

## Pesticide Exposure and Inherited Variants in Vitamin D Pathway Genes in Relation to Prostate Cancer

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### Abstract

**Background:** Vitamin D and its metabolites are believed to impede carcinogenesis by stimulating cell differentiation, inhibiting cell proliferation, and inducing apoptosis. Certain pesticides have been shown to deregulate vitamin D's anticarcinogenic properties. We hypothesize that certain pesticides may be linked to prostate cancer via an interaction with vitamin D genetic variants.

**Methods:** We evaluated interactions between 41 pesticides and 152 single-nucleotide polymorphisms (SNP) in nine vitamin D pathway genes among 776 prostate cancer cases and 1,444 male controls in a nested case-control study of Caucasian pesticide applicators within the Agricultural Health Study. We assessed  $P_{\text{interaction}}$  values using likelihood ratio tests from unconditional logistic regression and a false discovery rate (FDR) to account for multiple comparisons.

**Results:** Five significant interactions ( $P < 0.01$ ) displayed a monotonic increase in prostate cancer risk with individual pesticide use in one genotype and no association in the other. These interactions involved parathion and terbufos use and three vitamin D genes (*VDR*, *RXRβ*, and *GC*). The exposure-response pattern among participants with increasing parathion use with the homozygous *CC* genotype for *GC* rs7041 compared with unexposed participants was noteworthy [low vs. no exposure: OR, 2.58, 95% confidence interval (CI), 1.07–6.25; high vs. no exposure: OR, 3.09, 95% CI, 1.10–8.68;  $P_{\text{interaction}} = 3.8 \times 10^{-3}$ ].

**Conclusions:** In this study, genetic variations in vitamin D pathway genes, particularly *GC* rs7041, an SNP previously linked to lower circulating vitamin D levels, modified pesticide associations with prostate cancer risk.

**Impact:** Because our study is the first to examine this relationship, additional studies are needed to rule out chance findings. *Cancer Epidemiol Biomarkers Prev*; 22(9); 1557–66. ©2013 AACR.

### Introduction

The vitamin D endocrine system has the ability to generate biologic responses in more than 30 target tissues, including the prostate (1). Vitamin D and its metabolites are thought to impede carcinogenesis by stimulating cell differentiation, inhibiting cell proliferation, inducing apoptosis, suppressing tumor invasiveness, angiogenesis, and metastasis as well as reducing oxidative stress and inflammation (1–3). Vitamin D receptors (VDR) mediate

the biologic effect of the vitamin D steroid hormone, which has been shown to produce apoptotic, antiproliferative, and prodifferentiation activities in prostate cells *in vitro* and *in vivo* (2).

Sunlight, the major source of vitamin D, may have a direct effect on lowering prostate cancer risk (4–9). Evidence from ecologic studies has shown an inverse correlation between prostate cancer incidence and mortality and sunlight exposure (5, 6). Results from individual-based epidemiologic studies also suggest that higher sunlight exposure is associated with reduced prostate cancer risk (7, 8). In a recent U.S. case-control study, significant reductions in advanced prostate cancer risk for high-activity *VDR* polymorphic alleles were observed in the presence of high sunlight exposure (9). Higher serum 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] levels have also been observed, in a review of serum vitamin D levels, to be associated with lower incidence rates of aggressive prostate cancer (4). Although conflicting results have also been reported, experimental evidence coupled with epidemiologic findings indicate that vitamin D may play an important role in prostate cancer.

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Exposure to certain occupational hazards, such as pesticides, has been suggested as a possible risk factor for prostate cancer among farmers (10–14). Animal studies show that at high exposure levels, pesticides may be toxic to the prostate and could be indirectly mutagenic through free radical production (15–18); however, the mechanisms in humans are not understood. Certain pesticides, such as organochlorines, and those containing halogenated compounds have been shown to enhance the growth of initiated tumor cells (15, 19). They have also been reported to interfere with gap junction intercellular communication, which plays an essential role in the regulation of cell proliferation and differentiation and thus, also in the tumor growth process (19). Pesticides may also disrupt endocrine processes by modifying the activity of key enzymes involved in steroid metabolism and synthesis (15, 19). Therefore, certain pesticides may have the ability to disrupt the metabolism, synthesis, and ultimately anticarcinogenic properties of vitamin D and its metabolites.

Because certain pesticides may have the ability to deregulate the anticarcinogenic properties of vitamin D (15, 19), we conducted a nested case–control study of male pesticide applicators within the Agricultural Health Study (AHS) to evaluate interactions between pesticide use and genetic variation in nine vitamin D pathway genes and risk of prostate cancer.

## Materials and Methods

### Study population

Details of the AHS prostate cancer nested case–control study have been previously described (20). Briefly, eligible cases included all Caucasian pesticide applicators with biologic material (buccal cell) for analyses who were diagnosed with prostate cancer after enrollment in the AHS cohort between 1993 and 2004. Eligible controls included Caucasian male applicators with buccal cell material who were alive at the time of case diagnosis and had no previous history of cancer with the exception of nonmelanoma skin cancer. Controls were frequency-matched to cases at a ratio of 2:1 by date of birth ( $\pm 1$  year). After removing 280 participants ( $N = 215$  cases;  $N = 65$  controls) due to genotyping quality control constraints (insufficient/poor DNA quality or  $< 90\%$  completion rate for genotyping assays) and a genetic background that was inconsistent with European ancestry (i.e., African ancestry; ref. 20), the final study sample size consisted of 777 cases and 1,444 controls.

