Sun Exposure, Vitamin D Receptor Gene Polymorphisms, and Risk of Advanced Prostate Cancer

Esther M. John,[1] Gary G. Schwartz,[2] Jocelyn Koo,[1] David Van Den Berg,[3] and Sue A. Ingles[3]

[1]Northern California Cancer Center, Fremont, California; [2]Comprehensive Cancer Center, Wake Forest University, Winston-Salem, North Carolina; and [3]Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

Substantial experimental evidence indicates that the hormonal form of vitamin D promotes the differentiation and inhibits the proliferation, invasiveness, and metastasis of human prostatic cancer cells. Results from epidemiologic studies of vitamin D status and/or vitamin D receptor (*VDR*) polymorphisms and prostate cancer risk have been mixed. We conducted a population-based, case-control study of advanced prostate cancer among men ages 40 to 79 years from the San Francisco Bay area. Interview data on lifetime sun exposure and other risk factors were collected for 905 non-Hispanic White men (450 cases and 455 controls). Using a reflectometer, we measured constitutive skin pigmentation on the upper underarm (a sun-protected site) and facultative pigmentation on the forehead (a sun-exposed site) and calculated a sun exposure index from these measurements. Biospecimens were collected for 426 cases and 440 controls. Genotyping was done for *VDR* polymorphisms in the 5′ regulatory region (*Cdx-2*), exon 2 (*Fok*I), and the 3′ region (*Taq*I and *Bgl*I). Reduced risk of advanced prostate cancer was associated with high sun exposure determined by reflectometry [odds ratio (OR), 0.51; 95% confidence interval (95% CI), 0.33-0.80] and high occupational outdoor activity (OR, 0.73; 95% CI, 0.48-1.11). Significant risk reductions with the high-activity alleles *Fok*I *FF* or *Ff*, *Taq*I *tt*, and *Bgl*I *BB* genotypes and a nonsignificant reduction with *Cdx-2 AG* or *AA* genotype were observed in the presence of high sun exposure, with ORs ranging from 0.46 to 0.67. Our findings support the hypothesis that sun exposure and *VDR* polymorphisms together play important roles in the etiology of prostate cancer. (Cancer Res 2005; 65(12): 5470-9)

Introduction

In 1990, Schwartz and Hulka (1) noted that the descriptive epidemiology of prostate cancer (increasing incidence with age, Black race, and residence at northern latitudes) resembles the epidemiology of adult vitamin D deficiency and proposed that vitamin D deficiency increases the risk for prostate cancer ("the vitamin D hypothesis"). Vitamin D is produced in the skin after exposure to UV radiation or may be obtained from the diet and supplements. Vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D (25-OHD), the major circulating vitamin D metabolite. 25-OHD in turn is hydroxylated in the kidneys to form 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D], an endocrine hormone that functions to control serum levels of calcium and phosphorus. 1,25$(OH)_2$D is also produced by nonrenal tissues that possess 1α-hydroxylase (2), including human prostatic cells (3, 4), where it functions locally to control cellular growth and differentiation. In 1992, Miller et al. (5) showed that prostate cells possess specific high-affinity receptors for 1,25$(OH)_2$D [vitamin D receptors (VDR)]. Subsequent research has established that 1,25$(OH)_2$D promotes the differentiation and inhibits the proliferation, invasiveness, and metastasis of prostate cells (6–8). These findings have led to the active exploration of 1,25$(OH)_2$D and its analogues as therapeutic agents for prostate cancer (9).

In contrast to experimental studies, epidemiologic evidence pertinent to the vitamin D hypothesis is more limited. Prostate cancer risk has been inversely associated with sun exposure, the major source of vitamin D. In most individuals, ~90% of circulating levels of 25-OHD are derived from casual sun exposure (10). In the United States, high residential sun exposure has been associated with lower mortality rates in ecologic studies (1, 11), reduced mortality in a death certificate–based, case-control study (12), and reduced risk in a follow-up study (13). A cross-sectional study from South Carolina reported a significantly lower prevalence of abnormal prostate-specific antigen (PSA) levels in men with frequent sun exposure (14). A case-control study from England found a 3-fold increased risk associated with low lifetime sun exposure (15). The results from seroepidemiologic studies are mixed, with some (16, 17), but not others (18–23), reporting increased risks among men with low serum levels of 25-OHD.

The effects of 1,25$(OH)_2$D are mediated through the VDR, which is expressed in both normal and malignant prostatic cells (5). The expression and/or function of the VDR protein may be influenced by polymorphisms in the 3′ end (intron 8 and exon 9), the middle (exon 2), and the 5′ upstream regulatory region of the gene, which contains at least seven alternatively spliced first exons, labeled IA-IG. Following two reports of 3- to 4-fold increased risks of prostate cancer associated with 3′ polymorphisms (24, 25), subsequent studies assessing 3′ polymorphisms, *Taq*I, *Bsm*I, *Apa*I, and *poly-A*, or the exon 2 polymorphism, *Fok*I, produced reports of significant (26–32) and nonsignificant (15, 33–36) associations as well as no association (37–41). There is some suggestion that associations may be stronger for advanced prostate cancer (25, 34, 29, 32). Except for two reports, studies of prostate cancer have not considered the effect of *VDR* polymorphisms in conjunction with serum levels of their ligand (26) or with data on sun exposure (42).

Given the strong experimental yet sparse epidemiologic evidence, we conducted a population-based, case-control study of advanced prostate cancer in the San Francisco Bay area to assess its association with several sun exposure measures, including an index based on skin pigmentation measurements.

Requests for reprints: Esther M. John, Northern California Cancer Center, 2201 Walnut Avenue, Suite 300, Fremont, CA 94538. Phone: 510-608-5007; Fax: 510-608-5085. E-mail: ejohn@nccc.org.

We also examined polymorphisms in three regions of the *VDR* gene and explored the modifying effect of sun exposure on the associations between *VDR* genotype and prostate cancer risk. This is the largest study to date to focus on advanced disease.

