Vitamin D Receptor Polymorphisms and Breast Cancer Risk: Results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium

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Abstract

Background: **Vitamin D is hypothesized to lower the risk of breast cancer by inhibiting cell proliferation via the nuclear vitamin D receptor (VDR). Two common single nucleotide polymorphisms (SNP) in the *VDR* gene (*VDR*), rs1544410 (*Bsm*I), and rs2228570 (*Fok*I), have been inconsistently associated with breast cancer risk. Increased risk has been reported for the *Fok*I *ff* genotype, which encodes a less transcriptionally active isoform of *VDR*, and reduced risk has been reported for the *Bsm*I *BB* genotype, a SNP in strong linkage disequilibrium with a 3′-untranslated region, which may influence *VDR* mRNA stability.**

Methods: **We pooled data from 6 prospective studies in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium to examine associations between these SNPs and breast cancer among >6,300 cases and 8,100 controls for each SNP using conditional logistic regression.**

Results: **The odds ratio (OR) for the rs2228570 (*Fok*I) *ff* versus *FF* genotype in the overall population was statistically significantly elevated [OR, 1.16; 95% confidence interval (95% CI), 1.04-1.28] but was weaker once data from the cohort with previously published positive findings were removed (OR, 1.10; 95% CI, 0.98-1.24). No association was noted between rs1544410 (*Bsm*I) *BB* and breast cancer risk overall (OR, 0.98; 95% CI, 0.89-1.09), but the *BB* genotype was associated with a significantly lower risk of advanced breast cancer (OR, 0.74; 95% CI, 0.60-0.92).**

Conclusions: **Although the evidence for independent contributions of these variants to breast cancer susceptibility remains equivocal, future large studies should integrate genetic variation in *VDR* with biomarkers of vitamin D status.** (Cancer Epidemiol Biomarkers Prev 2009;18(1):297–305)

Introduction

The geographic gradient in breast cancer incidence in North America suggests the possibility that sunlight and vitamin D may reduce breast cancer risk (1). Higher circulating 25-hydroxyvitamin D [25(OH)D] levels,

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Note: J.D. McKay and M.L. McCullough contributed equally to this work. J.D. McKay is a C.J. Martin fellow (National Health and Medical Research Council Australia).

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Table 1. Descriptive characteristics of breast cancer cases and controls genotyped for the *VDR Fok*I or *Bsm*I SNP, by cohort

	CPS-II		EPIC	
	Cases	Controls	Cases	Controls
n	499	504	1,677	2,795
Race/ethnicity, *n* (%)				
White	488 (98)	497 (99)	1,677 (100)	2,795 (100)
Hispanic	4 (1)	1 (0)		
African American	4 (1)	4 (1)		
Asian	0	0		
Hawaiian	0	0		
Other/missing	1 (0)	0		
Age at diagnosis, mean (SD)	70 (6)		58 (8)	
Body mass index, kg/m^2 (mean, SD)	25 (5)	26 (5)	26 (4)	26 (5)
Menopausal status, *n* (%)*				
Premenopausal			411 (25)	779 (28)
Postmenopausal	499 (100)	504 (100)	1,123 (67)	1,771 (63)
Unknown/missing			143 (9)	245 (9)
Age at menarche, *n* (%) †				
≤12	232 (46)	226 (45)	568 (34)	981 (35)
13-14	215 (43)	234 (46)	784 (47)	1,234 (44)
≥15	46 (9)	40 (8)	257 (15)	496 (18)
Unknown/missing	6 (1)	4 (1)	68 (4)	84 (3)
Age at menopause, *n* (%) ‡				
≤44	100 (20)	110 (22)	127 (11)	235 (13)
45-49	100 (20)	139 (28)	253 (23)	481 (127)
50-54	226 (45)	195 (39)	419 (37)	663 (37)
≥55	61 (12)	49 (10)	88 (8)	133 (8)
Unknown/missing	12 (2)	11 (2)	236 (21)	259 (15)
Parity, *n* (%)				
Nulliparous	45 (9)	43 (9)	214 (13)	355 (13)
≤2 children	170 (34)	138 (27)	927 (55)	1,449 (52)
≥3 children	270 (54)	311 (62)	432 (26)	842 (30)
Unknown/missing	14 (3)	12 (2)	104 (6)	149 (5)
First-degree family history, *n* (%)				
Yes	101 (20)	75 (15)		
No	388 (78)	409 (81)		
Unknown	10 (2)	20 (4)	1,677 (100)	2,795 (100)
Postmenopausal hormone therapy, *n* (%) ‡				
Never	164 (33)	208 (41)	624 (56)	1,135 (64)
Ever	332 (67)	294 (58)	451 (40)	573 (32)
Unknown/missing	3 (1)	2 (0)	48 (4)	63 (4)
ER/PR status, *n* (%)				
ER+/PR+	219 (44)			
ER−/PR−	27 (5)			
ER+/PR−	33 (7)			
ER−/PR+	3 (1)			
ER/PR borderline	7 (1)			
Not available	210 (42)		1,677 (100)	
Stage of breast cancer, *n* (%)				
In situ	108 (22)		109 (6)	
Localized invasive	302 (61)		803 (48)	
Advanced	69 (14)		274 (16)	
Unknown	20 (4)		491 (29)	

