Original Article

Circulating Vitamin D, Vitamin D–Related Genetic Variation, and Risk of Fatal Prostate Cancer in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium

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BACKGROUND: Evidence from experimental animal and cell line studies supports a beneficial role for vitamin D in prostate cancer (PCa). Although the results from human studies have been mainly null for overall PCa risk, there may be a benefit for survival. This study assessed the associations of circulating 25-hydroxyvitamin D (25(OH)D) and common variations in key vitamin D–related genes with fatal PCa. **METHODS:** In a large cohort consortium, 518 fatal cases and 2986 controls with 25(OH)D data were identified. Genotyping information for 91 single-nucleotide polymorphisms (SNPs) in 7 vitamin D–related genes (vitamin D receptor, group-specific component, cytochrome P450 27A1 [*CYP27A1*], *CYP27B1*, *CYP24A1*, *CYP2R1*, and retinoid X receptor α) was available for 496 fatal cases and 3577 controls. Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations of 25(OH)D and SNPs with fatal PCa. The study also tested for 25(OH)D-SNP interactions among 264 fatal cases and 1169 controls. **RESULTS:** No statistically significant relationship was observed between 25(OH)D and fatal PCa (OR for extreme quartiles, 0.86; 95% CI, 0.65-1.14; *P* for trend = .22) or the main effects of the SNPs and fatal PCa. There was evidence suggesting that associations of several SNPs, including 5 related to circulating 25(OH)D, with fatal PCa were modified by 25(OH)D. Individually, these associations did not remain significant after multiple testing; however, the *P* value for the set-based test for *CYP2R1* was .002. **CONCLUSIONS:** Statistically significant associations were not observed for either 25(OH)D or vitamin D–related SNPs with fatal PCa. The effect modification of 25(OH)D associations by biologically plausible genetic variation may deserve further exploration. ***Cancer* 2015;121:1949-56.** © *2015 American Cancer Society.*

KEYWORDS: circulating 25-hydroxyvitamin D, fatal prostate cancer, gene-environment interaction, single-nucleotide polymorphisms, vitamin D genes.

INTRODUCTION

In addition to its role in bone health, vitamin D regulates the expression of 3% to 5% of genes, many of which are related to cancer.[1] Evidence from experimental animal and cell line studies supports a beneficial role for vitamin D in the prevention and treatment of prostate cancer (PCa),[2,3] but results from human epidemiologic studies are conflicting.

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Epidemiologic studies focusing on circulating 25-hydroxyvitamin D (25(OH)D) have not supported a protective association for higher 25(OH)D with overall PCa risk.[4-8] More recently, there has been growing interest in whether vitamin D may specifically influence cancer survival and prognosis.[9] This is particularly relevant for PCa because indolent and fatal disease may be etiologically different.[10] However, only a few studies have investigated the relationship between circulating 25(OH)D and PCa-specific mortality; some have found a protective association,[6,11,12] whereas others have not.[7,13]

At the same time, an increasing number of studies have explored whether common genetic variants among genes that play a role in vitamin D metabolism and signaling are associated with PCa risk. Most studies have focused on 5 specific single-nucleotide polymorphism (SNPs) in the vitamin D receptor (*VDR*) gene, and results have been inconsistent.[14] More comprehensive studies assessing common variations across *VDR* and several other vitamin D pathway genes and PCa risk have yielded few additional findings.[15-18] Furthermore, only 2 studies have assessed the relationship of common variations in these vitamin D pathway genes with the endpoint of fatal PCa; although both studies found suggestive associations, their results were not consistent.[6,19] A yet unexplored area is whether associations between vitamin D–related SNPs and fatal PCa may be modified by circulating levels of 25(OH)D. The identification of such interactions would lend mechanistic and causal support to an association of vitamin D with PCa.

Recently, the Health Professionals Follow-Up Study (HPFS) reported that higher prediagnostic 25(OH)D levels were associated with a statistically significant reduction (57%) in the risk of fatal PCa (highest quartile vs lowest quartile).[6] Using a pathway-based approach, HPFS also reported that common variations in 7 vitamin D–related genes (cytochrome P450 27A1 [*CYP27A1*], *CYP2R1*, *CYP27B1*, group-specific component [*GC*], *CYP24A1*, retinoid X receptor α [*RXRA*], and *VDR*) were related to fatal PCa (particularly *VDR* and *CYP27A1*).[6] The genes were chosen because of laboratory evidence showing that they are directly involved in vitamin D metabolism and signaling.