Study Design and Methods

Study Population

Cases. Patients with newly diagnosed prostate cancer were identified through the Greater San Francisco Bay Area Cancer Registry, which is part of the Surveillance, Epidemiology and End Results (SEER) Cancer Registry Program. Eligible cases ages 40 to 79 years included non-Hispanic White men diagnosed with primary advanced prostate cancer between July 1997 and February 2000 and African American men diagnosed with primary advanced prostate cancer between July 1997 and December 2000. Advanced prostate cancer was defined as a tumor invading and extending beyond the prostatic capsule and/or extending into adjacent tissue or involving regional lymph nodes or distant metastatic sites (Surveillance, Epidemiology and End Results 1995 clinical and pathologic extent of disease codes 41-85).

The cancer registry ascertained 1,015 advanced prostate cancer cases (799 with regional stage and 216 with distant stage), 95% of them with a histologic type of adenocarcinoma. Of these, 33 were enrolled in another study, 12 were declined contact by their physician, 106 (10%) were deceased at the time of contact (42 with regional stage and 64 with distant stage), and 76 did not meet the eligibility criteria (15 did not self-identify as non-Hispanic White or African American, 2 reported prior prostate cancer, 5 did not speak sufficient English, 43 had moved from the San Francisco Bay area, and 11 did not qualify for other reasons). Of 788 cases contacted, 568 (72%) completed the interview, including 450 (72%) Whites and 118 (73%) African Americans. Interviews were not completed due to refusal (n = 156), illness (n = 21), inability to locate (n = 20), and other reasons (n = 21). Of the 568 cases who completed the interview, 563 were alive when contacted for a blood or mouthwash sample. Of these, 533 (95%) cases provided a biospecimen sample, including 426 (95%) Whites and 107 (92%) African Americans.

Controls. Controls ages 40 to 79 years were identified through random-digit dialing using a modification of the Waksberg method (43). Using telephone numbers of recently diagnosed cancer patients, a bank of nearly 32,000 random numbers was generated by replacing the last two digits of patients' numbers with random numbers and generating 10 random telephone numbers for each patient number. Of the 18,489 telephone numbers assessed as residential (60% of generated numbers), 29% were called up to 10 times reaching answering machine only or never receiving any answer. Among the 13,152 numbers where a household member was reached, 10,892 (83%) participated in the enumeration of household members. Controls ages 65 to 79 years were also identified through random selections from the rosters of beneficiaries of the Health Care Financing Administration (HCFA).

Using frequency matching on race and 5-year age group, 1,081 controls were selected into the study (717 random-digit dialing and 364 HCFA controls). Of these, 123 did not meet the eligibility criteria (16 were deceased at the time of contact, 18 did not self-identify as non-Hispanic White or African American, 41 reported a history of prostate cancer, 8 did not speak sufficient English, 18 had moved from the San Francisco Bay area following selection into the study, and 22 did not qualify for other reasons). For 90 HCFA controls, no telephone number could be located. Of the 868 controls contacted, 545 (63%) completed the interview, including 455 (64%) Whites and 90 (57%) African Americans. Interviews were not completed due to refusal (n = 249), illness (n = 15), inability to locate (n = 32), and other reasons (n = 27). A blood or mouthwash sample was obtained for 525 (96%) controls, including 440 (97%) Whites and 85 (94%) African Americans.

Data and Biospecimen Collection

Trained professional interviewers conducted in-person interviews and administered a structured questionnaire that asked about demographic background, lifetime histories of residences, occupations, physical activity, sun exposure, use of medications and supplements, alcohol consumption and tobacco use, family history of prostate cancer, medical history, and screening for prostate cancer. A 74-item food frequency questionnaire adapted from Block's Health History and Habits Questionnaire assessed usual intake during the reference year, defined as the year before diagnosis for cases and the year before selection into the study for controls.

The interviewers also measured standing height and weight. Using a portable reflectometer (Minolta Chromameter CR-300), two measurements of skin pigmentation were taken at the upper underarm, a site that is generally not exposed to sunlight (constitutive pigmentation), and at the center of the forehead, a site that is generally exposed to sunlight (facultative pigmentation). The Chromameter measures skin color through skin reflectance ranging from 0 (perfect black) to 100 (perfect white). This instrument has been shown to characterize skin color and quantify small skin color changes (44) and to produce measurements of high intrarater and interrater reproducibility (45). Darker foreheads have been correlated with residence in regions of higher solar radiation (46), and the difference between constitutive and facultative skin pigmentation has been proposed as a quantitative index of sun exposure that is related to cumulative lifetime sun exposure (47). A fasting blood sample or a mouthwash sample (48) was collected. Frozen lymphocytes and mouthwash samples were stored at −70°C before DNA extraction. Study participants provided written informed consent, and the study was approved by the institutional review boards of the Northern California Cancer Center and the University of Southern California.

VDR Genotyping

We examined polymorphisms in three regions of the *VDR* gene: a single nucleotide polymorphism (SNP) in a Cdx-2 protein binding site (49) lying between exons ID and IG, a missense SNP [rs10735810, a *Fok*I restriction site length polymorphism (RFLP)] in the first of two potential start codons in exon 2, and two SNPs in exon 9 (rs731236, a synonymous *Taq*I RFLP, and a novel *Bgl*I RFLP lying 303 bp downstream of the stop codon). Genotyping of the four SNPs, *Cdx-2*, *Fok*I, *Taq*I, and *Bgl*I, was done by the TaqMan assay using the TaqMan Core Reagent kit (Applied Biosystems, Foster City, CA). PCR reactions were carried out using standard conditions recommended by the manufacturer. The following primer and minor groove binder probe sequences were used: for *Cdx-2*, forward primer 5′-CATTGTAGAACATCTTTTGTATCAGGAACT-3′, reverse primer 5′-GGTCTTCCCAGGACAGTATTTTTCA-3′, G allele FAM-AGGTCA-CA*G*TAAAAAC-3′, and A allele VIC-AGGTCACA*A*TAAAAAC-3′; for *Fok*I, forward primer 5′-GCACTGACTCTGGCTCTGACCG-3′, reverse primer 5′-GTCAAAGTCTCCAGGGTCAGGCA-3′, *A* allele VIC-TGCCTCC*A*TCCCTG-GTAA-3′, and *G* allele FAM-TGCCTCC*G*TCCCTGTA-3′; for *Fok*I, forward primer 5′-CTTCTCTATCCCCGTGCCC-3′, reverse primer 5′-ACGTCTG-CAGTGTGTTGGACA-3′, *T* allele VIC-GCGCTGAT*T*GAGGCCA-3′, and *C* allele FAM-CGCTGAT*C*GAGGCCA-3′; and for *Bgl*I, forward primer 5′-GCAGGGCCTTGCCCA-3′, reverse primer 5′-CACTAGGCGCTGGACAAGC-3′, *C* allele FAM-CGCTGCC*C*TAAGTGG-3′, and *A* allele VIC-CCGCTGC*A*-TAAGTGG-3′. Fluorescent signals were measured using an ABI 7900HT Detection System. Experimental samples were compared with nine previously sequenced controls (three of each genotype) to identify the three genotypes at each locus. Samples outside of the parameters defined by the controls were designated as noninformative. All PCR batches included water blanks. Technicians were blinded to case-control status.

