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Vitamin D Pathway and Other Related Polymorphisms and Risk of Prostate Cancer: Results from the Prostate Cancer Prevention Trial

Kathleen C. Torkko[a], **Cathee Till**[b], **Catherine M. Tangen**[b,c], **Phyllis J. Goodman**[b], **Xiaoling Song**[c], **Jeannette M Schenk**[c], **M. Scott Lucia**[a], **Ulrike Peters**[c], **Adrie van Bokhoven**[a], **Ian Thompson Jr.**[d], **Marian L. Neuhouser**[c]

[a]University of Colorado, Anschutz Medical Campus, Department of Pathology /MS 8104, 12801 E 17th Ave, Aurora, CO 80045

[b]SWOG Statistics and Data Management Center, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave North, Seattle, WA 98109-1024

[c]Cancer Prevention Program, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave North, Seattle, WA 98109-1024

[d]Christus Santa Rosa Hospital-Medical Center, 2827 Babcock Road, San Antonio, Texas 78229

Abstract

Vitamin D may influence prostate cancer risk, but evidence is inconsistent. We conducted a nested case-control study in the Prostate Cancer Prevention Trial (PCPT). Cases (n=1,128) and controls (n=1,205) were frequency matched on age, first-degree relative with prostate cancer and PCPT treatment arm (finasteride/placebo); African-Americans were oversampled and case/control status was biopsy-confirmed. We selected 21 SNPs in vitamin D-related genes *(VDR, GC, C10orf88, CYP2R1, CYP24A1, CYP27B1, DHCR7, NADSYN1*) to test genotype and genotype-treatment interactions in relation to prostate cancer. We also tested mean serum 25(OH)D differences by minor allele distributions and tested for serum 25(OH)D-genotype interactions in relation to prostate cancer risk. Log-additive genetic models (Bonferroni-corrected within genes) adjusted for age, BMI, PSA, and family history of prostate cancer revealed a significant interaction between treatment arm and *GC*/rs222016 (finasteride OR=1.37, placebo OR=0.85, p-interaction<0.05), *GC*/rs222014 (finasteride OR=1.36, placebo OR=0,85, p-interaction<0.05) and *CYP27B1*/rs703842 (finasteride OR=0.76, placebo OR=1.10, p-interaction<0.05) among Caucasians, and *C10orf88*/rs6599638 (finasteride OR=4.68, placebo OR=1.39, p-interaction<0.05) among African Americans. *VDR*/rs1544410 and *CYP27B1*/rs703842 had significant treatment interactions for high-grade disease among Caucasians (finasteride OR=0.81, placebo OR=1.40, p-interaction<0.05 and finasteride OR=0.70, placebo OR=1.28, p-interaction<0.05, respectively). Vitamin D-related SNPs influenced serum 25(OH)D, but gene-serum 25(OH)D effect modification for prostate cancer was marginally observed only for *CYP24A1*/rs2248359. In conclusion, evidence that

Corresponding Author: Marian L Neuhouser, PhD, RD, Cancer Prevention Program, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109-1024, Tel: 206-667-4797, Fax: 206-667-7850, mneuhous@fredhutch.org.

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vitamin D-related genes or gene-serum 25(OH)D associations influence prostate cancer risk is modest. We found some evidence for gene-finasteride interaction effects for prostate cancer in Caucasians and African-Americans. Results suggest only minimal associations of vitamin D with total or high-grade prostate cancer.

Keywords

vitamin D; prostate cancer; 25(OH)D; genetics

INTRODUCTION

Prostate cancer is a very common cancer in aging men (1), but the etiology remains elusive. Vitamin D is thought to play a role in prostate cancer development and progression since experimental evidence shows that vitamin D influences key carcinogenesis-related processes including promoting cellular differentiation and apoptosis and reducing cell proliferation (2–4). Further, prostate cancer cell-line studies show that the biologically active form of the vitamin, 1,25(OH)D, reduces metastatic potential by increasing E-cadherin expression that interferes with cell adhesion of the circulating cells (5). These and other findings in cell lines and preclinical animal models led to the hypothesis that vitamin D has anti-cancer properties and could be considered as a target for prostate cancer prevention.

Despite the *in vitro* and animal model data, observational studies in humans have been inconsistent regarding the relationship of circulating vitamin D with prostate cancer risk. Vitamin D status in these studies is usually determined by serum concentrations of 25(OH)D, the primary circulating form of vitamin D and the precursor to 1,25(OH)D. A study published in 2011 from a nested case–control analysis of 1,000 cases and 1,000 controls found that Finnish men in the Alpha-Tocopherol Beta-Carotene Cancer (ATBC) Prevention Study cohort with higher circulating serum 25(OH)D concentrations had a statistically significantly elevated risk for total prostate cancer (highest quintile vs. lowest OR= 1.56, 95%CI 1.15–2.12) (6). Three meta-analyses found was no association between serum 25(OH)D and prostate cancer risk (7–9). However, a later meta-analysis reported a significant 17% increase in prostate cancer risk for men with higher vs. lower serum 25(OH)D (10) (11). In addition, in our previous publication from the Prostate Cancer Prevention Trial, we showed no overall association of serum 25(OH)D with prostate cancer risk (10), results that could have been obscured by not considering genetic variation in vitamin D metabolism. PCPT vitamin D data were recently pooled with data from 19 cohorts (12). In those analyses, higher vs lower serum 25(OH)D were associated with a prostate cancer odds ratio = 1.22 (95% CI 1.13–1.31, P-trend<0.001) with noticeable risk increases for non-aggressive disease (OR=1.24, 95% CI 1.13–1.36) but not for aggressive disease (OR=0.94, 95% CI 0.78–1.15). This recent pooled analysis did not consider genetic influences on serum 25(OH)D.

