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Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk

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We systematically investigated the association of 48 SNPS in four vitamin D metabolizing genes [*CYP27A1*, *GC*, *CYP27B1* and *CYP24A1*] with serum 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] levels and the association of these SNPS and an additional 164 SNPS in eight downstream mediators of vitamin D signaling [*VDR*, *RXRA*, *RXRB*, *PPAR*, *NCOA1*, *NCOA2*, *NCOA3* and *SMAD3*] with prostate cancer risk in the 749 incident prostate cancer cases and 781 controls of the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. 25(OH)D (all cases and controls) and 1,25$(OH)_2$D (a subset of 150 controls) levels were measured by radioimmunoassay and SNP data were genotyped as part of a genome-wide scan. Among investigated SNPS, only four tag SNPS in *GC*, the major serum 25(OH)D carrier, were associated with 25(OH)D levels; no SNPS were associated with 1,25$(OH)_2$D levels. None of the 212 SNPS examined were associated with cancer risk overall. Among men in the lowest tertile of serum 25(OH)D (<48.9 nmol/l), however, prostate cancer risk was related to tag SNPS in or near the 3′ untranslated region (UTR) of *VDR*, with the strongest association for rs11574143 [odds ratio (95% confidence interval) for risk allele carriers versus wild-type: 2.49 (1.51–4.11), $P = 0.0007$]; the genotype associations were null among men in tertile 2 and tertile 3. Results from the most comprehensive evaluation of serum vitamin D and its related genes to date suggest that tag SNPS in the 3′ UTR of *VDR* may be associated with risk of prostate cancer in men with low vitamin D status.

Introduction

Vitamin D inhibits proliferation and differentiation *in vitro* of human prostatic cells (1) and limits growth of prostate tumors in animal models (2). However, results from epidemiological studies investigating the association between vitamin D serum levels and prostate cancer risk are largely inconsistent (3–12). It is possible that the association of serum vitamin D with cancer risk could be modified by common genetic variations in vitamin D-related genes, potentially explaining for some of the inconsistency.

25-Hydroxyvitamin D [25(OH)D], the pro-hormonal major circulating form of vitamin D, is metabolically activated to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) and is deactivated by cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1) in the kidney (13,14) (Figure 1). 25(OH)D and 1,25$(OH)_2$D are transported in serum by the vitamin D-binding protein (gene name: *GC*, group-specific component), although 1,25$(OH)_2$D can also be generated locally in some tissue, such as the prostate (15). 1,25$(OH)_2$D binds to vitamin D (1,25-dihydroxyvitamin D3) receptor (VDR), a nuclear receptor (16,17), at target organs, forming heterodimers with retinoid X receptor (RXR) (18) and recruiting other transcriptional cofactors [e.g. PPAR, nuclear receptor coactivator (NCOA) and SMAD] to regulate target gene transcription, including those involved in cell proliferation, differentiation and apoptosis (19).

The association between SNPS in *VDR*, *CYP27B1* and *CYP24A1* and prostate cancer has been investigated in previous studies. Meta-analyses for five restriction fragment length polymorphisms (RFLPs; BsmI, ApaI, TaqI, poly(A) and FokI) in *VDR* show null or inconsistent results (20); however, recent resequencing data show that these few SNPS cover the common genetic variation within this large gene only to a very limited extent (21). A recent population based case–control study, evaluating 22 *VDR* tag SNPS, reported associations between two tag SNPS in *VDR* and prostate cancer risk, but did not observe associations with tag SNPS in *CYP27B1* or *CYP24A1* (22). Three studies examined the interaction of the limited number of *VDR* RFLPs and serum vitamin D levels in relation to prostate cancer, but results were also inconsistent (10,11,23).

In this study, we comprehensively investigated prostate cancer risk in relation to serum 25(OH)D and polymorphic variants in genes encoding for enzymes that synthesize, carry and degrade vitamin D [*CYP27A1*, *GC*, *CYP27B1* and *CYP24A1* and in genes that encode for downstream mediators of vitamin D signaling [*VDR*, *RXRA*, *RXRB*, *PPAR*, *NCOA1*, *NCOA2*, *NCOA3* and *SMAD3*] in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. We also investigated the association of circulating vitamin D levels [25(OH)D and 1,25$(OH)_2$D] with genes encoding for enzymes that synthesize, carry and degrade vitamin D (*CYP27A1*, *GC*, *CYP27B1* and *CYP24A1*).

Abbreviations: CGEMS, Cancer Genetic Markers of Susceptibility; CYP24A1, cytochrome P450, family 24, subfamily A, polypeptide 1; CYP27A1, cytochrome P450, family 27, subfamily A, polypeptide 1; CYP27B1, cytochrome P450, family 27, subfamily B, polypeptide 1; FDR, false discovery rate; GC, group-specific component; LD, linkage disequilibrium; NCOA1, nuclear receptor coactivator 1; 25(OH)D, 25-hydroxyvitamin D; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; OR, odds ratio; PLCO, Prostate, Lung, Colorectal and Ovarian; RFLP, restriction fragment length polymorphism; RXRA, retinoid X receptor, alpha; RXRB, retinoid X receptor, beta; UTR, untranslated region; VDR, vitamin D (1,25-dihydroxyvitamin D3) receptor.

