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Xenobiotic metabolizing gene variants, pesticide use, and risk of prostate cancer

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Abstract

Background—To explore associations with prostate cancer and farming, it is important to investigate the relationship between pesticide use and single nucleotide polymorphisms (SNPs) in xenobiotic metabolic enzyme (XME) genes.

Objectives—We evaluated pesticide-SNP interactions between 45 pesticides and 1,913 XME SNPs with respect to prostate cancer among 776 cases and 1,444 controls in the Agricultural Health Study.

Methods—We used unconditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Multiplicative SNP-pesticide interactions were calculated using a likelihood ratio test.

Results—A positive monotonic interaction was observed between petroleum oil/petroleum distillate use and rs1883633 in the oxidative stress gene glutamate-cysteine ligase (*GCLC*) (p-interaction=1.0×10^{−4}); men carrying at least one variant allele (minor allele) experienced an increased prostate cancer risk (OR=3.7, 95% CI: 1.9–7.3). Among men carrying the variant allele for thioredoxin reductase 2 (*TXNRD2*) rs4485648, microsomal epoxide hydrolase 1 (*EPHX1*) rs17309872, or myeloperoxidase (*MPO*) rs11079344, increased prostate cancer risk was observed with high compared to no petroleum oil/petroleum distillate (OR=1.9, 95% CI: 1.1–3.2, p-interaction=0.01), (OR=2.1, 95% CI: 1.1–4.0, p-interaction=0.01), or terbufos (OR=3.0, 95% CI: 1.5–6.0 p-interaction=2.0×10^{−3}) use, respectively. No interactions were deemed noteworthy at the false discovery rate = 0.20 level; the number of observed interactions in XMEs was comparable to the number expected by chance alone.

Conclusions—We observed several pesticide-SNP interactions in oxidative stress and phase I/phase II enzyme genes and risk of prostate cancer. Additional work is needed to explain the joint contribution of genetic variation in XMEs, pesticide use, and prostate cancer risk.

Keywords

Prostate cancer; pesticides; xenobiotic metabolizing enzymes; single nucleotide polymorphism; interaction

INTRODUCTION

There have been few environmental factors that have been identified to alter prostate cancer risk. However, results from the Agricultural Health Study (AHS), a prospective cohort of licensed private and commercial pesticide applicators in Iowa and North Carolina, show that pesticide applicators have a significantly higher risk of prostate cancer than men in the general population of Iowa and North Carolina [1]. The metabolism and subsequent excretion of pesticides from the body requires a series of chemical reactions that depend on xenobiotic metabolic enzymes (XMEs) [2]. Phase I and phase II enzyme reactions are well-described processes for the clearance of xenobiotic substances. The enzymes responsible for mediating phase I reactions encompass members of the cytochrome P450 (CYPs) superfamily [3]. Substantial literature documents the principal involvement of CYPs in the metabolism of specific xenobiotic substrates including some herbicides and organophosphosphate (OP) insecticides [4–8]; still, the metabolism of many pesticides is not well characterized. Phase II enzymes also play a crucial role in xenobiotic metabolism and are a necessary part of the pathway to excretion. Phase II enzymes, such as sulfotransferases (SULTs), N-acetyltransferases (NATs), UDP-glucuronosyltransferases (UGTs), and glutathione S-transferases (GSTs) catalyze conjugation reactions of intermediates directly to form detoxification products, or further metabolize other reactive intermediates for future excretion [3]. In addition, key nuclear receptors including the aryl hydrocarbon receptor (AHR), pregnane X receptor (PXR or NR1I2) and the constitutive active/androstane receptor (CAR or NR1I3) can stimulate gene transcription in response to pesticides and affect their metabolism [9–11]. Accumulation of toxic pesticide intermediates can induce the production of reactive oxygen species (ROS), markers of oxidative stress, which can react with cellular DNA to cause mutation or gross DNA rearrangements [12;13]. For example, the roles of enzymatic antioxidants like superoxide dismutase (SOD) and catalase (CAT), or paraoxonase 1 (PON1) in the metabolism of highly toxic OP oxon metabolites, are well-described in the defense against ROS [12;14].

There is also evidence that genetic susceptibility plays a role in prostate cancer development. For example, twin studies have estimated that 42% of prostate cancer risk may be explained by genetic factors [15]. Recent genome-wide scans of prostate cancer have also identified high susceptibility loci in various gene regions [16;17]. Although none of the genome-wide scans to date have identified susceptibility loci in XMEs, these studies have not addressed the complex interactions that XMEs have with relevant exposures. Thus, in order to fully understand the relationship between pesticide use, genetic susceptibility in XMEs, and prostate cancer risk, it is important to investigate the role of the interaction between pesticides and genetic polymorphisms. Only one study has considered the joint contribution of two single nucleotide polymorphisms (SNPs) in XME genes and pesticide use on subsequent risk of prostate cancer; this study observed an elevated but non-significant risk among carriers of the allele variants [18].