Season, body weight, adiposity, vitamin D intake from both food and supplements, and UVB exposure all influence serum 25(OH)D concentrations (13). Estimates suggest that only about 25% of the variability in 25(OH)D is due to these identifiable factors and that serum

25(OH)D may have a heritability component (heritability estimates range from 28.8% to 38% in those with European ancestry) (14, 15). Given the important role that genetic characteristics may play in the determination of serum 25(OH)D, it is important to study the multiple genes that control its actions and metabolism. Vitamin D3 is transported in the blood to the liver, where it is converted into 25(OH)D by the enzyme 25-hydroxylase that is coded by the gene *CYP2R1*. It then travels to the kidneys where it is converted to 1, 25(OH)D by 1-α-hydroxylase, an enzyme coded by *CYP27B1*. Other tissues, including the prostate, also express 1-α-hydroxylase (16). *CYP24A1* codes for 24-hydroxylase, an enzyme that deactivates calcitriol. These genes and others not directly on the metabolic pathway have been found to be related to circulating 25(OH)D from candidate gene analysis (14) and genome-wide association studies (GWAS) (15, 17, 18). For example, *GC* encodes for vitamin D–binding protein (DBP), the major carrier of vitamin D in circulation. *DHCR7* encodes the enzyme that catalyzes the conversion of 7-dehydrocholesterol, a vitamin D3 precursor, to cholesterol. *DHCR7* is often combined with *NADSYN1* and was identified as a novel locus from a 2010 GWAS study (18). Finally, the region that includes the open-reading frame 88 (*C10orf88*) on chromosome 10q26.13 was also found to be associated with vitamin D concentrations in blood (17).

Selected polymorphisms from vitamin D-related genes have been studied in relation to their associations with prostate cancer. The most studied vitamin D gene is the vitamin D receptor (*VDR*), which is the critical mediator of vitamin D actions. While one review concluded that common polymorphisms in vitamin D pathway genes were not associated with prostate cancer risk (19), other studies found that interactions may exist between SNPs and 25(OH)D that affect prostate cancer risk (20).

Here we report on a nested case-control study in the Prostate Cancer Prevention Trial (PCPT). The overall goal of the PCPT was to test whether finasteride, a 5-α-reductase inhibitor that blocks the conversion of testosterone to its more active form of dihydrotestosterone, would reduce the period prevalence of prostate cancer (21). Using PCPT biospecimens, we investigated both circulating concentrations of 25(OH)D and vitamin D-related genes in relation to prostate cancer risk. In addition, we investigated whether finasteride influenced the relationship of polymorphisms in the vitamin D genes with prostate cancer risk since 25(OH)D interacts with androgen signaling, which is a key pathway for prostate cancer development.

MATERIALS AND METHODS

Data for this nested case-control study are from the Prostate Cancer Prevention Trial (PCPT), a randomized double-blind, placebo-controlled trial of finasteride for the primary prevention of prostate cancer (21). Briefly, 18,880 men aged 55 years or older with normal digital rectal examination (DRE) results, PSA ≤3 ng/mL and no history of prostate cancer, severe lower urinary tract symptoms, or clinically significant coexisting conditions were randomized to receive finasteride (5 mg/day) or placebo (21). During the PCPT, participants underwent DRE and PSA assessment annually, and men with a DRE suspicious for cancer or a PSA (adjusted for the effect of finasteride) above 4.0 ng/mL were referred for a prostate biopsy (21). At the end of seven years, all men not previously diagnosed with prostate

cancer were asked to undergo an end-of-study biopsy per the primary trial protocol to determine the presence or absence of prostate cancer (21). Six core samples were collected under transrectal ultrasonographic guidance, and biopsies were reviewed for adenocarcinoma by both the local study site pathologist and a central pathology laboratory (21). In the case of discordant results, a referee pathologist reviewed cases until concordance was reached (21). Clinical stage was assigned locally, and tumors were graded centrally using the Gleason scoring system. To be consistent with the primary trial report (21), tumors with Gleason scores < 7 were classified as low-grade and those with Gleason scores ≥ 7 were classified as high-grade. The Institutional Review Boards of all participating SWOG institutions approved this study and all participants signed written informed consent.

Case and control selection

Cases (n=1,128) were men with biopsy-confirmed prostate cancer, identified either by a 'for-cause' biopsy triggered by a PSA > 4 ng/mL or abnormal DRE during the trial (n=423) or at the end-of-study biopsy (n=705) and who had available DNA for genotyping analysis. Controls (n=1,205) were selected from men who had no evidence of prostate cancer on the end-of-study biopsy and had available DNA for genotyping. Controls were frequency matched to cases on distributions of age, first-degree family history of prostate cancer and treatment arm, and included all available nonwhites to increase the pool of minorities. The present analysis includes only Caucasian and African-American men. All other race and ethnic groups were excluded from this analysis due to very low numbers.

