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Original Contribution

Interactions Between Genome-Wide Significant Genetic Variants and Circulating Concentrations of 25-Hydroxyvitamin D in Relation to Prostate Cancer Risk in the National Cancer Institute BPC3

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Genome-wide association studies (GWAS) have identified over 100 single nucleotide polymorphisms (SNPs) associated with prostate cancer. However, information on the mechanistic basis for some associations is limited. Recent research has been directed towards the potential association of vitamin D concentrations and prostate cancer, but little is known about whether the aforementioned genetic associations are modified by vitamin D. We investigated the associations of 46 GWAS-identified SNPs, circulating concentrations of 25-hydroxyvitamin D (25(OH)D), and prostate cancer (3,811 cases, 511 of whom died from the disease, compared with 2,980 controls—from 5 cohort studies that recruited participants over several periods beginning in the 1980s). We used logistic regression models with data from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3) to evaluate interactions on the multiplicative and additive scales. After allowing for multiple testing, none of the SNPs examined was significantly associated with 25(OH)D concentration, and the SNP–prostate cancer associations did not differ by these concentrations. A statistically significant interaction was observed for each of 2 SNPs in the 8q24 region (rs620861 and rs16902094), 25(OH)D concentration, and fatal prostate cancer on both multiplicative and additive scales ($P \leq 0.001$). We did not find strong evidence that associations between GWAS-identified SNPs and prostate cancer are modified by circulating concentrations of 25(OH)D. The intriguing interactions between rs620861 and rs16902094, 25(OH)D concentration, and fatal prostate cancer warrant replication.

25-hydroxyvitamin D; BPC3; gene-environment interactions; genome-wide association studies; prostate cancer

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; BPC3, Breast and Prostate Cancer Cohort Consortium; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; GWAS, genome-wide association study; HPFS, Health Professionals Follow-up Study; OR, odds ratio; PHS, Physicians' Health Study; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; RERI, relative excess risk due to interaction; SNP, single nucleotide polymorphism.

Prostate cancer is the most common type of cancer in men and has large clinical heterogeneity, ranging from well-differentiated indolent tumors to aggressive and fatal disease. Genome-wide association studies (GWAS) have identified over 100 genetic variants associated with prostate cancer risk, explaining approximately 30% of the genetic variance of the disease, but have generally failed to identify variants specific for aggressive or fatal disease (1, 2). Some of these single nucleotide polymorphisms (SNPs) are located in intergenic regions, which are still under investigation for

their potential functions. A few studies have investigated the interaction of these genetic associations according to other established or suspected risk factors for prostate cancer, including age, ethnicity, family history, body mass index, diabetes, or circulating concentrations of insulin-like growth factors or steroid sex hormones, and have found no strong evidence for multiplicative interaction (3–6). However, little is known about whether these genetic associations are modified by circulating concentrations of 25-hydroxyvitamin D (25(OH)D). There is ample biological evidence of an anticancer role for 25(OH)D, as metabolites of vitamin D control cellular growth and differentiation (7), and administration of vitamin D analogs inhibits the progression of prostate cancer in animal models and in phase II trials (8–10). Two meta-analyses of epidemiologic studies—Yin et al. (11) in 2009 and Gilbert et al. (12) 2011—have observed null associations for circulating 25(OH)D and risk of prostate cancer overall or aggressive prostate cancer, while a more recent meta-analysis (Xu et al. (13) in 2014) observed a statistically significant positive association for all prostate cancer. To further investigate the mechanistic basis for the association of GWAS-identified SNPs and prostate cancer risk, we examined the additive and multiplicative interactions of 46 SNPs and circulating 25(OH)D concentrations in relation to total and fatal prostate cancer risk in the Breast and Prostate Cancer Cohort Consortium (BPC3).

METHODS

Source and study population

BPC3 is a consortium of 9 cohort studies being conducted in the United States and Europe that was established in 2004 to investigate genetic risk factors for breast and prostate cancer (14). The studies recruited patients over various periods beginning in the 1980s. BPC3 includes the following studies: the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC); the American Cancer Society Cancer Prevention Study II; the European Prospective Investigation into Cancer and Nutrition (EPIC); the Health Professionals Follow-up Study (HPFS); the Multi-Ethnic Cohort; the Nurses' Health Study; the Physicians' Health Study (PHS); the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO); and the Women's Health Study. Incident cancer cases were identified through linkage to cancer registries or through self-reports that were confirmed by medical records and/or pathology reports. Detailed information about this consortium and its component studies can be found elsewhere (14). All of these studies have been approved by the institutional review boards or ethics committees of their respective institutions.

The present study used 5 cohorts (ATBC, EPIC, HPFS, PHS, and PLCO) that enrolled male participants who provided genetic and circulating vitamin D data. Men were excluded if they had prevalent cancer at recruitment or if they were not of white European ancestry, resulting in a total number of 3,811 cases (511 of whom were known to have died from the disease) and 2,980 controls.

Genotyping

A total of 47 SNPs were genotyped based on published GWAS for prostate cancer; they were: rs13385191, rs1465618, rs721048, rs10187424, rs12621278, rs2292884, rs2660753, rs7629490, rs6763931, rs10936632, rs17021918, rs7679673, rs2242652, rs12653946, rs2121875, rs130067, rs1983891, rs339331, rs9364554, rs12155172, rs10486567, rs6465657, rs2928679, rs1512268, rs1016343, rs16901979, rs16902094, rs620861, rs6983267, rs4242382, rs1571801, rs10993994, rs7127900, rs12418451, rs10896449, rs10875943, rs902774, rs11649743, rs4430796, rs1859962, rs8102476, rs11672691, rs2735839, rs5759167, rs11704416, rs5945619, rs5919432. PHS and PLCO did not have data for rs10187424, rs6763931, rs10936632, rs2242652, rs2121875, rs130067, rs10875943, and rs5919432. Missing genotypes for all 47 SNPs were imputed by sampling from the observed frequency distribution in men without missing genotypes stratified by case-control status, as previously reported in detail (15). The allele frequencies and results from all statistical analyses in the present study were similar before and after the imputations, and thus only results using the directly genotyped information were presented.

Genotyping was performed using the TaqMan assay (Applied Biosystems, Foster City, California) in 6 genotyping laboratories in 3 countries: Cancer Genomics Research Laboratory at the National Cancer Institute, Harvard T.H. Chan School of Public Health, University of Southern California, German Cancer Research Center, University of Cambridge, and Imperial College London. The median genotyping success rate was 98.7% overall (interquartile range, 97.4%–99.6%; range, 82.4%–100%). Blinded duplicate samples (approximately 5%) were included within each study, and the concordance rate was greater than 99%. All but 1 autosomal SNP (rs1983891) were in Hardy-Weinberg equilibrium ($P > 0.001$), and that variant was removed from the analysis.