Materials and methods

Study setting

The PLCO Cancer Screening Trial is a large, randomized controlled multicenter trial in the USA (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO and Washington, DC) of ~155 000 men and women, designed to evaluate selected methods for the early detection of these four cancers (24,25). PLCO enrollment began in 1993 and ended in 2001. Participants have been randomized to either a screening or control arm. The men in the screening arm were offered prostate cancer screening by serum prostate-specific antigen at entry and annually for 5 years and digital rectal examination at entry and annually for 3 years. Men with a positive screening result (prostate-specific antigen test >4 ng/ml or digital rectal examination suspicious for prostate cancer) were referred to their medical care providers for prostate cancer diagnostic evaluation. Incident cases of prostate cancer were also ascertained from annually mailed questionnaires to participants. We acquired all medical and pathologic records related to prostate cancer diagnosis for all men with suspected prostate cancer by screening examination or annual questionnaire. Data were abstracted by trained medical record specialists. Screening arm participants were asked to provide a blood sample at

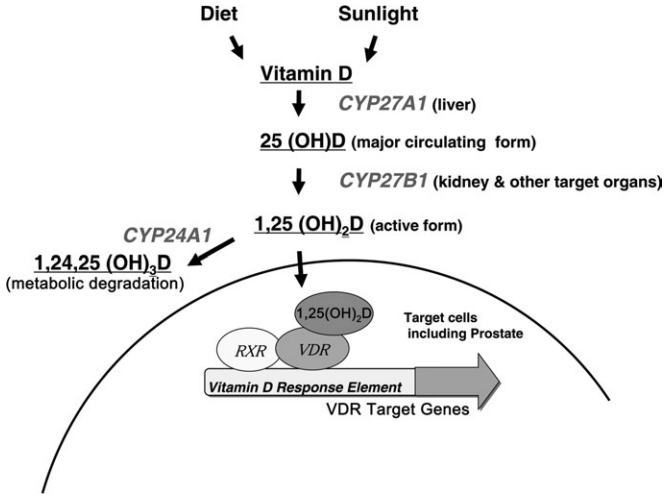

Fig. 1. Vitamin D metabolism and function.

each screening visit. The institutional review boards of the USA National Cancer Institute and the 10 study centers approved the trial and participants provided written informed consent.

Study population

Subjects for this study were participants in the National Cancer Institute Cancer Genetic Markers of Susceptibility (CGEMS) investigation of SNPS and prostate cancer risk; details of case and control selection have been described elsewhere (26). Briefly, of the 38 350 men randomized to the screening arm of the trial, cases and controls were selected from men who were of non-Hispanic white race/ethnicity, had no prior history of prostate of cancer before randomization, had at least one (PLCO) prostate cancer screen (prostate-specific antigen testing) before 1 October 2003, had completed a baseline questionnaire about risk factors for cancer and had provided a blood sample. Clinical stage I and II tumors with Gleason sum <7 were defined as non-aggressive. Clinical stage III and IV tumors and/or tumors with Gleason sum ≥ 7 were defined as aggressive. For CGEMS, 1172 prostate cancer cases were selected for study, including all eligible aggressive cancer cases and a randomly selected subset (70.4%) of non-aggressive cases. A total of 1157 controls were selected by incidence-density sampling (27) with a case–control ratio of 1:1 frequency matched by age at cohort entry (5 year intervals), time since initial screening (1 year time window) and calendar year of cohort entry. For the serum-based study of vitamin D, we excluded prevalent cases (defined as cases diagnosed within the first year of follow-up since initial screening) and their corresponding controls to reduce the potential influence of subclinical cancer on vitamin D concentrations, leaving 749 cases and 781 controls for study (Figure 2).

Further, in CGEMS, we examined significant main effects of SNPS associated with prostate cancer in PLCO and in four additional replication studies totaling 4020 cases and 4028 controls (American Cancer Society Cancer Prevention Study II, 1790/1797; the Health Professionals Follow-up Study, 619/620; the CeRePP French Prostate Case–Control Study, 671/671 and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, 940/940) (26).

Genotyping

Data on 212 tag SNPS in 12 genes were accessed from a whole-genome scan of PLCO prostate cancer cases and controls in the CGEMS Study, a genome-wide association study (Phase 1A with HumanHap300 and Phase 1B HumanHap240 assays, both from Illumina Corp., San Diego, CA), including 561 494 tag SNPS. The materials and methods of the genome-wide scan have been reported (26) and are also available at http://cgems.cancer.gov/data/. SNPS for analysis were selected from HapMap for minor allele frequencies ($>5\%$) and r^2 (>0.8), described by Carlson *et al.* (28). The VDR FokI polymorphism (rs10735810), not characterized in HapMap, was additionally genotyped by TaqMan assay. About 6% [13 SNPS; one for *CYP27B1*, one for *CYP24A1*, six for *NCOA1*, three for *RXRA*, one for *VDR* (rs1544410) and one for SMAD3; http://cgems.cancer.gov/data/] of the 212 tag SNPS selected for our study of vitamin D-related genetics had a significant ($P < 0.05$) departure from Hardy–Weinberg proportion, which is consistent with what would be expected by chance. We included all 212 tag SNPS for the subsequent analyses. Sixty-one additional SNPS in the *VDR* genomic region (from 2 kb upstream of the transcription start site to 10 kb beyond the last exon), listed in HapMap, including three commonly studied *VDR* RFLPs [ApaI (rs7975232), TaqI (rs731236) and Cdx2 (rs11568820)], were imputed using the observed genotypes, the HapMap CEPH European reference panel and the MACH imputation program (http://sph.umich.edu/csg/abecasis/MACH/). We included only imputed SNPS with a r^2 quality score >0.8.

Vitamin D assay

Non-fasting blood specimens collected at the clinical centers were processed and frozen within 2 h of blood draw and stored at -70°C. Baseline serum 25(OH)D concentrations in case and control participants and $1,25(OH)_2D$ concentrations in a subset of 150 randomly selected control participants were measured by radioimmunoassay (Heartland Assays, Ames, IA) (29). Laboratory personnel were blinded to case–control status. Multiple blinded quality control samples from four different individuals were included in all batches; the coefficients of variation were 5.9% for 25(OH)D and 8.5% for $1,25(OH)_2D$.

Assessment of questionnaire-based covariates

At enrollment, all participants were asked to complete a baseline questionnaire including age, ethnicity, education, current and past smoking behavior, history of cancer and other diseases, use of selected drugs, recent history of screening exams and prostate-related health factors. Usual dietary intake over the 12 months before enrollment was assessed with a 137-item food frequency questionnaire.

Statistical analysis

Gene-based associations were assessed by log-likelihood tests for each gene comparing models with and without terms for each SNP (genotype coded as 0, 1 and 2) in a given gene (degree of freedom $=1 \times$ number of SNPS per gene). For highly correlated SNPS ($r^2 > 0.9$) within a gene, only one of the SNPS was included in the model, to avoid collinearity.