### Exposure assessment

Two self-administered questionnaires, completed during cohort enrollment (1993–1997), collected information on lifetime use of 50 pesticides (<http://aghealth.org/questionnaires.html>). The first questionnaire inquired about ever/never use of 50 pesticides as well as duration (in years) and frequency (average days/year) of use for a subset of 22 of these pesticides. The second take-home questionnaire, completed by 60.4% of cases and 67.2% of

controls, solicited detailed information on the frequency and duration of use of the remaining 28 pesticides. For each pesticide, we calculated total lifetime days of application (number of years  $\times$  days/year applied). An intensity-weighted metric for each pesticide was also calculated by multiplying the total lifetime days by an intensity score, derived from an algorithm based on mixing status, application method, equipment repair, and use of personal protective equipment (21). We categorized pesticide exposure variables into a three-level ordinal-scale: none, low, and high. The low and high categories were defined by the median level ( $\leq 50\%$  and  $> 50\%$ ) of use among controls. Parathion use included both ethyl and methyl parathion. Crop and animal applications for permethrin were combined into one exposure variable. Because of statistical power limitations, we excluded pesticides with less than 10% prevalence among the controls (trichlorfon, ziram, aluminum phosphide, ethylene dibromide, maneb/mancozeb, chlorothalonil, carbon tetrachloride/carbon disulfide, and aldicarb), leaving 41 pesticides available for analysis (Supplementary Table S1).

### Genotyping and single-nucleotide polymorphism selection

Details about buccal cell collection and DNA extraction have been previously described (20). Candidate genes ( $N = 1,291$ ) were genotyped at the National Cancer Institute's (NCI; Frederick, MD) Core Genotyping Facility (CGF; <http://cgf.nci.nih.gov/operations/multiplex-genotyping.html>) using the Custom Infinium BeadChip Assays (iSelect) from Illumina Inc. as part of an array of 26,512 single-nucleotide polymorphisms (SNP). Blinded duplicate samples (2%) were included, and concordance of the duplicate samples ranged from 96% to 100%. Tag SNPs for candidate vitamin D pathway genes were chosen on the basis of Caucasian HapMap population samples [Data Release 20/Phase II, National Center for Biotechnology Information (NCBI) Build 36.1 assembly, dbSNPb126], using a modified version of the method described by Carlson and colleagues (22) as implemented in the Tagzilla (<http://www.p3g.org/biobank-toolkit/tagzilla>) software package. For each candidate gene, SNPs 20 kb upstream of the start of transcription to 10 kb downstream of the stop codon were grouped using a binning threshold of  $r^2 = 0.80$ , where one tag SNP per bin was selected. Also included were SNPs previously reported as being potentially functional (20).

We identified nine vitamin D–associated candidate genes from the iSelect platform based on their involvement in vitamin D binding, transport, metabolism, function, and/or expression, mechanisms through which vitamin D may influence cancer risk. The group specific component (GC) vitamin D–binding protein serves as the major carrier of vitamin D and its metabolites in plasma to target tissue (23). Cytochrome P450 enzymes (*CYP24A1*, *CYP27A1*, and *CYP27B1*) hydroxylate vitamin D to its active form, 1,25(OH)<sub>2</sub>D (21). The biologic effects of vitamin D are exerted when the vitamin binds to VDR after

dimerization with retinoid-x-receptor (*RXR-alpha* and *RXR-beta*) genes (24, 25). This VDR–RXR complex is directed to the vitamin D–responsive element in the promoter region of 1,25-regulated genes, where mediator complex subunit (*MED24* and *MED16*) genes can induce or suppress transcription by interacting with the VDR–RXR complex (25). Of the 190 vitamin D tag SNPs genotyped, 173 remained after quality control exclusions (completeness <90% or Hardy–Weinberg equilibrium  $P < 1 \times 10^{-6}$ ). Further restriction of SNPs with a minor allele frequency of at least 5% among controls due to limited power for interaction assessments with rarer variants, resulted in 152 SNPs (Supplementary Table S2). The genotype completion rate for these SNPs ranged from 98% to 100%.

### Statistical analysis

All analyses were conducted using STATA version 10, unless otherwise noted. To estimate main effects ORs and 95% confidence intervals (CI) for the 41 pesticides and 152 vitamin D pathway SNPs with prostate cancer, we used unconditional logistic regression models adjusted for age and state (Iowa and North Carolina). Additional adjustment for family history of prostate cancer did not modify results and was therefore not included in the final model. We assessed the relationship between prostate cancer and pesticide use using two exposure metrics, intensity-weighted (lifetime exposure days  $\times$  intensity score) and unweighted (years of use  $\times$  days/year) lifetime days of exposure. Only findings for intensity-weighted lifetime days of exposure, which took into account additional factors such as use of personal protective equipment (21), are presented; although, results were similar. Associations between exposure scores (low and high use) for the 41 pesticides were not highly correlated ( $r^2$  range, 0.0001–0.45 using Spearman rank correlation coefficient).

We calculated the association between vitamin D SNPs and prostate cancer assuming a dominant and codominant genetic model for SNPs. For linear test of trend, we coded the homozygous common, heterozygous, and homozygous rare groups as 0, 1, or 2, respectively, corresponding to the number of rare alleles. Associations between SNPs were evaluated among controls to assess correlated loci using the *pwld* program in STATA. Of the 152 SNPs genotyped, two pairs of SNPs in the *VDR* ( $r^2 = 0.98$  for rs4516035 and rs7139166;  $r^2 = 0.94$  for rs731236 and rs7975128) and *RXR* ( $r^2 = 0.97$  for rs1567464 and rs12526336;  $r^2 = 0.88$  for rs9277937 and rs1547387) genes, three pairs of SNPs in the *GC* gene ( $r^2 = 0.98$  for rs7041 and rs222040;  $r^2 = 0.93$  for rs705120 and rs222040;  $r^2 = 0.92$  for rs7041 and rs705120), and one pair of SNPs in the *RXRA* ( $r^2 = 0.90$  for rs3118571 and rs877954) and *CYP27B1* ( $r^2 = 0.96$  for rs10747783 and rs2072052) genes were found to be highly correlated ( $r^2 > 0.85$ ).