In this study we evaluated the interaction between pesticide use and 1,913 SNPs in genes that code XMEs, and risk of prostate cancer in 2,220 AHS participants.

MATERIALS AND METHODS

Study population

The AHS is a prospective cohort study that includes 55,747 male licensed pesticide applicators in Iowa and North Carolina. Applicators were recruited from 1993 through 1997; a detailed description of this cohort has been previously published [19]. During a follow-up telephone interview conducted in 1999–2003, applicators were asked for a mouthwash rinse

sample to provide DNA from buccal cells. Approximately 72% of all applicators who completed the follow-up interview returned a buccal sample. In addition, applicators with incident prostate cancer who did not return a sample at follow-up were asked separately to provide a mouthwash rinse sample, with 307/561 (55%) returning a sample. Men diagnosed with incident prostate cancer between 1993 and 2004 who also provided a buccal cell sample were included in the current nested-case-control study. Eligibility, inclusion and exclusion criteria have been previously described [20]. Briefly, cancer cases were coded using the *International Classification of Diseases for Oncology*, 2nd edition, and stage (local, regional, distant, unstaged) and grade (well differentiated, moderately differentiated, poorly differentiated, undifferentiated, missing) were abstracted by the state cancer registries in Iowa and North Carolina. Eligible controls were frequency matched 2:1 to cases by date of birth (+/- 1 year). Controls were male applicators who provided buccal cell material, were alive and not lost to follow-up at the time of case diagnosis, and had no previous cancer diagnosis except non-melanoma skin cancer. All participants for the nested case-control study are white. Based on these inclusion criteria, 841 cases (66% of total white cases in the cohort as of 2004) and 1,659 controls were identified (total N= 2,500). Due to genotyping space limitations 164 controls were excluded. Of the remaining samples, 108 were removed due to insufficient or poor DNA quality (N=20; 14 cases, 6 controls) or <90% completion rate (i.e. more than 10% of the SNP assays failed for a given sample, N=88; 47 cases, 41 controls). We further identified 5 individuals who were suspected to be non-white (<80% European ancestry using STRUCTURE software [21] or significant deviation from the first two components in principal components analysis [22]) leaving a final sample size of 776 cases and 1,444 controls. Informed consent was obtained and the study protocol was reviewed by all relevant Institutional Review Boards.

Genotyping, Gene/SNP selection, and Quality Control

DNA was extracted from buccal cells using the Autopure protocol (QIAGEN). Genotyping was performed at NCI's Core Genotyping Facility (<http://cgf.nci.nih.gov/operations/multiplex-genotyping.html>) [23], using the Custom Infinium® BeadChip Assays (iSelect™) from Illumina Inc. as part of a collaborative genotyping effort of an array of 26,512 SNPs in 1,291 candidate genes. TagSNPs were chosen to cover candidate genes for the three ancestry populations [Caucasian (CEU), Japanese Tokyo (JPT) + Chinese Beijing (CHB) and Yoruba people of Ibadan, Nigeria (YRI)] in the HapMap Project (Data Release 20/Phase II, NCBI Build 36.1 assembly, dbSNPb126) to allow use of this custom iSelect panel for studies containing different ethnic populations. TagSNPs were chosen using a modified version of the method described by Carlson et al. [24] as implemented in the Tagzilla (<http://tagzilla.nci.nih.gov/>) software package. For each original candidate gene, SNPs within the region spanning 20 kb 5' of the start of transcription to 10 kb 3' of the end of the last exon were grouped using a binning threshold of $r^2=0.80$, and tagSNPs chosen from these bins.

From the available data, we selected 149 candidate genes in the xenobiotic metabolism pathway, defined to include genes known to play a role in the biotransformation of xenobiotic substrates, including pesticides. Selected genes include those that encode phase I and phase II enzymes, receptors mediating the induction of xenobiotic biotransforming enzymes, and key enzymes in the regulation of the intracellular redox environment (oxidative stress). Additional genotyping for two glutathione transferase variants responsible for key conjugation reactions was also conducted; copy number variation assays for *GSTM1* (Ex4+10+>-) and *GSTT1* (Ex5-49+>-) deletions were performed separately using Applied Biosystems TaqMan® SNP Genotyping Assays. As a result, a total of 2,192 SNPs in 149 candidate genes in the xenobiotic metabolism pathway were identified. Exclusion for SNPs with low completion rate (<90% of samples) and SNPs showing evidence of deviation from

Hardy–Weinberg proportions ($p < 1 \times 10^{-6}$) were made. SNPs with $MAF < 0.05$ in the nested case-control samples were further excluded for analysis due to limited power. The overall genotyping rate was between 96% and 100% in the resultant $N=1,913$ SNPs in 149 candidate genes (Supplemental Table 1). Blinded duplicate samples (2%) were also included and concordance of these samples ranged from 96–100%.