The objectives of the current study were to follow up the findings from HPFS within a large consortium of prospective cohort studies. Specifically, we 1) assessed whether men with low circulating 25(OH)D are at increased risk for fatal PCa and 2) investigated the association of common variations in vitamin D pathway genes with fatal PCa. Furthermore, we also explored gene-environment interactions between circulating 25(OH)D, vitamin D pathway SNPs, and fatal PCa.

MATERIAL AND METHODS

Study Population

The Breast and Prostate Cancer Cohort Consortium is a collaboration of well-established prospective cohort studies investigating genetic risk factors for breast cancer and PCa, and it has been described in detail previously.[20] Studies that participated in this analysis included the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study,[7] the European Prospective Investigation into Cancer and Nutrition,[5] HPFS,[6] the Physicians' Health Study,[21] and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.[15] The respective local institutional review boards approved each study. Each cohort provided a nested series of PCa cases and controls; within each cohort, controls without a previous diagnosis of PCa were matched to cases by factors such as age and ethnicity (depending on the cohort).[20] In addition, covariate information, including the body mass index (kg/m^2), history of diabetes (yes or no), smoking status (never, current, or former), and age at blood draw and diagnosis, were available. We restricted the current study to men who self-reported being of European descent.

Outcome Ascertainment

Men with incident PCa were identified through population-based cancer registries or self-reports confirmed by medical records, including pathology reports. Data on the disease stage and grade at the time of diagnosis were noted when they were available. The primary outcome was fatal PCa risk. Cases were followed for overall mortality and PCa-specific mortality with a combination of death certificates, medical record review, and population registries.

25(OH)D Assessment

Prediagnostic circulating 25(OH)D levels were assayed separately in each study and were available for 518 fatal PCa cases and 2986 controls. Details of the 25(OH)D assessment specific to each cohort, including the type of assay and quality control measures, have been previously published[5-7,15,21] (see also Supporting Table 1 [see online supporting information]). Some studies conducted multiple batches of assays at different time points (eg, HPFS selected cases and controls in 4 separate batches, and each batch was assayed at a different time point: blood draw to January 1996, February 1996 to January 1998, February 1998 to January 2000, and February 2000 to January

2004). To account for study, season of blood draw, and laboratory variation due to multiple batches of assays conducted at different time points within a study, we created study, season, and batch quartile and median cut points based on levels in the control subjects. Seasons were defined as summer (June to August)/autumn (September to November) and spring (March to May)/winter (December to February). We did not analyze absolute cut points of 25(OH)D because of substantial variation among the different assays.

Genotyping

A subset of the Breast and Prostate Cancer Cohort Consortium cases with aggressive PCa (defined as a Gleason score of 8 to 10 or stage C/D) and controls were included in a genome-wide association study (GWAS) to identify novel risk variants for aggressive PCa (496 fatal PCa cases and 3577 controls).[20] Genotyping was performed for the majority of the subjects with the Illumina Human 610-Quad array, but some subjects from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial were genotyped with other Illumina arrays (ie, HumanHap 317K+240K or 550K). Quality control measures have been described in detail elsewhere.[20] In brief, samples were excluded if the genotyping call rate was <95% or autosomal heterozygosity was <0.25 or >0.35. Additional common variants and missing values were imputed with a Markov chain–based haplotyper (MACH) and phase 2 CEU (Utah residents with ancestry from northern and western Europe). HapMap data.[22] We were able to extract genotypes or a proxy ($R^2 \geq 0.8$) for 88 of the 95 SNPs included in the original study. Four of the SNPs, including the *VDR* SNPs rs2228570 (Fok1), rs11168275, and rs11574032 and the *RXRA* SNP rs34312136, were unavailable, and 3 SNPs (rs10875693 [*VDR*] and rs7853934 and rs41400444 [*RXRA*]) were partially tagged ($0.6 < R^2 < 0.8$). Among the men with GWAS data, there were 264 fatal PCa cases and 1169 controls who also had overlapping information on circulating 25(OH)D.