We used unconditional logistic regression analysis to assess the association of genotypes with overall prostate cancer (1 degree of freedom log-likelihood ratio test) for each of the tag SNPS, adjusting for age in 5 years, and study center. Results are similar when we used conditional logistic regression. Further adjustment for family history of prostate cancer (yes/no), body mass index (<25, 25–29.9 and ≥ 30 kg/m^2), vigorous physical activity (0, 1, 2, 3, 4 and 5+ h/week), daily aspirin and/or ibuprofen use (none, aspirin only, ibuprofen only and aspirin and ibuprofen both), smoking status (never, current, former and cigar or pipe only), total energy (quintile, kilocalories per day), dairy products, (quintile, servings per day), dietary vitamin D (quintile, international units per day), supplemental vitamin D (quintile, international units per day), dietary calcium (quintile, milligrams per day), supplemental calcium (quintile, milligrams per day) and history of diabetes did not materially affect the results ($<10\%$ change of the beta-coefficients; data not shown).

Haplotype frequencies, odd ratios (ORs) and 95% CIs were estimated for genes with multiple SNPS using Haplo Stats (30). This program reconstructs haplotypes and estimates ORs simultaneously based on a suitable expectation–maximization algorithm (31,32). We have also performed a sliding window analysis for associations of the *VDR* haplotypes with prostate cancer risk for a window of three adjacent tagging markers, sliding across the map in single-marker increments, to gain further insight into the potential causal region (30).

To explore whether the main effect of genotype on prostate cancer risk was modified by serum 25(OH)D level, genotype data were stratified by tertiles of serum 25(OH)D as defined from the distribution in controls, with associations between genetic variation and risk examined within each stratum. Because 25(OH)D concentrations varied by season of blood collection, we calculated season-standardized 25(OH)D using residuals from locally weighted polynomial regression models to describe the deviation of 25(OH)D from the predicted weekly average (12,33). We did not use season standardization for $1,25(OH)_2D$ because it did not vary by season of blood collection (data not shown). To test statistical interactions on a multiplicative scale, a cross product term of the ordinal score for each genotype and the 25(OH)D tertile variables was included in multivariate models. In a joint analysis of VDR SNPS and 25(OH)D (Table III), we additionally adjusted for diabetes in the model since diabetes was a confounder of the relationship between 25(OH)D and prostate cancer risk in our previous analysis (12).

The relationship between SNPS in for genes, *CYP27A1*, *GC*, *CYP27B1* and *CYP24A1* and serum 25(OH)D and 1,25(OH)2D concentrations were assessed in the control group using linear regression, after log transformation of serum values. Unless otherwise specified, statistical analyses were performed with SAS Version 9.1 (SAS Institute, Cary, NC). All statistical tests performed were two sided.

We evaluated the robustness of our results using the false discovery rate (FDR). FDR is the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypothesis among all the genes or SNPS analyzed in this report. The Benjamini and Hochberg method (34) was used to calculate FDR values using the 'multtest' package in the R project for statistical analyses (http://www.r-project.org).

Results

Genetic variation in genes related to vitamin D metabolism and serum vitamin D concentration

We examined the association of tag SNPS in four genes related to vitamin D metabolism (i.e. *CYP27A1*, *GC*, *CYP27B1* and *CYP24A1*) with serum 25(OH)D and $1,25(OH)_2D$ concentrations (Table I). Four SNPS in *GC* {rs12512631 [3′ untranslated region (UTR)], rs2282679 (intron 12), rs7041 (exon 11; Glu432Asp) and rs1155563 (intron 1)} with high linkage disequilibrium (LD) were associated with modest differences in serum 25(OH)D [≤8.2 nmol/l, $P \leq 0.0004$, Figure 3 (see LD structure); Table I]. When these SNPS were simultaneously included in the multivariate model, associations remained for rs2282679 and rs1155563, but others were no longer significantly associated with serum 25(OH)D concentrations. No other tag SNPS examined were associated with serum 25(OH)D concentrations. Tag SNPS examined were not associated with serum 1,25(OH)D concentrations; however, the sample size was limited ($n = 150$). Other tag

Table I. Adjusted mean of 25(OH)Vitamin D and $1,25(OH)_2$ Vitamin D concentrations in relation to vitamin D metabolism-related tag SNPS in control subjects of the PLCO Cancer Screening Trial[a]