Circulating vitamin D concentrations

Prediagnostic circulating 25(OH)D concentrations were measured in specialist laboratories. All laboratory personnel were blinded to the case-control status of the samples. Detailed information on assay methods and quality control statistics by the participating cohorts can be found elsewhere (16–20).

Blood samples were collected at different time points and assessed in different batches except for PLCO, for which all blood samples were assessed in a single batch. Because vitamin D concentrations are dependent on seasonality, and analysis in different batches normally induces laboratory variation, we created "cohort-, batch-, and season-specific" tertiles using the distribution of 25(OH)D concentrations among the controls. We also used a continuous measure of 25(OH)D concentration that was standardized for cohort, batch, and season using linear regression models as described in Rosner et al. (21).

Statistical analysis

The main aim of the present study was to examine the interactions of genes and circulating vitamin D with respect to

total and fatal prostate cancer risk. However, to properly assess interactions on the multiplicative and absolute scales, all possible marginal pairwise associations were also assessed and presented herein. In particular, we estimated the following: 1) the associations of the 46 SNPs with risk of total and fatal prostate cancer, 2) the associations of the 46 SNPs with circulating concentrations of 25(OH)D, and 3) the associations of 25(OH)D concentration with risk of total and fatal prostate cancer. We performed all analyses using cohort-, batch-, and season-specific tertiles of vitamin D concentrations and a Rosner-standardized continuous vitamin D variable.

Logistic regression models with adjustment for age (continuous) at blood draw, cohort, and country (within EPIC) were used to assess the associations between the 46 SNPs and the risk of total and fatal prostate cancer. Odds ratios and their 95% confidence intervals were calculated per carried allele that was associated with an increased risk of prostate cancer in the GWAS literature. Logistic regression models were also used to evaluate the association between 25(OH)D concentrations and risk of total and fatal prostate cancer after adjustment for age at blood draw, year of blood draw, cohort, and country (within EPIC) as well as diabetes, alcohol intake, and body mass index. Geometric means and 95% confidence intervals for the circulating 25(OH)D concentrations were calculated by genotype for each SNP (rare homozygote, heterozygote, common homozygote), using linear regression models for the natural logarithmic transformation of 25(OH)D. Models adjusted for age at blood draw, year of blood draw, case-control status, cohort, and country (within EPIC). The F-distribution was used to assess differences between the 3 geometric means.

Multiplicative and additive interactions of genes and vitamin D in relation to total and fatal prostate cancer risk were examined using several methods. For the multiplicative interactions, we employed both a case-control and a case-only design. The per-allele odds ratios for total and fatal prostate cancer for each SNP were compared across 25(OH)D concentrations using logistic regression models that adjusted for age at blood draw, year of blood draw, cohort, and country (within EPIC). The *P* values for interaction were calculated using likelihood ratio tests based on per-allele odds ratios. All reported *P* values were uncorrected for multiple hypotheses testing, but they are interpreted in view of the 46 independent comparisons made, as the examined SNPs were not in linkage disequilibrium. Using the Bonferroni correction and a significance level of 5%, only an uncorrected *P* value of less than 0.001 would be regarded as statistically significant. As a sensitivity analysis, the false-discovery-rate approach was also used to account for multiple testing, as it may have higher statistical power (22). The false-discovery-rate method gave similar results to the Bonferroni correction (data not shown), and thus results are shown throughout the text only for the latter approach.

A 2-step case-only approach was also used to evaluate multiplicative interactions with greater statistical power. SNPs were dichotomized according to the presence of the risk allele, and logistic regression models were fitted to assess the association between the binary SNPs and 25(OH)D concentrations among only the prostate cancer cases after adjustment for age at blood draw, year of blood draw, cohort, and country (within EPIC). Under the assumption that genes and 25(OH)D concentrations are independent in the overall population, a statistically significant association in this model indicates an interaction association (23). For the observed nominally significant interactions, we reverted to the less powerful case-control analysis to more fully assess interactions while reducing the number of tested comparisons.

As an attempt to further increase the power to detect multiplicative interactions, another sensitivity analysis (conditional logistic regression with counterfactuals) was employed. When the studied genes are independent of the exposure and the Hardy-Weinberg equilibrium holds, which is the case in the present analysis, it has been shown that this method is unbiased and achieves higher statistical power than the conventional analysis (24). This method gave results similar to our primary analysis (data not shown), and thus results are shown throughout the text only for the latter approach.

Additive interactions for cohort-, batch-, and season-specific tertiles and Rosner-standardized continuous 25(OH)D levels with dichotomized SNPs were estimated using the relative excess risk due to interaction (RERI) method, as described in Hosmer and Lemeshow (25). To estimate the 95% confidence intervals of the RERIs, we performed bootstrap sampling with 1,000 samples. Each time, samples were drawn separately for cases and controls to maintain the original numbers. Using the resulting bootstrap sampling distribution and, more specifically, its 2.5th and 97.5th percentiles, we estimated the confidence intervals (26). Although different methods have been suggested for the construction of confidence intervals for RERIs (25, 26), this method has been shown to have the best coverage, especially in cases of asymmetry (26–28).

The cumulative multiplicative and additive interactions for all 46 SNPs were assessed by creating an additive genetic score after summing the number of risk alleles across the 46 SNPs for each participant. We regarded missing genotypes as zero risk alleles and considered the gene score as a continuous variable. All analyses were performed in STATA, version 12 (StataCorp LP, College Station, Texas). We also implemented kernel machine models to better evaluate the aggregate association with all 46 SNPs. Logistic regression kernel machine models (29–31) treat each of the SNPs as a random effect, while the adjusting covariates are incorporated as fixed effects. Kernel machine models consider the joint association with the entire set of SNPs and take into account the between-SNPs correlation, thereby improving the power of the test and reducing the multiple testing burden. Kernel machine analysis was performed using the iSKAT package (https://www.hsph.harvard.edu/xlin/) in R (R Foundation for Statistical Computing, Vienna, Austria) within a Unix environment.

RESULTS

Selected characteristics of the 3,811 prostate cancer cases and 2,980 controls are shown by cohort in Web Table 1

(available at http://aje.oxfordjournals.org/). The cases were, on average, aged 63.2 years at the time of blood draw and 68.6 years at cancer diagnosis, while the controls had an average age of 63.5 years at blood draw. The mean concentrations of 25(OH)D differed by cohort, with smaller values observed in the ATBC Study, which was conducted in Finland.