Statistical Methods

All statistical tests were 2-sided and were conducted with SAS 9.3 (SAS Institute, Cary, NC) and R statistical packages (http://www.r-project.org/). We used logistic regression to calculate the odds ratios (ORs) and 95% confidence intervals (CI) for the associations of circulating 25(OH)D and vitamin D pathway SNPS with the risk of incident fatal PCa in comparison with controls. The primary models used data pooled across all 5 cohorts and were adjusted for the age at diagnosis (cases) or selection (controls) and cohort.

For 25(OH)D, we also created models adjusted for the year of blood draw, time from blood draw to diagnosis, body mass index (kg/m^2), diabetes history (yes or no), and smoking (never, current, or former). The lowest quartile of 25(OH)D was used as the reference category, and tests for trends used an ordinal variable (1-4) corresponding to the quartile in which the individual's circulating 25(OH)D fell. We conducted a sensitivity analysis excluding men who were diagnosed with PCa within the first 2 years after the blood draw. For the SNP analyses, we used an allele-dosage model. Finally, we conducted exploratory analyses to test for SNP-25(OH)D interactions with respect to fatal PCa risk among the subset of men with both circulating 25(OH)D and genetic data. We performed a stratified analysis for the association of the SNPs with fatal PCa in those men with high 25(OH)D versus low 25(OH)D (dichotomized at the median). We tested for a multiplicative interaction by adding a cross-product term to the model (SNP×25(OH)D) and assessed significance with the Wald test. To improve the power of the statistical test for interaction, we used a continuous measure of 25(OH)D that was standardized for the season, cohort, and batch with the method described by Rosner et al.[23] Briefly, with this method, β coefficients from a linear regression model of 25(OH)D with batch, season, and cohort indicators were averaged; for each specific cohort-season-batch combination, the difference between the corresponding beta coefficient from the model and the average coefficient was subtracted from the unadjusted 25(OH)D value to create a continuous measurement that was standardized to the average cohort-season-batch combination.

We implemented set-based tests across the entire pathway of 7 genes and at the individual gene level for the association with fatal PCa and for SNP-25(OH)D interactions. A set-based test may have enhanced power because it aggregates multiple signals within a set, leverages the correlation between SNPs, and reduces the multiple testing burden. The mathematical details of the logistic regression kernel machine models and gene-environment set-based association test (GESAT) are described elsewhere.[24-27] In brief, the kernel machine model treats each of the included SNPs as a random effect; desired covariates (eg, age and study) are entered as fixed effects. The joint effect of the entire SNP set are considered, and the degrees of freedom are calculated with respect to the correlation between the SNPs in the set; this improves the power of the hypothesis test. The null hypothesis that the variance of the SNP random effects is zero (ie, the SNPs individually or jointly are not associated

TABLE 1. ORs and CIs for Quartiles of Circulating 25(OH)D and Fatal Prostate Cancer Pooled Across 5 Cohorts

	25(OH)D Quartile				*P* for Trend
	First	Second	Third	Fourth	
Median 25(OH)D level, ng/mL[a]	14.4	20.1	24.8	33.0	
Cases/controls	141/740	131/751	120/757	126/738	
OR (95% CI)[b]	1.00 (reference)	0.87 (0.66-1.15)	0.79 (0.60-1.05)	0.86 (0.65-1.14)	.22
Results excluding cases diagnosed within 2 years of blood draw					
Cases/controls	113/740	112/751	103/757	110/738	
OR (95% CI)[b]	1.00 (reference)	0.92 (0.68-1.23)	0.85 (0.63-1.16)	0.94 (0.69-1.27)	.59

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio.
25(OH)D quartiles are batch-, season- (summer/fall vs winter/spring), and cohort-specific.
[a] Among controls, the median values for 25(OH)D were 35.9, 50.2, 61.9, and 82.4 nmol/L in the first, second, third, and fourth quartiles, respectively.
[b] Adjusted for the age at blood draw, the time from blood draw to diagnosis, cohort, and body mass index.