Statistical Analysis

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between SNPs and prostate cancer, pesticides and prostate cancer and the interaction between SNPs, pesticide use and prostate cancer risk. For SNP analyses, genotypes were coded as counts of the risk allele assuming a log-additive model and models were adjusted for age (10 yr-intervals) and state (Iowa or North Carolina). Exposure to pesticides was classified from responses to two self-administered questionnaires that were completed at enrollment. These questionnaires collected comprehensive data on lifetime use of 50 pesticides; pesticides with a prevalence of use less than 5% in the current nested case-control subgroup were excluded leaving 45 pesticides for analysis (17 herbicides, 21 insecticides, 2 fumigants, and 5 fungicides). Participants were asked how many years they applied each chemical (1 year or less, 2–5, 6–10, 11–20, 21–30, or more than 30 years) and how many days the applicator personally used it in an average year (less than 5, 5–9, 10–19, 20–39, 40–59, 60–150, or more than 150 days). Pesticides were categorized by lifetime exposure days (years of use $\times$ days per year) in this analysis from AHS data release version PIREL0712.04. Lifetime exposure days were categorized as non-exposed, low, and high exposed to a given chemical using the median cut-point based on distribution of lifetime days among cases and controls together for each chemical. Pesticide models were adjusted for confounders of the pesticide-prostate cancer relationship and include age, state and family history of prostate cancer in first degree relative (no, yes, missing).

SNP-pesticide interactions were examined using a multiplicative model. The p-value for each SNP-pesticide interaction was computed by comparing nested models with and without the cross-product terms using a likelihood ratio test. SNP-pesticide interactions with $p\text{-interaction} \leq 0.01$ were carried further for exploration with stratification by genotype using a dominant model for increased power. The top five interaction results, regardless of the direction of the association, from each XME pathway are presented. We also hypothesized that pesticides increase the risk of prostate cancer; thus in order to highlight the most interesting findings, additional stratified results (with $p\text{-interaction} \leq 0.01$) are presented if a monotonic increasing risk across pesticide exposure strata was evident. All interaction models were adjusted for age and state. Additional factors that were examined but ultimately not considered in the modeling because they did not change point estimates by more than 10% were family history of prostate cancer in first degree relative (no, yes, missing), type of applicator (private or commercial), and other pesticide adjustment based on the correlations between selected pesticides. We were not able to explore aggressive prostate cancer alone due to small numbers.

We applied the false discovery rate (FDR) (Benjamini–Hochberg adjustment) method to account for the expected proportion of false discoveries [25]. FDR values were calculated separately for each pesticide and separately from the results of 1,913 tests (i.e., total number of SNPs studied) in the evaluation of the association between each SNP-pesticide interaction and the risk of prostate cancer. Interactions were deemed noteworthy at an $FDR = 0.20$ level.

RESULTS

Cases and controls selected for the nested case-control study were similar to all prostate cancer cases from the cohort in age, state of residence, applicator type, presence of familial prostate cancer, and in prostate cancer disease characteristics for cases (data not shown) [20]. A list of all 149 genes and the number of SNPs in each gene identified in the xenobiotic metabolizing pathway is presented in Supplemental Table 1.

We examined SNPs involved in regulating a number of enzymes, including 1,203 in phase I/phase II enzymes, 61 in key xenobiotic receptors, and 649 in key enzymes that regulate the intracellular redox environment Table 1 shows a summary of the SNP results, as well as the interaction results. The number of observed main effects of SNPs in the oxidative stress pathway (at the $\alpha=0.01$ level) was slightly greater than the number expected by chance (1% of the 649 SNPs examined in this pathway); in contrast, we did not observe any departure from the expected numbers of main effects for SNPs in Phase I/Phase II enzyme genes or in receptor genes. When crossed with 45 pesticides, the number of observed interactions was comparable to the number expected by chance alone (at the $\alpha=0.01$ level).

Associations between SNPs in XME genes and prostate cancer that were statistically significant at the $p \leq 0.01$ level are presented in Table 2. P-values for all 1,913 SNPs are presented in Supplemental Table 2. The strongest association was seen for rs933271 in the oxidative stress gene thioredoxin reductase 2 (*TXNRD2*) with $OR_{\text{per allele}} = 1.33$ (95% CI: 1.16, 1.52), $p\text{-trend} = 4.62 \times 10^{-5}$. Five additional SNPs in this gene were associated with prostate cancer, rs5993882 ($p\text{-trend}=0.0014$), rs5746847 ($p\text{-trend}=0.0036$), rs4485648 ($p\text{-trend}=0.0065$), rs9606186 ($p\text{-trend}=0.0078$), rs6518591 ($p\text{-trend}=0.0086$). After simultaneous adjustment for these five *TXNRD2* SNPs, only rs933271 remained significant ($p=0.0075$), suggesting a single independent signal. Associations between several other oxidative stress genes and prostate cancer were observed. Two SNPs in myeloperoxidase (*MPO*) were significantly associated with prostate cancer, rs11079344, $OR_{\text{per allele}} = 1.59$ (95% CI: 1.22, 2.08) and rs8178406, $OR_{\text{per allele}} = 1.21$ (95% CI: 1.07, 1.37). Two SNPs in microsomal epoxide hydrolase 1 (*EPHX1*) were also significantly associated with prostate cancer, rs2740168 ($p\text{-trend}=0.0042$) and rs2292566, $OR_{\text{per allele}} = 1.27$ (95% CI: 1.07, 1.51). Additional associations in phase I/phase II enzyme genes including *CYP2C18*, and *CYP2C19* were also observed.