Twenty-nine SNPs (63%) were nominally statistically significantly associated with risk of any prostate cancer (Web Table 2); the directions of all associations were consistent with previous GWAS findings (2). Seventeen SNPs were not significantly associated with risk in this study, likely due to the smaller sample size compared with the published GWASs. Thirteen SNPs (28%) were nominally statistically significantly associated with risk of fatal prostate cancer (Web Table 3). Individuals in the top tertile of 25 (OH)D concentrations had a higher risk of any prostate cancer than did individuals in the bottom tertile (odds ratio (OR) = 1.16, 95% confidence interval (CI): 1.03, 1.31; *P* for trend = 0.01), whereas no association was observed for risk of fatal prostate cancer (Web Table 4; for the top vs. bottom tertile, OR = 0.96, 95% CI: 0.75, 1.22; *P* for trend = 0.73). These results did not differ by participating cohort (*P* for heterogeneity > 0.41).

The distribution of 25(OH)D concentrations by genotype for each of the 46 SNPs is shown in Table 1. Only 4 associations were nominally statistically significant, and none remained significant after adjusting for multiple testing. In order to investigate whether the genetic associations with risk of total and fatal prostate cancer were stronger for specific strata of 25(OH)D concentrations, we evaluated gene-environment interactions on the multiplicative (Tables 2 and 3) and additive scales (Web Tables 5 and 6). Table 2 presents the assessment of multiplicative interactions for total prostate cancer. Analyses included the case-control and the case-only approach using cohort-, batch-, and season-specific tertiles and Rosner-standardized continuous terms for 25(OH)D concentration. None of the multiplicative interactions was statistically significant for any SNP after allowing for multiple testing, and this was also evident when the additive genetic score and the kernel machine genetic score were used. Only one SNP, rs620861, had a marginally statistically significant interaction using the case-only approach. We used a *P* threshold of 0.01 for the case-control analyses, applying a correction to the standard threshold of 0.05 to account for multiple testing on the basis of finding 5 nominally significant SNPs for 25(OH)D tertiles in the case-only analyses. When interactions on the additive scale were evaluated, again no statistically significant association was found after adjustment for multiple testing (Web Table 5).

Multiplicative interactions for fatal prostate cancer are presented in Table 3. After correcting for multiple testing in the case-control approach with a Bonferroni threshold *P* value of 0.001, or after conducting the 2-step approach in the case-only design, we identified only 1 statistically significant interaction, for rs620861. The per-allele odds ratio for fatal prostate cancer was significantly lower for men in the lowest third of 25(OH)D (OR = 0.75, 95% CI: 0.58, 0.97), null for the second third (OR = 1.06, 95% CI: 0.81, 1.39), and significantly higher for the highest third (OR = 1.48, 95% CI: 1.11, 1.97; *P* for interaction = 0.001). In other words, the per-tertile association of 25(OH)D concentrations with fatal prostate cancer risk yielded a statistically significantly inverse odds ratio of 0.49 (95% CI: 0.33, 0.73) for men with the *TT* genotype, whereas the association was not significant for men with the *CC* genotype (OR = 1.15, 95% CI: 0.94, 1.41). A statistically significant interaction was observed for the kernel machine genetic score but not for the additive genetic score (Table 3).

When interactions on the additive scale were evaluated, nominal statistical significance was evident for 7 SNPs, but only rs620861 (*P* = 0.00001) and rs16902094 (*P* = 0.00001) survived the correction for multiple testing (Web Table 6). We observed the following marginal risk estimates for the association of rs620861 (dichotomized) with fatal prostate cancer (OR = 0.24) and the association of the cohort-, batch-, and season-specific tertiles of 25(OH)D (per tertile) with fatal prostate cancer (OR = 0.50), with an odds ratio for interaction of 2.10. Based on these estimates, we calculated a RERI of 0.51 (95% CI: 0.25, 0.70). The positive values indicate that the excess risk of fatal prostate cancer due to the presence of low concentrations of vitamin D is greater for those who have the risk allele (*T*) for rs620861 than those who do not have it, which agrees with the results from the interaction analysis on the multiplicative scale.

DISCUSSION

In this large pooled analysis, we investigated potential departure from multiplicative and additive interactions for 46 susceptibility SNPs, circulating concentrations of 25(OH)D, and the risk of total and fatal prostate cancer. We observed that the SNP and total prostate cancer associations did not differ by 25(OH)D concentrations on the multiplicative or additive scale after correction for multiple comparisons, although we found evidence of multiplicative and additive interaction between 25(OH)D and each of 2 SNPs in the 8q24 region (rs620861 and rs16902094) with risk of fatal prostate cancer.

The exact biological mechanisms behind such a potential interaction are unclear. The SNPs are located in chromosomal region 8q24, which is considered an intergenic region with pleiotropic associations with several cancers and other diseases (32). The mechanisms by which genetic variation in this region influences the risk of prostate cancer are not yet fully understood (33). Nonetheless, studies have shown that 8q24 physically interacts with the nearby proto-oncogene *MYC* (33–36), which is a well-defined oncogenic transcription factor and the most frequently amplified protein-coding gene across all cancer types (32). Loci on 8q24 act as tissue-specific regulators (enhancers) of *MYC* (34) and have been found to interact with the *MYC* promoter specifically in prostate cancer cell lines (35, 36).

There was no indication in the present analysis of an association of rs620861 or rs16902094 with concentrations of 25(OH)D, nor, to our knowledge, has such an association been mentioned in the literature, which could suggest that

Table 1. Distribution of 25-Hydroxyvitamin D Concentrations (1982–2004) According to the Genotype of 46 Single Nucleotide Polymorphisms Identified in Genome-Wide Association Studies, Breast and Prostate Cancer Cohort Consortium