We estimated ORs and 95% CIs for the joint effect between 41 pesticides and 152 vitamin D pathway SNPs and risk of prostate cancer risk using a common referent group. We calculated  $P_{\text{interaction}}$  values by comparing

regression models with and without interaction terms using a likelihood ratio test (LRT). In addition, a false discovery rate (FDR)–adjusted  $P$  value was calculated for each pesticide-specific interaction accounting for the 152 SNPs using SAS version 9.2 (SAS Institute; ref. 26). FDR-adjusted  $P$  values accounting for both the 152 vitamin D pathway SNPs and 41 different pesticides were not calculated given that this correction would have been too stringent to allow for detection of small effects. Interactions meeting FDR < 0.20 were considered robust to adjustment for multiple comparisons. A HaploStat package in R [version 2.13.0; <http://www.r-project.org>] was also used to conduct haplotype analyses for SNPs in linkage disequilibrium blocks within a gene. Linkage disequilibrium blocks among controls were identified in Haploview [<http://www.broad.mit.edu/mpg/haploview/index.php>]. No meaningful associations between cancer risk and exposure were observed from haplotype analyses.

In this article, we have presented results for intensity-weighted pesticide use and vitamin D pathway SNP interactions that show a monotonic increase ( $P_{\text{trend}} < 0.05$ ) in prostate cancer risk with increasing pesticide use in one genotype stratum (in either the dominant or codominant models) and no significant decrease in risk with pesticide use in the other stratum that met an FDR < 0.20 or an  $P_{\text{interaction}} < 0.01$ . Associations for pesticide use, vitamin D pathway SNPs, and prostate cancer risk not meeting these criteria with  $P_{\text{interaction}} < 0.01$  are presented in Supplementary Table S3.

### Results

Compared with the AHS cohort, applicators participating in the nested case–control study were similar with regards to state of residence, applicator type, family history of prostate cancer, and for cases, stage and grade of prostate cancer was similar to other prostate cancer cases not included in the case–control study (Table 1; ref. 20). Cases and matched controls in the nested case–control study were, as expected, older at enrollment than cohort members in the AHS, which reflects the incidence of prostate cancer in older men.

Associations between pesticide use and prostate cancer risk, shown in Supplementary Table S1, were largely null within this case–control set; though, we did observe inverse associations for some pesticides: dicamba, cyanazine, paraquat, 2,4,5-T, lindane, carbaryl, and chlordane.

Of the 152 SNPs examined from the nine vitamin D pathway genes, we found noteworthy associations with prostate cancer for 13 SNPs across six genes (Table 2). Relative to the more common homozygous genotype, we observed significant inverse trend associations for the effect of each added allele for five SNPs across *VDR* (rs4334089, rs7299460, rs7970314, rs7305180, and rs10459217), two SNPs across *CYP27A1* (rs645163 and rs6436094), and one SNP across *MED16* (rs1651896). We also observed significant increased trends for the effect of

**Table 1.** Characteristics of male participants from the AHS cohort and nested case–control study

Characteristic	Nested case–control		AHS cohort	
	Prostate cases N (%)	Controls N (%)	Prostate cases N (%)	Non-cases N (%)
Participants	776	1,444	1,275	48,286
Age at enrollment, y				
<50	3 (0.4)	5 (0.4)	9 (0.7)	17,801 (36.9)
50–59	74 (9.5)	144 (10.0)	111 (8.7)	13,592 (28.2)
60–69	259 (33.4)	491 (34.0)	409 (32.1)	9,515 (19.7)
70–79	355 (45.8)	634 (43.9)	573 (44.9)	5,657 (11.7)
≥80	85 (11.0)	170 (11.8)	173 (13.6)	1,721 (3.6)
State of residence				
Iowa	520 (67.0)	991 (68.6)	789 (61.9)	32,740 (67.8)
North Carolina	256 (33.0)	453 (31.4)	486 (38.1)	15,546 (32.2)
Applicator type				
Private	741 (95.5)	1,363 (94.4)	1,219 (95.6)	43,895 (90.9)
Commercial	35 (4.5)	81 (5.6)	56 (4.4)	4,391 (9.1)
Family history of prostate cancer <sup>a</sup>				
No	575 (74.1)	1,193 (82.6)	924 (72.5)	41,365 (85.7)
Yes	130 (16.8)	145 (10.0)	212 (16.6)	3,748 (7.8)
Prostate cancer stage				
Local	579 (74.3)		945 (74.1)	
Regional	156 (20.1)		247 (19.4)	
Distant	12 (1.5)		33 (2.6)	
Not staged	29 (3.7)		50 (3.9)	
Prostate cancer grade				
Well differentiated	38 (4.9)		60 (4.7)	
Moderately differentiated	547 (70.5)		855 (67.1)	
Poorly differentiated	168 (21.6)		302 (23.7)	
Undifferentiated	4 (0.5)		6 (0.5)	
Not graded	19 (2.4)		52 (4.1)	

<sup>a</sup>Family history of prostate cancer in first-degree relative.

each added allele for two SNPs across *VDR* (rs3782905 and rs7132324), and one in each of the following genes: *RXRA* (rs6537944), *RXRB* (rs421446), and *CYP24A1* (rs2426498). Supplementary Table S2 presents the associations for the remaining 139 vitamin D pathway SNPs evaluated.