Associations between pesticide use and prostate cancer are presented in Table 3. Although no statistically significant positive associations between pesticides and prostate cancer were observed, there was suggestive evidence of increased risk (OR greater than 1.0) with increasing number of days of use of petroleum oil/petroleum distillate used as herbicide, terbufos, fonofos, phorate, and methyl bromide. Among chemicals with a lower prevalence of use (<30 %) in the nested case-control study (dieldrin, lindane, 2,4,5-T and others), some significant ($p\text{-trend}<0.05$) inverse associations were observed. A list of all 45 pesticides, their prevalence of use, and the median level of lifetime days of use of each chemical is presented in Supplemental Table 3.

Stratified odds ratios for the association between pesticides and prostate cancer for the top five interactions from each XME pathway are presented in Table 4. In general, the p-values for SNPs in Phase I/II enzymes and for oxidative stress SNPs were smaller compared with those for receptors. Of the fifteen interaction results presented, fourteen were qualitative (involving increased risk with exposure in one genotype stratum and decreased risk in the other) or showed a significant protective association with prostate cancer. However, one interaction showed evidence for a positive interaction with a monotonic trend of increasing risk among variant allele (minor allele) carriers; the risk of prostate cancer associated with

low petroleum oil/petroleum distillate use was 1.6 times those with no use (OR=1.6, 95% CI: 0.9, 2.9) and for high petroleum oil/petroleum distillate use was 3.7 times those with no use (OR=3.7, 95% CI: 1.9–7.3) among men carrying at least one variant allele in the glutamate-cysteine ligase (*GCLC*) SNP rs1883633. None of the interactions presented in Table 4 were noteworthy after adjustment using the FDR method.

In Table 5, we present all results that show a positive monotonic trend with a p-interaction for pesticides and prostate cancer <0.01, regardless of pathway. Several SNP-pesticide combinations in oxidative stress genes and phase I/phase II enzyme genes displayed this pattern, but none in the receptor pathway, although fewer tests were conducted for receptor SNPs. In addition to the positive interaction between petroleum oil/petroleum distillate and rs1883633, in the oxidative stress gene *GCLC* (p-interaction= 1.0×10^{-4}), other notable pesticide interactions among variants which also showed independent associations with prostate cancer were observed. Among men carrying the variant allele for *TXNRD2* rs4485648, *EPHX1* rs17309872, or *MPO* rs11079344, increased prostate cancer risk was observed with high compared to no petroleum oil/petroleum distillate (OR=1.9, 95% CI: 1.1–3.2, p-interaction=0.01), (OR=2.1, 95% CI: 1.1–4.0, p-interaction=0.01), or terbufos (OR=3.0, 95% CI: 1.5–6.0 p-interaction= 2.0×10^{-3}) use, respectively. None of the interactions presented in Table 5 were noteworthy after adjustment using the FDR method.

DISCUSSION

In this nested case-control study, we evaluated the interaction between genes coding for XMEs and pesticide use on the risk of prostate cancer. We used two approaches to present interaction results. The first approach highlights the top five interactions observed in each XME pathway (Table 4). Among these interactions, the majority showed biologically less plausible inverse associations, qualitative interactions, or unstable point estimates due to small cell counts. For the second approach, we presented interactions (Table 5) where at least one genotype stratum showed a significant positive pesticide effect on prostate cancer and a monotonic pattern across pesticide use strata (from low to high use). The interaction between petroleum oil/petroleum distillate and the promoter region SNP rs1883633, in *GCLC* was identified in both approaches. Several other notable interactions in oxidative stress and phase I/phase II enzyme genes based on the stratified pattern of pesticide use by genotype were observed, including several interactions with SNPs that also showed a main effect on prostate cancer in our study; however, none were deemed noteworthy at the FDR = 0.20 level. Furthermore, the number of observed interactions was comparable to the number expected by chance alone.