Blood collection, processing, genotyping and serum 25(OH)D measures

Non-fasting whole blood samples were collected and shipped overnight in a chilled container, processed at Esoterix (Calabasas CA) and shipped on dry ice for storage at the University of Colorado (21). White blood cells were aliquoted and stored at –70°C until extraction of DNA from the WBC samples using Qiagen M48 robot (Valencia, CA). SNPs were selected based on prior literature that was available at the time of our laboratory analysis (20, 22–24). Genotypes were determined using the Illumina VeraCode GoldenGate genotyping assay (Illumina Inc.; San Diego, CA). The list of SNPs to be genotyped was submitted to Illumina and scored with the Assay Design Tool (ADT). Those SNPs with acceptable scores were developed into an oligonucleotide pool assay (OPA) designed for a VeraCode GoldenGate panel. Two hundred fifty nanograms of DNA were used as the template for the assay. The assay was performed in 96 well plates following the established protocol (Illumina). The plates were scanned using an Illumina BeadXpress reader and the genotypes were analyzed using GenomeStudio software (Illumina). Interplate and intraplate replicates were included as quality control measures, and duplicate concordance was ≥98% for all SNPs (mean=99%). Baseline serum was assayed via a chemiluminescent assay for 25(OH)D using LIAISON 25OHVitaminDTOTAL Assay (DiaSorin Inc.) at the Fred Hutchinson Cancer Research Center Biomarker Laboratory (10). The lower limit of quantitation for this assay is 4 ng/mL and no specimens had results below this level (10). All batches were balanced by case and control status and laboratory personnel at the Biomarker Laboratory were blinded to participant status. The coefficient of variation (CV) for 86 blinded duplicate quality control samples was 8.3% (10).

Other data collection

Data on age, race/ethnicity and family history of prostate cancer in first-degree relatives were collected at baseline using standardized self-administered questionnaires (21). Participants' height and weight were measured at baseline and body mass index (BMI) was calculated as weight (kg)/height (m^2). PSA was measured at baseline as part of the trial protocol (21).

Statistical analysis

Participant characteristics were summarized and compared between cases and controls using t-tests for continuous variables and chi-square tests for categorical variables, separately by Caucasian and African-American self-reported race/ethnicity. Chi-square tests were used to compare minor allele frequencies between cases and controls, stratified by race (Supplementary Table 1). Hardy-Weinberg equilibrium (HWE) was tested for all SNPs by race. Those SNPs not meeting HWE were not included in the analyses. For each SNP, the most frequent genotype in Caucasian men was used as the referent genotype as it provided the greatest model stability. To account for the frequency-matched case-control study design, unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for risk of total prostate cancer, and polytomous logistic regression was used to estimate ORs and 95% CIs for both low-grade and high-grade cancer. A log-additive genetic model was used, so that the OR represents the risk of cancer or high-grade cancer per each additional minor allele. Model covariates were selected based on *a priori* information about potential confounding and included age (continuous), BMI (continuous), baseline PSA (continuous), and first-degree family history of prostate cancer (yes/no). To evaluate whether the vitamin D genotype-prostate cancer relationship differentially influenced finasteride's effect on risk, tests for interaction were conducted by entering cross-product terms of SNPs and finasteride and testing these terms with the Wald test. To determine whether a participant's baseline serum 25(OH)D concentration influenced the genotype-cancer relationship, we first created season-adjusted and log-transformed values for serum 25(OH)D as previously described for Caucasian participants (10). We then calculated geometric means of serum 25(OH)D by genotype, and calculated p-values comparing 25(OH)D values by SNP genotype using unadjusted linear regression with season-adjusted log[serum 25OH)D] as the dependent variable. We next created tertiles of baseline season-adjusted serum 25(OH)D and tested whether the genotype-cancer relationships varied by tertile of serum 25(OH)D. Trend interaction p-values between tertiles of baseline serum 25(OH)D and genotype with prostate cancer were calculated using an ordinal variable corresponding to tertile rank, from lowest to highest. These analyses involving serum 25(OH)D were restricted to Caucasian men (due to small numbers of African-Americans) on the placebo arm only for both sample size consideration and to reduce the potential for multiple comparisons.

All statistical tests are 2-sided. 95% confidence intervals are included as a uniform descriptive measure, and statistical significance for main effects was determined using a Bonferroni adjustment within each gene. Thresholds for statistical significance each gene were: *VDR* =0.013; *GC* =0.008; *C10corf88*, *CYP2R1, CYP27B1*, and *DHCR7* =0.05;

CYP24A1 =0.01; and *NADSYN1* =0.025. Tests for interaction were considered significant with p<0.05. SAS (version 9.4, Cary NC) and R were used for all statistical analyses.

RESULTS

The 2,333 men (1128 cases and 1205 controls) who met study criteria were predominantly Caucasian (92.4%); the remainder were African American (Table 1). Due to the study's frequency matching criteria, cases and controls in both racial groups did not differ by age or family history of prostate cancer. Caucasian men (both cases and controls) were slightly older than African-American men (both cases and controls). The mean BMI for both race groups and for cases and controls were all in the overweight range (BMI=25.0–29.9 kg/m^2). The proportion of cases with Gleason ≥ 7 was slightly higher in African-Americans than Caucasians.

Twenty-one vitamin D-related SNPs were included in this study (Supplementary Table 1). For many genes, African-American genotype distributions were different from those for Caucasians, particularly when contrasting the cases and controls. In some cases, the rare allele for Caucasians is the common allele in African-Americans (e.g., rs222016). For data presentation purposes, the risk allele for both groups was defined as the rare allele in Caucasians.