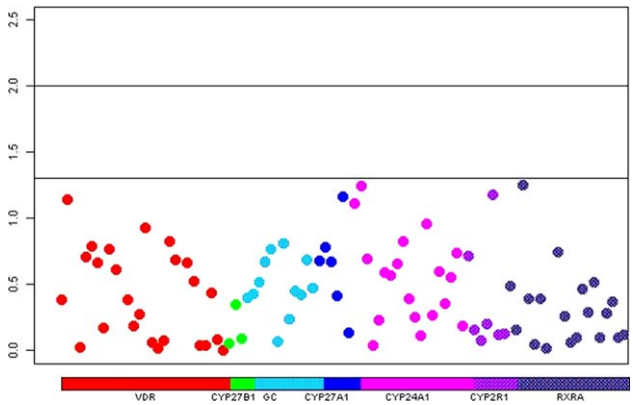

Figure 1. Manhattan plot showing *P* values for the association of each individual vitamin D single-nucleotide polymorphism and fatal prostate cancer risk. Each single-nucleotide polymorphism is color-coded by gene and is represented as a circle on the plot. *CYP* indicates cytochrome P450; *GC*, group-specific component; *RXRA*, retinoid X receptor α; *VDR*, vitamin D receptor.

with disease) can be tested with a score test. Similarly, the GESAT[27] considers the coefficients of the gene-environment interaction terms as random effects and develops a variance component score test within the induced generalized linear mixed model framework.

We report nominal *P* values, but we also calculated the effective number of independent tests for the individual SNP associations.[28] After we accounted for linkage disequilibrium, the 91 SNPs corresponded to 77 independent tests; therefore, a *P* value significance threshold of .0006 controls the experiment-wide type I error rate at the .05 level.

RESULTS

Supporting Table 2 (see online supporting information) describes the characteristics of the fatal PCa cases and controls for those with 1) circulating 25(OH)D data, 2) genotype data, and 3) both circulating 25(OH)D and GWAS information. Those participating in the GWAS were more heavily weighted to a higher stage and grade because of the selection criteria described previously.

25(OH)D and Fatal PCa

Among the men with prediagnostic circulating 25(OH)D data, 518 died from PCa over a median follow-up time of 8.1 years after their diagnosis. The median levels of 25(OH)D in the first and fourth quartiles were 14.4 ng/mL (35.9 nmol/L) and 33.0 ng/mL (82.4 nmol/L), respectively. Table 1 shows the ORs and 95% CIs by quartiles of circulating 25(OH)D for the risk of fatal PCa adjusted for the age at blood draw, time from blood draw to diagnosis and cohort, and body mass index. There was no significant association of 25(OH)D and the risk of fatal PCa across the 5 cohorts combined (OR for highest quartile vs lowest quartile, 0.86; 95% CI, 0.65-1.14; *P* for trend = .22). Three of the 5 cohorts showed an inverse trend, but only 1 cohort (HPFS) was statistically significant (Supporting Table 3 [see online supporting information]). When we excluded HPFS, the pooled results remained nonsignificant (OR for highest quartile vs lowest quartile, 0.96; 95% CI, 0.70-1.31; *P* for trend = .73; data not shown). There was no evidence for heterogeneity between studies when we looked at the estimates for the highest quartile versus the lowest quartile with Cochran's Q test. Additional adjustments for diabetes status and smoking did not change the results.

Common Genetic Variation in Vitamin D Pathway and Fatal PCa

Among the men with available genotype information, 496 died of PCa. Figure 1 shows the *P* value for the per-

TABLE 2. Associations of Nominally Significant Vitamin D Pathway SNPs and Lethal Prostate Cancer Stratified by 25(OH)D (High Vs Low)