SNP	Chromosome	Gene	Zygosity and 25(OH)D Concentration[a]						*P* Value[b]
			Rare Homozygote		Heterozygote		Common Homozygote		
			GM	95% CI	GM	95% CI	GM	95% CI	
rs13385191	2	*C2orf43*	22.4	21.5, 23.4	21.8	21.4, 22.2	22.2	21.8, 22.5	0.18
rs1465618	2	*THADA*	21.8	20.9, 22.9	22.2	21.8, 22.6	21.9	21.7, 22.2	0.59
rs721048	2	*EHBP1*	22.0	20.9, 13.2	21.9	21.5, 22.3	22.1	21.8, 22.4	0.82
rs10187424	2	*GGCX/VAMP8*	22.4	20.5, 24.4	21.3	19.6, 23.2	21.7	19.9, 23.6	0.05
rs12621278	2	*ITGA6*	19.0[c]	15.4, 23.5	22.3	21.6, 23.0	22.0	21.8, 22.2	0.32
rs2292884	2	*MLPH*	22.7	21.6, 23.8	22.1	21.7, 22.6	21.9	21.5, 22.3	0.29
rs2660753	3	Unknown	21.8	20.0, 23.7	22.1	21.6, 22.6	22.0	21.8, 22.3	0.94
rs7629490	3	Unknown	21.7	21.0, 22.4	22.0	21.6, 22.4	22.1	21.7, 22.5	0.66
rs6763931	3	*ZBTB38*	21.5	19.8, 23.4	21.7	20.0, 23.6	21.5	19.7, 23.4	0.71
rs10936632	3	*CLDN11/SKIL*	21.6	19.9, 23.5	21.7	20.0, 23.6	21.7	20.0, 23.6	0.95
rs17021918	4	*PDLIM5*	21.9	21.2, 22.6	22.1	21.8, 22.5	22.1	21.7, 22.4	0.80
rs7679673	4	*TET2*	21.8	21.2, 22.3	22.2	21.8, 22.5	22.0	21.6, 22.4	0.45
rs2242652	5	*TERT*	19.8	17.3, 22.6	21.1	18.8, 23.7	21.0	18.7, 23.6	0.21
rs12653946	5	*IRX4*	22.0	21.4, 22.5	21.9	21.6, 22.3	22.2	21.7, 22.6	0.66
rs2121875	5	*FGF10*	22.1	20.1, 24.2	21.3	19.6, 23.2	22.2	20.4, 24.2	0.01
rs130067	6	*CCHCR1*	21.6	18.9, 26.7	21.3	18.9, 23.9	20.8	18.5, 23.4	0.22
rs339331	6	*RFX6*	22.1	21.3, 23.0	22.0	21.7, 22.4	22.0	21.7, 22.3	0.97
rs9364554	6	*SLC22A3*	22.7	21.9, 23.6	21.9	21.5, 22.2	22.1	21.8, 22.4	0.16
rs12155172	7	*SP8*	22.0	21.0, 23.0	21.9	21.6, 22.3	22.1	21.8, 22.3	0.91
rs10486567	7	*JAZF1*	21.2	20.3, 22.1	22.0	21.7, 22.4	22.1	21.8, 22.4	0.18
rs6465657	7	*LMTK2*	22.0	21.5, 22.4	22.1	21.8, 22.4	22.0	21.6, 22.4	0.87
rs2928679	8	*SLC25A37*	22.3	21.8, 22.8	21.9	21.6, 22.2	22.1	21.7, 22.5	0.44
rs1512268	8	*NKX3.1*	22.0	21.5, 22.5	21.9	21.6, 22.2	22.2	21.8, 22.6	0.54
rs1016343	8	Unknown	22.0	21.1, 23.0	22.3	21.9, 22.7	21.9	21.7, 22.2	0.38
rs16901979	8	Unknown	23.0[c]	18.3, 28.9	21.8	21.0, 22.7	22.1	21.9, 22.3	0.79
rs16902094	8	Unknown	22.2	20.8, 23.6	22.1	21.7, 22.5	22.0	21.7, 22.3	0.92
rs620861	8	Unknown	21.5	20.9, 22.1	22.1	21.8, 22.4	22.0	21.6, 22.4	0.26
rs6983267	8	Unknown	21.9	21.4, 22.3	22.0	21.7, 22.3	22.2	21.8, 22.6	0.53
rs4242382	8	Unknown	24.0	22.2, 25.9	22.0	21.6, 22.5	22.0	21.8, 22.3	0.11
rs1571801	9	*DAB21P*	22.5	21.7, 23.4	21.9	21.5, 22.3	22.1	21.7, 22.4	0.40
rs10993994	10	*MSMB*	22.0	21.5, 22.6	22.3	22.0, 22.6	21.8	21.4, 22.1	0.12
rs7127900	11	Unknown	21.9	20.9, 23.0	22.0	21.7, 22.4	22.0	21.7, 22.3	0.96
rs12418451	11	Unknown	22.3	21.6, 23.1	21.9	21.5, 22.2	22.2	21.8, 22.5	0.37
rs10896449	11	Unknown	22.1	21.7, 22.6	22.1	21.8, 22.4	21.9	21.5, 22.4	0.82
rs10875943	12	*TUBA1C/PRPH*	21.1	18.7, 23.9	21.0	18.6, 23.6	20.9	18.6, 23.5	0.86
rs902774	12	*KRT8*	22.5	21.1, 23.9	22.1	21.6, 22.5	22.1	21.8, 22.4	0.86
rs11649743	17	*HNF1B*	21.8	20.6, 23.0	21.8	21.4, 22.2	22.1	21.9, 22.4	0.40
rs4430796	17	*HNF1B*	22.5	22.0, 23.0	21.9	21.6, 22.2	22.0	21.6, 22.4	0.10
rs1859962	17	Unknown	21.8	21.3, 22.2	22.1	21.8, 22.4	22.2	21.8, 22.7	0.32
rs8102476	19	Unknown	22.1	21.6, 22.6	22.1	21.7, 22.4	21.9	21.5, 22.3	0.75
rs11672691	19	Unknown	22.2	21.2, 23.2	22.1	21.7, 22.5	22.0	21.6, 22.3	0.79
rs2735839	19	*KLK2/KLK3*	19.9	18.5, 21.4	22.0	21.5, 22.5	22.1	21.8, 22.3	0.02
rs5759167	22	*BIL/TTLL1*	21.6	21.2, 22.1	22.2	21.8, 22.5	22.1	21.6, 22.5	0.19
rs11704416	22	*TNRC6B*	21.9	20.8, 23.0	22.2	21.8, 22.7	21.9	21.6, 22.2	0.50

Table continues

Table 1. Continued

SNP	Chromosome	Gene	Zygosity and 25(OH)D Concentration[a]						*P* Value[b]
			Rare Homozygote		Heterozygote		Common Homozygote		
			GM	95% CI	GM	95% CI	GM	95% CI	
rs5945619	X	*NUDT11*	22.3	22.0, 22.7			21.9	21.6, 22.2	0.06
rs5919432	X	*AR*	20.3	18.0, 22.9			21.1	18.8, 23.8	0.03

Abbreviations: CI, confidence interval; GM, geometric mean; SNP, single nucleotide polymorphism.

[a] From a linear regression analysis between natural log-transformed 25-hydroxyvitamin D concentrations (Rosner-standardized) for cohort, batch, and season and further adjusted for age at blood draw, year of blood draw, case-control status, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition).