With only a few exceptions, the majority of SNPs in vitamin D-related genes had neither overall associations nor any interactions with finasteride (PCPT treatment arm) in relation to prostate cancer risk (Table 2). *GC*/rs222016 in Caucasians had a per minor allele prostate cancer odds ratio =1.37 (95%CI 1.06–1.79) for the finasteride arm and a per minor allele odds ratio = 0.85 (95%CI0.68–1.05) for the placebo arm, p-interaction <0.05. Another *GC* SNP, rs222014, in Caucasians had a per minor allele prostate cancer odds ratio =1.36 (95% CI 1.00–1.86) on the finasteride arm and per minor allele odds ratio =0.85 (95% CI 0.65–1.08) for the placebo arm, p-interaction<0.05. *CYP27B1*/rs703842 in Caucasians had a per allele prostate cancer odds ratio = 0.76 (95%CI 0.62–0.95) for the finasteride arm and odds ratio = 1.10 (95%CI 0.94–1.30) for the placebo arm, p-interaction<0.05. Among African-Americans, *C10orf88* /rs6599638 had a per minor allele prostate cancer odds ratio=4.88 (95%CI 1.96–12.13) for the finasteride arm and OR= 1.39 (95%CI 0.65–2.97) for the placebo arm, p-interaction<0.05.

We next investigated vitamin D-related genotype associations and their potential interactions with PCPT-treatment arm restricted to high-grade prostate cancer (Gleason ≥7) (Table 3). These analyses were conducted only in Caucasians due to sample size considerations. Most of these relationships were null with no apparent differences in the magnitude or direction of associations by PCPT treatment arm. The two exceptions were *VDR* (rs1544410), which was associated with a per minor allele increased risk of 1.40 (95% CI 1.05–1.86) for the placebo arm whereas the finasteride arm showed an inverse association (OR=0.81, 95%CI 0.62–1.06), p-interaction<0.01; and *CYP27B1* (rs703842), which was associated with a per minor allele increased risk of 1.28 (95% CI 0.96–1.70) on the placebo arm, and a per minor allele decreased risk of 0.79 (95% CI 0.59–1.07) on the finasteride arm, p-interaction<0.05.

We explored associations between SNPs and baseline season-adjusted serum 25(OH)D as well as whether any SNP-prostate cancer risk associations were modified by serum 25(OH)D (placebo arm only) (Table 4). Five of the 21 genes examined exhibited a per minor allele significant association with serum 25(OH)D after Bonferroni adjustment. Some SNPs conferred increases in serum 25(OH) D while others conferred decreases. For example, two *GC* SNPs (rs7041 and rs2282679) were associated with significantly lower serum 25(OH) as the number of minor alleles increased, whereas *GC* (rs12512631) was associated with higher serum 25(OH) as the number of minor alleles increased. *CYP2R1* (rs2060793) was associated with higher serum 25(OH)D and minor alleles for *NADSYN1* (rs12785878) were associated with lower serum 25(OH)D. Despite the influence of these SNPs on serum 25(OH)D, there was only one borderline significant SNP-serum 25(OH)D interaction in relation to prostate cancer risk, for *CYP24A1*/fs2248359, p-interaction=0.049.

DISCUSSION

In this nested case-control study of 2,156 Caucasian and 177 African American men enrolled in the Prostate Cancer Prevention Trial (PCPT), we investigated the associations of 21 SNPs in vitamin D pathway genes with prostate cancer risk by trial-randomization treatment group and by race. We also examined SNP-cancer associations with risk for high-grade disease, but only among Caucasians due to small numbers of African-Americans and we examined whether SNP-serum 25(OH)D interactions influenced prostate cancer risk. Our main findings are: 1) characteristics in vitamin D pathway genes vary slightly between men of Caucasian and African ancestry; 2) for a few select genes, both the magnitude and direction of association with prostate cancer varies by finasteride vs. placebo use; 4) two SNPs – one in *VDR* and one in *CYP27B1* conferred increased prostate cancer risks on the placebo arm but inverse associations on the finasteride arm; and 3) some but not all vitamin D pathway genes and their variants influence serum 25(OH)D. However, the ensuing relationships with prostate cancer were not clear because the serum 25(OH)-genotype interaction tests in relation to prostate cancer were either marginally significant or not statistically significant.

One of the important design aspects of this study is that it leveraged data and specimens from a randomized, placebo-controlled trial testing a 5-α-reductase inhibitor vs. placebo for primary prevention of prostate cancer (21). All participants had standardized PSA at baseline and all participants had protocol-defined end-of-study biopsies read by a centralized pathologist. Modest but noticeable differences emerged in gene-prostate cancer risk associations that varied slightly by trial treatment arm. For example, three SNPs were associated with per minor allele increased prostate cancer risk only for those in the finasteride arm (*GC*/rs222016 and *GC*/rs222014 in Caucasians and *C10orf88*/rs6599638 in African-Americans) while one SNP (*CYP27B1*/rs703842) in Caucasians was associated with lower risk in the finasteride arm. We were particularly interested in the two SNPs that demonstrated inverse associations for high-grade prostate cancer on the finasteride arm and increased risk on the placebo arm (*VDR*/rs1544410 *CYP27B1*/rs703842). While we are unsure of the underlying biological reasons for these observed associations, it is possible that there is shared biology between the genotypes and the metabolism and disposition of finasteride, thereby conferring the significant treatment-interaction effects. High-grade

prostate cancer is harder to treat, often becoming castrate-resistant so the results are somewhat intriguing.