Gene	dbSNP ID[b]	Risk (variant) allele	Adjusted geometric mean 25(OH)D (nmol/l) ($N = 781$)					Adjusted geometric mean $1,25(OH)_2D$ (pg/ml) ($N = 150$)				
			Wild-type	Heterozygote	Homozygote variant	*P* value	*n* (wild-type, heterozygote, homozygote variant)	Wild-type	Heterozygote	Homozygote variant	*P* value	*n* (wild-type, heterozygote, homozygote variant)
CYP27A1	rs4674338	A	55.12	56.96	56.22	0.41	269, 345, 126	35.4	37.9	38.2	0.33	47, 68, 30
	rs7568196	A	55.69	57.41	56.18	0.57	283, 326, 117	38.4	36.2	31.9	0.11	78, 59, 10
	rs13013510	A	55.84	58.02	54.11	0.36	183, 369, 183	37.3	37.5	36.2	0.82	50, 65, 29
	rs11677711	G	56.45	56.11	62.14	0.94	661, 74, 1	37.4	37.4	36	0.79	46, 71, 30
	rs4674345	A	55.47	57.56	54.24	0.51	186, 373, 182	37	39.5	—	0.43	134, 11, 0
	rs6436094	G	56.77	55.13	56.62	0.43	407, 290, 41	35.4	37.7	38.5	0.32	47, 69, 31
GC	rs12512631	C	54.51	56.18	62.74	**0.0004**	313, 326, 97	37.2	36.8	38.9	0.92	109, 35, 3
	rs705117	G	56.31	54.69	61.00	0.78	540, 180, 21	37.9	38	35.9	0.93	134, 12, 1
	rs2282679	C	59.21	53.47	52.63	**0.000043**	371, 300, 65	37.3	37.1	34.5	0.85	105, 36, 4
	rs16846912	G	56.43	56.04		0.96	729, 6	37	37.8	—	0.76	127, 20, —
	rs1491709	T	56.18	55.24	62.81	0.95	633, 102, 6	37.1	43.2	—	0.38	143, 2, —
	rs7041	T	59.80	54.66	53.36	**0.0004**	233, 358, 150	38.4	35.8	34.9	0.44	98, 42, 5
	rs4752	C	56.42	56.05		0.96	730, 6, 0	38.4	34.6	—	0.06	110, 37, —
	rs222014	A	55.71	57.07	58.02	0.41	592, 137, 7	38.7	36.8	35.2	0.34	47, 77, 23
	rs222016	G	56.41	55.62	66.65	0.47	536, 182, 18	37.1	43.2	—	0.35	143, 2, —
	rs222020	C	56.01	55.63	66.06	0.36	538, 185, 18	37.5	36	—	0.49	115, 32, —
	rs1155563	C	59.35	53.00	54.54	**0.0002**	375, 288, 74	35.8	38.3	36.8	0.35	53, 74, 18
	rs1352844	T	55.40	58.78	57.54	0.07	579, 150, 12	37.8	37.8	33.1	0.31	77, 56, 12
	rs2298849	C	55.99	57.06	60.56	0.23	499, 210, 26	37.2	37.3	34.5	0.86	105, 387, 4
	rs3733359	T	56.23	55.30	45.54	0.43	662, 76, 3	38.4	36.3	32.4	0.10	80, 54, 12
	rs843006	T	56.10	56.77	61.95	0.24	507, 203, 25	37.9	35.6	34.9	0.40	100, 40, 5
CYP27B1	rs1048691	T	56.26	56.55	48.47	0.16	452, 234, 33	37.6	37.4	29.7	0.30	84, 52, 4
	rs4646537	C	56.40	56.86	50.73	0.94	687, 48, 1	37.1	39.1	—	0.39	136, 9, —
	rs703842	A	56.40	56.86	50.73	0.46	340, 310, 91	37.5	35.9	39.2	0.39	72, 55, 20
	rs8176345	C	55.19	56.60	57.80	0.18	693, 48, 0	37.4	33.2	—	0.2	13, 10, —
	rs10877013	T	55.44	56.98	58.21	0.15	339, 306, 91	37.4	36.1	39.2	0.49	72, 53, 20
CYP24A1	rs6127112	A	56.03	57.80	59.46	0.26	579, 148, 9	37.6	36.1	40.1	0.46	80, 59, 8
	rs2762926	T	55.45	56.85	56.91	0.40	226, 366, 143	37	37.7	36.1	0.77	53, 68, 24
	rs2585413	A	56.06	56.06	56.57	0.90	387, 298, 56	36.9	37.7	36.4	0.85	43, 74, 28
	rs6097797	C	55.30	58.80	57.42	0.06	554, 164, 23	37	37.9	30.3	0.70	106, 38, 1
	rs2762929	C	55.70	56.41	57.72	0.34	274, 349, 113	36.8	40.5	—	0.17	132, 15, —
	rs8124792	A	56.10	56.06	60.58	0.93	664, 75, 2	37.5	36.1	29.3	0.63	110, 36, 1
	rs6097801	A	55.29	58.99	56.59	0.06	556, 163, 21	37.1	36.5	38.4	0.63	38, 72, 37
	rs2762932	C	57.20	54.43	55.52	0.11	547, 177, 12	38.3	37.4	34.5	0.82	108, 33, 5
	rs927650	T	55.98	55.43	58.00	0.36	202, 382, 157	36.8	38.5	30.8	0.07	78, 60, 9
	rs912505	G	55.05	57.62	58.35	0.06	446, 256, 39	37.1	37.5	38.3	0.89	108, 35, 2
	rs6068816	T	55.91	57.33	54.38	0.59	609, 123, 9	37.5	36.3	33.6	0.61	109, 34, 4
	rs4809958	G	55.83	56.78	57.66	0.48	531, 191, 19	37.5	35.2	40.3	0.04	44, 67, 36
	rs3787557	C	56.15	56.56	63.81	0.52	535, 193, 8	37.2	37	38.9	0.92	107, 34, 4
	rs4809959	A	57.02	54.80	57.88	0.73	199, 369, 173	37.2	37.5	32.2	0.50	87, 55, 5
	rs4809960	C	56.51	55.04	58.80	0.95	391, 295, 55	37.2	37.3	31.3	0.70	123, 22, 2
	rs2248359	T	56.63	55.47	56.73	0.84	268, 356, 117	37.6	35.6	—	0.32	116, 29, —
	rs2585423	G	55.62	57.83	57.14	0.20	554, 174, 13	37.2	37.5	34.6	0.76	108, 34, 5
	rs2585422	C	56.50	55.78	64.00	0.93	558, 169, 9	37.3	37	37	0.98	73, 60, 14
	rs765058	A	55.92	56.66	54.63	0.99	384, 307, 50	37.1	37.6	35.9	0.79	61, 64, 22
	rs6023009	T	58.13	54.79	56.52	0.17	284, 342, 110	37.1	36.8	39.5	0.72	93, 44, 10
	rs1555439	T	57.34	53.98	55.33	0.05	457, 241, 42	37.4	37.3	36.4	0.90	62, 60, 20
	rs6068824	C	57.66	55.57	56.35	0.37	257, 352, 131	36.8	36.7	39.2	0.47	57, 63, 26

[a]Used only controls subjects and the 1 degree of freedom trend from a generalized linear regression model adjusted for age in 5 year intervals, vitamin D intake, diabetes and study center. Used season-standardize 25(OH)D.
[b]SNP identifier based on NCBI dbSNP; we included SNPS occurring between 2 kb before each genes transcriptional start site and up to 10 kb after the last exon.

SNPS in genes that encode for downstream mediators of vitamin D signaling (*VDR*, *RXRA*, *RXRB*, *PPAR*, *NCOA1*, *NCOA2*, *NCOA3* and *SMAD3*) were not associated with 25(OH)D or 1,25(OH)D concentrations in *post hoc* exploratory analyses.