[b] Test of the difference of 3 geometric means using the F distribution. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing.

[c] The total sample size in this genotype subgroup was ≤50 observations.

the observed interaction may be due to chance. However, there is ample literature on the regulatory role of vitamin D on the *MYC* gene. Vitamin D signaling can suppress expression of genes regulated by *c-MYC*, providing a molecular basis for the cancer-preventive actions of vitamin D (37). Furthermore, it has been shown that the active regulator of vitamin D—1,25$(OH)_2$D3—down-regulates *c-MYC* and its transcription factor *E2F*, subsequently resulting in reduced growth of several prostate cancer cell lines (38–40). Future studies are needed—first to verify the observed intriguing interactions between 8q24 rs620861 and rs16902094, 25(OH)D concentration, and fatal prostate cancer risk, and second to shed light on the potential underlying biological mechanisms.

Reports of associations between circulating concentrations of vitamin D metabolites and risk of prostate cancer are inconsistent in the epidemiologic literature. A meta-analysis of 25 studies published in 2011 provided little evidence that 25(OH)D concentrations were associated with the risk of total or aggressive prostate cancer (12). Some recent prospective studies have also reported null associations between 25(OH)D and risk of any prostate cancer but inverse associations for aggressive or lethal disease (18, 41). However, other recent prospective studies observed positive associations for total disease and null associations for lethal disease (16, 42, 43) or a statistically significant U-shaped association for total and aggressive disease (44). A meta-analysis published in 2014 observed a statistically significant, 17% elevated risk of any prostate cancer for individuals with higher levels of 25(OH)D, but the investigators did not explore associations by stage and grade of the disease. Potential reasons for the inconsistencies in the literature addressing vitamin D and prostate cancer have been described in detail elsewhere (43); briefly they include the use of different 25(OH)D assays, single (instead of multiple) measurement of 25(OH)D, the different screening practices by country, and the large clinical heterogeneity of prostate cancer. Future studies should include consideration of prostate cancer mortality as the most clinically relevant prostate cancer endpoint (45).

To our knowledge, this is the first study to investigate potential interactions between GWAS-identified SNPs related to prostate cancer risk and circulating levels of 25(OH)D. We evaluated the strength of the evidence for the observed statistically significant interactions based on published guidelines (46). The strength of the literature evidence for the main association of 25(OH)D concentrations with risk of fatal prostate cancer can be considered weak, as few studies have examined fatal prostate cancer as an outcome, whereas the strength of the evidence for the association between the 2 SNPs in 8q24 (rs620861 and rs16902094) and risk of fatal disease is considered moderate; several GWASs have confirmed these findings for total prostate cancer, but results are sparse for fatal disease. In summary, this corresponds to a moderate a priori likelihood for the existence of an interaction. However, the overall strength of the evidence for an interaction is weak, given that replication is currently lacking and the evidence in the present analysis is based on only approximately 500 fatal prostate cancer cases.

These results imply a lack of robust interactions on the multiplicative or additive scales associating 46 prostate cancer susceptibility SNPs and 25(OH)D concentration with risk of either total or fatal prostate cancer. However, lack of statistical interaction does not imply lack of biological (causal) interaction. We cannot exclude the possibility that there may be modest or weak gene–vitamin D interactions that this study had insufficient statistical power to detect. Moreover, we tested only for 2-level interactions in the present study, and higher-order interactions may have been missed, although power to detect such interactions would be lower. BPC3 investigators are in a unique position to explore gene-environment interactions because BPC3 consists of 9 well-established cohort studies (of which 5 were included in this analysis) with prospectively collected blood specimens, high-quality biomarker assays, and genotyping data for thousands of participants. With 3,811 cases and 2,980 controls, this study had more than 80% power to detect a multiplicative interaction association of 1.7, assuming an allele frequency of 30% and a SNP or 25(OH)D main association with total prostate cancer of 1.1, but the power was reduced

Table 2. Per-Allele Associations Between 46 Single Nucleotide Polymorphisms Identified in Genome-Wide Association Studies and Risk of Total Prostate Cancer According to 25-Hydroxyvitamin D Concentrations (1982–2004), Breast and Prostate Cancer Cohort Consortium