Five interactions met the FDR < 0.20 criterion and showed a monotonic increase in prostate cancer risk with increasing pesticide use in one genotype stratum and no significant decrease in risk with use in the other. The joint effects for these interactions, presented in Table 3, involved two pesticides, parathion and terbufos, and three vitamin D pathway genes, *RXRB*, *GC*, and *VDR*. The most striking association was observed between parathion and *RXRB* rs1547387. Compared with unexposed men with the *CC* homozygous referent genotype, we observed a more than 4-fold increase in prostate cancer risk in men with at least one *G* allele with high levels of parathion use (OR, 4.27; 95% CI, 1.32–13.78;  $P_{\text{interaction}} = 2.4 \times 10^{-3}$ ; FDR-adjusted  $P = 0.19$ ). A significant increase in cancer risk was also found with increasing parathion use for subjects with the *CC* homozygous genotype for *GC*

rs7041 compared with unexposed subjects (low vs. no use: OR, 2.58, 95% CI, 1.07–6.25; high vs. no use: OR, 3.09, 95% CI, 1.10–8.68;  $P_{\text{interaction}} = 3.8 \times 10^{-3}$ ; FDR-adjusted  $P = 0.19$ ). In addition, we saw a similar interaction pattern for the highly correlated *GC* rs222040 SNP ( $r^2 = 0.98$ ) and parathion use ( $P_{\text{interaction}} = 3.0 \times 10^{-3}$ ; FDR-adjusted  $P = 0.19$ ). Another *GC* SNP, rs12512631, was also found to significantly interact with terbufos; compared with unexposed subjects, men with the *TT* homozygous rs12512631 genotype had an increased risk for prostate cancer with both low (OR, 1.58; 95% CI, 1.09–2.28) and high (OR, 1.73; 95% CI, 1.20–2.49) levels of terbufos use ( $P_{\text{interaction}} = 9.5 \times 10^{-4}$ ; FDR-adjusted  $P = 0.07$ ). Furthermore, compared with unexposed subjects with the *TT* referent genotype in the *VDR* SNP rs4328262, we found a significant 39% (95% CI, 1.00–1.95) increase in risk for men with high levels of terbufos use with at least one *G* allele ( $P_{\text{interaction}} = 8.5 \times 10^{-4}$ ; FDR-adjusted  $P = 0.07$ ).

We observed eight significant interactions that did not meet the FDR < 0.20 criterion, but showed a monotonic increase in prostate cancer risk with increasing pesticide

Table 2. Association between prostate cancer risk and 13 SNPs across six vitamin D pathway genes

Gene	SNP	Location	Referent			Codominant model			Dominant model			
			Case/control			Case/control			Case/control			
			Genotype	N/N	OR (95% CI)	Genotype	N/N	OR (95% CI)	$P_{\text{trend}}$	Genotype	N/N	OR (95% CI)
VDR	rs3782905	IVS4+6584C>G	GG	343/705	1.19 (0.99–1.43)	CC	89/140	1.31 (0.97–1.76)	0.03	CG+CC	417/708	1.21 (1.02–1.45)
VDR	rs4334089	IVS2+7605G>A	GG	440/766	0.90 (0.75–1.08)	AA	44/115	0.66 (0.46–0.96)	0.04	AG+AA	334/677	0.86 (0.72–1.02)
VDR	rs7299460	IVS1+2470C>T	CC	400/705	0.96 (0.80–1.15)	TT	58/154	0.66 (0.48–0.92)	0.04	CT+TT	376/739	0.90 (0.75–1.07)
VDR	rs7132324	-34412C>T	CC	316/651	1.18 (0.98–1.43)	TT	105/174	1.24 (0.94–1.63)	0.06	CT+TT	460/792	1.19 (1.00–1.43)
VDR	rs7970314	-35277A>G	AA	508/864	0.79 (0.65–0.95)	GG	34/83	0.69 (0.45–1.04)	5.3E-03	AG+GG	255/561	0.77 (0.64–0.93)
VDR	rs7305180 <sup>a</sup>	-42038G>T	GG	559/969	0.81 (0.66–0.99)	TT	22/55	0.69 (0.41–1.14)	0.01	GT+TT	217/473	0.79 (0.65–0.96)
VDR	rs10459217 <sup>a</sup>	-43364C>T	TT	523/885	0.80 (0.66–0.97)	CC	24/70	0.58 (0.36–0.93)	2.5E-03	CT+CC	245/538	0.77 (0.64–0.93)
RXRA	rs6537944 <sup>a</sup>	IVS5-694T>C	TT	629/1197	1.19 (0.92–1.52)	CC	11/9	2.35 (0.97–5.71)	0.04	CT+CC	129/199	1.24 (0.97–1.58)
RXRB	rs421446	-6529G>A	AA	353/708	1.16 (0.96–1.40)	GG	62/93	1.34 (0.95–1.89)	0.05	AG+GG	382/647	1.18 (0.99–1.42)
CYP24A1	rs2426498 <sup>a</sup>	-6562G>C	CC	575/1108	1.08 (0.88–1.34)	GG	23/19	2.32 (1.26–4.30)	0.04	CG+GG	201/336	1.15 (0.94–1.41)
CYP27A1	rs645163 <sup>a</sup>	*2503T>C	CC	581/996	0.72 (0.59–0.89)	TT	18/27	1.14 (0.62–2.09)	0.02	CT+TT	195/447	0.75 (0.61–0.91)
CYP27A1	rs6436094	Ex14+203A>G	AA	453/759	0.81 (0.67–0.98)	GG	45/92	0.82 (0.56–1.19)	0.04	AG+GG	299/618	0.81 (0.68–0.97)
MED16	rs1651896	*2265C>T	CC	383/647	0.85 (0.70–1.02)	TT	63/136	0.78 (0.56–1.08)	0.04	CT+TT	367/743	0.83 (0.70–1.00)

NOTE: Adjusted for age and state.