Genetic variation in the vitamin D pathway and prostate cancer risk

Overall, variation in the 12 vitamin D-related genes examined was not associated with prostate cancer risk among prostate cancer cases and controls in PLCO with serum measurements (Table II, global $P \geq 0.05$). Also, none of the individual tag SNPS, including the two previously implicated *VDR* BsmI (rs1544410) and FokI (rs10735810), were strongly associated with prostate cancer risk. Although several SNPS in *NCOA2* (rs3812430, rs2926703, rs6472520 and rs7823221), one SNP in *GC* (rs705117) and one SNP in *VDR* (rs11574143) were weakly associated with risk (*P* values ranged 0.02–0.06), these weak associations were not significant in the CGEMS replication follow-up studies (data not shown; all *P* values > 0.05). Since genetic variation in *VDR* has been implicated in prostate cancer risk in several studies, we imputed the additional common variants in *VDR* available from HapMap and explored their association with prostate cancer risk. None of the 61 imputed SNPS in *VDR*, including those known by an older nomenclature, ApaI (rs7975232), TaqI (rs731236) and Cdx2 (rs11568820), were associated with prostate cancer in PLCO overall (data not shown). The association results did not differ from the null for disease aggressiveness (Table II), age at diagnosis (data not shown) or family history of prostate cancer (data not shown).

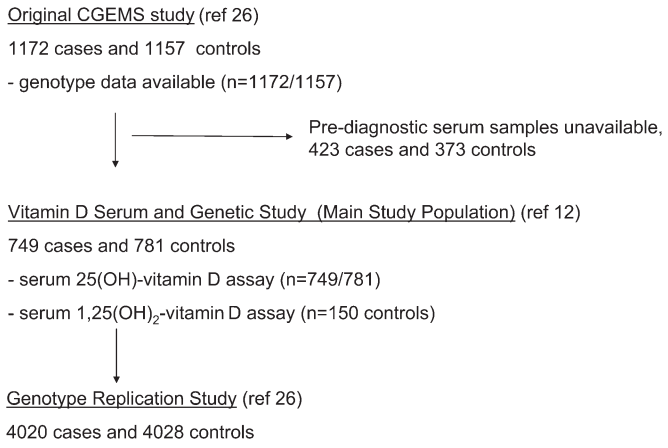

Fig. 2. Consort flow diagram of the study.

Genetic variation in the vitamin D pathway and prostate cancer risk, stratified by serum vitamin D concentrations

A significant association was observed between *VDR* genetic variation and prostate cancer risk in men in the lowest tertiles of serum 25(OH)D (<48.9 nmol/l) (global $P = 0.03$; Table II); these associations were null for men in the higher two tertiles (global $P = 0.47$ for tertile 2 and 0.45 for tertile 3). Individual SNP analyses (Figure 4, Table III) showed significant associations in men in the lowest tertiles of serum 25(OH)D with three tag SNPS in or near the 3′ UTR of *VDR* [OR (95% CI) for risk allele carriers = 2.49 (1.51–4.11, *P* trend = 0.0007) for

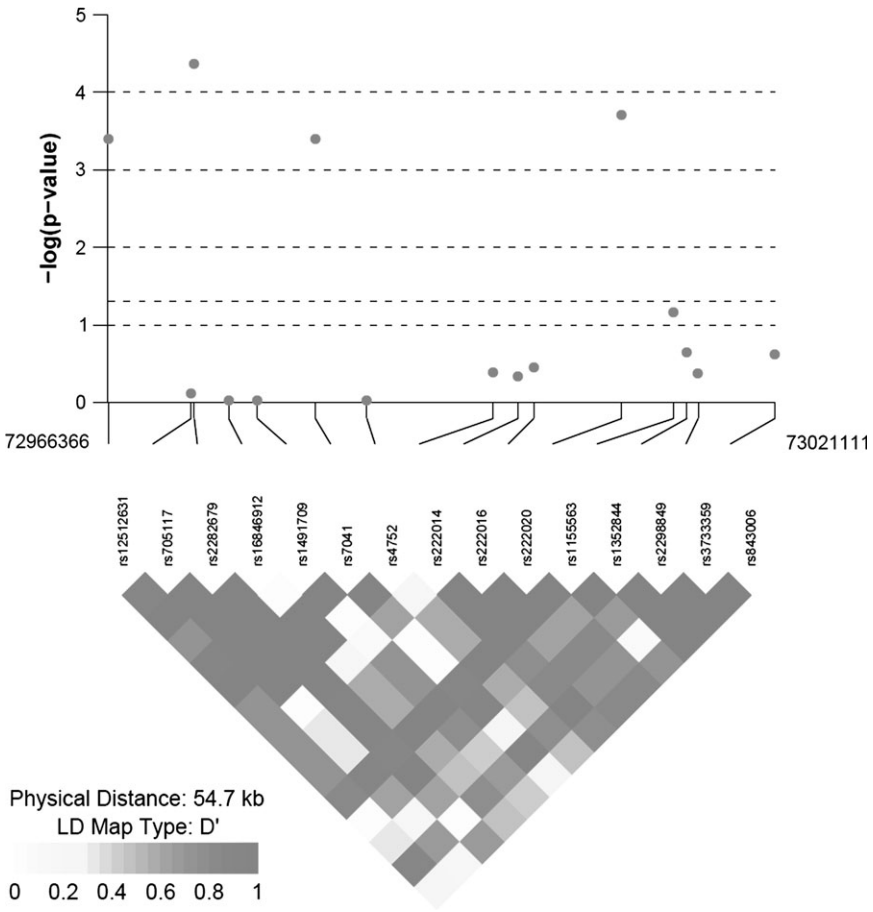

Fig. 3. *P* values for the association between tag SNPS in *GC* and 25(OH) vitamin D concentrations in the control group of the PLCO Trial (chromosome 4; shown 3′ → 5′ direction).