Gene:SNP (Major/Minor Allele)	OR (95% CI) for Low 25(OH)D Strata	*P*	OR (95% CI) for High 25(OH)D Strata	*P*	*P* for Interaction[a]
VDR:rs2239186(A,G)	1.26 (0.88-1.78)	.20	1.44 (1.01-2.05)	.04	.44
VDR:rs2189480(G,T)	1.44 (1.05-1.97)	.02	1.12 (0.83-1.50)	.47	.60
VDR:rs2283342(A,G)	1.07 (0.72-1.57)	.75	1.59 (1.09-2.32)	.02	.15
VDR:rs12721364(G,A)	1.34 (0.89-2.02)	.16	0.67 (0.44-1.02)	.06	.05
VDR:rs10875693(T,A)	0.66 (0.46-0.96)	.03	1.16 (0.82-1.64)	.42	.23
VDR:rs4760648(C,T)	0.85 (0.63-1.14)	.27	1.23 (0.90-1.69)	.19	.03
GC:rs1155563(T,C)	0.96 (0.69-1.33)	.81	1.54 (1.10-2.14)	.01	.12
GC:rs1491716(G,A)	0.50 (0.25-0.98)	.04	1.25 (0.68-2.30)	.48	.70
GC:rs6817912(C,T)	1.93 (1.00-3.70)	.05	0.93 (0.48-1.81)	.83	.72
GC:rs12640179(C,G)	1.81 (0.99-3.31)	.05	0.72 (0.36-1.48)	.37	.03
CYP27A1:rs647952(A,G)	0.20 (0.05-0.74)	.02	1.28 (0.48-3.41)	.63	.51
CYP2R1:rs2060793(G,A)	1.34 (1.00-1.79)	.05	1.13 (0.84-1.52)	.42	.03
CYP2R1:rs12794714(G,A)	0.69 (0.51-0.93)	.01	0.86 (0.63-1.16)	.32	.04
CYP2R1:rs1562902(T,C)	1.41 (1.04-1.90)	.03	1.05 (0.79-1.42)	.72	.004
CYP2R1:rs10832312(T,C)	1.65 (1.02-2.65)	.04	0.88 (0.52-1.49)	.64	.04
CYP2R1:rs11023374(T,C)	0.75 (0.53-1.07)	.11	0.97 (0.68-1.38)	.85	.01
CYP24A1:rs2585413(G,A)	0.93 (0.67-1.29)	.67	0.71 (0.51-0.98)	.04	.48
CYP24A1:rs2585415(G,A)	0.99 (0.72-1.35)	.93	0.70 (0.51-0.96)	.03	.21
CYP24A1:rs927650(C,T)	0.99 (0.73-1.33)	.94	1.50 (1.11-2.04)	.009	.19
CYP24A1:rs3787555(C,A)	1.40 (1.00-1.96)	.05	0.81 (0.57-1.16)	.25	.78
RXRA:rs1536475(G,A)	1.09 (0.73-1.61)	.69	0.79 (0.51-1.20)	.27	.03

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; *CYP*, cytochrome P450; GC, group-specific component; OR, odds ratio; *RXRA*, retinoid X receptor α; SNP, single-nucleotide polymorphism; *VDR*, vitamin D receptor.
OR per minor allele for fatal prostate cancer (adjusted for the age at diagnosis).
[a] Wald *P* values for the SNP coded (0,1,2)×25(OH)D (continuous). 25(OH)D was standardized by the cohort, season, and laboratory assay batch.

allele associations of each SNP and fatal PCa, and Supporting Table 4 (see online supporting information) provides the OR and 95% CI for each SNP. None of the SNPs were significantly associated with fatal PCa, and the global *P* value across the pathway from the kernel machine test was not significant ($P = .44$).

SNP-25(OH)D Interactions and Fatal PCa

In the subset of men who had both circulating 25(OH)D and genotype data, there were 264 PCa deaths. Table 2 details the SNPs that either were nominally significant in strata of high vitamin D (n = 6) or low vitamin D (n = 11) or had a *P* for interaction value < .05 (n = 9); none of the SNPs remained statistically significant after we considered multiple testing, so these associations may be due to chance. However, the results of the GESAT across the entire pathway was $P = .06$, and at the gene-level, *CYP2R1* was statistically significant ($P = .002$). Supporting Table 5 (see online supporting information) includes the results for all of the SNPs and GESATs. We also assessed whether the vitamin D pathway SNPs were associated with circulating 25(OH)D levels. Our results replicated SNPs in the genes *GC* and *CYP2R1* known to be related to circulating 25(OH)D from a previous GWAS[15] (Supporting Table 6 [see online supporting information]). Five of these SNPs (*GC*, rs1155563; *CYP2R1*, rs2060793, rs12794714, rs1562902, and rs11023374) were among the SNPs whose association with fatal PCa differed with the circulating 25(OH)D level (Table 2).