Abbreviation: REF, reference.

<sup>a</sup>Homozygous rare allele frequency less than 5% among the controls.

use in one genotype group with no significant decrease in risk with use in the other (Table 4). These interactions involved parathion, terbufos, petroleum oil, atrazine, and metribuzin, and *RXRB*, *GC*, *VDR*, and *RXRA* vitamin D genes. Parathion was shown to interact with *RXRB* rs9277937, which is highly correlated with rs1547387 ( $r^2 = 0.90$ ), and thus it exhibited a similar interaction pattern ( $P_{\text{interaction}} = 9.9 \times 10^{-3}$ ). Parathion also interacted with *GC* rs705120, which is highly correlated with rs222040 ( $r^2 = 0.92$ ), and therefore displayed a similar interaction pattern ( $P_{\text{interaction}} = 8.6 \times 10^{-3}$ ). The interaction between terbufos and two *VDR* SNPs, rs7139166 and rs7132324, indicated that participants with high levels of use with either the homozygous common *CC* rs7139166 (OR, 1.72; 95% CI, 1.12–2.62;  $P_{\text{interaction}} = 7.0 \times 10^{-3}$ ) or heterozygous variant *CT+TT* rs7132324 (OR, 1.51; 95% CI, 1.08–2.11;  $P_{\text{interaction}} = 9.0 \times 10^{-3}$ ) genotype observed a significantly increased prostate cancer risk compared with unexposed men. Those with high levels of use of petroleum oil/distillate with the *VDR* rs7132324 homozygous common *TT* genotype also observed a more than 5-fold increase in prostate cancer risk (OR, 5.50; 95% CI, 1.73–17.49) when compared with unexposed applicators with the *CC* homozygous referent genotype ( $P_{\text{interaction}} = 1.7 \times 10^{-3}$ ). In addition, high levels of use of petroleum oil/distillate with the homozygous referent *G* allele for *RXRA* rs3132300 was associated with a significant increase in prostate cancer risk (OR, 1.66; 95% CI, 1.07–2.57;  $P_{\text{interaction}} = 5.9 \times 10^{-3}$ ). The interaction between atrazine and *VDR* rs17721101 revealed that high levels of use among participants with the *AC/CC* genotype observed a more than 2-fold increase in risk compared with unexposed men (OR, 1.26/0.47 = 2.68;  $P_{\text{interaction}} = 9.4 \times 10^{-3}$ ). We observed a comparable increase in risk for participants with high use of metribuzin with the homozygous rare *GG* genotype for *VDR* rs731236 (OR, 2.11; 95% CI, 1.19–3.74;  $P_{\text{interaction}} = 6.6 \times 10^{-3}$ ).

## Discussion

In this nested case-control study, we evaluated interactions between pesticide use and SNPs across nine vitamin D pathway genes. We observed five interactions that were robust to multiple comparison adjustment of an FDR < 0.20, and displayed a significant monotonic increase in prostate cancer risk with increasing pesticide use in one genotype stratum but no significant decrease in risk in the other genotype stratum. These interactions were observed between parathion and terbufos, two organophosphate pesticides, and three vitamin D pathway genes (*VDR*, *RXRB*, and *GC*).

According to the U.S. Environmental Protection Agency, terbufos and ethyl parathion are both classified as extremely toxic organophosphate insecticides (27, 28), though only parathion is classified as a class C possible human carcinogen (terbufos class E, a noncarcinogen human agent; ref. 29). Evidence from epidemiologic studies evaluating the carcinogenic potential of parathion and

**Table 3.** The joint effects between pesticide exposure, SNPs in the vitamin D pathway genes, and prostate cancer risk that met an FDR <0.20