Table II. Associations between prostate cancer and common genetic variation in selected vitamin D-related genes in the PLCO Cancer Screening Trial

Associated genes[a]	Number of SNPS per gene	Global *P* value for genotype association[b,c]				
		Total prostate cancer (n = 749 cases and 781 controls)				Aggressive prostate cancer[c] (n = 466 case and 781 controls)
		Total population	Low serum 25(OH)D	Medium serum 25(OH)D	High serum 25(OH)D	Total population
CYP27A1	6	0.44	0.64	0.45	0.26	0.75
GC	15	0.96	0.86	0.95	0.20	0.94
CYP27B1	5	0.83	0.36	0.79	0.63	0.66
CYP24A1	22	0.70	0.21	0.35	0.83	0.35
VDR	28	0.30	**0.03**	0.47	0.45	0.08
RXRA	11	0.93	0.73	0.18	0.79	0.92
RXRB	12	0.47	0.45	0.25	0.76	0.55
PPAR	3	0.63	0.52	0.13	0.23	0.42
NCOA1	11	0.23	0.63	**0.02**	0.10	0.24
NCOA2	29	0.05	0.05	0.10	0.06	0.15
NCOA3	19	0.14	0.31	0.68	0.88	0.15
SMAD3	46	0.59	0.66	0.08	0.76	0.69

[a]GC, vitamin D-binding protein (alias: VDBP), CYP24A1, 20q13; PPAR-binding protein (aliases: DRIP205); SMAD3, SMAD, mothers against DPP homolog 3.
[b]Global *P* value was calculated by log-likelihood ratio test for each gene comparing models with and without terms for each SNP (genotype coded as 0, 1 and 2) in a given gene (degree of freedom = 1 × number of SNPS per gene).
[c]Global *P* values for aggressive disease (defined as Gleason score 7+ or Stage III+) are shown in parentheses.
Note: VDR global P values based on 89 SNPS (28 genotyped and 61 imputed SNPS) were 0.40 and 0.21 for total and aggressive prostate cancers.

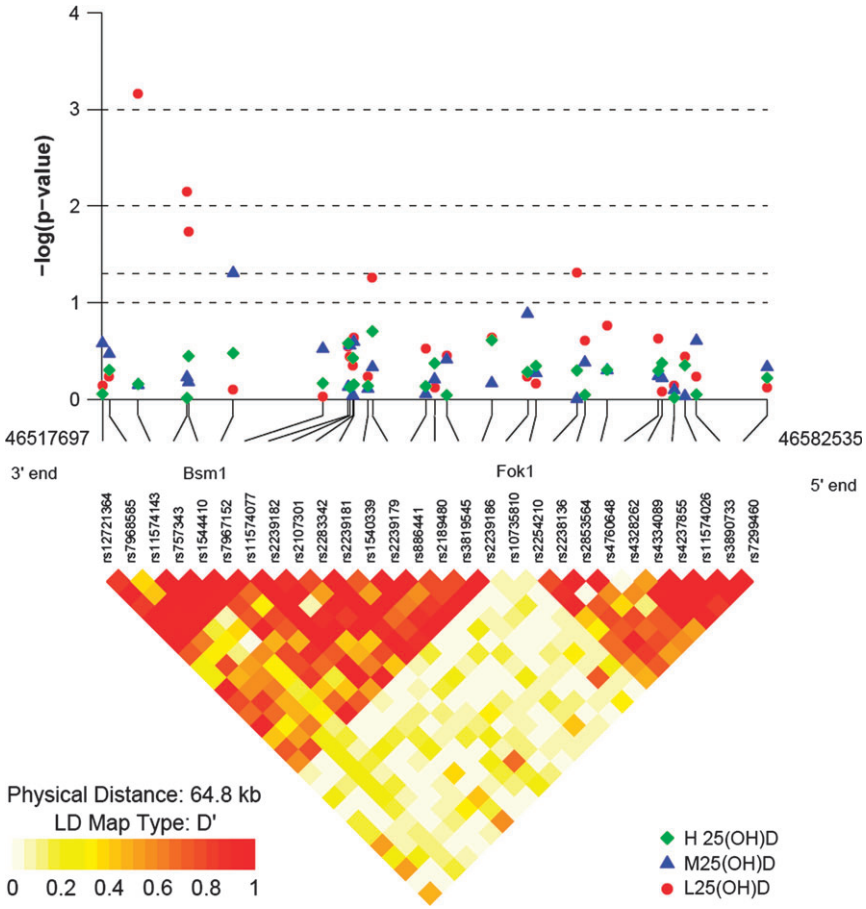

Fig. 4. *P* values for the association between tag SNPS in *VDR* and prostate cancer risk according to tertiles of 25(OH) vitamin D. Each red dot represents –log (*P* value) among men in the lowest tertile of 25(OH) vitamin D and each triangle and each square represents among men in the second and third tertile of 25(OH) vitamin D, respectively (chromosome 12; shown 3′ → 5′ direction). Note: ApaI and TaqI are located between rs757343 and rs11574143 (see supplementary Figure 1, available at *Carcinogenesis* Online).

Table III. Multivariate ORs and 95% CIs for the association between *VDR* tag SNPS and prostate cancer according to 25(OH)D concentrations in the PLCO Cancer Screening Trial ($n = 749$ cases and 781 controls)