Consistent with previous literature, genotypes for the three RFLP polymorphisms are reported using standard nomenclature for RFLP assays (using lower and upper case letters to indicate the presence or absence of a restriction site, respectively). The *Fok*I *A* and *G* alleles are indicated by *f* and *F*, respectively; the *Taq*I *T* and *C* alleles by *T* and *t*, respectively; and the *Bgl*I *A* and *C* by *B* and *b*, respectively.

Exposure Variables

We derived several measures of sun exposure, including residential solar radiation, outdoor activity, constitutive pigmentation, facultative

pigmentation, and a sun exposure index based on the difference between constitutive and facultative pigmentation. To assess the effect of residential solar radiation, we assigned a solar radiation level to each state of residence reported in the residential history using data from 235 National Weather Service Stations (50). Solar radiation in each state was classified as low (<305), medium (305-365), or high (≥366) based on the tertile distribution of average daily total global radiation measured in Langleys. We examined residential solar radiation in the state of birth and years lived in states of low solar radiation before age 21 years, before age 41 years, and over the lifetime. Time spent outdoors was assessed from lifetime histories of jobs, physical activity (i.e., walking or bicycling to school or work, outdoor exercise, and moderate to strenuous outdoor chores), and sedentary activities (e.g., sunbathing and watching sporting events). Total outdoor activity was estimated for ages 15 to 20 years, ages 21 to 40 years, age 15 years to the reference year, and during the 20 years before the reference year. Skin reaction to summer midday sun exposure (i.e., severe, moderate, mild, or no sunburn) was assessed as an indirect measure of sun exposure, as individuals who burn rather than tan spend less time outdoors (51). The sun exposure index was calculated as the relative difference between the two measurements (i.e., facultative pigmentation minus constitutive pigmentation divided by constitutive pigmentation multiplied by 100; ref. 47).

Statistical Analysis

Because skin pigmentation is an important determinant of vitamin D synthesis, and because the relatively small number of African Americans (107 cases and 85 controls) was insufficient to permit race-specific analysis, we restricted this analysis to Whites (450 cases and 455 controls). Unconditional logistic regression modeling was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) associated with sun exposure measures and *VDR* genotype. We evaluated known and suspected risk factors for prostate cancer as potentially confounding variables and used multivariate logistic regression to adjust for age and family history of prostate cancer. Dose-response trends were assessed across ordinal values of categorical variables. To determine whether the association with *VDR* genotype is modified by factors affecting vitamin D status, we stratified the analyses by tertiles of the sun exposure index. Tests of interaction were conducted by including cross-product terms in the logistic models and conducting a 1 *df* Wald test.

Haplotype frequencies and linkage disequilibrium (LD) coefficients (D') were estimated using the Estimation-Maximization algorithm as implemented in Haploview (Whitehead Institute of Biomedical Research, 2003). ORs associated with haplotypes at the 3′ locus were estimated by logistic regression. To determine whether variation at the 3′ locus acts differently in combination with the two *Fok*I alleles (52), we categorized *Fok*I/*Taq*I genotype combinations by the number of high-activity (*Fok*I *F* and *Taq*I *t*) alleles. We did separate analyses for cases with regional or distant stage disease.

Results

Characteristics of cases and controls are shown in Table 1. The median age at diagnosis was 64 years. Most cases had regional disease (88%), and similar proportions of cases (75%) and controls (71%) reported at least one PSA screening test in the past 5 years. Family history of prostate cancer was significantly associated with increased risk (Table 1). Obesity (body mass index ≥30) was associated with reduced risk, and high intake of total fat and saturated fat and current smoking were associated with nonsignificant risk increases. Risk did not vary with education, history of benign prostatic hyperplasia, caloric intake, height, or alcohol consumption.

One third of cases and controls had always lived in a high solar radiation region, and 88% of cases and 89% of controls lived in a high solar radiation region during the two decades before the interview. Residential sun exposure was not associated with prostate cancer risk (Table 2). There was no evidence that cases were more likely to be born in a region of low solar radiation than controls. Lifetime duration of residence in a low solar radiation region did not differ between cases and controls. Similarly, duration of residence in a low solar radiation region before age 21 years and before age 41 years was not associated with prostate cancer risk (data not shown).

Self-reported lifetime outdoor activity did not differ between cases and controls (Table 2). Similarly, no differences were seen for outdoor activity before age 21 years, before age 41 years, and during the two decades before the interview (data not shown). Nonsignificant reductions in risk were found among men in the highest quartile of occupational outdoor activity (OR, 0.73; 95% CI, 0.48-1.11) and men who do not burn when exposed to summer midday sun (OR, 0.72; 95% CI, 0.46-1.11).

Among controls, scores for constitutive pigmentation (upper underarm) ranged from 23.9 (darker) to 91.1 (lighter), with a mean of 39.6. Facultative pigmentation (forehead) ranged from 17.1 to 63.5, with a mean of 26.5. Constitutive pigmentation did not differ between cases and controls (Table 3). However, controls had significantly darker facultative pigmentation, and increasing darkness was associated with a trend of decreasing risk (P = 0.03). Similarly, the pigmentation-based sun exposure index was inversely associated with risk (P = 0.02). Prostate cancer risk was reduced by half among men in the highest quintile of the sun exposure index (OR, 0.51; 95% CI, 0.33-0.80).