SNP	No. of Cases	No. of Controls	Tertiles of 25(OH)D[a]						*P* for Interaction[b]	*P* for Interaction[c]	*P* for Interaction[d]	*P* for Interaction[e]
			First		Second		Third					
			OR	95% CI	OR	95% CI	OR	95% CI				
rs13385191	3,377	2,556	1.09	0.94, 1.26	1.15	0.99, 1.33	1.01	0.87, 1.16	0.48	0.23	0.04	0.24
rs1465618	3,648	2,816	1.12	0.96, 1.30	1.15	0.99, 1.33	1.06	0.92, 1.23	0.62	0.81	0.33	0.63
rs721048	3,675	2,849	1.14	0.97, 1.34	1.12	0.97, 1.31	1.23	1.05, 1.43	0.50	0.79	0.57	0.57
rs10187424	2,252	1,782	1.11	0.95, 1.31	1.03	0.88, 1.20	1.04	0.89, 1.21	0.53	0.54	0.02	0.10
rs12621278	3,682	2,834	0.98	0.75, 1.30	1.00	0.76, 1.31	1.25	0.95, 1.64	0.26	0.87	0.42	0.33
rs2292884	3,109	2,455	1.12	0.96, 1.30	0.99	0.85, 1.15	1.02	0.88, 1.19	0.47	0.39	0.42	0.65
rs2660753	3,692	2,884	1.06	0.87, 1.28	1.20	1.00, 1.44	1.14	0.94, 1.37	0.51	0.33	0.23	0.14
rs7629490	3,115	2,441	1.15	1.00, 1.32	1.09	0.95, 1.25	1.09	0.95, 1.25	0.62	0.56	0.37	0.23
rs6763931	2,318	1,837	0.98	0.85, 1.14	1.03	0.88, 1.20	1.06	0.91, 1.23	0.52	0.43	0.26	0.23
rs10936632	2,348	1,858	0.96	0.83, 1.12	1.03	0.89, 1.20	1.09	0.94, 1.27	0.26	0.84	0.09	0.40
rs17021918	3,656	2,831	1.07	0.94, 1.21	1.00	0.88, 1.14	1.20	1.06, 1.37	0.18	0.18	0.19	0.34
rs7679673	3,677	2,824	1.15	1.02, 1.30	1.21	1.07, 1.37	1.09	0.96, 1.23	0.54	0.26	0.64	1.00
rs2242652	2,350	1,858	1.11	0.92, 1.33	1.29	1.06, 1.57	1.43	1.19, 1.73	0.05	0.04	0.80	0.24
rs12653946	3,365	2,548	1.10	0.97, 1.26	1.06	0.94, 1.21	0.99	0.87, 1.12	0.25	0.41	0.24	0.25
rs2121875	2,285	1,739	1.06	0.90, 1.26	0.92	0.78, 1.08	1.09	0.93, 1.29	0.81	0.77	0.19	0.18
rs130067	2,340	1,856	1.06	0.87, 1.28	1.20	0.99, 1.45	0.95	0.79, 1.15	0.43	0.50	0.60	0.87
rs339331	3,387	2,564	1.11	0.97, 1.28	0.99	0.85, 1.14	1.07	0.93, 1.23	0.74	0.44	0.38	0.55
rs9364554	3,694	2,876	1.08	0.94, 1.25	1.06	0.92, 1.21	1.07	0.94, 1.22	0.91	0.14	0.38	0.73
rs12155172	3,650	2,830	0.97	0.84, 1.13	1.11	0.96, 1.28	1.05	0.91, 1.21	0.47	0.43	0.75	0.67
rs10486567	3,677	2,873	1.22	1.05, 1.41	1.12	0.97, 1.29	1.19	1.03, 1.37	0.85	0.56	0.08	0.21
rs6465657	3,681	2,868	1.13	1.00, 1.27	1.08	0.96, 1.22	1.02	0.91, 1.15	0.21	0.42	0.77	0.49
rs2928679	3,692	2,828	1.05	0.93, 1.19	1.02	0.91, 1.16	0.98	0.87, 1.11	0.48	0.46	0.94	0.80
rs1512268	3,709	2,851	1.09	0.96, 1.23	1.02	0.91, 1.16	1.11	0.98, 1.25	0.88	0.81	0.32	0.54
rs1016343	3,694	2,875	1.27	1.10, 1.46	1.02	0.88, 1.17	1.23	1.07, 1.42	0.79	0.39	0.62	0.31
rs16901979	3,655	2,852	1.38	1.00, 1.90	1.21	0.87, 1.67	1.87	1.34, 2.60	0.20	0.14	0.42	0.42
rs16902094	3,362	2,621	1.11	0.94, 1.32	1.00	0.85, 1.18	1.19	1.01, 1.41	0.61	0.02	0.23	0.59
rs620861	3,460	2,673	0.97	0.85, 1.11	1.07	0.94, 1.21	1.25	1.10, 1.43	0.01	0.10	0.04	0.06
rs6983267	3,670	2,847	1.20	1.06, 1.35	1.13	1.00, 1.28	1.31	1.16, 1.48	0.32	0.80	0.28	0.62
rs4242382	3,755	2,921	1.33	1.10, 1.62	1.46	1.21, 1.77	1.58	1.31, 1.90	0.27	0.83	0.17	0.48
rs1571801	3,598	2,773	1.02	0.88, 1.17	1.23	1.07, 1.42	1.04	0.91, 1.20	0.89	0.84	0.98	0.65
rs10993994	3,653	2,852	1.20	1.06, 1.36	1.18	1.05, 1.34	1.18	1.04, 1.33	0.91	0.69	0.83	0.47
rs7127900	3,666	2,815	1.12	0.96, 1.30	1.09	0.94, 1.27	1.30	1.12, 1.51	0.16	0.14	0.20	0.35
rs12418451	3,773	2,932	1.10	0.96, 1.26	1.09	0.96, 1.24	1.14	1.01, 1.30	0.66	0.35	0.72	0.10

Table continues

Table 2. Continued

SNP	No. of Cases	No. of Controls	Tertiles of 25(OH)D[a]						*P* for Interaction[b]	*P* for Interaction[c]	*P* for Interaction[d]	*P* for Interaction[e]
			First		Second		Third					
			OR	95% CI	OR	95% CI	OR	95% CI				
rs10896449	3,635	2,850	1.22	1.08, 1.37	1.18	1.04, 1.33	1.21	1.08, 1.36	0.99	0.49	1.00	0.59
rs10875943	2,363	1,867	1.02	0.87, 1.20	1.08	0.92, 1.28	0.99	0.85, 1.16	0.81	0.77	0.51	0.86
rs902774	3,574	2,752	1.02	0.86, 1.22	1.16	0.98, 1.37	1.06	0.90, 1.25	0.88	0.71	0.83	0.48
rs11649743	3,661	2,858	1.24	1.06, 1.45	1.19	1.02, 1.39	1.08	0.92, 1.26	0.18	0.12	0.46	0.68
rs4430796	3,596	2,802	1.30	1.14, 1.47	1.29	1.14, 1.46	1.17	1.04, 1.32	0.19	0.44	0.05	0.18
rs1859962	3,701	2,882	1.21	1.07, 1.37	1.37	1.22, 1.55	1.08	0.96, 1.22	0.16	0.99	0.99	0.49
rs8102476	3,622	2,796	0.88	0.78, 0.99	1.13	1.00, 1.28	1.08	0.96, 1.22	0.01	0.07	0.15	0.22
rs11672691	3,338	2,549	1.24	1.06, 1.44	0.96	0.83, 1.12	1.12	0.96, 1.29	0.37	0.10	0.61	0.73
rs2735839	3,614	2,795	1.13	0.95, 1.36	1.20	1.01, 1.43	1.07	0.90, 1.28	0.63	0.47	0.55	0.14
rs5759167	3,661	2,819	1.08	0.96, 1.22	1.14	1.01, 1.28	1.26	1.12, 1.42	0.08	0.08	0.20	0.02
rs11704416	3,354	2,564	1.16	0.99, 1.36	1.15	0.98, 1.34	1.01	0.87, 1.18	0.23	0.17	0.79	0.58
rs5945619	3,703	2,871	1.18	1.08, 1.29	1.04	0.96, 1.14	1.10	1.01, 1.20	0.29	0.23	0.33	0.38
rs5919432	2,336	1,851	1.02	0.89, 1.16	1.00	0.87, 1.14	1.19	1.04, 1.36	0.13	0.19	0.01	0.04
Additive SNP score[f]	3,811	2,980	1.05	1.04, 1.06	1.03	1.02, 1.05	1.04	1.03, 1.06	0.50	0.97	0.60	0.63
Kernel machine score[g]									0.21	0.28	<0.001	<0.001

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

[a] From a logistic regression model of SNPs and total prostate cancer risk by cohort-, batch-, and season-specific tertiles of 25(OH)D concentration adjusted for age at blood draw, year of blood draw, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition).