We know of no other studies that have examined drug-vitamin D SNP interactions in relation to prostate cancer risk. However, some of our results are generally consistent with other studies that have found overall modest relationships of SNPs in vitamin D pathway genes with risk of both total and high-grade prostate cancer. Gilbert et al conducted a nested case control study (n=1,275 cases and 2,062 controls) using data and specimens from the UK-based ProtecT trial (8). Sixteen vitamin-D related SNPs were tested, five of which overlapped with those we tested here in the PCPT. Of those five, results were similar to our results showing no overall associations and no differences by disease grade where they defined high-grade in the same manner as PCPT (Gleason score ≥ 7). In the prostate cancer portion of the Breast and Prostate Cancer Cohort Consortium (BPC3), Mondul et al used data from published GWAS to identify vitamin-related SNPs (25). Two SNPs in that analysis overlapped with our analysis (rs2282679 and rs6013897) and similarly, the BPC3 data show no clear pattern of association of genetic variation with overall prostate cancer risk. With regards to high grade disease, our observed association of *VDR*/rs1544410 SNP (also known as *BSM1*) in high-grade cancer in the placebo arm only is consistent with another study where the minor allele was associated with lethal prostate cancer and marginally associated with high-grade cancer in Caucasians (22). This finding is further supported by a meta-analysis of 13 studies showing that *BSMI* GG was associated with high Gleason score (≥7) (26). In contrast, another study found no associations with prostate cancer specific death or recurrence (23).

Contrary to our *a priori* hypothesis, while we found that some SNPs influenced serum 25(OH)D, there was only one marginal SNP-serum 25(OH)D interaction in relation to prostate cancer risk. These PCPT findings are generally consistent with other published studies where there do not appear to be clear or consistent associations of vitamin D pathway genes, and their interaction with serum 25(OH)D in relation to prostate cancer risk (27). Shui et al used data and specimens from the Prostate Cancer Cohort Consortium to test associations of vitamin D pathway genes with fatal prostate cancer as defined from death certificates, medical records and cancer registries (28). Twenty-one genes were examined, only one of which overlapped with our analysis (rs2060793). The investigators reported a fatal prostate cancer odds ratio of 1.34 (95% CI 1.0–1.79) for those with low serum 25(OH)D, whereas we found no overall or high-grade association per minor allele for this SNP nor any differential association by baseline serum 25(OH)D despite our demonstration of significantly higher serum 25(OH)D among those with 2 minor alleles. However, our results are in contrast to Ahn et al. who reported the strongest gene-serum 25(OH)D risk associations for study participants with the minor allele of rs11574143 who were in the lowest tertile of serum 25(OH)D (20). One possible explanation for our lack of SNP-serum 25(OH)D-prostate cancer interactions is that when we stratified the genotype groups across the low, middle and high tertiles of serum 25(OH)D, the cell sizes became very small. Unlike many studies, we used season-adjusted measures of serum 25(OH), a strategy that we have previously found to be very helpful when blood samples are collected across the four seasons, which is know to contribute to vitamin D variability (10, 29). Lack of consistency between published studies may also be attributable to differences in assays used to measure

serum 25(OH)D (30, 31) It is quite likely that to further the understanding of vitamin D pathway genes and their relationship to prostate cancer risk, pooled data from multiple cohorts, particularly those enriched with African-American men, will be needed. It is also possible that these relationships are much more complex than the current science is able to unravel or that unmeasured or uncontrolled confounding prevented detection of meaningful associations that could be applied to general population prevention strategies. Other strategies such as Mendelian randomization may be particularly useful as recently shown by Dimitraopoulou et al who reported no associations of vitamin-D related SNPs with prostate cancer (27).

This study has several strengths. Our study sample was derived from a completed phase III clinical trial (21). As such, the data were uniformly and rigorously collected at more than 200 clinical sites across the United States. Importantly, both prostate cancer cases and controls have definitive evidence for presence or absence of disease based on the biopsy protocol. This characteristic of the study design minimizes any disease misclassification and the contamination of the control group with undiagnosed cases. We also used season-adjusted serum 25(OH)D measures, which reduces the well- known variation in this measure when including blood samples that have been collected across the four calendar seasons (10, 29). Limitations include that while we did include African-Americans, the sample size for that group was still smaller than ideal, particularly when applying a within-gene Bonferroni adjustment. The study design stipulated that we oversample African-Americans who were PCPT participants and who met the eligibility criteria for the case-control study. Even with this design feature, the number of African American participants was low and these findings should be viewed as exploratory. Genetic variation conferring risk in African-American men may vary from that for Caucasian men and this is an area that deserves further study (32–34) as most genetic discovery studies have been conducted in European ancestry individuals, (15), limiting inferences that can be applied to African-Americans. Genetic epidemiology is a constantly changing field; it is possible that some informative vitamin D-related SNPs emerged after our laboratory analyses were completed (15). New panels of GWAS-identified SNPs (15) have different SNPs than those used in the present study; it is possible that results of the present study would differ using some of the recently identified SNPs. Another limitation is that we examined many candidate, *a priori* SNPs. While applied within-gene multiple comparisons tests, but the possibility exists that false positives could still be present. We believe that this risk is minimal given that most of the associations were null. We urge readers to view results with caution and to be guided by sample size and the ORs and 95% confidence intervals, which provide the magnitude of association and precision of the estimates. Finally, we examined genetic variation in germline DNA; results may differ when investigating vitamin D-related gene expression in both cancer and adjacent normal tissue in the prostate (35, 36).

In conclusion, a very limited number of vitamin D pathway SNPs were associated with both total and high-grade prostate cancer. We found suggestive evidence for a limited number of genotype-finasteride interactions where the direction of the prostate cancer risk varied by PCPT treatment arm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1.