*Menopausal status at time of blood donation.
†Age at menarche in PLCO; categories for ages 12 to 13 were modeled as ages 13 to 14, and ages 14 to 15 and ≥16 were combined with ≥15.
‡Among postmenopausal women only.

which reflect vitamin D status from dietary intake, vitamin supplements, and sun exposure combined, have been associated with lower risk of breast cancer in a retrospective (2) and one (3) of two (3, 4) prospective studies. 25(OH)D can be converted to its active form, 1,25-hydroxyvitamin D in breast tissue, where it then binds to the vitamin D receptor (VDR), a nuclear transcription factor that regulates the expression of multiple genes, including some responsible for cell cycle regulation, differentiation, and apoptosis (5). The receptor is present in most cell types, including normal and neoplastic breast tissue (6). In the MMTV-neu transgenic mouse model of breast cancer, animals totally lacking the *VDR* gene exhibited abnormal mammary duct morphology and had reduced survival, whereas, in animals heterozygous for *VDR*, the incidence of mammary tumors was increased and the latency was shortened (7).

Two common genetic polymorphisms in the *VDR*, rs2228570 (*Fok*I) and rs1544410 (*Bsm*I), have been inconsistently associated with breast cancer risk in previous studies. In a large nested case-control study, the rs2228570 (*Fok*I) *ff* genotype was associated with a

Table 1. Descriptive characteristics of breast cancer cases and controls genotyped for the VDR *Fok*I or *Bsm*I SNP, by cohort (Cont'd)

MEC		NHS		PLCO		WHS	
Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
1,598	1,952	1,257	1,748	1,073	1,100	685	683
399 (25)	437 (22)	1,179 (94)	1,641 (94)	975 (91)	991 (90)	654 (95)	653 (96)
332 (21)	383 (20)	3 (0)	6 (0)	10 (1)	13 (1)	4 (1)	4 (1)
338 (21)	426 (22)	10 (1)	11 (1)	45 (4)	43 (4)	5 (1)	5 (1)
422 (26)	419 (21)	3 (0)	9 (1)	36 (3)	44 (4)	7 (1)	6 (1)
107 (7)	287 (15)	0	0	6 (1)	3 (0)	0	0
0	0	62 (5)	81 (5)	1 (0)	6 (1)	15 (2)	15 (2)
65 (9)		63 (7)		66 (6)		60 (8)	
27 (6)	27 (6)	25 (5)	26 (5)	27 (5)	27 (5)	25 (4)	26 (5)
174 (11)	319 (16)	259 (21)	326 (19)			148 (22)	145 (21)
1,385 (87)	1,600 (82)	869 (69)	1,271 (73)	1,063 (99)	1,091 (99)	435 (64)	408 (60)
39 (2)	33 (2)	129 (10)	151 (9)	10 (1)	9 (1)	102 (15)	130 (19)
845 (53)	961 (49)	638 (51)	844 (48)	213 (20)	213 (20)	388 (57)	354 (52)
558 (35)	747 (38)	514 (41)	748 (43)	592 (55)	605 (55)	253 (37)	290 (42)
166 (10)	226 (12)	97 (8)	145 (8)	268 (25)	279 (25)	44 (6)	39 (6)
29 (2)	18 (1)	8 (1)	11 (1)	0	3 (0)	0	0
419 (20)	554 (35)	184 (21)	283 (22)	273 (26)	288 (26)	70 (16)	88 (22)
354 (26)	432 (27)	243 (28)	363 (29)	224 (21)	263 (24)	129 (30)	123 (30)
441 (32)	451 (28)	395 (45)	551 (43)	413 (39)	395 (36)	180 (41)	142 (35)
124 (9)	114 (7)	47 (5)	74 (6)	153 (14)	145 (13)	31 (7)	32 (8)
47 (3)	49 (3)					25 (6)	23 (6)
230 (14)	218 (11)	95 (8)	119 (7)	113 (11)	93 (8)	106 (15)	94 (14)
579 (36)	676 (35)	412 (33)	543 (31)	368 (34)	329 (30)	270 (39)	238 (35)
767 (48)	1,038 (53)	737 (59)	1,077 (62)	592 (55)	676 (61)	309 (45)	351 (51)
22 (1)	20 (1)	13 (1)	9 (1)	0	2 (0)	0	0
273 (17)	217 (11)	244 (19)	243 (14)	209 (19)	175 (16)	137 (20)	110 (16)
1,318 (82)	1,732 (89)	1,013 (81)	1,505 (86)	856 (80)	920 (84)	539 (79)	566 (83)
7 (0)	3 (0)	0	0	8 (1)	5 (0)	9 (1)	7 (1)
493 (36)	647 (40)	204 (23)	360 (28)	281 (26)	326 (30)	133 (31)	152 (37)
880 (63)	930 (58)	665 (77)	911 (72)	778 (73)	757 (69)	283 (65)	234 (57)
12 (1)	23 (2)	0	0	4 (0)	8 (1)	19 (4)	22 (5)
763 (48)		597 (47)		434 (40)		475 (69)	
216 (14)		160 (13)		69 (6)		83 (12)	
135 (8)		124 (10)		54 (5)		60 (9)	
37 (2)		33 (3)		4 (0)		17 (2)	
21 (1)		27 (2)		35 (3)		8 (1)	
426 (27)		315 (25)		477 (44)		42 (6)	
15 (1)		209 (17)		172 (16)		0	
1,160 (73)		658 (52)		394 (37)		476 (69)	
417 (26)		368 (29)		223 (21)		170 (25)	
6 (0)		22 (2)		284 (26)		39 (6)	