The *GCLC* gene is shown to have oxidative stress-responsive elements in the promoter/enhancer region [26;27] and polymorphisms that are associated with decreased *GCLC* expression are suggested to be important determinants of susceptibility to oxidative stress and DNA damage [28]. While this SNP was not independently associated with prostate cancer, there does appear to be a modifying effect of *GCLC* genotype on the association between petroleum oil/petroleum distillate use and prostate cancer. The use of petroleum oil/petroleum distillate has not been previously associated with prostate cancer in AHS analyses [29], however, there appears to be a non-significant but positive association with petroleum oil herbicide use in this subset of nested case-control participants. Historically, petroleum oils and distillates, hydrocarbons derived from petroleum, have been used as herbicides [30]. It is difficult to interpret the effect of the use of petroleum oil-based pesticides on prostate cancer in this study because of the wide variability in use and structure as well as the lack of specificity about its use in the questionnaire data. Nonetheless, several other interactions with this chemical were observed in oxidative stress pathway SNPs, implicating this mechanism in pesticide-related prostate carcinogenesis.

Other interactions with petroleum oil were also observed. We observed an increased risk of prostate cancer associated with increasing levels of petroleum oil use among variant allele carriers of the *TXNRD2* SNP rs4485648, which also had an association with prostate cancer (SNP p-trend=0.0065). *TXNRD2* is a key enzyme in the regulation of the intracellular redox environment [31]. Thus, polymorphisms in these enzymes may result in an imbalance in the oxidative stress/antioxidant status [32]. These oxygen radicals may cause damage to DNA and chromosomes, induce epigenetic alterations, and interact with oncogenes or tumor suppressor genes [33;34] and increase prostate cancer risk. It is important to consider, however, that alternative mechanisms might explain the increased prostate cancer risk observed with *TXNRD2*. This gene partially overlaps the catechol-O-methyltransferase (*COMT*) gene on chromosome 22 that has been well described in androgen and estrogen metabolism [35;36]. The role of androgen biosynthesis and metabolism in prostate cancer growth, proliferation, and progression is well established [37] and the putative role of pesticides as endocrine disruptors [38], suggests that pesticides may act via a hormonal mechanism to influence prostate cancer development.

Several of the pesticides with significant interactions in this study have been previously linked to prostate cancer in the AHS. For example, use of the fumigant methyl bromide was significantly associated with prostate cancer risk in AHS applicators in an early analysis of the cohort [29]. Also, use of the organophosphate insecticides fonofos and terbufos has been linked with excesses of prostate cancer in applicators among those with a family history of prostate cancer [29;39;40]. The association between terbufos and prostate cancer is of particular interest since it is still registered for use and has a relatively high prevalence of ever use (~40% in AHS cohort), unlike fonofos which is no longer registered for use and methyl bromide which has a low prevalence of use. In the present study terbufos use was associated with prostate cancer in the presence of the variant genotype of the *MPO* promoter SNP rs11079344. This SNP was also significantly associated with prostate cancer in the current study. *MPO* is an oxidative stress gene that codes for myeloperoxidase which has been described to influence cancer risk in response to xenobiotics, including pesticides [41–43]. Another *MPO* promoter region SNP which was not genotyped here, with a variant resulting in decreased *MPO* expression [44], has been extensively studied in lung, bladder, and breast cancer [45]. Thus, our results suggest that when we consider these two potential risk factors together, the risk of prostate cancer may be potentiated.

In addition to variants in oxidative stress related genes, SNPs in Phase I/Phase II enzyme genes showed positive associations with prostate cancer. For example, among men carrying the variant allele in the *EPHX1* gene rs2292566, the risk of prostate cancer in the highest category of petroleum oil use was 2.1 times those with no use. In addition, the independent association of rs2292566 appears to be an important risk factor for prostate cancer in this population. Epoxide hydrolase functions in both the activation and detoxification of epoxides and low *EPHX1* activity has been associated with increased cytogenetic damage in the presence of pesticide exposure [3;41]. Variations in direct pesticide metabolizing enzymes, including those in the CYP2C family appear also to be important genetic markers of prostate cancer susceptibility in this study. Although we did not observe any positive interactions, several SNP associations were observed in *CYP2C18* and *CYP2C19*. A variety of organophosphate and carbamate compounds have been shown to be directly metabolized by phase I enzymes CYP2C8, CYP2C9, CYP2C18, and CYP2C19 [46]. Thus, polymorphisms in the genes that code for these Phase I/Phase II enzymes may alter levels of toxic pesticide intermediates and influence prostate cancer risk.

Several strengths and limitations of our study should be recognized. High quality genotype and pesticide information is available in the AHS. For many gene-exposure studies, the key limitation is the quality of the exposure information. The quality of information on pesticide

use among AHS participants is high; self-reported pesticide use information has been found to be reliable in this cohort [47;48]. Furthermore, the ability of the AHS to look at individual pesticides rather than groups (herbicides or insecticides or chemical classes) is critical because observed cancer risks appear to be chemical-specific. Although the numbers within some strata were small, to our knowledge there are no other studies with more power to examine this interaction. In addition, we considered many interactions and none of the observed interactions were deemed noteworthy at the FDR=0.20 level; it is possible, however, that multiple testing issues have masked any true interactions present in this dataset. The numbers of interactions were what we might have expected by chance alone although some interesting patterns were observed. Similarly, we observed interesting SNP associations, however, the number of significant associations for SNPs in the oxidative stress genes was only slightly greater than would be expected by chance, while the number of significant associations for SNPs in Phase I/Phase II enzyme genes and in receptor genes were as expected. Also, we were not able to examine disease aggressiveness due to small numbers. Finally, all participants in this study were white, which limits the generalizability of the results to other racial/ethnic groups.

