Seasonal Variation in Vitamin D, Vitamin D-binding Protein, and Dehydroepiandrosterone: Risk of Prostate Cancer in Black and White Men[1]

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Abstract

Our previous study provided evidence that higher serum levels of the active form of vitamin D, 1,25-dihydroxyvitamin D (1, 25-D), might possibly slow the progression of subclinical to clinically significant prostate cancer in both black and white men, especially after age 57. This paper extends the prior study by contrasting seasonal variation in 1,25-D and its precursor, 25-hydroxyvitamin D (25-D), in case and control subjects. In addition, the risk of prostate cancer is related to serum levels of vitamin D-binding protein (VDBP) and total dehydroepiandrosterone and to polymorphic variation in VDBP. The expected elevated summer levels of 25-D were seen in case and control subjects and, as expected, 1,25-D did not vary throughout the year in the control subjects. Unexpectedly, lower case levels of 1,25-D were limited largely to the summer months ($P = 0.01$) in both black and white cases and to cases greater than or equal to the median age of 57 years. Levels of VDBP and dehydroepiandrosterone and the frequencies of VDBP polymorphisms were similar in case and control subjects, although striking differences were seen in allelic frequencies in black and white men. These observations provide additional evidence that vitamin D metabolism may impact the risk of prostate cancer.

Introduction

PCa[3] continues to be the most frequently diagnosed cancer in American men and the second leading cause of cancer death (1). An estimated 165,000 men were diagnosed with PCa and 35,000 died from PCa in 1993. The risk of PCa increases with age and is higher in black men than in white men (2).

Both normal and cancerous prostate cells have vitamin D receptors, indicating that they are target cells that can respond to vitamin D. The active form of vitamin D, 1,25-D, inhibits the growth of normal and cancerous prostate cells *in vitro* (3–6). Thus, higher levels of 1,25-D might possibly delay the progression of subclinical to clinically significant PCa.

Our previous prospective study is consistent with this hypothesis. We found that men diagnosed with PCa had significantly lower prediagnostic levels of 1,25-D compared to control subjects of the same age and race with sera stored on approximately the same day (7). This difference was attributable to men greater than or equal to the median baseline age of 57 years, and similar differences were found for black and white men.

There was no overall difference between case and control subjects in prediagnostic levels of 25-D, the immediate precursor to 1,25-D. However, predictive models were more informative when an interaction term (*i.e.*, the product of 25-D and 1,25-D) was included with 25-D and 1,25-D, allowing risk for each metabolite to depend on the level of the other metabolite. The seasonal elevation of 25-D in the summer months in the Northern hemisphere when more pre-vitamin D is formed in the skin in response to sunlight (8) raised the possibility that the statistical interaction might involve seasonal differences in vitamin D metabolism in case and control subjects.

Both 25-D and 1,25-D are transported in the circulation largely bound to VDBP or albumin (9, 10). Less than 1% of 1,25-D circulates unbound and is able to interact with target tissues. Free 1,25-D, as a proportion of *all* 1,25-D, is inversely related to serum VDBP concentration (9). The relevance of this observation to PCa is indicated by the higher serum concentration of VDBP in case compared to control subjects in Schwartz *et al.* (11), implying that free 1,25-D might be lower in men with PCa.

The three most common phenotypes of VDBP, $Gc1_{fast}$, $Gc1_{slow}$, and Gc2 (9, 10), combine to form six genotypes designated $1_F/1_F$, $1_F/1_S$, $1_F/2$, $1_S/1_S$, $1_S/2$, and 2/2. Each polymorphism is thought to have similar affinity for 1,25-D (12). Nonetheless, subtle differences in affinity or other attributes might influence the level of free 1,25-D and, hence, the risk of PCa.

DHEA is a hormone hypothesized to reduce cancer risk (13, 14). A number of studies demonstrate that the administration of DHEA to rodents conveys some protection against spontaneous and implanted tumors, including PCa, and against chemical carcinogenesis (14–16). Low serum levels of DHEA are associated with the presence and risk of development of human cancers (17–19). Comstock *et al.* (20) find lower (12%) prediagnostic DHEA-S, sulfated plus unsulfated, in men with PCa compared to control subjects. The lack of statistical sig-

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[3] The abbreviations used are: PCa, prostatic carcinoma; 25-D, 25-hydroxyvitamin D_2 or D_3; 1,25-D, 1,25-dihydroxyvitamin D_2 or D_3; VDBP, vitamin D-binding protein; DHEA, dehydroepiandrosterone; DHEA-S, total dehydroepiandrosterone, sulfated and unsulfated; DOY, day of year.