Baseline characteristics of prostate cancer cases and controls[1] in the Prostate Cancer Prevention Trial by race

Characteristics	Caucasian (n=2156)		African American (n=177)	
	Case	Control	Case	Control
Number	1081	1075	47	130
Age, mean years (range)	63.5 (55–82)	64.0 (55–84)	61.9 (55–77)	61.7 (55–77)
Finasteride Treatment Arm, n (%)	446 (41.3)	419 (39.0)	20 (42.6)	81 (62.3)
Family History of prostate cancer, n (%)	235 (21.7)	244 (22.7)	7 (14.9)	24 (18.5)
BMI (kg/m^2), mean (range)	27.3 (19–52)	27.6 (19–46)	29.4 (21–47)	28.5 (17–42)
Gleason ≥7, n (% of all cases)	269 (24.9)	N/A	17 (36.2)	N/A

1. Nested case control selection frequency matched on age, first degree family history of prostate cancer and treatment arm with oversampling for eligible African-Americans.

Table 2.

Evaluating Interactions of Vitamin D Related Genotypes and Finasteride with Prostate Cancer in the Prostate Cancer Prevention Trial, Stratified by Race.

					Caucasian				African American			
					N case/control				N case/control			
			Allele		Number of minor alleles			OR (95% CI)[1]	Number of minor alleles			OR (95% CI)[1]
Gene	rs number	Treatment Arm	Major	Minor	0	1	2		0	1	2	
VDR	rs1544410	Finasteride	G	A	156/129	212/189	75/97	0.83 (0.69–1.01)	8/37	11/38	1/6	1.11 (0.48–2.56)
		Placebo			222/233	301/315	100/101	1.00 (0.85–1.18)	8/28	13/18	5/3	2.48 (1.07–5.78)
VDR	rs11568820	Finasteride	G	A	284/259	139/146	20/13	1.01 (0.79–1.28)	2/6	6/27	11/48	0.88 (0.40–1.92)
		Placebo			405/418	206/207	22/26	1.01 (0.83–1.24)	2/5	9/14	16/30	1.13 (0.49–2.59)
VDR	rs757343	Finasteride	G	A	349/325	84/81	7/4	0.99 (0.73–1.35)	17/69	3/11	0/1	1.12 (0.29–4.35)
		Placebo			482/489	138/153	8/8	0.93 (0.73–1.18)	24/41	3/7		0.93 (0.19–4.45)
VDR	rs11574143	Finasteride	G	A	369/347	71/68	4/3	0.97 (0.69–1.37)	18/66	2/13	0/2	0.50 (0.11–2.37)
		Placebo			523/525	107/122	3/6	0.84 (0.64–1.10)	23/40	4/9		1.22 (0.29–5.09)
GC	rs7041	Finasteride	C	A	149/142	215/191	79/81	0.97 (0.80–1.17)	2/6	5/23	13/52	0.89 (0.40–1.95)
		Placebo			186/199	312/314	127/139	1.00 (0.85–1.17)	0/1	10/21	17/26	1.22 (0.43–3.47)
GC	rs222014	Finasteride	G	A	339/339	93/74	8/4	1.36 (1.00–1.86) [+,**]	12/64	7/16	0/1	1.83 (0.65–5.10)
		Placebo			508/509	117/134	7/9	0.84 (0.65–1.08) [**]	18/44	9/4	0/1	3.16 (0.92–10.89)
GC	rs222016	Finasteride	A	G	296/310	131/99	15/10	1.37 (1.06–1.79) [+,**]	2/20	8/43	8/17	2.12 (0.92–4.88)
		Placebo			455/460	166/169	11/24	0.85 (0.68–1.05) [**]	3/12	16/24	6/11	1.26 (0.56–2.87)
GC	rs705117	Finasteride	A	G	318/307	110/95	14/14	1.03 (0.79–1.34)	1/11	10/31	9/39	1.10 (0.52–2.30)
		Placebo			466/466	152/171	14/16	0.91 (0.73–1.13)	7/5	12/27	8/17	0.79 (0.34–1.81)
GC	rs2282679	Finasteride	A	C	240/223	173/163	28/33	0.94 (0.75–1.17)	16/66	3/14	0/1	0.72 (0.19–2.72)
		Placebo			311/325	264/270	60/60	1.02 (0.86–1.21)	17/38	10/11		1.07 (0.32–3.53)
GC	rs12512631	Finasteride	A	G	175/166	223/196	43/53	0.95 (0.77–1.17)	10/28	7/45	2/8	0.60 (0.26–1.38)
		Placebo			287/313	281/267	64/72	1.05 (0.89–1.24)	15/23	9/21	3/5	1.12 (0.49–2.57)
C10orf88	rs6599638	Finasteride	G	A	132/123	219/204	87/85	0.92 (0.76–1.12)	3/37	9/39	7/5	4.88 (1.96–12.13) [+,**]
		Placebo			179/206	304/309	141/129	1.11 (0.95–1.30)	9/23	13/18	5/8	1.39 (0.65–2.97) [**]