34% higher breast cancer risk (95% CI, 1.06-1.69; ref. 8), but other studies, mostly smaller in size (9-14), have not reported similar associations. The presence of the rs2228570 (*Fok*I) *f* allele in the 5′-promoter region of the *VDR* results in production of a VDR protein that is three amino acids longer and less effective as a transcriptional activator (15).

Initial epidemiologic studies of the rs1544410 (*Bsm*I) polymorphism suggested a potentially important role in breast cancer of variants in this single nucleotide polymorphism (SNP), especially for more aggressive forms of the disease (9, 20). The intronic rs1544410 (*Bsm*I) SNP is located at the 3′-end of the *VDR* gene. This SNP is in strong linkage disequilibrium with a poly(A) microsatellite repeat in the 3′-untranslated region (12, 17, 18), which may influence *VDR* mRNA stability. Six (2, 9, 16, 19-21) of 11 (2, 8, 9, 11, 12, 16, 19-23) studies have reported higher breast cancer risk associated with the rs1544410 (*Bsm*I) *bb* genotype.

There are several potential explanations for inconsistencies in findings for these common SNPs. Many of the individual studies have been based on <200 cases (9, 11, 19, 20, 22, 23). Allelic frequencies of the *VDR* polymorphisms, particularly *Bsm*I, vary by ethnicity (16, 17, 20, 21), and few studies have been large enough to examine risk by ethnicity with precision. Associations that vary by tumor characteristics might also be missed in studies combining all cases. Effect modification by

environmental factors, including calcium intake (12), which may influence vitamin D metabolism, has also been suggested. We pooled data from six cohorts collaborating in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (24) to determine in a very large series of cases and controls if these two widely studied SNPs in the *VDR* gene, rs2228570 (*Fok*I) and rs1544410 (*Bsm*I), contribute to susceptibility to breast cancer.

Materials and Methods

Study Population. The Breast and Prostate Cancer Cohort Consortium, particularly the breast cancer component (25), has been described in detail elsewhere (24). Briefly, the consortium includes large prospective cohorts (or consortia of smaller cohorts) assembled in the United States and Europe that have DNA for genotyping and extensive questionnaire data on all participants. This analysis included 6,473 cases and 8,397 controls for rs2228570 (*Fok*I) and 6,355 breast cancer cases and 8,149 controls for rs1544410 (*Bsm*I) from six cohorts that had genotyped these SNPs on the *VDR* gene. Cohorts included the American Cancer Society Cancer Prevention Study II (CPS-II) Nutrition Cohort, the European Prospective Investigation into Cancer and Nutrition (EPIC); the Harvard Nurses' Health Study (NHS); the Hawaii-Los Angeles Multiethnic Cohort (MEC); the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) cohort, and the Women's Health Study (WHS). With the exception of MEC, most women in these cohorts are Caucasian. The MEC includes U.S. Caucasians, African Americans, Latinos, Japanese Americans, and Native Hawaiians. Each study has been approved by its respective institutional review board.