DISCUSSION

In our large cohort consortium, we did not find evidence to support associations of circulating 25(OH)D or common variations in key vitamin D pathway genes with the risk of fatal PCa. In our exploratory analysis for SNP-25(OH)D interactions, several nominally significant associations between vitamin D pathway SNPs and fatal PCa were observed in the stratified analysis at either high or low circulating 25(OH)D levels, and a significant gene-level association was observed for *CYP2R1*.

The pooled association across all 5 cohorts between higher circulating 25(OH)D and the risk of fatal PCa was in the protective direction, but it was not statistically significant. Three of the 5 cohorts showed an inverse trend, but only HPFS (as published previously[6]) was statistically significant. Other studies have published findings on circulating 25(OH)D and fatal PCa.[7,12,13] The Alpha-

Tocopherol, Beta-Carotene Cancer Prevention Study (294 fatal PCa cases) did not find a statistically significant association between prospectively collected circulating 25(OH)D and fatal PCa.[7] Two studies assessed the association of progression to PCa-specific mortality and 25(OH)D in postdiagnostic blood. A population-based study performed in 1476 men with PCa in the metropolitan Seattle–Puget Sound area found no significant association for progression to PCa-specific mortality,[13] whereas a different study based on 160 Norwegian men observed a statistically significant 67% decreased risk of progression with medium to high 25(OH)D levels versus low levels (<50 nmol/L), especially in men receiving hormone therapy.[12] A study combining data from HPFS and the Physicians' Health Study also found a protective association between prediagnostic circulating 25(OH)D and PCa mortality among cases, but the association was mostly driven by HPFS.[11] A few studies have generated concern that there may be an increased risk of aggressive PCa with higher circulating 25(OH)D[15,29]; our study does not support an increased risk of fatal PCa with higher levels of circulating 25(OH)D.

Varying results by cohort could be due to differences in screening practices, the severity of disease at diagnosis, the prevalence of factors that could modify the association, or chance. The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial and HPFS both involved highly screened populations and showed the strongest protective associations for vitamin D with fatal PCa. It is difficult to explain this observation, but one potential hypothesis is that vitamin D may be more effective in preventing progression in prostate-specific antigen–screened populations in which disease is treated early; cancers detected at a later point in the natural history of the disease could be more likely to be resistant to this vitamin D effect. There were no major differences in the range of vitamin D levels in each cohort; in particular, the median levels in the first and fourth quartiles in the controls were comparable among the cohorts (Supporting Table 3 [see online supporting information]). Overall, the difference between the medians of the first and fourth quartiles of 25(OH)D in the controls was 18.6 ng/mL (46.5 nmol/L), with more than a quarter of the men classified as at risk for having inadequate vitamin D levels (<20 ng/mL or <50 nmol/L; Table 2). Although a single measurement of circulating 25(OH)D has been shown to have reasonable validity over time,[30] multiple measurements would more accurately reflect long-term exposure. In addition, the 25(OH)D assays differed across cohorts and were not calibrated to a common assay, nor they were standardized to an accepted gold standard; this prevented the use of absolute cut points for comparisons.

We did not observe evidence for confounding by body mass index, diabetes, or smoking, but we did not have information on other potential confounders such as physical activity. For example, if higher levels of physical activity are associated with higher circulating 25(OH)D levels[6] and a lower risk for PCa mortality, the observed results could be biased away from the null.

We did not observe any statistically significant main effects of common variations in key vitamin D pathway–related genes on the risk for fatal PCa. We did not replicate the findings in the prior HPFS study[6] or the findings from a case-only study that assessed 48 tag SNPs in *VDR*, *CYP27B1*, and *CYP24A1* and progression to PCa mortality.[19]