In conclusion, we observed several positive interactions in oxidative stress and phase I/phase II enzyme genes. None, however, were deemed noteworthy at the FDR=0.20 level and the overall number of observed interactions was comparable to the number expected by chance alone. Nonetheless, some XMEs did modify the association between pesticide use and prostate cancer. Interactions of pesticides and SNPs in oxidative stress genes and phase I/phase II enzyme genes implicate these mechanisms in particular. More evidence for the pesticide-prostate association is needed to fully explain the mechanisms by which pesticides might influence cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SNP	Single Nucleotide Polymorphism
XME	Xenobiotic Metabolizing Enzyme
OR	Odds Ratio
CI	Confidence Interval
AHS	Agricultural Health Study
PNCC	Prostate Nested Case-Control Study
FDR	False Discovery Rate

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Table 1

Summary results table for observed and expected single nucleotide polymorphism associations and pesticide interactions in xenobiotic metabolizing enzymes.

XME pathway	Total SNPs	Expected SNP Associations ≤ 0.01	Observed SNP Associations ≤ 0.01	Pesticides Examined	Expected Interactions ≤ 0.01	Observed Interactions ≤ 0.01
Phase I/Phase II	1203	12	10	45	541	537
Receptors	61	<1	0	45	28	31
Oxidative Stress	649	7	13	45	292	295

Xenobiotic Metabolizing Enzyme [XME], Single Nucleotide Polymorphism (SNP).

Table 2

Selected results (p-trend ≤ 0.01) for the association between prostate cancer and SNPs in xenobiotic metabolizing enzyme genes in the AHS PNCC

SNP	SNP Region	Risk allele	RAF*	Chromosome	Gene	XME pathway	OR [†] 95% CI	p-trend
rs933271	-2080T>C	C	0.26	22	<i>TXNRD2</i>	Oxidative Stress	1.33 (1.16, 1.52)	4.62E-05
rs11079344	-16363A>G	A	0.92	17	<i>MPO</i>	Oxidative Stress	1.59 (1.22, 2.08)	0.0007
rs5993882	-8206 T>G	G	0.22	22	<i>TXNRD2</i>	Oxidative Stress	1.26 (1.09, 1.45)	0.0014
rs3760808	IVS1-1326C>T	C	0.68	19	<i>SULT2B1</i>	Phase I, Phase II Enzymes	1.24 (1.08, 1.42)	0.0023
rs8178406	*3573T>C	T	0.58	17	<i>MPO</i>	Oxidative Stress	1.21 (1.07, 1.37)	0.0030
rs12979328	-12597A>C	C	0.26	19	<i>DHHDH</i>	Oxidative Stress	1.22 (1.07, 1.40)	0.0034
rs5746847	IVS1-2382A>G	C	0.54	22	<i>TXNRD2</i>	Oxidative Stress	1.20 (1.06, 1.36)	0.0036
rs2740168	IVS3+1328G>A	G	0.58	1	<i>EPHX1</i>	Phase I, Phase II Enzymes	1.20 (1.06, 1.37)	0.0042
rs2287833	-15250T>A	A	0.33	19	<i>DHHDH</i>	Oxidative Stress	1.20 (1.06, 1.37)	0.0044
rs10424237	IVS2+2144C>G	C	0.52	19	<i>SULT2B1</i>	Phase I, Phase II Enzymes	1.20 (1.06, 1.35)	0.0047
rs854555	IVS8+1126C>A	C	0.64	7	<i>PONI</i>	Oxidative Stress	1.21 (1.06, 1.38)	0.0049
rs2292566	Ex3-8G>A	A	0.13	1	<i>EPHX1</i>	Phase I, Phase II Enzymes	1.27 (1.07, 1.51)	0.0057
rs2547238	IVS3-93G>C	G	0.71	19	<i>SULT2A1</i>	Phase I, Phase II Enzymes	1.21 (1.06, 1.40)	0.0060
rs854550	Ex9-255C>T	C	0.79	7	<i>PONI</i>	Oxidative Stress	1.25 (1.07, 1.47)	0.0065
rs4485648	IVS1-784A>G	G	0.18	22	<i>TXNRD2</i>	Oxidative Stress	1.24 (1.06, 1.44)	0.0065
rs7659526	-17127T>C	T	0.85	4	<i>GSTCD</i>	Oxidative Stress	1.29 (1.07, 1.56)	0.0071
rs9606186	IVS1-1738G>C	G	0.53	22	<i>TXNRD2</i>	Oxidative Stress	1.18 (1.04, 1.33)	0.0078
rs7874112	IVS4-5165A>G	G	0.11	9	<i>MTAP</i>	Oxidative Stress	1.30 (1.07, 1.57)	0.0079
rs2281891	Ex8+5C>T	C	0.83	10	<i>CYP2C18</i>	Phase I, Phase II Enzymes	1.26 (1.06, 1.50)	0.0080
rs1322179	IVS5-5011C>T	C	0.83	10	<i>CYP2C19</i>	Phase I, Phase II Enzymes	1.26 (1.06, 1.49)	0.0084
rs6518591	IVS1+5203T>C	C	0.18	22	<i>TXNRD2</i>	Oxidative Stress	1.23 (1.05, 1.44)	0.0086
rs8102683	-7433C>T	C	0.74	19	<i>CYP2A6</i>	Phase I, Phase II Enzymes	1.22 (1.05, 1.41)	0.0086
rs2296680	IVS5+221G>A	G	0.83	10	<i>CYP2C18</i>	Phase I, Phase II Enzymes	1.25 (1.06, 1.49)	0.0089
rs12768009	IVS1+3235G>A	G	0.83	10	<i>CYP2C19</i>	Phase I, Phase II Enzymes	1.25 (1.05, 1.48)	0.0100