[b] *P* for interaction was calculated based on the case-control analysis and a variable for cohort-, batch-, and season-specific tertiles of 25(OH)D concentration. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing (*P* threshold = 0.001).

[c] *P* for interaction was calculated based on the case-control analysis and a continuous 25(OH)D variable (Rosner-standardized) for cohort, batch, and season. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing (*P* threshold = 0.001).

[d] *P* for interaction was calculated based on the case-only analysis for dichotomized SNPs and cohort-, batch-, and season-specific tertiles of 25(OH)D concentration. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing. We used a *P* threshold of 0.01 for the case-control analyses, applying a correction to the standard threshold of 0.05 to account for multiple testing on the basis of finding 5 nominally significant SNPs for 25(OH)D tertiles in the case-only analyses.

[e] *P* for interaction was calculated based on the case-only analysis for dichotomized SNPs and a continuous 25(OH)D variable (Rosner-standardized) for cohort, batch, and season. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing. We used a *P* threshold of 0.01 for the case-control analyses, applying a correction to the standard threshold of 0.05 to account for multiple testing on the basis of finding 5 nominally significant SNPs in the case-only analyses.

[f] From a logistic regression model of a continuous additive genetic score (after summing the number of risk alleles across the 46 SNPs for each participant) and total prostate cancer risk. For the case-only analysis, the additive genetic score was dichotomized at the median among controls.

[g] From a logistic regression kernel machine model across the entire set of 46 SNPs and total prostate cancer risk.

Table 3. Per-Allele Association Between 46 Single Nucleotide Polymorphisms Identified in Genome-Wide Association Studies and Risk of Fatal Prostate Cancer According to 25-Hydroxyvitamin D Concentrations (1982–2004), Breast and Prostate Cancer Cohort Consortium

SNP	No. of Cases	No. of Controls	Tertiles of 25(OH)D[a]						*P* for Interaction[b]	*P* for Interaction[c]	*P* for Interaction[d]	*P* for Interaction[e]
			First		Second		Third					
			OR	95% CI	OR	95% CI	OR	95% CI				
rs13385191	419	2,556	1.07	0.78, 1.46	1.03	0.76, 1.40	0.92	0.66, 1.27	0.50	0.46	0.75	0.88
rs1465618	459	2,816	1.07	0.79, 1.45	1.23	0.91, 1.66	1.11	0.81, 1.51	0.83	0.86	0.62	0.71
rs721048	489	2,849	0.97	0.69, 1.35	0.98	0.71, 1.35	1.34	0.97, 1.85	0.19	0.30	0.58	0.77
rs10187424	307	1,782	1.19	0.87, 1.65	1.24	0.87, 1.75	1.07	0.76, 1.51	0.58	0.87	0.91	0.91
rs12621278	475	2,834	0.84	0.49, 1.45	1.11	0.63, 1.98	1.45	0.77, 2.72	0.21	0.56	0.47	0.008
rs2292884	403	2,455	1.26	0.92, 1.73	0.73	0.51, 1.05	1.13	0.83, 1.56	0.67	0.91	0.75	0.71
rs2660753	486	2,884	0.84	0.54, 1.30	1.08	0.73, 1.61	0.90	0.59, 1.37	0.87	0.18	0.51	0.20
rs7629490	400	2,441	1.10	0.83, 1.47	1.05	0.79, 1.40	0.94	0.70, 1.26	0.45	0.45	0.54	0.75
rs6763931	321	1,837	0.88	0.65, 1.19	0.78	0.56, 1.09	1.36	0.99, 1.86	0.07	0.07	0.34	0.28
rs10936632	315	1,858	0.85	0.61, 1.17	1.26	0.90, 1.77	1.05	0.77, 1.44	0.30	0.89	0.89	0.90
rs17021918	469	2,831	1.01	0.78, 1.31	0.85	0.64, 1.12	1.05	0.81, 1.38	0.85	0.31	0.69	0.55
rs7679673	472	2,824	1.27	0.99, 1.62	1.32	1.01, 1.72	1.29	0.99, 1.68	0.99	0.89	0.96	0.86
rs2242652	324	1,858	0.87	0.61, 1.23	0.97	0.64, 1.46	1.63	1.07, 2.49	0.03	0.42	0.86	0.80
rs12653946	420	2,548	1.33	1.02, 1.75	0.94	0.71, 1.25	1.15	0.88, 1.50	0.41	0.95	0.64	0.79
rs2121875	309	1,739	1.00	0.72, 1.38	1.12	0.78, 1.60	1.27	0.90, 1.78	0.34	0.42	0.37	0.58
rs130067	323	1,856	0.97	0.66, 1.42	0.93	0.60, 1.42	0.80	0.53, 1.19	0.52	0.85	0.83	0.93
rs339331	421	2,564	1.26	0.94, 1.69	0.75	0.55, 1.02	1.03	0.76, 1.39	0.32	0.08	0.28	0.43
rs9364554	489	2,876	1.09	0.83, 1.44	1.03	0.78, 1.37	1.14	0.86, 1.51	0.87	0.60	0.71	0.64
rs12155172	472	2,830	0.86	0.64, 1.17	1.27	0.95, 1.70	1.05	0.78, 1.43	0.35	0.42	0.35	0.72
rs10486567	500	2,873	0.81	0.62, 1.06	1.00	0.75, 1.33	1.09	0.81, 1.47	0.16	0.23	0.59	0.72
rs6465657	478	2,868	1.06	0.83, 1.36	1.03	0.80, 1.33	0.82	0.63, 1.06	0.18	0.24	0.45	0.36
rs2928679	477	2,828	1.12	0.87, 1.45	0.99	0.77, 1.29	0.96	0.74, 1.24	0.32	0.68	0.16	0.58
rs1512268	482	2,851	1.07	0.84, 1.37	0.92	0.71, 1.18	0.92	0.72, 1.19	0.42	0.59	0.35	0.27
rs1016343	487	2,875	1.60	1.21, 2.10	1.17	0.87, 1.57	1.10	0.81, 1.50	0.07	0.06	0.28	0.30
rs16901979	490	2,852	1.06	0.55, 2.06	1.45	0.77, 2.73	1.90	1.01, 3.58	0.20	0.11	0.40	0.17
rs16902094	450	2,621	0.96	0.68, 1.34	1.31	0.95, 1.80	1.60	1.15, 2.23	0.03	0.002	0.05	0.11
rs620861	463	2,673	0.75	0.58, 0.97	1.06	0.81, 1.39	1.48	1.11, 1.97	0.001	0.009	0.001	0.02
rs6983267	498	2,847	1.27	1.00, 1.60	1.19	0.92, 1.52	1.22	0.95, 1.57	0.79	0.94	0.72	0.83
rs4242382	505	2,921	1.08	0.73, 1.59	1.97	1.39, 2.80	1.35	0.95, 1.91	0.44	0.40	0.29	0.17
rs1571801	482	2,773	1.22	0.94, 1.60	0.98	0.73, 1.31	1.17	0.88, 1.55	0.86	0.48	0.45	0.90
rs10993994	494	2,852	0.88	0.68, 1.13	1.24	0.97, 1.59	1.36	1.06, 1.76	0.02	0.03	0.04	0.007
rs7127900	474	2,815	1.11	0.82, 1.50	0.90	0.65, 1.25	1.14	0.83, 1.56	0.86	0.88	0.41	0.66
rs12418451	504	2,932	1.10	0.84, 1.43	0.96	0.73, 1.26	1.21	0.93, 1.58	0.60	0.42	0.34	0.78