Associations for cases with regional stage disease were similar to those for all cases combined (data not shown), but the association with high occupational outdoor activity became significant (OR, 0.62; 95% CI, 0.40-0.97) and a nonsignificant risk reduction emerged for men in the highest quintile of lifetime outdoor activity (OR, 0.80; 95% CI, 0.51-1.25).

Among controls, distributions of all four *VDR* genotypes were in Hardy-Weinberg equilibrium. As reported previously (53), the *Fok*I polymorphism was not in LD with either 5′ or 3′ loci. D' statistics (and 95% CI) were 0.00 (−0.01 to 0.16) and 0.01 (−0.01 to 0.12) between *Fok*I and Cdx-2 and *Taq*I, respectively. In the 3′ region, *Fok*I and *Bgl*I were tightly linked, with a D' (and 95% CI) of 0.98 (0.95-1.00). *Taq*I/*Bgl*I haplotype frequencies were 0.40 for *tB*, 0.46 for *Tb*, and 0.14 for *TB*.

Cdx-2 genotype was not associated with prostate cancer risk (Table 4). Approximately 30% risk reductions were found for *Fok*I *FF* (versus ff; OR, 0.72; 95% CI, 0.47-1.08), *Taq*I *tt* (versus *TT*; OR, 0.69; 95% CI, 0.46-1.02), and *Bgl*I *BB* (versus *bb*; OR, 0.69; 95% CI, 0.47-1.02). Using haplotypes to assess variation in the 3′ locus, the OR (95% CI) was 0.63 (0.41-0.99) for *tB/tB* versus *Tb/Tb* haplotype. Risk was reduced by half among men with 4 versus <2 high-activity (*Fok*I *F* and *Taq*I *t*) alleles (OR, 0.48; 95% CI, 0.27-0.87). Analyses limited to cases with regional disease produced similar results (data not shown).

In the presence of high sun exposure, *VDR* genotypes were more strongly associated with reduced risk (Table 4), with OR (95% CI) of 0.67 (0.40-1.11) for *Cdx-2 AA* or *AG* (versus *GG*), 0.46 (0.23-0.92) for *Fok*I *FF* or *Ff* (versus *ff*), 0.48 (0.24-0.95) for *Taq*I *tt* (versus *TT* or *Tt*), and 0.58 (0.33-1.00) for *Bgl*I *BB* (versus *bb* or *bB*). Joint effects of sun exposure and *VDR* genotype are presented in Table 5. Compared with men with low sun exposure and lacking protective genotypes, risk reductions of 40% to 65% were seen for men with both high sun exposure and protective *VDR* genotypes, with OR (95% CI) of 0.50 (0.31-0.83) for *Cdx-2 AG* or

Table 1. Risk factors for advanced prostate cancer in non-Hispanic White men

	Advanced cases (n = 450), n (%)	Controls (n = 455), n (%)	Age-adjusted OR (95% CI)
Age (y)			
40-49	19 (4)	12 (3)	
50-59	133 (30)	122 (27)	
60-69	186 (41)	204 (45)	
70-79	112 (25)	117 (26)	
Median age (y)	64	65	
Tumor stage			
Regional	395 (88)		
Distant	55 (12)		
Tumor grade*			
Well differentiated	11 (2)		
Moderately differentiated	271 (60)		
Poorly differentiated	160 (36)		
Undifferentiated	1		
Unknown	7 (2)		
PSA screening past 5 y[†]			
No	109 (24)	78 (17)	
Yes	338 (75)	321 (71)	
Unknown	3 (1)	56 (12)	
Education			
High school graduate or less	94 (21)	84 (18)	1.0
Some college	113 (25)	130 (29)	0.76 (0.52-1.13)
College graduate	243 (54)	241 (53)	1.15 (0.85-1.57)
			P_{trend} = 0.3
Family history of prostate cancer in first-degree relatives			
No	370 (82)	403 (89)	1.0
Yes	80 (18)	52 (11)	1.67 (1.15-2.44)
			P = 0.01
Benign prostatic hyperplasia[†]			
No	335 (74)	339 (75)	1.0
Yes	100 (22)	111 (24)	0.94 (0.68-1.30)
Unknown	15 (3)	5 (1)	P = 0.7
Vasectomy			
No	316 (70)	297 (65)	1.0
Yes	134 (30)	158 (35)	0.79 (0.60-1.05)
			P = 0.1
Energy intake[‡] (kcal/d)			
<1,847	112 (25)	113 (25)	1.0
1,847-2,409	101 (22)	114 (25)	0.89 (0.61-1.29)
2,410-3,072	107 (24)	113 (25)	0.94 (0.65-1.37)
≥3,073	125 (28)	114 (25)	1.08 (0.75-1.57)
Unknown[§]	5 (1)	1	P_{trend} = 0.6
Total fat intake[‡] (g/d)			
<56	81 (18)	113 (25)	1.0
56-83	133 (30)	114 (25)	1.62 (1.11-2.37)
84-116	107 (24)	113 (25)	1.31 (0.89-1.93)
≥117	124 (28)	114 (25)	1.49 (1.02-2.20)
Unknown[§]	5 (1)	1	P_{trend} = 0.1
Saturated fat intake[‡] (g/d)			
<18	86 (19)	113 (25)	1.0
18-27	130 (29)	114 (25)	1.49 (1.02-2.17)
28-38	111 (25)	113 (25)2	1.27 (0.86-1.87)
≥39	118 (26)	114 (25)	1.34 (0.91-1.97)
Unknown[§]	5 (1)	1	P_{trend} = 0.3
Measured height (cm)			
<170	85 (19)	96 (21)	1.0
170-174.9	117 (26)	117 (26)	1.12 (0.76-1.66)
175-179.9	115 (26)	109 (24)	1.18 (0.80-1.75)
≥180	99 (22)	105 (23)	1.05 (0.70-1.58)
Unknown[‖]	34 (8)	28 (6)	P_{trend} = 0.8
Body mass index[‡,¶]			
<25	124 (28)	114 (25)	1.0
25-29.9	230 (51)	211 (47)	1.01 (0.73-1.38)
≥30	96 (21)	129 (28)	0.68 (0.47-0.99)
Unknown		1	P_{trend} = 0.2
Alcohol consumption[‡] (g/d)			
0	120 (27)	123 (27)	1.0
0.1-4.9	86 (19)	80 (18)	1.11 (0.75-1.64)
5.0-9.9	44 (10)	55 (12)	0.81 (0.51-1.30)
10.0-19.9	71 (16)	73 (16)	0.99 (0.66-1.51)
≥20.0	129 (29)	124 (27)	1.07 (0.75-1.53)
			P_{trend} = 0.8
Smoking status[‡]			
Never	126 (28)	138 (30)	1.0
Former	241 (54)	248 (55)	1.08 (0.80-1.47)
Current	83 (18)	69 (15)	1.31 (0.88-1.95)
			P_{trend} = 0.2

*Well differentiated: Gleason's score 2, 3, or 4; moderately differentiated: Gleason's score 5, 6, or 7; poorly differentiated: Gleason's score 8, 9, or 10.
†Before the reference year.
‡During the reference year.
§Missing or unreliable dietary data.
‖Declined measurements.
¶Based on measured height or self-reported height if measurements were declined and self-reported weight during the reference year.