Pesticide	Gene	SNP	Alleles	Unexposed			Low-level exposure <sup>a</sup>			High-level exposure <sup>a</sup>			LRT	FDR
				N/N	OR (95% CI)		N/N	OR (95% CI)		N/N	OR (95% CI)			
Parathion	RXRB	rs1547387 <sup>b</sup>	CC	513/944	REF	22/35	1.12 (0.65–1.94)	12/39	0.54 (0.28–1.04)					
			CG+GG <sup>c</sup>	113/232	0.89 (0.70–1.15)	8/8	1.82 (0.68–4.89)	10/4	<b>4.27 (1.32–13.78)</b>	2.4 × 10 <sup>-3</sup>	0.19			
Parathion	GC	rs7041 <sup>b</sup>	CC <sup>c</sup>	186/367	REF	12/9	<b>2.58 (1.07–6.25)</b>	10/6	<b>3.09 (1.10–8.68)</b>					
			AC	313/595	1.04 (0.83–1.30)	14/24	1.12 (0.56–2.22)	7/27	0.48 (0.20–1.14)					
			AA	128/207	1.22 (0.92–1.62)	4/10	0.77 (0.24–2.50)	5/10	0.93 (0.31–2.79)	7.0 × 10 <sup>-3</sup>	0.32			
			AC+AA	441/802	1.09 (0.88–1.34)	18/34	1.02 (0.56–1.85)	12/37	0.60 (0.31–1.19)	3.8 × 10 <sup>-3</sup>	0.19			
Parathion	GC	rs222040 <sup>b</sup>	AA <sup>c</sup>	190/373	REF	11/10	2.14 (0.89–5.12)	11/6	<b>3.39 (1.23–9.36)</b>					
			AG	311/597	1.02 (0.82–1.28)	14/23	1.16 (0.58–2.31)	6/27	0.41 (0.17–1.02)					
			GG	126/206	1.20 (0.91–1.59)	4/10	0.77 (0.24–2.49)	5/10	0.93 (0.31–2.78)	6.1 × 10 <sup>-3</sup>	0.32			
			AG+GG	437/803	1.07 (0.87–1.32)	18/33	1.04 (0.57–1.90)	11/37	0.55 (0.27–1.12)	3.0 × 10 <sup>-3</sup>	0.19			
Terbufos	GC	rs12512631	TT <sup>c</sup>	166/347	REF	69/94	<b>1.58 (1.09–2.28)</b>	72/89	<b>1.73 (1.20–2.49)</b>					
			CT	188/382	1.03 (0.80–1.33)	62/123	1.08 (0.75–1.55)	49/133	0.78 (0.54–1.15)					
			CC	52/74	1.47 (0.99–2.19)	14/33	0.91 (0.47–1.75)	10/26	0.82 (0.38–1.74)	1.3 × 10 <sup>-3</sup>	0.20			
			CT+CC	240/456	1.10 (0.87–1.41)	76/156	1.04 (0.75–1.46)	59/159	0.79 (0.55–1.13)	9.5 × 10 <sup>-4</sup>	0.07			
Terbufos	VDR	rs4328262	TT	139/280	REF	58/75	<b>1.60 (1.07–2.30)</b>	35/108	0.66 (0.43–1.02)					
			GT	192/384	1.01 (0.77–1.31)	67/120	1.15 (0.80–1.66)	68/106	1.32 (0.91–1.91)					
			GG	75/139	1.08 (0.76–1.53)	20/55	0.75 (0.43–1.30)	27/34	1.63 (0.94–2.81)	0.23	0.65			
			GT+GG <sup>d</sup>	267/523	1.02 (0.80–1.32)	87/175	1.03 (0.73–1.43)	95/140	<b>1.39 (1.00–1.95)</b>	8.5 × 10 <sup>-4</sup>	0.07			

NOTE: Adjusted for age and state.

Abbreviation: REF, reference.

<sup>a</sup>Intensity-weighted lifetime days of exposure: low and high categories defined by the median among exposed controls.<sup>b</sup>Correlation between: GC SNPs rs7041 and rs222040,  $r^2 = 0.98$ ; RXRB SNPs rs9277937 and rs1547387,  $r^2 = 0.90$ .Test of the monotonic trend of prostate cancer risk across increasing tertiles of pesticide use: <sup>c</sup> $P_{\text{trend}} < 0.01$ ; <sup>d</sup> $P_{\text{trend}} < 0.05$ .

terbufos in humans have generally been null (28, 30–33); though in most studies, limited power may have made it difficult to detect an association if one existed. Compared with these previously published reports, our study has approximately double the number of exposed cases. Despite the fact that these specific agents are not established human carcinogens, recent epidemiologic findings, some based on AHS data, show increased cancer risk associated with exposure (13, 20, 34–38). In 2010, a significant increase in overall cancer risk was observed among AHS participants exposed to terbufos (HR, 1.21; 95% CI, 1.06–1.37); when risk was evaluated by cancer site, a suggestive association with prostate cancer was also found for those in the highest category of exposure (HR, 1.21; 95% CI, 0.99–1.47; ref. 13). Terbufos has been significantly linked to aggressive prostate cancer risk in the AHS (34) and shown to interact with variants in xenobiotic metabolism genes (35), as well as with the 8q24 region (20). For parathion exposure, a significant exposure–response increase in risk for cutaneous melanoma ( $P_{\text{trend}} = 0.003$ ) was reported among AHS participants in 2010 (36). In 2007, Calaf and Roy reported that ethyl parathion exposure influenced the *in vitro* transformation of human breast epithelial cells and initiator factors in the transformation process of breast cancer (37). Increase in risk of adrenal cortical tumors, thyroid follicular adenoma, and

pancreatic islet cell carcinoma has also been associated with methyl parathion exposure in rodents (38).

The strongest interaction observed in our study was between the *RXRB* gene variant rs1547387 and parathion; however, to our knowledge, no previously published study has evaluated the association between this specific SNP and cancer. Also of particular interest were the significant interactions observed between GC gene variants rs7041, rs222040, rs12512631, and rs705120, prostate cancer and use of both terbufos and parathion. Epidemiologic evidence has shown that these specific GC gene variants may influence circulating levels of 25(OH)D. In a large cohort study, investigating prostate cancer risk and vitamin D genes, rs12512631 and rs7041 GC SNPs were significantly associated with 25(OH)D levels; subjects with the rs12512631 C allele and those with the rs7041 A allele were found to have lower 25(OH)D levels ( $P = 0.0004$ ; ref. 39). Although no elevated prostate cancer risk was observed with rs7041 and rs12512631 (39), in our study, marginally significant and significantly elevated prostate cancer risk was observed among unexposed participants with these specific GC alleles. Reduced 25(OH)D levels associated with these particular GC SNP variants have also been shown in other epidemiologic studies (40, 41) as well as in two genome-wide association studies (42, 43). Although there is strong evidence linking