Single Nucleotide Polymorphism (SNP); Xenobiotic Metabolizing Enzyme (XME); Odds Ratio (OR); Confidence Interval (CI); Agricultural Health Study (AHS); Prostate Nested Case-Control Study (PNCC).

* Risk Allele Frequency (RAF) among controls.

[†] OR per risk allele assuming a log-additive model. Adjusted for age and state.

Table 3
Odds ratios and 95% CI for the association between prostate cancer and 45 pesticides in the AHS PNCC

Lifetime exposure days	None		Low		High		P trend
	Ca/Co		Ca/Co	OR* (95% CI)	Ca/Co	OR* (95% CI)	
Carbaryl	352/633	REF	110/224	0.84 (0.64, 1.10)	107/256	0.62 (0.46, 0.84)	0.003
2,4,5-T	500/898	REF	93/158	1.07 (0.81, 1.42)	50/148	0.60 (0.42, 0.84)	0.004
Lindane	606/1,089	REF	38/94	0.69 (0.47, 1.03)	29/80	0.64 (0.41, 0.99)	0.031
Diazinon	513/964	REF	68/117	1.03 (0.74, 1.42)	47/122	0.67 (0.47, 0.96)	0.032
Phorate	462/846	REF	79/155	0.73 (0.54, 0.99)	76/195	1.16 (0.92, 1.44)	0.041
Dieldrin	660/1,203	REF	21/52	0.78 (0.46, 1.31)	26-Jun	0.44 (0.18, 1.07)	0.044
DDT	373/699	REF	100/205	0.89 (0.68, 1.18)	104/239	0.77 (0.59, 1.01)	0.064
Chlordane	505/888	REF	77/190	0.69 (0.51, 0.92)	52/112	0.79 (0.55, 1.11)	0.092
2,4,5-TP	662/1,222	REF	17/31	0.96 (0.52, 1.75)	13/41	0.58 (0.31, 1.10)	0.095
Cyanazine	391/698	REF	151/309	0.84 (0.65, 1.07)	141/305	0.78 (0.60, 1.00)	0.104
Metolachlor	369/712	REF	179/303	1.14 (0.91, 1.44)	133/303	0.84 (0.65, 1.07)	0.109
Imazethapyr	411/773	REF	143/232	1.16 (0.89, 1.50)	127/293	0.81 (0.62, 1.05)	0.119
Heptachlor	545/1,003	REF	48/108	0.85 (0.59, 1.23)	52/124	0.77 (0.55, 1.10)	0.123
Malathion	225/399	REF	173/351	0.85 (0.67, 1.09)	142/310	0.80 (0.62, 1.04)	0.149
Permethrin (all)	575/1,100	REF	78/117	1.24 (0.92, 1.69)	55/128	0.80 (0.57, 1.12)	0.224
Petroleum oil/Petroleum Distillate	488/964	REF	56/113	1.00 (0.71, 1.41)	57/93	1.24 (0.87, 1.76)	0.236
2,4-D	135/218	REF	309/597	0.83 (0.64, 1.08)	288/582	0.79 (0.61, 1.04)	0.245
Pendimethalin	474/856	REF	73/173	0.74 (0.55, 0.99)	79/165	0.85 (0.63, 1.14)	0.247
Chlorothalonil	711/1,320	REF	23/54	0.74 (0.45, 1.24)	17/39	0.73 (0.40, 1.32)	0.257
Maneb/mancozeb	664/1,238	REF	15/35	0.77 (0.41, 1.45)	18/40	0.72 (0.40, 1.30)	0.279
Paraquat	592/1,082	REF	39/105	0.65 (0.44, 0.96)	34/67	0.82 (0.53, 1.29)	0.296
Alachlor	277/546	REF	223/400	1.08 (0.86, 1.35)	176/378	0.91 (0.72, 1.15)	0.298
Butylate	501/903	REF	43/122	0.64 (0.44, 0.93)	82/169	0.86 (0.64, 1.15)	0.305
Dicamba	324/573	REF	171/368	0.78 (0.61, 1.00)	181/360	0.82 (0.63, 1.05)	0.318
Metribuzin	433/792	REF	83/183	0.84 (0.62, 1.12)	92/192	0.87 (0.66, 1.16)	0.351
Toxaphene	585/1,084	REF	36/77	0.82 (0.54, 1.24)	43/86	0.87 (0.59, 1.28)	0.354