To our knowledge, this is the first study to assess interactions between a comprehensive set of SNPs in key genes related to vitamin D signaling and metabolism and levels of circulating 25(OH)D with respect to fatal PCa. An overall GESAT pathway *P* value of .06 and suggestive effect modification by circulating 25(OH)D on the association of a number of SNPs and fatal PCa were observed. Several of these SNPs were located in *GC* (the vitamin D–binding protein) and *CYP2R1* (a 25-hydroxylase), genes that have been observed to influence circulating 25(OH)D levels.[31] The strongest evidence for effect modification was in the *CYP2R1* gene; 5 SNPs had nominally significant *P* values for interaction, and the gene-level GESAT was statistically significant ($P = .002$). Interestingly, 4 of these 5 *CYP2R1* SNPS were associated with levels of circulating 25(OH)D in our study. On average, men who carried an allele that was associated with higher circulating 25(OH)D levels in the general population but who still had low circulating 25(OH)D levels were at higher risk for fatal PCa. The other SNPs were mainly located in *CYP24A1*, an enzyme critical for the catabolism of vitamin D, and *VDR*, the key nuclear receptor that mediates the genomic effects of vitamin D. Overexpression of *CYP24A1* has been shown to correlate with worse outcomes in several solid tumors, including prostate tumors.[32] Conversely, higher VDR expression has been associated with better PCa survival.[33] Because of the strong biological plausibility of the idea that circulating 25(OH)D may affect the association of these SNPs with fatal PCa, future study is warranted.

A few studies have assessed circulating 25(OH)D-SNP interactions for overall PCa incidence. Most of these studies were small candidate gene studies that focused mainly on *VDR* polymorphisms, including Bsm1

(rs1544410),[15,34,35] Cdx2 (rs11568820),[15,35] and Fok1 (rs2228570/rs10735810),[15,21,35-37] and the results have been inconclusive. Our study was unable to assess Fok1, and we did not observe any gene-environment interactions with Cdx2 or Bsm1. Recently, a study of 1514 participants found that a variant in *VDR* (rs7968585) modified the association of 25(OH)D and a composite clinical outcome (incident hip fracture, myocardial infarction, cancer incidence, and total mortality). This finding was further replicated through a meta-analysis of 3 independent cohorts.[38] Although not statistically significant, our data were consistent with this finding. rs7967152 ($r^2 = 0.87$ with rs7968585) showed a stronger association with fatal PCa in men with low 25(OH)D (OR for low 25(OH)D, 1.30; 95% CI, 0.97-1.74; $P = .08$) versus those with high 25(OH)D (OR for high 25(OH)D, 0.89; 95% CI, 0.66-1.20; $P = .46$; Supporting Table 6 [see online supporting information]).

The relationship between circulating 25(OH)D and the prostate environment is complex and could be mediated by the expression of several vitamin D–related genes. It is possible that circulating levels of 25(OH)D do not adequately reflect the bioavailability in the prostate tissue because *CYP27B1* is expressed in the prostate and can synthesize 1,25(OH)2D from 25(OH)D within the prostate. Nonetheless, a clinical trial of vitamin D supplementation indicated that levels of vitamin D metabolites (25(OH)D and 1,25(OH)2D) in prostate tissue correlated positively with serum circulating levels.[39] The influence of 25(OH)D in the prostate environment may be further mediated by the expression of *VDR* or *CYP24A1* in prostate tissue. Higher expression of *VDR* in prostate tumor tissue has been associated with a decreased risk of PCa progression,[33] and increased expression of *CYP24A1* has been observed to be associated with advanced-stage PCa and resistance to vitamin D–based therapies.[32] Studies integrating circulating 25(OH)D with tumor molecular profiling could shed light on the inconsistent results to date.

A major strength of this study was the combination of several large cohort studies with prospective blood collection and relatively long-term and complete follow-up; this allowed the assessment of fatal PCa, an endpoint that has been traditionally difficult to study epidemiologically with substantial numbers of outcomes because of the long natural history of the disease. With a median follow-up of 8.1 years, we were able to capture 518 fatal PCa cases. Even so, longer follow-up would allow greater accrual of deaths further in the natural history of the disease. Finally, after the subsetting of the men who had both levels of circulating 25(OH)D and genotyping data, our sample size was reduced, and this limited our power to detect interactions.

In conclusion, we did not find strong evidence to support the hypothesis that circulating 25(OH)D or common variation in key vitamin D pathway genes is related to a decreased risk of fatal PCa. We observed suggestive modification of the association between some of the SNPs and fatal PCa by circulating 25(OH)D levels, especially in *CYP2R1*. These latter findings could be further assessed in other cohort studies or in clinical trials of vitamin D supplementation or vitamin D agonists.

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CONFLICT OF INTEREST DISCLOSURES

Peter Kraft has served as a consultant to Merck.

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