Table 2. Association of the rs2228570 (*Fok*I) SNP with breast cancer risk by cohort, overall, and by ethnicity

Cohort	Genotype	Cases	Controls	HWE controls	Minor allele frequency controls	OR (95% CI)	P_{trend}	$P_{heterogeneity}$
CPS-II	*FF*	185	178			1		
	Ff	200	214	0.9	0.38	0.90 (0.68-1.18)		
	ff	73	66			1.05 (0.71-1.56)	0.93	
EPIC	*FF*	643	1,070			1		
	Ff	754	1,245	0.5	0.37	1.00 (0.88-1.14)		
	ff	224	383			0.97 (0.80-1.18)	0.83	
MEC	*FF*	657	844			1		
	Ff	668	834	0.44	0.34	1.04 (0.89-1.21)		
	ff	201	223			1.17 (0.93-1.46)	0.22	
NHS	*FF*	422	655			1		
	Ff	578	765	0.85	0.37	1.16 (0.98-1.36)		
	ff	205	228			1.40 (1.11-1.75)	0.003	
PLCO	*FF*	380	441			1		
	Ff	505	512	0.69	0.36	1.16 (0.96-1.40)		
	ff	180	141			1.49 (1.15-1.93)	0.003	
WHS	*FF*	225	219			1		
	Ff	292	288	0.82	0.39	0.99 (0.77-1.26)		
	ff	81	91			0.87 (0.62-1.23)	0.5	
All cohorts, excluding NHS	*FF*	2,090	2,752			1		
	Ff	2,419	3,093	0.45	0.36	1.03 (0.95-1.11)		
	ff	759	904			1.10 (0.98-1.24)	0.12	
All cohorts	*FF*	2,512	3,407			1		
	Ff	2,997	3,858	0.45	0.36	1.05 (0.98-1.13)		
	ff	964	1,132			1.16 (1.04-1.28)	0.006	0.03
Ethnic-specific results from multiethnic cohort only								
Hispanic	*FF*	106	134			1		
	Ff	146	180	0.8	0.41	1.05 (0.75-1.48)		
	ff	66	64			1.34 (0.87-2.07)	0.22	
African American	*FF*	197	257			1		
	Ff	115	135	0.22	0.23	1.13 (0.83-1.56)		
	ff	13	27			0.65 (0.32-1.30)	0.81	
Japanese American	*FF*	163	173			1		
	Ff	164	179	0.67	0.35	1.06 (0.78-1.45)		
	ff	74	53			1.63 (1.07-2.49)	0.05	
Hawaiian	*FF*	39	121			1		
	Ff	52	126	0.77	0.34	1.02 (0.61-1.71)		
	ff	13	31			1.02 (0.46-2.27)	0.94	
Caucasian	*FF*	152	159			1		
	Ff	191	214	0.06	0.37	0.94 (0.69-1.28)		
	ff	35	48			0.80 (0.48-1.33)	0.42	0.31
All cohorts combined								
Caucasian	*FF*	1,900	2,576			1		
	Ff	2,406	3,085	0.89	0.38	1.05 (0.97-1.14)		
	ff	774	917			1.15 (1.02-1.28)	0.02	0.008

Breast cancer cases were identified in each cohort primarily by self-report and subsequently verified by medical records or linkage with population-based tumor registries. Controls were individually or frequency matched to cases by age at entry and, depending on the cohort, additional criteria, as described below. Information on estrogen receptor (ER) and progesterone receptor (PR) status and on localized versus metastatic cancers was obtained for most cohorts. Information on breast cancer risk factors was obtained by questionnaire before cancer diagnosis in all cohorts. Diet was assessed using validated food frequency questionnaires; dietary and total calcium intake estimates, adjusted for calories by the residual method (26), were calculated using nutrient databases and analytic programs specific to each cohort's food frequency questionnaire. Information on vitamin D status or intake was not available for all cohorts. Blood samples were collected before diagnosis in all cohorts, except for MEC and CPS-II, in which most were collected after diagnosis.

Two *VDR* SNPs, rs2228570 (*Fok*I) *F/f* and rs1544410 (*Bsm*I) *b/B*, were genotyped in the breast cancer cases and controls. For CPS-II and NHS, genotyping was conducted as described previously (8, 12). For EPIC, MEC, PLCO, and WHS, genotyping was done in four laboratories (IARC; University of Southern California; Core Genotyping Facility, National Cancer Institute; and Harvard School of Public Health, respectively). A fluorescent 5-endonuclease assay and the ABI-PRISM 7900 for sequence detection (TaqMan) were used, with an assay success rate of >97% in each laboratory and a replication rate of >99% for the blinded duplicates inserted within each study's samples (5-10% depending on study). Assay characteristics for the two *VDR* SNPs are available on a public Web site.[23] No interlaboratory variation in genotyping among IARC/University of Southern California/Core Genotyping Facility/Harvard School of Public Health, assessed by genotyping a designated set of 94 samples from the Coriell Biorepository (27) in each laboratory, was noted. We used a χ^2 test to assess whether the rs2228570 (*Fok*I) and rs1544410 (*Bsm*I) genotype distributions were in Hardy-Weinberg equilibrium (HWE) within populations defined by cohort and ethnicity.