AA, 0.59 (0.30-1.14) for *Fok*I *FF* or *Ff*, 0.35 (0.18-0.70) for *Taq*I *tt*, and 0.42 (0.24-0.73) for *Bgl*I *BB*. The interactions between *VDR* genotype and sun exposure index, however, did not reach statistical significance.

Discussion

This population-based case-control study adds to the emerging epidemiologic evidence that vitamin D from sun exposure and *VDR* genotype play a role in the development of prostate cancer. Reduced risks were associated with a high sun exposure index, high occupational outdoor activity, and putatively high-activity *VDR* genotypes in the presence of high sun exposure. These findings are consistent with other studies that assessed sun exposure based on pigmentation measurements (54), self-reports (15), residential solar radiation (1, 11–13), or serum 25-OHD (16, 17) and a recent study that assessed the joint effect of sun exposure and *VDR* variants (42).

Skin pigmentation measurements, which quantify a biological effect (i.e., skin response to UV radiation), are likely to be more

accurate measures of sun exposure than self-reports, which depend on participants' recall. Given the increase in facultative pigmentation with age, the sun exposure index was proposed as a measure of cumulative lifetime sun exposure (47). Compared with sun-sensitive individuals who burn, those who tan spend more time outdoors (51, 55), and Japanese women residing in high solar radiation regions had darker foreheads than those residing in lower solar radiation regions (46). Together, these data support the use of the pigmentation-based index as a measure of cumulative sun exposure. To our knowledge, the only previous epidemiologic study to use the same pigmentation measurements reported similar results for non-Hispanic White men (54). In that pilot study of 52 prostate cancer and 33 hospital-based controls with benign prostatic hypertrophy, cases had significantly lower sun exposure (difference between facultative and constitutive pigmentation).

Low sun exposure from self-reported recreational and occupational activities since age 20 years was associated with a 3-fold increased risk of prostate cancer in an English case-control study (15). In that study, most men with high cumulative sun exposure had outdoor occupations (55), which is consistent with our finding of reduced risk associated with high occupational outdoor activity. In our study, total outdoor activity was associated with a nonsignificant risk reduction for regional stage prostate cancer. It is possible that our assessment of outdoor activities as a surrogate measure of sun exposure was not as sensitive as the measure used by Luscombe et al. (15) that asked specifically about sun exposure. Consistent with reports that men with sun-sensitive skin spend less time outdoors (51, 55), we found a nonsignificant reduced risk in men who do not burn. Conversely, in English men with skin type 2 to 4, risk was increased (55).

Usual residence in a high solar radiation region or being born in the South were associated with reduced risk in the National Health and Nutrition Examination Survey I follow-up study (13). Similarly, lower mortality rates were associated with high

Table 2. Residential sun exposure, outdoor activity, and advanced prostate cancer risk in non-Hispanic White men

	Advanced cases (n = 450), n (%)	Controls (n = 455), n (%)	Age-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)*
Solar radiation in state of birth[†]				
Low	117 (26)	121 (27)	1.0	1.0
Medium	88 (20)	89 (20)	1.01 (0.69-1.50)	0.99 (0.67-1.47)
High	211 (47)	213 (47)	1.01 (0.74-1.39)	1.01 (0.73-1.39)
Foreign-born	33 (7)	32 (7)	1.09 (0.63-1.88)	1.08 (0.62-1.87)
				P_{trend} = 0.9
Duration of residence in states of low solar radiation (y)				
≥15	102 (23)	101 (22)	1.0	1.0
1-14	98 (22)	97 (21)	0.99 (0.67-1.47)	0.98 (0.66-1.46)
0[‡]	99 (22)	103 (23)	0.94 (0.64-1.39)	0.91 (0.61-1.35)
0[§]	151 (34)	154 (34)	0.96 (0.67-1.37)	0.95 (0.66-1.35)
				P_{trend} = 0.7
Lifetime outdoor activities (h/wk)				
<2.7	85 (19)	91 (20)	1.0	1.0
2.7-5.6	99 (22)	91 (20)	1.16 (0.77-1.75)	1.15 (0.76-1.73)
5.7-10.4	92 (20)	91 (20)	1.08 (0.72-1.64)	1.09 (0.72-1.65)
10.5-19.8	94 (21)	91 (20)	1.11 (0.73-1.67)	1.10 (0.73-1.67)
≥19.9	80 (18)	91 (20)	0.94 (0.62-1.44)	0.95 (0.62-1.45)
				P_{trend} = 0.8
Lifetime outdoor jobs (h/wk)				
0	123 (27)	120 (26)	1.0	1.0
1.4	84 (19)	84 (18)	0.99 (0.67-1.47)	0.96 (0.65-1.43)
1.4-5.6	100 (22)	83 (18)	1.19 (0.81-1.75)	1.20 (0.81-1.77)
5.7-14.7	81 (18)	84 (18)	0.94 (0.63-1.40)	0.95 (0.64-1.41)
≥14.8	62 (14)	84 (18)	0.73 (0.48-1.10)	0.73 (0.48-1.11)
				P_{trend} = 0.3
Skin reaction to summer midday sun exposure				
Moderate to severe sunburns	184 (41)	163 (36)	1.0	1.0
Mild sunburns	214 (48)	228 (51)	0.83 (0.63-1.10)	0.86 (0.65-1.14)
No sunburns	47 (11)	60 (13)	0.70 (0.45-1.08)	0.72 (0.46-1.11)
				P_{trend} = 0.1

*Adjusted for age and family history of prostate cancer.