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					Caucasian				African American			
					N case/control				N case/control			
			Allele		Number of minor alleles			OR (95% CI)[1]	Number of minor alleles			OR (95% CI)[1]
CYP2R1	rs2060793	Finasteride	G	A	170/163	211/181	62/73	0.93 (0.76–1.13)	8/34	11/36	1/11	0.82 (0.38–1.77)
		Placebo			237/249	294/304	103/99	1.02 (0.87–1.20)	14/24	10/22	2/3	1.68 (0.65–4.35)
CYP24A1	rs2248359	Finasteride	G	A	166/148	200/197	76/74	0.97 (0.80–1.17)	3/14	6/35	9/32	1.29 (0.61–2.73)
		Placebo			241/224	309/303	79/122	0.81 (0.69–0.95)	3/8	11/21	12/20	1.69 (0.75–3.81)
CYP24A1	rs2762942	Finasteride	A	G	398/382	42/36	2/1	1.13 (0.72–1.77)	20/77	0/4		
		Placebo			570/572	60/81	5/1	0.87 (0.62–1.21)	26/46	1/3		0.31 (0.02–4.90)
CYP24A1	rs4809958	Finasteride	A	C	306/302	125/108	12/7	1.17 (0.89–1.53)	17/68	2/13		0.63 (0.12–3.25)
		Placebo			460/466	159/174	13/13	0.96 (0.77–1.20)	26/41	1/8		0.17 (0.02–1.77)
CYP24A1	rs6013897	Finasteride	G	A	275/260	144/141	23/16	1.04 (0.82–1.31)	7/50	12/25	1/6	1.80 (0.84–3.83)
		Placebo			391/378	210/243	31/30	0.92 (0.76–1.11)	19/27	7/21	1/1	0.68 (0.24–1.92)
CYP24A1	rs6022999	Finasteride	A	G	244/235	170/159	28/23	1.09 (0.87–1.36)	4/10	8/42	8/29	0.96 (0.46–1.99)
		Placebo			364/382	238/228	31/44	0.98 (0.81–1.17)	3/10	15/22	9/16	1.37 (0.63–2.97)
CYP27B1	rs703842	Finasteride	A	G	212/174	199/187	31/54	0.76 (0.62–0.95)[+ **]	10/42	10/28	0/10	0.77 (0.35–1.70)
		Placebo			282/303	271/279	81/67	1.10 (0.94–1.30)[**]	12/17	14/25	1/6	0.69 (0.29–1.65)
DHCR7	rs1790349	Finasteride	A	G	318/295	111/116	14/6	0.98 (0.75–1.29)	12/53	8/23	0/5	1.01 (0.43–2.41)
		Placebo			443/472	167/168	22/14	1.15 (0.93–1.42)	19/32	8/15	0/2	0.61 (0.20–1.84)
NADSYN1	rs3829251	Finasteride	G	A	315/285	100/116	18/10	0.92 (0.71–1.19)	11/48	8/25	0/7	0.85 (0.37–1.96)
		Placebo			442/457	159/173	21/15	1.05 (0.85–1.30)	16/26	11/20	0/3	0.76 (0.28–2.04)
NADSYN1	rs12785878	Finasteride	A	C	250/221	159/172	33/26	0.94 (0.75–1.17)	2/4	8/39	10/38	0.89 (0.39–2.04)
		Placebo			349/350	240/251	45/52	0.98 (0.82–1.16)	3/3	12/18	12/28	0.77 (0.34–1.76)

All models adjusted for age, BMI, PSA, and family history of prostate cancer

[+] Main effects p-value significant after a within gene Bonferroni adjustment. Significance thresholds among genes were : *VDR* = 0.013 (4 tests), *GC*= 0.008 (6 tests), *C10corf88* = 0.05 (1 test), *CYP2R1* = 0.05 (1 test), *CYP24A1* = 0.01 (5 tests), *CYP27B1* = 0.05 (1 test), *DHCR7*= 0.05 (1 test), and *NADSYN1* = 0.025 (2 tests).

[**] p-interaction<0.05.

[1] Per minor allele

Table 3.

Evaluating Interactions of Vitamin D Related Genotypes and Finasteride with high-grade prostate cancer[1] in Caucasian men in the PCPT.

				Finasteride				Placebo			
				N case/control				N case/control			
		Allele		Number of minor alleles				Number of minor alleles			
Gene	**rs number**	**Major**	**Minor**	**0**	**1**	**2**	**OR (95% CI)**	**0**	**1**	**2**	**OR (95% CI)**
VDR	rs1544410	G	A	53/129	72/189	24/97	0.81 (0.62–1.06)**	30/233	62/315	26/101	1.40 (1.05–1.86)**
VDR	rs11568820	G	A	101/259	41/146	7/13	0.91 (0.64–1.28)	66/418	51/207	2/26	1.31 (0.93–1.85)
VDR	rs757343	G	A	116/325	32/81	1/4	0.98 (0.64–1.50)	93/489	22/153	1/8	0.74 (0.46–1.18)
VDR	rs11574143	G	A	121/347	27/68	1/3	1.03 (0.65–1.64)	97/525	22/122	0/6	0.86 (0.52–1.40)
GC	rs7041	C	A	52/142	64/191	33/81	1.03 (0.79–1.35)	26/199	69/314	23/139	1.17 (0.88–1.55)
GC	rs222014	G	A	119/339	27/74	2/4	1.14 (0.73–1.77)	93/509	24/134	2/9	0.95 (0.61–1.48)
GC	rs222016	A	G	105/310	37/99	7/10	1.25 (0.87–1.80)	82/460	31/169	5/24	1.02 (0.71–1.49)
GC	rs705117	A	G	105/307	38/95	6/14	1.08 (0.76–1.55)	78/466	40/171	1/16	1.19 (0.83–1.73)
GC	rs2282679	A	C	82/223	56/163	10/33	0.95 (0.70–1.29)	57/325	50/270	12/60	1.07 (0.79–1.44)
GC	rs12512631	A	G	60/166	76/196	12/53	0.90 (0.67–1.20)	56/313	54/267	8/72	0.91 (0.67–1.24)
C10orf88	rs6599638	G	A	52/123	66/204	30/85	0.82 (0.62–1.08)	38/206	54/309	24/129	0.97 (0.73–1.29)
CYP2R1	rs2060793	G	A	50/163	76/181	23/73	1.07 (0.82–1.41)	47/249	55/304	17/99	0.92 (0.69–1.23)
CYP24A1	rs2248359	G	A	57/148	64/197	27/74	0.98 (0.74–1.28)	46/224	57/303	16/122	0.81 (0.60–1.08)
CYP24A1	rs2762942	A	G	133/382	15/36	0/1	1.11 (0.60–2.06)	106/572	12/81	1/1	0.91 (0.50–1.65)
CYP24A1	rs4809958	A	C	102/302	41/108	5/7	1.21 (0.83–1.74)	81/466	34/174	4/13	1.22 (0.84–1.79)
CYP24A1	rs6013897	T	A	94/260	44/141	10/16	1.05 (0.76–1.46)	80/378	37/243	2/30	0.69 (0.48–1.00)
CYP24A1	rs6022999	A	G	87/235	52/159	9/23	0.97 (0.70–1.33)	69/382	48/228	2/44	0.85 (0.60–1.19)
CYP27B1	rs703842	A	G	71/174	65/187	12/54	0.79 (0.59–1.07)**	50/303	48/279	21/67	1.28 (0.96–1.70)**
DHCR7	rs1790349	A	G	113/295	26/116	10/6	0.93 (0.64–1.36)	77/472	38/168	3/14	1.34 (0.93–1.93)
NADSYN1	rs3829251	G	A	111/285	26/116	9/10	0.84 (0.58–1.21)	77/457	35/173	3/15	1.19 (0.82–1.73)
NADSYN1	rs12785878	A	C	79/221	54/172	15/26	1.06 (0.79–1.44)	62/350	45/251	12/52	1.15 (0.84–1.56)

