N = 316$		$N = 880$	
	N (%)	*N* (%)	OR (95% CI)	*N* (%)	OR (95% CI	*N* (%)	OR (95% CI)
TT	333 (40%)	512 (43%)	1.0 (ref.)	133 (42%)	1.00 (ref.)	379 (43%)	1.00 (ref.)
TC	379 (45%)	525 (44%)	1.00 (0.82–1.22)	141 (45%)	0.99 (0.73–1.34)	384 (44%)	0.95 (0.77–1.17)
CC	125 (15%)	159 (13%)	0.97 (0.73–1.28)	42 (13%)	0.94 (0.61–1.46)	117 (13%)	0.90 (0.67–1.22)
			$P = 0.86$		$P = 0.82$		$P = 0.47$

NOTE: Total calcium from diet and supplements.

All models adjusted for age, study site, SES and family history of prostate cancer.

men with the VDR Cdx2 GG genotype that has been linked to low calcium absorption (16, 17). Thus, low calcium intake (<604 mg/d) and low absorption genotype (VDR Cdx2 GG) seem to jointly confer markedly reduced risk.

We observed a threshold for calcium intake below which prostate cancer risk declined sharply for both African Americans and Whites. No reduction in risk was seen for intake in the middle 2 quartiles compared with men in the highest quartile of calcium intake (>1,258 mg/d). Conversely, men in the lowest quartile (<604 mg/d) had a 30% to 50% reduction in risk.

The Institute of Medicine (Washington, D.C.) recently set the recommended daily intake (RDI) of calcium for men 51 to 70 years of age at 1,000 mg/d and at 1,200 mg/d for men older than 70 years (18, 19). The tolerable upper limit (TUL) for calcium intake for both age groups is 2,000 mg/d. Notably, an increased risk of prostate cancer was one of the considerations leading to the selection of the TUL for men. Our data indicate that only calcium intake

Table 4. VDR Cdx-2 polymorphism and risk of prostate cancer, stratified by calcium intake

Low calcium (<604 mg/d)				High calcium (≥604 mg/d)			
African Americans	*N* (%)	*N* (%)	OR (95% CI)	African Americans	*N* (%)	*N* (%)	OR (95% CI)
	N = 91		*N* = 104		*N* = 132		*N* = 310
AA	50 (55%)	70 (67%)	1.0 (ref.)	*AA*	73 (55%)	182 (59%)	1.0 (ref.)
AG	30 (33%)	31 (30%)	0.68 (0.35–1.29)	*AG*	48 (36%)	106 (34%)	0.96 (0.61–1.51)
GG	11 (12%)	3 (3%)	0.18 (0.05–0.73)	*GG*	11 (8%)	22 (7%)	0.86 (0.39–1.90)
Per G allele			0.54 (0.33–0.88)	Per G allele			0.94 (0.68–1.31)
LRT *P* for interaction *P* = 0.06							
White Americans	*N* (%)	*N* (%)	OR (95% CI)	White Americans	*N* (%)	*N* (%)	OR (95% CI)
	N = 165		*N* = 170		*N* = 630		*N* = 947
AA	7 (4%)	17 (10%)	1.0 (ref.)	*AA*	36 (6%)	7 (5%)	1.0 (ref.)
AG	50 (30%)	64 (38%)	0.53 (0.20–1.41)	*AG*	221 (30%)	283 (30%)	1.02 (0.63–1.66)
GG	108 (65%)	89 (52%)	0.34 (0.31–0.88)	*GG*	373 (59%)	617 (65%)	1.26 (0.79–2.02)
Per G allele			0.61 (0.43–0.88)	Per G allele			1.18 (0.99–1.41)
LRT *P* for interaction *P* = 0.001							

NOTE: Calcium = diet plus supplements.
All models adjusted for age, study site, SES, and family history of prostate cancer.

below the RDI (and well below the TUL) is associated with decreased risk, which suggests that with respect to prostate cancer, both the RDI and the TUL are too high.