[†]Low: Maine, Vermont, New Hampshire, Massachusetts, Connecticut, Rhode Island, New York, Pennsylvania, Ohio, Michigan, Minnesota, and Washington; medium: New Jersey, Delaware, Maryland, District of Columbia, West Virginia, Virginia, North Carolina, South Carolina, Indiana, Kentucky, Tennessee, Wisconsin, Illinois, Iowa, Missouri, North Dakota, South Dakota, Montana, Oregon; and high: Georgia, Florida, Alabama, Mississippi, Arkansas, Louisiana, Nebraska, Kansas, Oklahoma, Texas, Wyoming, Colorado, New Mexico, Idaho, Utah, Arizona, Nevada, and California.

[‡]Residence in states of high and/or medium solar radiation.

[§]Residence in states of high solar radiation only.

Table 3. Constitutive and facultative pigmentation, sun exposure index, and advanced prostate cancer risk in non-Hispanic White men

	Advanced cases (n = 447), n (%)	Controls (n = 452), n (%)	Age-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)*
Constitutive skin pigmentation				
1 Light	95 (21)	91 (20)	1.0	1.0
2	65 (14)	91 (20)	0.69 (0.45-1.05)	0.69 (0.45-1.05)
3	96 (21)	91 (20)	1.01 (0.67-1.52)	1.03 (0.69-1.55)
4	108 (24)	91 (20)	1.14 (0.76-1.70)	1.13 (0.76-1.70)
5 Dark	83 (19)	88 (20)	0.90 (0.59-1.36)	0.89 (0.59-1.35)
				P_{trend} = 0.6
Facultative skin pigmentation				
1 Light	100 (22)	90 (20)	1.0	1.0
2	107 (24)	90 (20)	1.08 (0.73-1.61)	1.08 (0.73-1.62)
3	86 (19)	91 (20)	0.85 (0.56-1.28)	0.83 (0.55-1.26)
4	86 (19)	91 (20)	0.85 (0.56-1.28)	0.83 (0.55-1.25)
5 Dark	68 (15)	90 (20)	0.68 (0.44-1.03)	0.66 (0.43-1.01)
				P_{trend} = 0.03
Sun exposure index†				
1 Low	106 (24)	89 (20)	1.0	1.0
2	93 (21)	90 (20)	0.85 (0.57-1.28)	0.87 (0.58-1.30)
3	89 (20)	92 (20)	0.81 (0.54-1.21)	0.80 (0.53-1.20)
4	103 (23)	91 (20)	0.94 (0.63-1.40)	0.95 (0.64-1.42)
5 High	56 (12)	90 (20)	0.52 (0.33-0.80)	0.51 (0.33-0.80)
				P_{trend} = 0.02

*Adjusted for age, family history of prostate cancer, and month of pigmentation measurements.
†Relative difference between constitutive and facultative skin pigmentation.

residential solar radiation exposure in a death certificate–based, case-control study (12). Although we failed to find an association with residential sun exposure, these U.S.-wide studies had a much broader range of exposure than our San Francisco Bay area–based study, which did not include any men with lifelong residence in a low solar radiation region.

Prediagnostic serum levels of 25-OHD have been assessed in several prospective studies. A 3-fold increased risk was observed in Finnish men with low 25-OHD (<40 nmol/L or <16 ng/mL; ref. 16), although the associations were somewhat weaker in Swedish and Norwegian men and risk was also elevated in men with high 25-OHD levels (17). Several U.S. studies conducted in the San Francisco Bay area (18), Hawaii (19), Maryland (20), the Southeast (23), and among physicians (21) and health professionals (22) from various U.S. regions did not observe an association with serum 25-OHD levels, although there was some suggestion of an inverse association for advanced disease (18, 21, 22). It is noteworthy that in these studies the cut points for low 25-OHD ranged from <21.4 ng/mL (21) to <24.1 ng/mL (20) and <34 ng/mL (19), approximately twice those in the Finnish study (<12 ng/mL; ref. 16). It is possible that increased risks are detected only when lower 25-OHD levels are used to define the low exposure category.

The evidence in support of the vitamin D hypothesis appears strongest for studies with a wide range of sun exposure (i.e., U.S.-wide studies) or studies conducted at high latitudes (i.e., England and Scandinavia) where the prevalence of vitamin D deficiency and insufficiency is much higher. In the Finnish study (16), half the men had 25-OHD levels below 40 nmol/L (16 ng/mL), which is near a common clinical indicator of vitamin D deficiency (<15 ng/mL). Conversely, in the U.S. studies, considerably smaller proportions of men had deficient 25-OHD levels ranging from 5% (20) and 6.5% (21) to 11% (22) and 13.3% (18). In the Hawaiian study, none of the men had 25-OHD levels below 21 ng/mL (19). Therefore, increased risks associated with low 25-OHD may be difficult to detect in populations with a low prevalence of vitamin D deficiency.

A complementary approach to studying the role of vitamin D in prostate cancer is to examine genetic polymorphisms in vitamin D pathway genes, such as the *VDR* gene. Although two initial studies found 3- to 4-fold increased risks of prostate cancer associated with *VDR* polymorphisms in the 3′ end of the gene (24, 25), a recent meta-analysis involving 17 studies that assessed the *Taq*I, *Bsm*I, and *poly-A* repeat polymorphisms as well as the *Fok*I polymorphism in exon 2 concluded that none of these variants were likely to be a major determinant of prostate cancer risk (56).

There is some suggestion that *VDR* polymorphisms may be more strongly associated with advanced disease (25–27, 29, 34). Consistent with these studies, we found reduced risks associated with the putatively high-activity *VDR* alleles. Most previous studies included a mix of cases with localized and advanced disease. If the effects are indeed stronger for advanced disease, the inclusion of localized cases would attenuate risk estimates, which may explain some of the inconsistent findings.