Statistical Analyses. We used conditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for disease by SNP genotype using PROC PHREG in SAS version 9.1. The heterozygous and homozygous carriers of the minor allele were each compared with the homozygous carriers of the more prevalent allele, which leads to a 2 *df* test for association between SNP genotypes and risk of disease. P_{trend} values were calculated assuming a log-additive genetic model with 1 *df*. As noted, controls were individually or frequency matched to cases on age at entry and, depending on the cohort, additional characteristics, which could include study center, race/ethnicity, menopausal status, exogenous hormone use, phase of menstrual cycle, date of blood collection, time of day at blood collection, and fasting status at blood collection. Because PLCO did not match on race/ethnicity, this variable was included as a covariate in all models.

We considered conditional logistic regression models both without adjustment and with adjustment for known breast cancer risk factors, including age at menarche (≤12, 13-14, ≥15 years), parity (ever/never full-term pregnancy), menopausal status at blood draw (pre/post/unknown or missing), use of postmenopausal hormone therapy at blood draw (ever/never/unknown or missing), and body mass index (kg/m^2 as a continuous variable). Data on other breast cancer risk factors, such as family history, history of benign breast disease, and age at menopause, were not available from all cohorts. Because the results were essentially unchanged with adjustment, we present results from the conditional model controlling only for race/ethnicity. The analyses presented include invasive and *in situ* breast cancer cases; exclusion of the *in situ* breast cancers produced similar results.

We examined the heterogeneity of associations across the cohorts and across racial/ethnic groups using the *Q* statistic (28). Logistic regression models to examine breast cancer associations with genotype by specific hormone receptor status included only cases classified as ER+/PR+, ER−/PR−, and their matched controls. We similarly examined risk of breast cancer by genotype among women with localized and advanced disease. For all cohorts, advanced disease was defined as metastases to distant organs (''distant'' by Surveillance, Epidemiology and End Results Program staging) or regional metastases to lymph nodes or other adjacent tissues (''regional'' by Surveillance, Epidemiology and End Results Program staging). The NHS and PLCO cohorts also included breast tumors >2 cm in diameter without lymph node involvement or other regional spread (American Joint Committee on Cancer stage II) among advanced cases according to American Joint Committee on Cancer staging guidelines. In the various EPIC recruitment centers, advanced tumors were defined as distant metastases only or regional plus distant metastases combined due to different coding practices at the cancer registries. Consequently, we conducted a sensitivity analysis restricting analysis of advanced cases from EPIC centers with >10% of cases in this category. We also examined results stratified by menopausal status at blood draw. To test for heterogeneity by outcome, we conducted case-only analyses using unconditional logistic regression with the tumor characteristic or menopausal status as the dependent variable.

Dietary and total calcium intakes were combined across cohorts using cohort-specific quintiles. We tested for heterogeneity in genetic effects across extremes of dietary and total calcium intake by comparing a model containing indicator variables for heterozygous and homozygous minor allele genotypes, two categories of increased calcium intake (the three middle quintiles combined and the top quintile), and their product interaction terms to a model with only the genotype and calcium intake variables (a 4 *df* test).

Results

Descriptive characteristics of study participants are provided for each cohort in Table 1. The majority of women were postmenopausal and White, except for the MEC, in which there were roughly equal numbers of