dbSNP ID	Allele	Total population		25(OH)D tertile 1[a]		25(OH)D tertile 2		25(OH)D tertile 3		*P* trend	Interaction
		Case/control	OR (95% CI)	Case/control	OR (95% CI)[b]	Case/control	OR (95% CI)	Case/control	OR (95% CI)		
rs11574143	AA	565/628	1.00 (reference)	147/216	1.00 (reference)	210/202	1.50 (1.12–2.00)	208/210	1.42 (1.06–1.90)		
	GA	138/101	1.48 (1.12–1.97)	51/29	2.53 (1.52–4.19)	50/41	1.70 (1.07–2.72)	37/31	1.66 (0.98–2.82)		
	GG	6/7	1.01 (0.34–3.04)	1/1	1.50 (0.09–24.48)	3/3	1.54 (0.30–7.87)	2/3	1.04 (0.17–6.39)		
	AA	565/628	1.00 (reference)	147/216	1.00 (reference)	210/202	1.50 (1.12–2.00)	208/210	1.42 (1.06–1.90)	**0.0085**	**0.02**
	GA + GG	144/108	1.45 (1.10–1.91)	52/33	2.49 (1.51–4.11)	53/44	1.69 (1.07–2.67)	39/34	1.61 (0.97–2.69)	**0.10**	
	P trend for genotype		**0.04**		**0.0007**		**0.71**		**0.69**		
rs757343	AA	534/592	1.00 (reference)	138/200	1.00 (reference)	201/194	1.48 (1.10–1.99)	195/198	1.40 (1.04–1.89)		
	AG	166/139	1.29 (0.99–1.67)	59/46	1.86 (1.19–2.90)	60/47	1.76 (1.13–2.75)	47/46	1.42 (0.89–2.27)		
	GG	13/10	1.46 (0.63–3.38)	2/2	1.49 (0.21–10.79)	6/5	1.76 (0.52–5.94)	5/3	2.31 (0.54–9.91)		
	AA	534/592	1.00 (reference)	138/200	1.00 (reference)	201/194	1.48 (1.10–2.00)	195/198	1.40 (1.04–1.89)	**0.01**	**0.16**
	AG + GG	179/149	1.30 (1.02–1.67)	61/48	1.84 (1.19–2.86)	66/52	1.76 (1.15–2.70)	52/49	1.48 (0.94–2.32)	**0.50**	
	P trend for genotype		**0.11**		**0.007**		**0.59**		**0.96**		
rs1544410 (BsmI)	AA (BB)	98/114	1.00 (reference)	22/42	1.00 (reference)	47/37	2.36 (1.20–4.66)	29/35	1.58 (0.77–3.27)		
	AG (Bb)	358/379	1.10 (0.81–1.49)	94/129	1.41 (0.79–2.54)	124/123	1.86 (1.04–3.32)	140/127	2.10 (1.18–3.73)		
	GG (bb)	257/247	1.20 (0.87–1.67)	83/77	2.05 (1.12–3.77)	96/86	2.17 (1.19–3.94)	78/84	1.70 (0.93–3.12)		
	AA	98/114	1.00 (reference)	22/42	1.00 (reference)	47/37	2.37 (1.20–4.67)	29/35	1.59 (0.77–3.27)	**0.084**	**0.05**
	AG + GG	615/626	1.14 (0.85–1.53)	177/206	1.66 (0.95–2.90)	220/209	1.99 (1.14–3.46)	218/211	1.94 (1.11–3.38)	**0.56**	
	P trend for genotype		**0.24**		**0.005**		**0.98**		**0.66**		

[a]Serum 25(OH) vitamin D based on tertiles (low, intermediate and high, respectively) of the control group, 48.9 and 63.4 nmol/l were the cutoffs between tertiles.
[b]Logistic regression adjusted for age in 5 year intervals, study center and diabetes.

rs11574143 (3611 bp 3′ of A > G), 1.84 (1.19–2.86, *P* trend = 0.007) for rs757343 (IVS10+443A>G) and 1.66 (0.95–2.90, *P* trend = 0.0005) for rs1544410 (IVS10+283A>G)]; the genotype-related association was null among men in the tertile 2 and 3. These three SNPS were in high LD (Figure 4); when adjusted for simultaneously in a multivariate model, associations remained for men with low vitamin D for rs11574143 ($P = 0.03$), but the others were no longer significantly associated with disease risk. FDR-adjusted *P* values for the *VDR* association with prostate cancer risk, taking into account all 27 SNPS and three 25(OH)D groups (total 81 tests), were 0.06 for rs11574143, 0.28 for rs757343 and 0.50 for rs1544410. Examining risks within genotype groups (Table III), greater serum vitamin D was related to increased risk of prostate cancer in A allele carriers only (*P* trend = 0.0085, 0.01 and 0.08, respectively, for rs11574143, rs757343 and rs1544410), as supported by interaction tests for risk (Table III). These patterns were similar for both aggressive and non-aggressive prostate cancer (data not shown).

Consistent with the SNP-based analysis, haplotype analysis revealed the strongest risks related to carriage of the GGG haplotype of the three *VDR* SNPS (rs11574143, rs757343 and rs1544410; Table IV). Sliding window haplotype analysis (supplementary Figure 1 is available at *Carcinogenesis* Online), based on 89 genotyped or imputed SNPS in *VDR* in men with low vitamin D status, suggested that additional unmeasured risk variants, around rs11574143, rs757343 and rs1544410, may influence prostate cancer risk. Prostate cancer risk associations for other SNPS examined did not differ according to serum 25(OH)D (all *P* interactions > 0.05; data not shown).

Discussion

In a comprehensive analysis of common polymorphic variation in vitamin D pathway genes, we found only weak associations with prostate cancer risk overall. However, in men with low serum 25(OH)-vitamin D status, tag SNPS in or near the 3′ UTR of *VDR* were associated with prostate cancer risk, suggesting an important gene–environment interaction. In addition, SNPS in *GC*, which encodes the major carrier protein for 25(OH)D, were moderately associated with serum 25(OH)D concentration, but not with prostate cancer risk.

VDR, a key mediator of the biological actions of $1,25(OH)_2D$, possesses adenylate/uridylate-rich elements (AUUUA) in the 3′ UTR that regulate messenger RNA stability (35–37), potentially altering *VDR* expression and the cellular response to changing circulating vitamin D levels. SNPS in this region have been linked to higher *VDR* messenger RNA stability and higher gene transcription activity in some but not all *in vitro* studies (38–40). Of the three SNPS (rs11574143, rs757343 and rs1544410) in the 3′ UTR region related to prostate cancer in men with low serum 25(OH)D in our study, findings were strongest for rs11574143 ($P = 0.0007$). However, our sliding window haplotype analysis suggested that the effect might be due to additional unmeasured risk variants, in LD with rs11574143, rs757343 and rs1544410. Future studies are warranted to examine comprehensively the effect of SNPS in the 3′ UTR on VDR expression.