The observed genotypic associations are consistent with functional data. In the 5′ regulatory region, the polymorphic Cdx-2 transcription factor binding site influences VDR-mediated intestinal calcium and phosphate absorption (57), with the *G* allele exhibiting decreased Cdx-2 binding and decreased VDR transcriptional activity compared with the *A* allele (47). We found

the high-activity *Cdx-2 AG* and *AA* genotypes to be associated with reduced risk. Only one other study has examined this polymorphism, finding men with high sun exposure and the *Cdx-2 AA* genotype to be at increased risk (42).

In exon 2, use of the second start codon, as occurs in the *F* polymorphic variant lacking the first start codon (58), results in a VDR protein with an activation domain shortened by three amino acids (59). This protein is more efficient at transactivating a vitamin D–regulated target gene (60). In our study, *Fok*I *FF* or *Ff* genotype was associated with reduced risk but only in the presence of high sun exposure. Previous studies in men from Spain (37) and U.S. Whites (32) as well as a study of advanced disease in Chinese men (38) found no association with *Fok*I genotype. In African Americans, *Fok*I *FF* (versus *ff* or *Ff*) genotype was associated with a 2-fold increase in risk (32). Our findings are consistent with those by Bodiwala et al. (42) who reported a substantial risk reduction associated with *Fok*I *FF* (versus *ff*) genotype in the presence of high sun exposure.

Because known polymorphisms in the 3′ region of the *VDR* gene do not alter the amino acid sequence of the VDR protein, the functional significance of these variants is unclear. 3′ Untranslated region sequence variants may interact differently with other upstream sequences in the *VDR* gene to regulate transcription, translation, or RNA processing (reviewed in refs. 52, 61). Of those studies that found an association, reduced prostate cancer risk was

Table 4. *VDR* gene polymorphisms and risk of advanced prostate cancer in non-Hispanic White men, stratified by sun exposure index

	Advanced cases (n = 425), n (%)	Controls (n = 437), n (%)	Age-adjusted OR (95% CI)	Multivariate-adjusted OR (95% CI)*	Low sun exposure,† OR (95% CI)*	Medium sun exposure,† OR (95% CI)*	High sun exposure,† OR (95% CI)*
VDR Cdx-2							
GG	268 (64)	263 (60)	1.0	1.0			
AG	129 (31)	149 (34)	0.85 (0.64-1.14)	0.86 (0.64-1.15)			
AA	20 (5)	23 (5)	0.86 (0.46-1.61)	0.90 (0.48-1.68)			
				P_{trend} = 0.4			
GG	268 (64)	263 (60)	1.0	1.0	1.0	1.0	1.0
AG or AA	149 (36)	172 (39)	0.85 (0.65-1.12)	0.86 (0.65-1.14)	0.81 (0.50-1.30)	1.14 (0.71-1.83)	0.67 (0.40-1.11)
				P = 0.3	P = 0.4	P = 0.6	P = 0.1
*VDR Fok*I							
ff	69 (16)	57 (13)	1.0	1.0			
Ff	203 (48)	209 (48)	0.81 (0.54-1.20)	0.79 (0.53-1.19)			
FF	153 (36)	171 (39)	0.74 (0.49-1.11)	0.72 (0.47-1.08)			
				P_{trend} = 0.1			
ff	69 (16)	57 (13)	1.0	1.0	1.0	1.0	1.0
FF or Ff	356 (84)	380 (87)	0.77 (0.53-1.13)	0.76 (0.52-1.11)	0.98 (0.50-1.89)	0.92 (0.47-1.79)	0.46 (0.23-0.92)
				P = 0.2	P = 0.9	P = 0.8	P = 0.03
*VDR Taq*I							
TT	164 (39)	153 (35)	1.0	1.0			
Tt	200 (47)	200 (46)	0.94 (0.70-1.26)	0.95 (0.70-1.27)			
tt	60 (14)	83 (19)	0.68 (0.46-1.01)	0.69 (0.46-1.02)			
				P_{trend} = 0.1			
Tt or TT	364 (86)	353 (81)	1.0	1.0	1.0	1.0	1.0
tt	60 (14)	83 (19)	0.71 (0.49-1.01)	0.71 (0.49-1.02)	0.78 (0.43-1.41)	0.91 (0.47-1.77)	0.48 (0.24-0.95)
				P = 0.06	P = 0.4	P = 0.8	P = 0.03
*VDR Bgl*I							
bb	100 (24)	86 (20)	1.0	1.0			
Bb	209 (50)	202 (47)	0.89 (0.63-1.26)	0.89 (0.63-1.26)			
BB	112 (27)	139 (33)	0.70 (0.48-1.02)	0.69 (0.47-1.02)			
				P_{trend} = 0.05			
Bb or bb	309 (73)	288 (67)	1.0	1.0	1.0	1.0	1.0
BB	112 (27)	139 (33)	0.75 (0.56-1.02)	0.75 (0.56-1.01)	0.78 (0.47-1.28)	0.93 (0.56-1.56)	0.58 (0.33-1.00)
				P = 0.06	P = 0.3	P = 0.8	P = 0.05
No. high-activity (*Fok*I *F* and *Taq*I *t*) alleles‡							
<2	143 (34)	120 (27)	1.0	1.0	1.0	1.0	1.0
2	151 (36)	172 (39)	0.74 (0.53-1.02)	0.72 (0.52-1.00)	0.66 (0.37-1.16)	0.93 (0.54-1.61)	0.60 (0.32-1.12)
3	109 (26)	107 (25)	0.86 (0.60-1.23)	0.84 (0.59-1.21)	0.79 (0.43-1.48)	1.04 (0.56-1.96)	0.81 (0.42-1.56)
4	21 (5)	37 (8)	0.48 (0.27-0.86)	0.48 (0.27-0.87)	0.85 (0.33-2.19)	0.38 (0.12-1.17)	0.25 (0.07-0.81)
				P_{trend} = 0.05	P_{trend} = 0.5	P_{trend}=0.4	P_{trend} = 0.09

*Adjusted for age and family history of prostate cancer.

†Tertiles of sun exposure index.

‡<2 (*ffTT, ffTt,* and *FfTT*), 2 (*fftt, FfTt,* and *FFTT*), 3 (*Fftt* and *FFTt*), and 4 (*FFtt*).