[23] http://www.uscnorris.com/mecgenetics/CohortGCKView.aspx

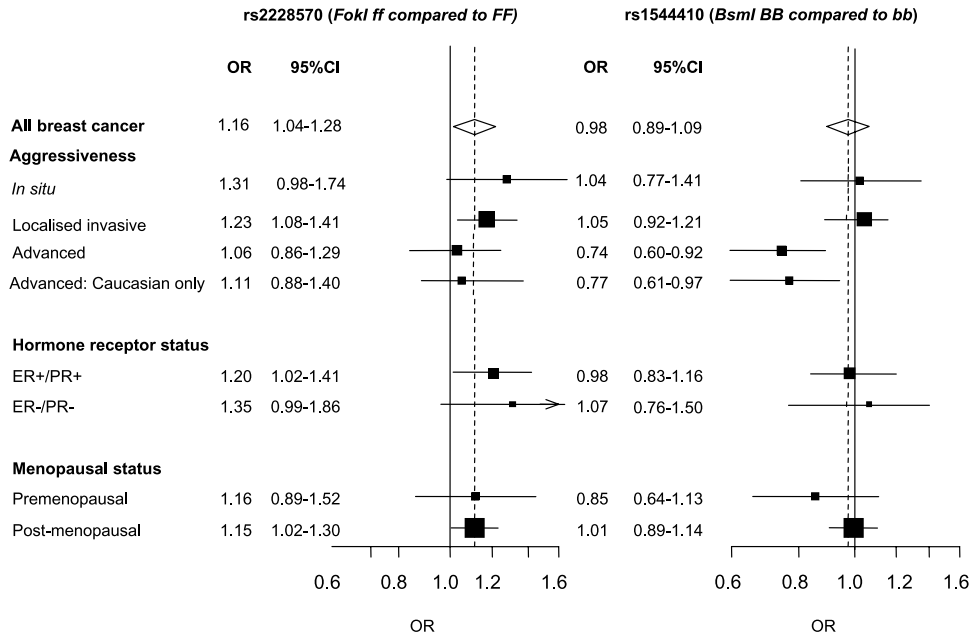

Figure 1. Risk of breast cancer by tumor characteristics and menopausal status for rs2228570 (*Fok*I) and rs1544410 (*Bsm*I). OR (95% CI) were calculated by conditional logistic regression and compared subjects homozygous for the less common variant (*ff* and *BB*, respectively) with subjects homozygous for the more common variant (*FF* and *bb*, respectively). *Diamond* and *dashed line,* overall OR.

White, Hispanic, African American, and Japanese American women. The genotype distribution in controls of the rs2228570 (*Fok*I) polymorphism obeyed HWE in all cohorts combined (P = 0.58) and in each of the five racial/ethnic subgroups within the MEC. In controls from all the cohorts, the distribution of genotypes for the rs1544410 (*Bsm*I) polymorphism deviated from that expected by HWE (P = 0.0004). However, the minor allele frequency for this SNP varied noticeably among the White, Hispanic, African American, Japanese American, and Hawaiian controls in the MEC (Table 2). Within each of these racial/ethnic subgroups, genotype distributions respected HWE ($P > 0.05$ for all).

Associations for individual SNPs are displayed in Table 2 by cohort, by race/ethnicity, and overall; ORs are shown by tumor characteristics and menopausal status in Fig. 1. We observed a modest, positive, statistically significant association between the rs2228570 (*Fok*I) *ff* genotype and relative risk of breast cancer (OR, 1.16; 95% CI, 1.04-1.28; P_{trend} = 0.006; $P_{heterogeneity}$ = 0.03). However, this association was no longer statistically significant (OR, 1.10; 95% CI, 0.98-1.24; P_{trend} = 0.12) after exclusion of the NHS, in which a positive association with the rs2228570 (*Fok*I) *f* polymorphism was reported previously (7). Exclusion of the 616 and 603 *in situ* breast cancers included in the *Fok*I and *Bsm*I analyses, respectively, produced similar results (OR, 1.15; 95% CI, 1.03-1.28 and OR, 0.98; 95% CI, 0.88-1.09, respectively).

In analyses within the racial/ethnic subgroups in the MEC, the association for the *Fok*I *ff* genotype was highest among Japanese American women (OR, 1.63; 95% CI, 1.07-2.49); however, the $P_{heterogeneity}$ across racial/ethnic subgroups was not statistically significant (P = 0.31; Table 2). In our complete data set, we observed for the *Fok*I *ff* genotype a marginally stronger association for localized invasive tumors than advanced tumors (OR, 1.23; 95% CI, 1.08-1.41 and OR, 1.06; 95% CI, 0.86-1.29, respectively) and for ER−/PR− tumors than for ER+/PR+ tumors (OR, 1.35; 95% CI, 0.99-1.86 and OR, 1.20; 95% CI, 1.02-1.41, respectively (Fig. 1). However, the $P_{heterogeneity}$ values by tumor characteristic were all >0.05).

No association was seen between the rs1544410 (*Bsm*I) SNP and breast cancer in all races combined or analyses confined to Caucasians (Table 3). Japanese American women with the B allele were at lower breast cancer risk, and the $P_{heterogeneity}$ across racial/ethnic groups was borderline (P = 0.08). In a subanalysis, women of all races with the rs1544410 (*Bsm*I) *BB* genotype had a statistically significantly lower risk of advanced breast cancer (OR, 0.74; 95% CI, 0.60-0.92; P_{trend} = 0.015), which persisted when only Caucasian women were considered (OR, 0.77; 95% CI, 0.61-0.97; P_{trend} = 0.045; Fig. 1). These results were identical in a sensitivity analysis that included only EPIC centers with (>10%) advanced cases (regional and distant metastases, combined).