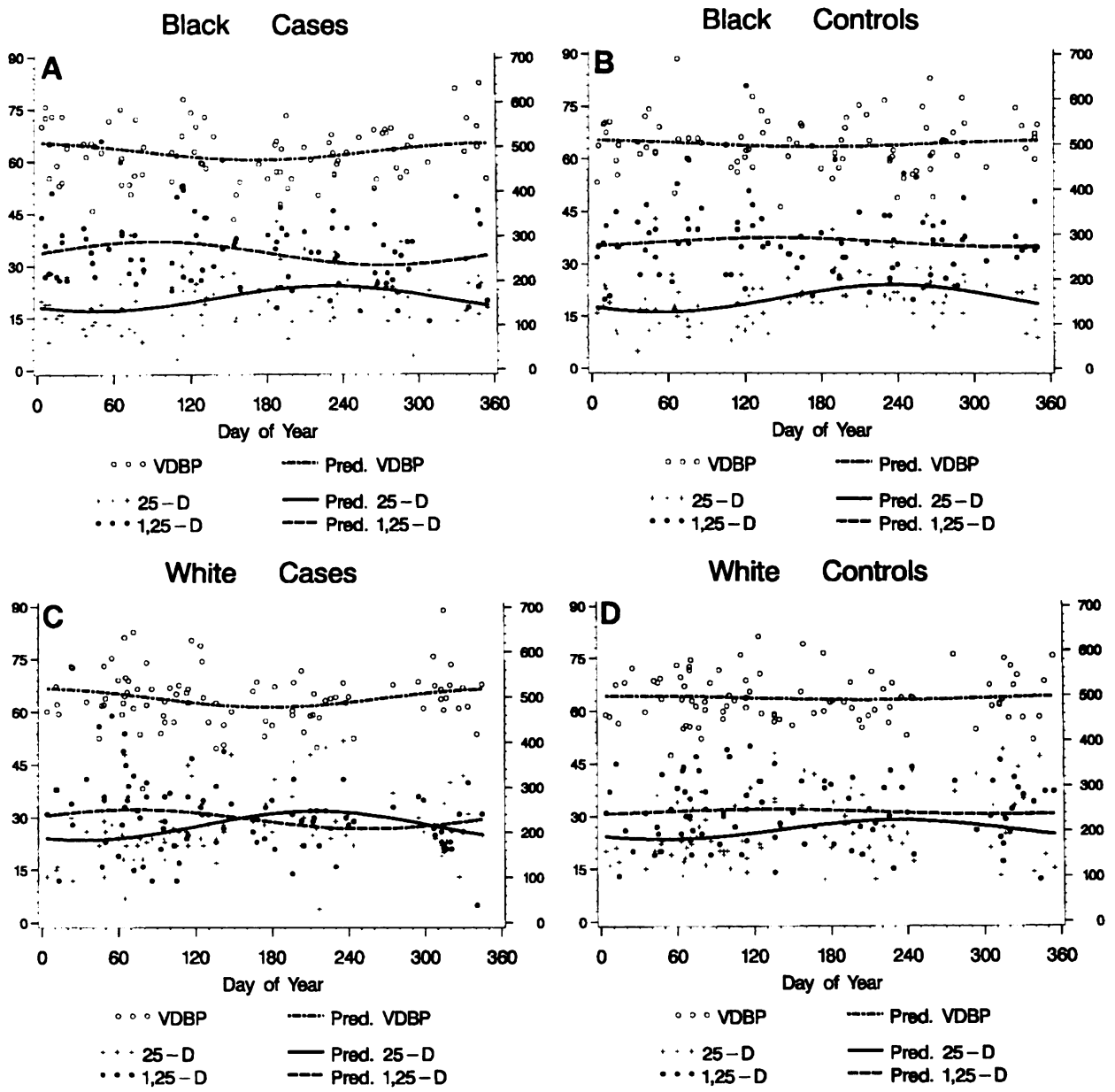

Fig. 1. Seasonal variation in serum levels of 25-D (ng/ml; *left axis*), 1,25-D (pg/ml; *left axis*), and VDBP (mg/l; *right axis*) in black cases (*A*), black controls (*B*), white cases (*C*), and white controls (*D*).

nificance in that study might be attributed to measurement error via diurnal variation, decreases with age, and laboratory measurement error (21–23), especially because the study was not large.