Table continues

Table 3. Continued

SNP	No. of Cases	No. of Controls	Tertiles of 25(OH)D[a]						*P* for Interaction[b]	*P* for Interaction[c]	*P* for Interaction[d]	*P* for Interaction[e]
			First		Second		Third					
			OR	95% CI	OR	95% CI	OR	95% CI				
rs10896449	493	2,850	1.05	0.83, 1.33	1.04	0.81, 1.34	1.29	1.00, 1.65	0.28	0.17	0.33	0.73
rs10875943	321	1,867	1.13	0.81, 1.58	1.08	0.77, 1.51	1.11	0.81, 1.51	0.98	0.57	0.98	0.75
rs902774	469	2,752	0.91	0.64, 1.29	1.41	1.01, 1.96	1.22	0.87, 1.70	0.23	0.47	0.17	0.55
rs11649743	496	2,858	1.41	1.01, 1.97	1.39	0.99, 1.96	0.93	0.67, 1.30	0.09	0.08	0.64	0.35
rs4430796	489	2,802	1.67	1.28, 2.18	1.43	1.10, 1.85	1.05	0.82, 1.35	0.02	0.10	0.005	0.007
rs1859962	495	2,882	1.32	1.03, 1.69	1.30	1.01, 1.67	1.06	0.83, 1.36	0.26	0.51	0.89	0.45
rs8102476	495	2,796	0.91	0.72, 1.15	1.04	0.81, 1.33	1.14	0.88, 1.46	0.25	0.43	0.29	0.08
rs11672691	418	2,549	1.72	1.22, 2.42	0.80	0.58, 1.10	1.45	1.04, 2.01	0.46	0.52	0.46	0.91
rs2735839	483	2,795	1.21	0.83, 1.74	0.76	0.55, 1.06	0.92	0.63, 1.35	0.31	0.37	0.39	0.15
rs5759167	471	2,819	0.99	0.78, 1.26	1.08	0.84, 1.40	1.30	1.01, 1.69	0.12	0.17	0.11	0.03
rs11704416	422	2,564	1.32	0.98, 1.79	1.17	0.84, 1.65	1.24	0.90, 1.70	0.78	0.91	0.52	0.70
rs5945619	490	2,871	1.22	1.03, 1.46	1.05	0.87, 1.26	1.26	1.06, 1.51	0.77	0.76	0.40	0.58
rs5919432	320	1,851	0.88	0.68, 1.13	0.95	0.71, 1.26	1.27	0.94, 1.71	0.06	0.05	0.26	0.06
Additive SNP score[f]	511	2,980	1.03	1.01, 1.05	1.03	1.01, 1.06	1.05	1.03, 1.08	0.22	0.04	0.66	0.95
Kernel machine score[g]									0.005	0.005	<0.001	<0.001

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

[a] From a logistic regression model of SNPs and total prostate cancer risk by cohort-, batch-, and season-specific tertiles of 25(OH)D concentrations, adjusted for age at blood draw, year of blood draw, cohort, and country (within the European Prospective Investigation into Cancer and Nutrition).

[b] *P* for interaction was calculated based on the case-control analysis and a variable for cohort-, batch-, and season-specific tertiles of 25(OH)D concentrations. Conventional *P* values are shown; only 1 *P* value was marginally statistically significant for rs620861 after allowance for multiple testing (*P* threshold = 0.001).

[c] *P* for interaction was calculated based on the case-control analysis and a continuous 25(OH)D variable (Rosner-standardized) for cohort, batch, and season. Conventional *P* values are shown; all *P* values were nonsignificant after allowance for multiple testing (*P* threshold = 0.001).

[d] *P* for interaction was calculated based on the case-only analysis for dichotomized SNPs and cohort-, batch-, and season-specific tertiles of 25(OH)D. Conventional *P* values are shown; only 2 *P* values were statistically significant (for rs620861 and the kernel machine score) after allowance for multiple testing. We used a *P* threshold of 0.0125 for the case-control analyses, applying a correction to the standard threshold of 0.05 to account for multiple testing on the basis of finding 4 nominally significant SNPs in the case-only analyses.

[e] *P* for interaction was calculated based on the case-only analysis for dichotomized SNPs and a continuous 25(OH)D variable (Rosner-standardized) for cohort, batch, and season. Conventional *P* values are shown; only 2 *P* values were statistically significant (for rs620861 and the kernel machine score) after allowance for multiple testing. We used a *P* threshold of 0.01 for the case-control analyses, applying a correction to the standard threshold of 0.05 to account for multiple testing on the basis of finding 5 nominally significant SNPs in the case-only analyses.

[f] From a logistic regression model of a continuous additive genetic score (after summing the number of risk alleles across the 46 SNPs for each participant) and fatal prostate cancer risk. For the case-only analysis, the additive genetic score was dichotomized at the median among controls.

[g] From a logistic regression kernel machine model across the entire set of 46 SNPs and fatal prostate cancer risk.

for fatal prostate cancer. Recently published GWASs have identified several prostate cancer SNPs other than the 46 SNPs studied here. Therefore, more studies with a larger number of participants are needed to reexamine our findings, to study untested GWAS-identified SNPs, and to evaluate the gene and vitamin D interactions for total and fatal prostate cancer in individuals with European ancestry and other ethnicities.

Overall, we did not find strong evidence that associations between GWAS-identified SNPs and prostate cancer are modified by circulating concentrations of 25(OH)D. The intriguing multiplicative interactions between rs620861 and rs16902094, 25(OH)D concentration, and fatal prostate cancer warrant replication.

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