Calcium intake was lower in African Americans than in Whites, a finding that is consistent with many other reports (20–22). Only half as many Whites as African Americans (21% vs. 41%) consumed a diet sufficiently low in calcium (i.e., <604 mg/d) to be associated with a reduced risk. Thus, among Whites, the low calcium absorption genotype did not seem to be protective when considered in isolation from calcium intake.

The prevalence of the low calcium absorption genotype is much lower in African Americans than Whites (10% vs. 61% among controls). Despite the difference in genotype prevalence by race, results for the interaction of genotype and diet were consistent across both groups. In numerous studies and in diverse ethnicities, the low calcium absorption genotype (VDR Cdx2 GG) has been associated with markers of reduced calcium availability, for example, lower bone mineral density, and osteoporosis (23–25). The Cdx2 polymorphism interrupts a transcription factor–binding site that is thought to be important for intestinal VDR expression and calcium absorption (16, 17). Although a direct effect of the Cdx2 SNP on calcium absorption has not been shown *in vivo*, functional differences have been shown *in vitro* (15). Thus, effect

Table 5. Calcium intake and risk of prostate cancer, stratified by VDR Cdx2 polymorphism

		All cases vs. controls *N* = 1,531	
All races	**Controls *N* = 1,018**		
VDR Cdx2	***N* (%)**	***N* (%)**	**OR (95% CI)**
AA genotype	*N* = 166	*N* = 316	
High calcium	109 (66%)	229 (72%)	1.0 (ref.)
Low calcium	57 (34%)	87 (28%)	0.76 (0.50–1.15)
AG genotype	*N* = 349	*N* = 484	
High calcium	269 (77%)	389 (80%)	1.0 (ref.)
Low calcium	80 (23%)	94 (20%)	0.80 (0.57–1.14)
GG genotype	*N* = 503	*N* = 731	
High calcium	384 (76%)	639 (87%)	1.0 (ref.)
Low calcium	119 (24%)	92 (13%)	0.49 (0.36–0.67)
LRT *P* for interaction		*P* = 0.05	

Abbreviation: LRT, likelihood ratio test.
NOTE: Total calcium from diet and supplements.
Low calcium (<604 mg/d); high calcium (≥604 mg/d).
All models adjusted for age, study site, SES, and family history of prostate cancer.

modification of calcium intake by the Cdx2 genotype, which governs calcium physiology, is biologically plausible (7).

The VDR polymorphisms examined in this study are located in 3 independent regions of the gene: the upstream promoter (Cdx2), the start codon (FokI), and the exon containing the 3′UTR (TaqI). The presumed mechanisms of any effects of the FokI and TaqI polymorphisms are believed to be unrelated to calcium. The FokI polymorphism changes the length of the VDR protein, affecting transactivation ability (26), whereas 3′UTR polymorphisms (marked by TaqI) have been proposed to influence stability of the gene transcript (27). Neither of these polymorphisms modified the association between calcium intake and prostate cancer risk.

The FokI and TaqI polymorphisms, considered alone, were not associated with prostate cancer risk, except for an increased risk of advanced prostate cancer among Whites homozygous for the variant start codon genotype (previously reported by John and colleagues; ref. 8). This genotype was present among only 4% of African Americans (10 controls and 10 advanced cases), thus statistical power to detect an association in this group was limited. Our findings are consistent with recent meta-analyses (28, 29), which found only weak associations of borderline significance between VDR TaqI and FokI genotypes and prostate cancer risk.

The mechanisms underlying the association between high dietary calcium intake and increased risk of prostate cancer remain uncertain (1, 30–32). High dietary calcium has been proposed to inhibit the renal hydroxylation of 25-OHD into the active hormone, 1,25-dihydroxyvitamin D^5, but serum levels of either 25-OHD or 1,25-dihdroxyvitamin D have not been consistently associated with prostate cancer risk (33, 34). An attractive, alternate hypothesis is that calcium in diet increases levels of ionized calcium in serum and that serum-ionized calcium affects prostate cancer cells directly (35). Both the calcium-sensing receptor as well as calcium-dependent voltage gated channels is expressed in prostate cancer cells (33, 36, 37). Stimulation of these receptors by extracellular calcium is known to increase prostate cancer cell proliferation *in vitro* and metastasis *in vivo*. This interpretation is consistent with our previous findings for fatal prostate cancer from 2 prospective studies. Relative risks for fatal cancer were increased 2-fold among men with high versus low total serum calcium (38) and 3-fold among those with high versus low ionized serum calcium (39). An attractive aspect of the serum calcium model is that this mechanism, increases in ionized calcium stimulating cancer cell proliferation, can accommodate the findings from both the dietary and serum studies. This is because, although levels of total calcium in serum vary little following calcium intake, increases in dietary calcium cause an increase in serum levels of ionized calcium, which remain elevated for several hours after dietary intake (40, 41).