Here, we extend earlier work by contrasting seasonal variation in vitamin D metabolites in men diagnosed with PCa and control subjects. In addition, the risk of PCa is evaluated in relation to serum levels of VDBP and DHEA-S and to VDBP polymorphism.

Materials and Methods

Study Subjects. Case and control subjects were selected from men who were members of the Kaiser Permanente Medical Care Program of Northern California with stored serum samples obtained during 1964–1971 (7). One hundred eighty-one case subjects, 90 black and 91 white, were selected from Kaiser members diagnosed with PCa before December 31, 1987. These included all black cases diagnosed at the Oakland and San Francisco Kaiser clinics, plus 6 cases diagnosed at the San Rafael clinic, and a random sample of white cases diagnosed at the Oakland and San Francisco clinics. Case status was verified in 97% of cases, and primary and secondary Gleason grade assigned, by histopathological review of biopsy materials. Each case was matched to one control subject of the same age, sex, and race having stored sera obtained on approximately the same day, who was alive and a Kaiser member on the date of case

diagnosis. The study subjects and comparisons involving 25-D and 1,25-D have been described elsewhere (7).

Laboratory Determinations. Competitive protein binding and calf thymus receptor assays were used to measure the total (bound plus free) serum levels of 25-D (ng/ml; Ref. 24) and 1,25-D (pg/ml; Refs. 25, 26) in retrieved sera. VDBP (mg/l) and DHEA-S (ng/ml) concentrations were measured by radioimmunoassay (23). VDBP genotype was identified by use of isoelectric focusing (9).

Statistical Analysis. Linear models were used to explore seasonal variation. More specifically, DOY of sera storage as expressed by cyclical $\sin(2\pi * \text{DOY}/365)$ and $\cos(2\pi * \text{DOY}/365)$ terms was used to predict sera level, controlling for race (29). Wilcoxon's signed rank test for paired samples was used to compare case and control subjects. Conditional logistic models were used to estimate the risk of PCa given 25-D, 1,25-D, the interaction of 25-D with 1,25-D, VDBP, and DHEA-S, each as continuous measured quantities (27). Age heterogeneity in risk was tested by comparison of the model χ^2 statistic for a model constructed by using all subjects with the sum of model χ^2 statistics for models constructed for men less than and greater than or equal to the age of the median baseline age of 57 years. The resulting statistic is asymptotically distributed as a χ^2 with degrees of freedom equal to the number of model-predicting variables (28). Tests are two-sided with significance declared at the 0.05 level.

Results

Seasonal Variation. There was evidence of seasonal variation in serum levels of 1,25-D in case, but not control, subjects. Fig. 1 shows levels of 1,25-D, 25-D, and VDBP throughout the year. Serum 1,25-D was essentially constant throughout the year in control subjects, whereas case subjects, both black and white, had summer decreases in 1,25-D. In linear models, serum 1,25-D concentration was significantly related to season in case ($P = 0.01$) but not control subjects ($P = 0.63$) controlling for race. Looking more closely at the case subjects, there was strong seasonal variation in 1,25-D in cases age ≥57 years at baseline ($P = 0.01$) but not in those with younger baseline ages ($P = 0.07$).

As expected, summer increases in 25-D were found in case and control subjects, both black and white (Fig. 1.). The lower 25-D and, paradoxically, higher 1,25-D levels found in black compared to white subjects throughout the year are consistent with the intense skin pigmentation of black men.

Serum levels of VDBP were essentially constant throughout the year in control subjects. In case subjects, small decreases in VDBP, that were not statistically significant, were seen in the spring and summer.

VDBP Phenotype. VDBP allelic frequencies were strikingly different in black and white men. However, there was no evidence that they differed in the case and control subjects. Allele frequencies were 0.14 *versus* 0.15 (1_F), 0.55 *versus* 0.51 (1_S), and 0.30 *versus* 0.34 (2) for the white case and control subjects, respectively. In black subjects, allele frequencies were 0.69 *versus* 0.67 (1_F), 0.19 *versus* 0.24 (1_S), and 0.11 *versus* 0.08 (2), respectively, in the case and control subjects. Within each race, genotypic frequencies were similar in case and matched control subjects (Table 1), although there were obvious differences in genotypic frequencies in black and white men.