We evaluated whether the associations between the two *VDR* polymorphisms and breast cancer risk were modified by total calcium intake. Although risk was highest among women with the rs2228570 (*Fok*I) *ff* genotype in the top study-specific quintile of total calcium intake (OR, 1.37; 95% CI, 1.04-1.81) versus *FF*/bottom quintile of total calcium, the test for interaction was of borderline statistical significance (P = 0.08; Table 4A). Analyses stratified by extreme quintiles of total calcium intake showed no effect modification of the association between the rs1544410 (*Bsm*I) genotype and breast cancer risk (Table 4B).

Discussion

In this large pooled analysis of data from 6 prospective studies, we observed a small, statistically significant increase in breast cancer risk associated with the *ff* genotype of the *VDR* rs2228570 (*Fok*I) SNP, with a 16% increase in risk in homozygotes for the minor allele, relative to homozygotes for the more common allele (*FF*).

However, there was evidence of heterogeneity of findings across cohorts ($P = 0.03$), which was not explained by differences in race/ethnicity. In addition, the association was weakened and lost statistical significance when excluding the NHS, in which a positive association had been reported previously.

The presence of the rs2228570 (*Fok*I) *f* allele in the 5′-promoter region of the *VDR* results in production of a VDR protein that is less effective as a transcriptional activator (15). The cellular consequences of the less active *ff* genotype would be expected to mimic those of lower vitamin D status. Both the geographic gradient in breast cancer incidence and prospective and retrospective studies of circulating 25(OH)D and breast cancer risk suggest an inverse relationship between vitamin D status and breast cancer risk (2, 3), although findings are mixed (4). Our results, which found a modest increase in breast cancer risk associated with the *ff* genotype, although far from dramatic, are consistent with a role for vitamin D in breast cancer etiology.

The lack of an association between the rs1544410 (*Bsm*I) SNP and breast cancer risk argues against a major role of this polymorphism in breast cancer susceptibility, a result consistent with the indeterminate associations observed between this SNP and breast cancer risk (2, 8, 9, 11, 12, 16, 19-23). Although 6 studies reported increased risk of breast cancer with the *Bsm*I *bb* genotype (2, 9, 16, 19-21), mostly among Caucasian women, 5 other studies did not report a similar association (8, 11, 12, 22, 23). Two studies have suggested a higher risk of metastatic breast disease among homozygotes for the more common *b* allele (9, 20). In support of these findings, we did find that the *BB* genotype was statistically significantly and inversely associated with risk of advanced breast cancer tumors overall and when we restricted the analysis to Caucasian women. This result is consistent with preliminary reports suggesting a protective role for vitamin D in lung cancer survival and prognosis (29) and ecologic correlations of cancer survival with greater sun exposure or season of diagnosis (30, 31). The hypothesized mechanisms for

Table 3. Association of rs1544410 (*Bsm*I) SNP with breast cancer risk by cohort, overall, and by ethnicity

Cohort	Genotype	Cases	Controls	HWE controls	Minor allele frequency controls	OR (95% CI)	P_{trend}	$P_{heterogeneity}$
CPS-II	*bb*	142	162			1		
	Bb	212	200	0.53	0.39	1.22 (0.90-1.66)		
	BB	78	70			1.28 (0.86-1.92)	0.16	
EPIC	*bb*	573	951			1		
	Bb	767	1,219	0.08	0.4	1.02 (0.89-1.18)		
	BB	256	450			0.93 (0.77-1.13)	0.59	
MEC	*bb*	903	1,051			1		
	Bb	518	672	0.0009	0.26	0.93 (0.79-1.08)		
	BB	115	158			0.89 (0.68-1.17)	0.26	
NHS	*bb*	407	550			1		
	Bb	555	723	0.86	0.4	1.01 (0.85-1.20)		
	BB	160	242			0.91 (0.72-1.15)	0.55	
PLCO	*bb*	405	407			1		
	Bb	468	533	0.41	0.39	0.87 (0.72-1.05)		
	BB	192	157			1.21 (0.94-1.56)	0.43	
WHS	*bb*	201	200			1		
	Bb	303	298	0.78	0.42	1.01 (0.78-1.31)		
	BB	100	106			0.94 (0.68-1.32)	0.77	
All cohorts	*bb*	2,631	3,321			1		
	Bb	2,823	3,645	0.0004	0.37	0.98 (0.91-1.06)		
	BB	901	1,183			0.98 (0.89-1.09)	0.66	0.5
Ethnic-specific results from multiethnic cohort only								
Hispanic	*bb*	184	207			1		
	Bb	115	141	0.26	0.25	0.91 (0.66-1.26)		
	BB	24	21			1.32 (0.70-2.48)	0.85	
African American	*bb*	163	217			1		
	Bb	126	155	0.34	0.29	1.10 (0.80-1.51)		
	BB	27	40			0.91 (0.53-1.57)	0.94	
Japanese American	*bb*	341	299			1		
	Bb	71	106	0.86	0.14	0.60 (0.42-0.85)		
	BB	3	5			0.52 (0.12-2.26)	0.003	
Hawaiian	*bb*	65	175			1		
	Bb	35	86	0.7	0.2	1.23 (0.74-2.04)		
	BB	4	13			1.05 (0.31-3.53)	0.52	
Caucasian	*bb*	150	153			1		
	Bb	171	184	0.08	0.41	1.02 (0.74-1.41)		
	BB	57	79			0.85 (0.56-1.30)	0.55	0.08
All cohorts combined								
Caucasian	*bb*	1,751	2,271			1		
	Bb	2,381	3,010	0.14	0.41	1.01 (0.93-1.10)		
	BB	821	1,077			0.99 (0.89-1.11)	0.97	0.62