**Table 4.** The joint effects between pesticide exposure, SNPs in the vitamin D pathway genes, and prostate cancer risk

Pesticide	Gene	SNP	Alleles	Unexposed			Low-level exposure <sup>a</sup>		High-level exposure <sup>a</sup>		LRT	FDR
				N/N	OR (95% CI)		N/N	OR (95% CI)	N/N	OR (95% CI)		
				Case/control		Case/control		Case/control				
Parathion	RXRB	rs9277937 <sup>b</sup>	TT	515/965	REF	22/36	1.11 (0.65–1.92)	12/37	0.58 (0.30–1.12)			
			CT+CC <sup>c</sup>	111/211	0.98 (0.76–1.27)	8/7	2.12 (0.76–5.88)	10/5	<b>3.48 (1.17–10.31)</b>	9.9 × 10 <sup>-3</sup>	0.30	
Parathion	GC	rs705120 <sup>b</sup>	CC <sup>c</sup>	206/403	REF	13/11	<b>2.27 (1.00–5.16)</b>	11/8	<b>2.53 (1.00–6.43)</b>			
			AC	306/576	1.04 (0.84–1.30)	13/23	1.08 (0.53–2.18)	7/25	0.52 (0.22–1.23)			
			AA	115/197	1.14 (0.86–1.52)	4/9	0.85 (0.26–2.80)	4/10	0.74 (0.23–2.40)	0.02	0.51	
			AC+AA	421/773	1.07 (0.87–1.31)	17/32	1.01 (0.55–1.87)	11/35	0.58 (0.29–1.18)	8.6 × 10 <sup>-3</sup>	0.30	
Terbufos	VDR	rs7139166	CC <sup>c</sup>	123/281	REF	36/87	0.97 (0.62–1.52)	50/68	<b>1.72 (1.12–2.62)</b>			
			CG	201/367	1.26 (0.96–1.65)	80/119	<b>1.58 (1.10–2.27)</b>	62/128	1.14 (0.78–1.66)			
			GG	82/155	1.22 (0.87–1.71)	29/44	1.55 (0.92–2.61)	19/52	0.85 (0.48–1.50)	0.11	0.57	
			CG+GG	283/522	1.25 (0.96–1.61)	109/163	<b>1.57 (1.13–2.18)</b>	81/180	1.05 (0.75–1.49)	7.0 × 10 <sup>-3</sup>	0.34	
Terbufos	VDR	rs7132324	CC	169/355	REF	66/99	1.43 (0.99–2.07)	45/126	0.76 (0.52–1.12)			
			CT	178/337	1.11 (0.86–1.43)	65/119	1.17 (0.82–1.67)	63/102	1.32 (0.92–1.91)			
			TT	59/111	1.11 (0.77–1.60)	14/32	0.94 (0.49–1.81)	23/20	<b>2.45 (1.31–4.58)</b>	0.04	0.57	
			CT+TT <sup>c</sup>	237/448	1.11 (0.87–1.41)	79/151	1.12 (0.80–1.56)	86/122	<b>1.51 (1.08–2.11)</b>	9.0 × 10 <sup>-3</sup>	0.34	
Petroleum oil/distillate	VDR	rs7132324	CC	215/424	REF	20/50	0.82 (0.48–1.42)	14/50	0.56 (0.30–1.03)			
			CT	207/407	1.00 (0.79–1.26)	27/45	1.21 (0.73–2.00)	36/49	1.49 (0.94–2.36)			
			TT <sup>c</sup>	66/132	0.98 (0.70–1.37)	5/8	1.21 (0.39–3.76)	11/4	<b>5.50 (1.73–17.49)</b>	1.7 × 10 <sup>-3</sup>	0.26	
			CT+TT <sup>d</sup>	273/539	0.99 (0.80–1.24)	32/53	1.21 (0.76–1.93)	47/53	<b>1.79 (1.17–2.74)</b>	4.4 × 10 <sup>-3</sup>	0.43	
Petroleum oil/distillate	RXRA	rs3132300	GG <sup>c</sup>	299/612	REF	38/54	1.47 (0.95–2.28)	40/50	<b>1.66 (1.07–2.57)</b>			
			AG+AA	184/342	1.10 (0.87–1.37)	14/49	0.60 (0.33–1.11)	21/52	0.85 (0.50–1.44)	5.9 × 10 <sup>-3</sup>	0.43	
Atrazine	VDR	rs17721101	AA	172/310	REF	240/448	0.98 (0.76–1.26)	228/450	0.92 (0.72–1.19)			
Metribuzin	VDR	rs731236	AC+CC <sup>d</sup>	17/65	<b>0.47 (0.27–0.83)</b>	34/69	0.90 (0.57–1.42)	45/65	1.26 (0.82–1.93)	9.4 × 10 <sup>-3</sup>	0.96	
			AA	149/307	REF	30/66	0.96 (0.60–1.56)	25/65	0.80 (0.49–1.33)			
			AG	221/345	<b>1.32 (1.02–1.70)</b>	43/95	0.96 (0.63–1.46)	33/94	0.74 (0.47–1.15)			
			GG <sup>c</sup>	63/136	0.96 (0.67–1.37)	15/27	1.18 (0.60–2.28)	27/27	<b>2.11 (1.19–3.74)</b>	6.6 × 10 <sup>-3</sup>	0.49	
			AG+GG	284/481	1.22 (0.95–1.55)	58/122	1.02 (0.70–1.48)	60/121	1.05 (0.72–1.52)	0.84	0.99	

NOTE: Adjusted for age and state.

Abbreviation: REF, reference.