VDBP and DHEA-S. Serum levels of VDBP and DHEA-S were comparable in the case and control subjects (Table 2).

Table 1 VDBP genotypic frequencies in case-control pairs

Control genotype	Case genotype						
	$1_F/1_F$	$1_F/1_S$	$1_F/2$	$1_S/1_S$	$1_S/2$	2/2	
Black subjects							
$1_F/1_F$	21	9	9	2	1	2[a]	44
$1_F/1_S$	13	6	3	2	0	0	24
$1_F/2$	6	3	0	0	0	0	9
$1_S/1_S$	2	2	2	0	1	0	7
$1_S/2$	2	3	0	1	0	0	6
2/2	0	0	0	0	0	0	0
	44	23	14	5	2	2	90
White subjects							
$1_F/1_F$	0	0	0	0	0	0	0
$1_F/1_S$	1	2	1	2	5	1	12
$1_F/2$	0	3	1	7	3	1	15
$1_S/1_S$	0	3	1	8	12	1	25
$1_S/2$	1	4	4	8	10	4	31
2/2	1	0	1	4	1	1	8
	3	12	8	29	31	8	91

[a] One case subject had an uncommon polymorphism with slow electrophoretic mobility.

Mean levels of VDBP were 494 and 499 mg/l and those of DHEA-S were 192 and 199 ng/ml, respectively, for cases and controls. Matched differences in VDBP and DHEA-S were small and not statistically significant: −2.4 mg/l for VDBP ($P = 0.39$), −7.2 ng/ml for DHEA-S ($P = 0.66$), and −0.06 for log (DHEA-S; $P = 0.52$).

Looking at subgroups formed by age and race, there were no statistically significant matched case-control differences in either VDBP or DHEA-S (Table 2). Moreover, in the 80 case-control pairs in which the case's tumor had a Gleason grade between 7–10 (indicative of, at best, only a moderate degree of tumor differentiation) there was no evidence of case-control differences in either metabolite: −4.0 mg/l for VDBP ($P = 0.55$) and −4.9 ng/ml for DHEA-S ($P = 0.65$).

Conditional Logistic Models. We next constructed conditional logistic models to determine whether levels of VDBP or DHEA-S were predictive of the risk of PCa given information on 25-D, 1,25-D, and their interaction, which was highly predictive of risk ($P < 0.0001$; Ref. 7). In this extended model, as in the prior study, there was significant age heterogeneity in the risk of PCa ($P = 0.02$) such that these terms were predictive of risk only in men ≥57 years of age at baseline. In this older subgroup, VDBP and DHEA-S were not predictive of the risk of PCa, controlling for 25-D, 1,25-D, and the interaction of 1,25-D and 25-D (Table 3). The model fit the data significantly better when the interaction term was included in the model ($P = 0.007$). Modification of the model by allowing for interaction between 1,25-D and VDBP or by replacing 1,25-D by the product of 1,25-D with VDBP did not essentially alter the relationships shown in Table 3.

1,25-D Concentration. Despite these negative results, there was a small piece of evidence obtained from linear models that suggested that VDBP and DHEA-S might be indirectly related to risk. Namely, both VDBP and DHEA-S were correlated with 1,25-D in case subjects ≥57 years of age at baseline, controlling for race and season. Prediagnostic 1,25-D concentration increased by 0.044 pg/ml for each mg/ml increment of VDBP ($P = 0.001$) and by 0.024 pg/ml for each ng/ml increment of DHEA-S ($P = 0.004$). Overall, this linear model predicted 29% of the variation in prediagnostic serum levels of 1,25-D in case

Table 2 Serum levels of VDBP and DHEA-S[a]