Table 4.

(A) Association among *VDR FokI* genotype, total calcium intake, and breast cancer risk

*Fok*I genotype	Quintiles of total calcium intake		
	1	2-4	5
FF	368/468	1,066/1,277	306/425
	1.00	1.04 (0.88-1.22)	0.83 (0.67-1.02)
Ff	405/469	1,236/1,455	449/494
	1.09 (0.90-1.33)	1.05 (0.89-1.23)	1.07 (0.89-1.30)
ff	125/127	404/438	157/135
	1.25 (0.94-1.67)	1.13 (0.93-1.38)	1.37 (1.04-1.81)

(B) Association among *VDR BsmI* genotype, total calcium intake, and breast cancer risk

*Bsm*I genotype	Quintiles of total calcium intake		
	1	2-4	5
bb	417/457	1,139/1,325	370/428
	1.00	0.91 (0.78-1.07)	0.85 (0.70-1.04)
Bb	366/471	1,147/1,336	390/435
	0.82 (0.68-1.00)	0.89 (0.76-1.04)	0.91 (0.74-1.10)
BB	100/115	357/410	141/149
	0.94 (0.69-1.28)	0.90 (0.74-1.11)	0.96 (0.73-1.26)

NOTE: $P_{interaction} = 0.08$. $P_{interaction} = 0.43$.

better prognosis with more favorable vitamin D status are based on animal models and involve modulation of cell cycle progression, apoptosis, and cell signaling leading to reduced tumor invasiveness and angiogenesis (32). The ethnic differences in allele frequency for *Bsm*I also raise the possibility of confounding by population stratification, particularly as ethnic differences in breast cancer survival have been suggested (33, 34).

The potential modification of genotype-breast cancer associations by environmental factors, such as diet, is worthy of consideration. Calcium and vitamin D metabolism are closely linked and both nutrients have favorable effects on cell proliferation and differentiation of several cancer cell lines *in vitro* (35). Dietary factors including calcium are known to affect the vitamin D endocrine system (36), and diet may also influence autocrine/paracrine vitamin D metabolism (37). In previous studies of breast cancer (11) and colorectal adenoma (38), the risk by *VDR Bsm*I genotype varied by calcium intake, but no studies have reported an interaction with the *VDR FokI* SNP. We did not observe an interaction with the *Bsm*I SNP, and observed only a weak interaction ($P = 0.08$) between total calcium intake and *Fok*I genotype, using a conservative test for interaction. The association of the *Fok*I *ff* genotype with increased risk of breast cancer was seen across all levels of total calcium intake, but the association between total calcium and breast cancer was limited to the *Fok*I *FF* genotype. Although this finding may be due to chance, it may also indicate an interplay between calcium intake and VDR function. We were unable to examine *VDR* genotype interactions with circulating levels of 25(OH)D, the integrated marker of vitamin D status from diet, supplements, and UVB exposure. However, in a population-based case-control study (14) and a nested case-control study (8) no significant interaction among *Fok*I genotype, 25(OH)D, and breast cancer risk was observed.

These results from a pooled analysis of data from six large cohorts suggest that the rs2228570 (*Fok*I) and rs1544410 (*Bsm*I) polymorphisms in *VDR* may have a small role in breast cancer susceptibility. Although both genetic findings support the hypothesis that vitamin D status plays a role in breast cancer etiology, the associations were modest and may be due to chance. In future studies, *VDR* genetic variation should be integrated with prediagnostic biomarkers of vitamin D status.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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