Our results should be considered in light of several potential limitations. First, the retrospective design may have introduced recall bias in participants' reporting of calcium intake. However, our findings are consistent with those from several prospective studies in which recall bias is not an issue. Second, selection bias cannot be completely ruled out as participation rates were slightly lower for controls than for cases. However, this would occur only if the probability of inclusion in the study was related to calcium intake or absorption. We did not detect any heterogeneity in effect estimates by race/ethnicity, SES, or study site. Third, our recording of disease stage may be subject to misclassification. It is well known that prostate cancer that is staged clinically may be understaged relative to its true extent, as determined by pathologic stage. This limitation applies principally to early-stage disease, which may include a mixture of localized and advanced disease.

The relatively small number of Hispanics (101 controls, 119 localized cases, and 174 advanced cases), precluded ethnic-specific analyses among White men. Few Hispanics (11 controls, 10 localized cases, and 15 advanced cases) had low calcium intake (lowest quartile), which was associated with decreased risk. Average calcium intake was higher among Hispanics than among non-Hispanic Whites (1,342 vs. 1,043 mg/d). However, the low absorption Cdx2 (G) allele was common among Hispanics. The frequency of the G allele did not vary by ethnicity [81% vs. 80% reported by HapMap (42); 79% vs. 77% in our study, for Hispanics and non-Hispanic Whites, respectively]. Future, larger studies are needed to examine prostate cancer risk in Hispanics in relation to calcium intake.

Conversely, our study has several strengths, including its sample size, 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. Furthermore, few epidemiologic studies of diet have included large numbers of advanced-stage prostate cancer among African Americans.

Racial/ethnic disparities in prostate cancer are well documented. In the United States, incidence rates are 60% higher and mortality rates are 150% higher among African Americans than Whites (SEER 2000–2009; ref. 43). The reasons for these differences remain unexplained. We are impressed by the fundamental differences in calcium biology among African Americans and Whites. For example, despite consuming less calcium than Whites, African Americans have higher bone mineral density. Thus, African Americans are more efficient at calcium absorption (44, 45). We observed substantial differences in Cdx2 allele frequencies by race. The high-absorption allele is more common in populations of African origin (98% in Yorubans in Ibadan, 89% in Luhya in Kenya, 71% in African Americans of the U.S. Southwest) than in men of Northern or Western European ancestry (20% in Utah residents; ref. 42). We suggest that these genetic differences in calcium absorption, which are known to influence

racial differences in bone mineral density, may contribute to racial/ethnic differences in prostate cancer incidence and mortality.

Although the genetics of calcium absorption are not modifiable, the dietary intake of calcium is. Our results suggest that a subset of men, that is, those with the Cdx2 GG genotype, may be able to substantially reduce their risk of prostate cancer by reducing their calcium intake. Before making such a decision, men might want to consider their personal risk of other diseases that have been linked to lower calcium intake, such as osteoporosis and colorectal cancer. In addition, if the association between high calcium absorption and increased risk of prostate cancer is confirmed by additional studies, medicines that lower calcium absorption may have a role as chemopreventive agents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed. Mention of trade names, commercial products, specific equipment, or organizations does not constitute endorsement, guarantee, or warranty by the State of California Department of Health Services or the U.S. Government, nor does it imply approval to the exclusion of other products. The views expressed in this publication represent those of the authors and do not necessarily reflect the position or policies of the Northern California Cancer Center, the California Public Health Institute, the State of California Department of Health Services, or the U.S. Department of Health and Human Services.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.A. Ingles
Study supervision: S.A. Ingles

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