Subjects	*n*	VDBP (mg/l)			DHEAS (ng/ml)		
		Mean	Median	(Range)	Mean	Median	(Range)
Black <57 (yrs)							
Case	59	487	478	(357–763)	225	216	(65–467)
Control	60	495	491	(361–690)	229	197	(55–764)
Black ≥57 (yrs)							
Case	31	494	502	(339–644)	146	127	(48–384)
Control	30	515	507	(382–647)	162	148	(28–318)
White <57 (yrs)							
Case	32	503	496	(409–649)	239	206	(104–612)
Control	30	500	496	(369–616)	253	235	(102–547)
White ≥57 (yrs)							
Case	59	497	498	(298–694)	156	130	(31–381)
Control	61	493	486	(406–632)	163	150	(42–386)

	n	Matched case-control comparisons VDBP		DHEAS	
		mg/l	(*P*)	ng/ml	(*P*)
Black men					
<57 years	59	−5.2	(0.38)	−4.3	(0.84)
≥57 years	29	−18.6	(0.13)	−11.1	(0.49)
White men					
<57 years	30	4.8	(0.77)	−7.0	(0.91)
≥57 years	59	4.5	(0.63)	−8.2	(0.71)

[a] Black and white controls were dichotomized at the median age at the time of serum storage (<57 and ≥57). Wilcoxon's signed rank test was used to compare case and matched control subjects.

Table 3 Odds of PCa in men ages ≥57 years at baseline[a]

Predictor	Odds ratio (95% CI[b])	*P*
VDBP (mg/l)	1.00 (0.99–1.01)	0.65
DHEA-S (ng/ml)	1.00 (1.00–1.01)	0.90
1,25-D (pg/ml)	0.77 (0.66–0.89)	0.0004
25-D (ng/ml)	0.85 (0.72–0.99)	0.04
25-D1,25-D	1.01 (1.00–1.01)	0.007

[a] A conditional logistic model was constructed by using case-control pairs ≥57 years of age at baseline when sera was obtained for storage. The above variables were jointly used to predict case-control status. Parameter estimates were exponentiated to quantify the increased (odds ratio, >1.0) or decreased (odds ratio, <1.0) risk of PCa per unit change in the respective variables. The negative association of 25-D with PCa noted here differs in direction from the nonsignificant positive association in Table 3 of Ref. 7 because of the inclusion of the interaction term for 25-D and 1,25-D.
[b] CI, confidence interval.

subjects ≥57 years of age. These findings were not altered by the addition of age at baseline to the model to control for residual confounding (in DHEA-S) due to age or by the addition of 25-D. Replacement of 'season' terms with 25-D concentration did not essentially alter the relationship of VDBP and DHEA-S with 1,25-D.

Discussion

This study extends our prior study (7) that suggested that higher serum levels of 1,25-D might possibly be protective for PCa by attributing the observed protective effect to seasonally lower summer levels of 1,25-D in case subjects, black and white. Additionally, the seasonal depression of 1,25-D was more pronounced after age 57 years, consistent with greater protection related to 1,25-D in men above the median baseline age of 57 years. These findings provide additional evidence that vitamin D metabolism in men at high risk of PCa differs from that found in men at lower risk and may account for the statistical interaction of 25-D with 1,25-D that was found in our prior study.

In contrast to Schwartz *et al.* (30), we did not find higher VDBP concentrations in case subjects, indicative of lower free 1,25-D concentration in men with PCa, nor did VDBP information improve the predictiveness of the vitamin D metabolite concentrations for PCa. Chance or differences in study design and analyses may have led to differing results in the two studies. VDBP might possibly be indirectly involved in the risk of PCa by influencing serum 1,25-D concentration, although this evidence is weak. There was no evidence that the various polymorphisms of VDBP are related to the risk of PCa in either black or white men, although allelic and genotypic frequencies were obviously different in black and white men.

Consistent with Comstock *et al.* (20), we found that serum levels of DHEA-S were not significantly related to the risk of PCa nor did DHEA-S information improve the predictiveness of the vitamin D metabolite concentrations for PCa. Nonetheless, the positive correlation of DHEA-S and 1,25-D in case subjects ≥57 years of age at baseline, even after controlling for residual confounding by age, implies that DHEA-S might, indirectly, influence the risk of PCa. However, this evidence is weak and entirely speculative.

In summary, this study suggests the possibility that men at elevated risk of PCa have, on average, lower summer levels of serum 1,25-D compared to other men, for whom aggregate

levels of 1,25-D remained constant throughout the year. This result is consistent with the hypothesis that regulation of vitamin D metabolism, or behaviors that influence the conversion of 25-D to 1,25-D, impact the risk of PCa.

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