Table 5. Sun exposure index, VDR genotype, and advanced prostate cancer risk in non-Hispanic White men

VDR genotype	Low sun exposure,* OR (95% CI)†	Medium sun exposure,* OR (95% CI)†	High sun exposure,* OR (95% CI)†	$P_{interaction}$
VDR Cdx-2				
GG	1.0	0.82 (0.54-1.23)	0.74 (0.48-1.13)	0.7
AG or *AA*	0.81 (0.51-1.30)	0.93 (0.58-1.49)	0.50 (0.31-0.83)	
*VDR Fok*I				
ff	1.0	0.98 (0.41-2.33)	1.32 (0.55-3.15)	0.1
FF or *Ff*	0.99 (0.51-1.90)	0.91 (0.47-1.75)	0.59 (0.30-1.14)	
*VDR Taq*I				
Tt or *TT*	1.0	0.90 (0.63-1.28)	0.74 (0.51-1.07)	0.3
tt	0.80 (0.44-1.44)	0.82 (0.42-1.59)	0.35 0.18-0.70	
*VDR Bgl*I				
Bb or *bb*	1.0	0.88 (0.60-1.29)	0.74 (0.49-1.11)	0.4
BB	0.78 (0.48-1.28)	0.81 (0.48-1.36)	0.42 (0.24-0.73)	

*Tertiles of sun exposure index.
†Adjusted for age and family history of prostate cancer.

always associated with the *Taq*I *t* allele or an allele in LD with *Taq*I *t* (*Bsm*I *B, Apa*I *A*, or *poly-A S*). We found reduced risks associated with both *Taq*I *tt* and *Bgl*I *BB* genotypes but only in the presence of high sun exposure. Similarly, Ma et al. (26) reported reduced risk associated with the *Taq*I *tt* genotype but, conversely, only among men with low serum 25-OHD levels. Although our result for *Taq*I is not consistent with other null findings (56), none of the other studies (with one exception: ref. 42) considered the modifying effect of sun exposure.

In summary, the results of our study and those by Bodiwala et al. (42) suggest the importance of considering both *VDR* genotype and sun exposure when assessing prostate cancer risk. Compared with men with low sun exposure and lacking protective genotypes, we found risk reductions of 33% to 54% in men with both high sun exposure and protective *VDR* genotypes. Further investigations are warranted in study populations of sufficient size to detect statistically significant interactions.

Several limitations need to be considered when interpreting our results. A large proportion of cases (22%) identified through the cancer registry were not available for the study (deceased, relocation, or participation in another study). A similarly large proportion of controls (20%) did not meet the eligibility criteria. Among those contacted, participation was lower among controls (63%) than cases (72%), thus raising concern about potential selection bias.

There was a delay between diagnosis and interview (22 months for regional stage cases and 17 months for distant stage cases), resulting in a fairly large proportion of distant stage cases who were deceased (30% compared with 5% among regional stage cases). Thus, the distant stage cases who participated in this study may not be representative of all such cases. If deceased cases were more likely to have had low sun exposure, then the association with sun exposure may have been underestimated for distant stage disease.

Except for the pigmentation-based sun exposure index, all exposure histories were based on self-report. Because the potential relation between sun exposure and prostate cancer risk is not widely recognized, it is unlikely that errors in reporting lifetime sun exposure histories differed by case-control status, thus potentially biasing the results toward the null.

Several strengths are noteworthy. This case-control study was population-based and is the largest to date to examine sun exposure and *VDR* variants in relation to advanced prostate cancer risk. The focus on advanced disease is likely to have produced a clearer picture of the association with vitamin D–related exposures than had we included early-stage cases (62). Unlike most other studies, ours is the only one to examine variants in all three *VDR* loci and is one of the first to assess the combined effect of sun exposure and *VDR* variants on prostate cancer risk. Several measures of sun exposure were considered, including a pigmentation-based index that is less prone to exposure misclassification than self-reported sun exposure.

Besides age, race/ethnicity, and country of birth, few risk factors for prostate cancer have been consistently identified. The prevalence of vitamin D deficiency is higher in populations from high latitude regions and among the elderly (63). National Health and Nutrition Examination Survey III, a recent nationwide U.S. survey, indicated that the prevalence of vitamin D deficiency and insufficiency is relatively common even among young adults (64).

The proposed mechanism for the anticancer effects of sunlight exposure on prostate cancer risk involves the conversion of the prohormonal form of vitamin D, 25-OHD, into the active hormone by prostatic cells. Although noncancerous human prostate cells have been shown to express high levels of 1α-hydroxylase, cancerous prostate cells have lower 1α-hydroxylase expression (65, 66). However, both laboratory and clinical evidence indicate that some 1α-hydroxylase activity remains (67).

It is important to note that the historical definition of vitamin D deficiency (25-OHD levels of <15 ng/mL or <37.5 nmol/L) was based on the presence or absence of bone disease (rickets in children and osteomalacia in adults). The recognition that other organs, such as the prostate gland, possess VDR and 1α-hydroxylase and respond to the hormone and prohormone strongly suggests that vitamin D is essential for the development of these tissues as well. The level of vitamin D for sufficiency in these sites is unknown but is likely to be higher than for bone. It is also currently not known when during life sun exposure may have its greatest effect on reducing the risk of prostate cancer. Our findings and those by others (12, 13, 15) suggest that long-term sun exposure

may be important. Further studies in large populations, including non-Whites, are warranted to confirm the combined effects of sun exposure and *VDR* genotype and define the exposure period that is important in influencing prostate cancer risk.

From a public health perspective, it is important to emphasize that the possible benefits of sun exposure must be weighed against the risks of sun-induced skin cancer, especially melanoma (68). If future studies continue to show reductions in prostate cancer risk associated with sun exposure, increasing vitamin D intake from diet and supplements may be the safest solution to achieve an adequate vitamin D status.

Acknowledgments

Received 8/30/2004; revised 1/6/2005; accepted 3/1/2005.

Grant support: Cancer Research Fund grant 99-00527V-10182 (E.M. John) under Interagency Agreement 97-12013 (University of California contract 98-00924V) with the Department of Health Services Cancer Research Program. Cancer incidence data used in this publication have been collected by the Greater Bay Area Cancer Registry of the Northern California Cancer Center under contract N01-PC-35136 with the National Cancer Institute, NIH, and with support of the California Cancer Registry, a project of the Cancer Surveillance Section, California Department of Health Services, under subcontract 1006128 with the Public Health Institute.

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