Our results for rs1544410 (BsmI) are consistent with the Physician's Health Study's findings, both showing that carriage of the rs1544410 G allele is associated with higher risks of prostate cancer only in men with lower 25(OH)D levels (23), whereas in the Health Professionals Follow-up Study risks tended also to be increased with carriage of the G allele, but differences by serum 25(OH) were not seen (10).

A recent prostate cancer case–control study by Holick *et al.* (22) examined 22 tag SNPS across the *VDR* region and observed associations with two tag SNPS and prostate cancer risk: rs2107301, which we genotyped and found to be null, and rs2238135, which is in high LD with our genotyped rs2853564 ($D' = 1.0$; ~300 bp distance) and also found to be null. rs2107301 is, however, located in intron 4 and in weak LD with the risk-related SNPS in our study (Figure 3), whereas rs2238135 is located upstream in a different LD block. Our findings of no association between tag SNPS in *CYP27B1* or *CYP24A1* with prostate cancer risk are consistent with the Holick *et al.* study (22).

Table IV. Multivariate ORs and 95% CIs for the association between inferred *VDR* haplotypes and prostate cancer according to serum 25(OH)D concentration in the PLCO Cancer Screening Trial (n = 749 cases and 781 controls)

Haplotypes[a]	25(OH)D tertile 1				25(OH)D tertile 2				25(OH)D tertile 3			
	Case %	Control %	OR (95% CI)	*P* value	Case %	Control%	OR (95% CI)	*P* value	Case %	Control %	OR (95% CI)	*P* value
AAA	0.35	0.43	1.00 (reference)		0.41	0.40	1.00 (reference)		0.40	0.40	1.00 (reference)	
AAG	0.49	0.47	1.34 (1.00–1.80)	0.05	0.46	0.48	0.93 (0.71–1.21)	0.60	0.48	0.49	0.98 (0.74–1.30)	0.88
AGG	0.03	0.04	0.86 (0.38–1.97)	0.80	0.03	0.02	1.34 (0.60–3.00)	0.48	0.03	0.03	0.96 (0.46–1.98)	0.91
GGG	0.13	0.06	2.89 (1.71–4.89)	0.00008	0.11	0.10	1.10 (0.71–1.68)	0.68	0.08	0.07	1.18 (0.71–1.91)	0.54
Rare haplotypes[b]	0.00	0.00	—		0.00	0.00	—		0.01	0.01	—	
Global *P*			0.0004				0.72				0.63	

Note: Estimates are based on an additive effects model.
[a]Loci of tag SNPS are written 3′ to 5′ and include the following SNPS: rs11574143, rs757343 and rs1544410.
[b]Rare haplotypes are defined as those occurring <1% of the control population.

Other genetic studies have been limited to selected *VDR* RFLP SNPS [Bsm1 (rs1544410), ApaI (rs7975232), Fok1 (rs10735810) and Cdx2 (rs11568820)] and have shown inconsistent results (20).

Interactions between two additional *VDR* RFLPs (FokI and Cdx2) and serum vitamin D levels have been examined in relation to prostate cancer risk (10,11,23). Fok I (rs10735810), located in exon 2 of the *VDR* gene, may confer altered VDR transcriptional activity (41). The *Cdx2* SNP (rs11568820), located in the 5′ regulatory region of the VDR gene, may alter the affinity of the Cdx2 transcription factor for the VDR promoter, potentially affecting VDR expression (42). We found no overall association or significant interactions between FokI (rs10735810) and serum vitamin D in relation to prostate cancer risk. Other studies have been inconsistent, finding no association (10), lower risks related to the variant allele, in men with low serum 25(OH)D status (11), and stronger risk in men with high sunlight exposure, presumably correlated with higher 25(OH)D status (42,43). We also found no overall association or interactions with 25(OH)D for Cdx2 (rs11568820), although the Health Professionals Follow-up Study reported lower risks related to the Cdx2 (rs11568820) variant allele in men with low 25(OH)D (10).

Serum 25(OH)D concentration in blood is largely determined by non-genetic factors (15), including sunlight and diet; we found only modest differential in season-standardized 25(OH)D concentrations by variants in *GC*, which encodes vitamin D-binding protein, the major protein carrier of 25(OH)D. Our finding that associations remained for rs2282679 and rs1155563 in the simultaneously adjusted model may indicate additional unmeasured variants linked to rs2282679 and rs1155563 may play an important role in determining serum 25(OH)D concentrations. Serum $1,25(OH)_2D$ is tightly regulated (15), and we found no relationship with the studied gene variants; however, with only a subset of controls for this analysis (n = 150), we may not have had sufficient power to detect SNP–serum $1,25(OH)_2D$ associations.

Although tag SNPS in *GC* were related to serum 25(OH)D, these SNPS were not associated with total or aggressive prostate cancer risk. We recently reported no statistically significant differences in the PLCO Trial in the risk of prostate cancer with increasing serum 25(OH)D concentration, although there was some evidence of increasing risks for aggressive disease (12). Considering the modest impact of *GC* polymorphisms on serum 25(OH)D (i.e. mean difference: 8 nmol/l for GC rs2282679 homozygous versus variant homozygous), substantial gene–prostate cancer associations would not be expected.

This is the first large-scale evaluation of both serum vitamin D status and candidate genes in the vitamin D pathway in relation to prostate cancer. Since this study was conducted in a cancer screening trial, study participants had the same protocol for prostate cancer detection irrespective of lifestyle factors, substantially reducing the likelihood of screening-related detection bias. Other strengths include the use of prediagnostic serum vitamin D measurements. A potential limitation of our study is measurement of only a single serum sample; 25(OH)D measures at multiple time points would have resulted in more precise estimates of exposure.

In a broad evaluation of genetic variation in vitamin D-related genes, we found no overall associations with prostate cancer; however, genetic variants in the 3′ region of *VDR*, particularly rs11574143, may be associated with risk of this disease in men with low vitamin D status, indicating that knowledge of both serum 25(OH)D levels and VDR genotype may be needed to understand the complex relationship between vitamin D and prostate cancer risk.

Supplementary material

Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

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