[1] Gleason 7–10

All models adjusted for age, BMI, PSA, and family history of prostate cancer

† Main effects p-value significant after a within gene Bonferroni adjustment. Significance thresholds among genes were: *VDR* =0.013 (4 tests), *GC* =0.008 (6 tests), *C10corf88* =0.05 (one test), *CYP2R1* =0.05 (one test), *CYP24A1* = 0.01 (5 tests), *CYP27B1* = 0.05 (1 test), *DHCR7* = 0.05 (1 test), and *NADSYN1* = 0.025 (2 tests).

** p-interaction <0.05.

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Table 4.

Associations of genotypes with season-adjusted serum 25(OH)D among Caucasian men in the PCPT placebo arm.

			Number of minor alleles					
rs number	**Allele**		**0**		**1**		**2**	
	Major	**Minor**	**Case/Ctl**	**Mean 25(OH)D[1]**	**Case/Ctl**	**Mean 25(OH)D[1]**	**Case/Ctl**	**Mean 25(OH)D[1]**
rs1544410	G	A	222/233	59.44	301/315	58.75	100/101	60.61
rs11568820	G	A	405/418	58.19	206/207	61.67	22/26	59.45
rs757343	G	A	482/489	59.51	138/153	58.59	8/8	61.61
rs11574143	G	A	523/525	59.26	107/122	59.83	3/6	54.10
rs7041	C	A	186/199	62.48**	312/314	59.14**	127/139	55.60**
rs222014	G	A	508/509	59.23	117/134	59.52	7/9	63.86
rs222016	A	G	455/460	59.02	166/169	60.10	11/24	59.80
rs705117	A	G	466/466	59.30	152/171	58.61	14/16	66.53
rs2282679	A	C	311/325	61.90**	264/270	58.03**	60/60	52.13**
rs12512631	A	G	287/313	57.14**	281/267	60.46**	64/72	64.86**
rs6599638	G	A	179/206	59.42	304/309	59.31	141/129	59.70
rs2060793	G	A	237/249	57.34**	294/304	59.79**	103/99	63.04**
rs2248359	G	A	241/224	59.67	309/303	59.96	79/122	56.74†
rs2762942	A	G	570/572	59.24	60/81	60.18	5/1	59.31
rs4809958	A	C	460/466	59.50	159/174	59.08	13/13	56.21
rs6013897	T	A	391/378	60.07	210/243	58.06	31/30	59.10
rs6022999	A	G	364/382	59.71	238/228	59.40	31/44	54.98
rs703842	A	G	282/303	59.18	271/279	58.57	81/67	62.58
rs1790349	A	G	443/472	59.47	167/168	58.53	22/14	63.21
rs3829251	G	A	442/457	59.91	159/173	57.13	21/15	64.36
rs12785878	A	C	349/350	60.97**	240/251	57.71**	45/52	56.20**

[1] Means are geometric means.

[2] <49.5 nmol/L

[3] 49.5–66.9 nmol/L

[4] ≥66.9 nmol/L

[**] P-value comparing mean serum 25(OH)D concentration by genotype significant after a within gene Bonferroni adjustment, calculated using linear regression with log[25(OH)D] as dependent variable. Significance thresholds among genes were: *VDR* = 0.013 (4 tests), *GC* = 0.008 (6 tests), *C10corf88* = 0.05 (1 test), *CYP2R1* = 0.05 (1 test), *CYP24A1* = 0.01 (5 tests), *CYP27B1* = 0.05 (1 test), *DHCR7* = 0.05 (1 test), and *NADSYN1* = 0.025 (2 tests).

[†] P-interaction = 0.049 for test of serum 25(OH)D x SNP interaction in relation to prostate cancer risk. All other p-interaction tests>0.10

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