<sup>a</sup>Intensity-weighted lifetime days of exposure: low and high categories defined by the median among exposed controls.<sup>b</sup>Correlation between: RXRB SNPs rs9277937 and rs1547387 SNPs  $r^2 = 0.90$ ; GC SNPs rs705120 and rs222040 SNPs  $r^2 = 0.92$ .Test of the monotonic trend of prostate cancer risk across increasing tertiles of pesticide use: <sup>c</sup> $P_{\text{trend}} < 0.05$ ; <sup>d</sup> $P_{\text{trend}} < 0.01$ .

these GC variants to 25(OH)D, the underlying mechanism of action remains unclear. With the exception of VDR variants rs731236 and rs7139166, which have been linked to increased risk of prostate cancer (rs731236 C allele; ref. 44), breast cancers (rs731236 C allele; ref. 45), and cutaneous melanoma (rs731236 C allele and rs7139166 G allele; ref. 46), no other epidemiologic investigations involving vitamin D pathway SNPs, which were observed to modify associations between pesticide use and prostate cancer risk in our study, regardless of the FDR criterion, were found.

Because genes in the vitamin D pathway play a key role in cell processes related to differentiation, proliferation, and apoptosis (1–3) as well as in the synthesis of steroid hormones in the adrenal glands and gonads (46), our findings that vitamin D pathway genes could modify associations between parathion and terbufos organophosphate insecticides are biologically plausible. The mechanisms of this interaction are not understood, but could

possibly include acetylcholinesterase inhibition or the dysregulation of hormonal functions. Organophosphates generally exert their toxic and possibly carcinogenic effects by inhibiting acetylcholinesterase, an enzyme shown to play an important role in noncholinergic cell processes such as mitosis, proliferation, differentiation, and apoptosis (47). In addition, organophosphates have been shown to exhibit antiandrogenic activity (48, 49), such as altering serum testosterone levels (50, 51), which has been directly linked to prostate cancer (52, 53).

Significant interactions in our study were also observed for exposures to three herbicides, metribuzin, atrazine, and petroleum oil/petroleum distillate. The toxicologic evidence implicating these pesticides as human carcinogens is weak (32, 54–57). To date, only three epidemiologic studies have assessed metribuzin exposure in relation to cancer in humans (32, 43, 55); with the exception of glioma (54) and lymphohematopoietic cancers (55), findings have primarily been null (32, 55). Elevated risk of non-Hodgkin

lymphoma (32), glioma (54), and thyroid cancer (58) with exposure to atrazine were suggested in a few small studies. Recent gene-exposure analyses from the AHS have also shown atrazine to interact with genes in lipid metabolism (59), base excision repair (60), and xenobiotic metabolism (35) pathways in relation to prostate cancer. Studies of atrazine exposure in humans have generally shown no evidence of an association with cancer (56), although one study did report a significant elevated association between non-Hodgkin's lymphoma risk and atrazine exposure (32). The risk assessment for this pesticide is still incomplete and ongoing for cancer (56). The effect of petroleum oil exposure on cancer risk is difficult to understand given the lack of specificity about its use and its wide variability in composition (35). Nevertheless, exposure to this pesticide has been shown to interact with prostate cancer risk and genes in lipid (59) and xenobiotic metabolism (35) pathways in AHS. Because significant interactions have been reported between the aforementioned pesticides and genes in pathways other than vitamin D, these findings suggest that any relationship that might exist between pesticides and prostate cancer may involve multiple biologic processes. However, none of the other pathway SNPs for which an association was reported were correlated with SNPs in our study. Given the lack of association between these pesticides and prostate cancer risk, as well as the fact that no other study has evaluated vitamin D pathway genes in relation to pesticide exposure and prostate cancer risk, the novel results of our study need replicating and should be considered hypothesis generating.

Several strengths as well as limitations of our study should be acknowledged. The AHS collected in-depth information on potential confounders as well as high-quality detailed pesticide use data using self-administered questionnaires. Although exposure misclassification is a concern for many gene-exposure studies, the reliability of pesticide usage (61) and the accuracy of duration (62) and intensity (63) of exposure have been found to be high in this cohort. Moreover, the effect of exposure misclassification in this cohort study would most likely bias risk estimates for exposure interactions toward the null (64). Furthermore, details about the use of individual pesticides from a wide range of chemical and functional classes in our study are valuable as observed cancer risks seem to be chemical specific. The availability of genotyping data for a large number of SNPs allowed for comprehensive assessment of genes across the vitamin D pathway. Yet, because we restricted assessment of SNPs to those with a minor allele frequency more than 5%, we may have potentially excluded important SNPs that modify risk. The multitude of interactions that were assessed increased the possibility of chance findings. To reduce the likelihood of false-positive results, several steps were taken such as focusing on interactions that met an FDR > 0.20 and that resulted in a positive monotonic association between pesticide use and prostate cancer in one genotype and no significant association in the other, as the

biologic mechanism for qualitative interactions is unclear. On the other hand, we recognize that these criteria may have also concealed some true-positive findings. In addition, power for some stratified analyses is limited given the small number of cases that may have led to some false-positive or -negative associations. To our knowledge, however, no other study has had greater power to evaluate pesticide-gene interaction with prostate cancer. Finally, while we were limited in our ability to explore interactions with aggressive prostate cancers, we did assess interactions by family history of cancer given the previous observed effect modification on the association between pesticides and prostate cancer in this cohort (30). Although similar risk estimates were observed among those in our study with and without a family history of cancer, it is possible that other genes, multiple genes, or nongenetic factors that track in families might account for this previously observed association (30).

In this nested case-control study, we observed interaction between organophosphate insecticides, terbufos and parathion, and vitamin D pathway gene variants with respect to prostate cancer. Although the results of our study are novel, there are some biologically plausible explanations. However, because this is the first study to assess prostate cancer risk in relation to vitamin D pathway genes and pesticide use, additional well-powered studies among populations with detailed information on pesticide use are needed to extend and further evaluate findings to rule out chance and help clarify the potential biologic mechanisms underlying pesticide associations with cancer, such as exploring interactions by cancer aggressiveness.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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