Lifetime exposure days	None		Low		High		P trend
	Ca/Co		Ca/Co	OR* (95% CI)	Ca/Co	OR* (95% CI)	
Coumaphos	610/1,144	REF	36/66	1.00 (0.66, 1.52)	30/66	0.83 (0.53, 1.30)	0.426
DDVP	603/1,123	REF	42/90	0.81 (0.55, 1.20)	44/93	0.85 (0.58, 1.24)	0.428
Captan	623/1,144	REF	30/68	0.81 (0.52, 1.27)	31/62	0.85 (0.54, 1.33)	0.491
Chlorimuron-ethyl	487/955	REF	97/173	1.10 (0.83, 1.44)	47/106	0.83 (0.58, 1.20)	0.504
Atrazine	189/375	REF	277/494	1.13 (0.89, 1.42)	275/545	1.00 (0.79, 1.27)	0.512
Benomyl	662/1,242	REF	19/33	1.03 (0.58, 1.86)	17/36	0.84 (0.46, 1.52)	0.556
Fonofos	511/992	REF	104/181	1.12 (0.85, 1.47)	74/132	1.07 (0.78, 1.46)	0.605
Aldicarb	675/1,269	REF	13/23	0.97 (0.48, 1.96)	14/28	0.84 (0.43, 1.64)	0.607
Parathion	627/1,176	REF	28/36	1.36 (0.82, 2.26)	24/50	0.83 (0.50, 1.38)	0.609
Aldrin	481/896	REF	63/148	0.80 (0.57, 1.10)	83/165	0.93 (0.70, 1.26)	0.628
Metolaxyl	590/1,113	REF	46/85	0.98 (0.67, 1.44)	36/67	0.92 (0.58, 1.44)	0.705
Carbon Disulfide/Carbon Tetrachloride	674/1,244	REF	21/39	1.02 (0.59, 1.76)	21/42	0.91 (0.53, 1.55)	0.742
Trifluralin	312/583	REF	186/362	0.95 (0.76, 1.20)	185/357	0.95 (0.75, 1.21)	0.762
EPTC	530/1,063	REF	80/112	1.41 (1.03, 1.93)	63/129	0.95 (0.68, 1.31)	0.781
Methyl bromide	637/1,215	REF	52/108	0.88 (0.60, 1.28)	56/97	1.05 (0.71, 1.53)	0.799
Chlorpyrifos	451/854	REF	137/248	1.02 (0.80, 1.30)	164/313	0.98 (0.78, 1.23)	0.838
Carbofuran	433/857	REF	129/212	1.18 (0.92, 1.51)	121/237	0.98 (0.77, 1.26)	0.845
Glyphosate	182/333	REF	285/561	0.90 (0.71, 1.14)	274/527	0.93 (0.73, 1.18)	0.886
Terbufos	406/803	REF	156/256	1.21 (0.95, 1.54)	124/246	1.01 (0.78, 1.30)	0.927

Confidence Interval (CI); Agricultural Health Study (AHS); Prostate Nested Case-Control Study (PNCC).

* Adjusted for age, state, and family history of prostate cancer.

Table 4

Stratified odds ratios and 95% CI, adjusted for age and state, for associations between pesticides and prostate cancer for top five interactions from each pathway

						Pesticide Use						
PHASE I/PHASE II ENZYMES						None		Low		High		
SNP	Gene	SNP p-trend	Pesticide	Genotype	Ca/Co		Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	P-interaction	
rs1808682	CYP2B6	0.5846	DDT	AA	204/432	REF	62/110	1.3 (0.9, 1.8)	60/125	1.0 (0.7, 1.5)	6.3 × 10 ⁻⁵	
				AG+GG	169/267	REF	38/95	0.6 (0.4, 0.9)	44/114	0.6 (0.4, 0.8)		
rs9367980	TPMT	0.4652	Alachlor	CC	168/256	REF	116/221	0.8 (0.6, 1.1)	92/199	0.7 (0.5, 1.0)	8.7 × 10 ⁻⁵	
				CT+TT	105/276	REF	105/170	1.7 (1.2, 2.3)	82/166	1.3 (0.9, 1.9)		
rs2331564	UGT2A3	0.5114	Carbofuran	CC	312/584	REF	89/149	1.1 (0.8, 1.5)	71/181	0.7 (0.5, 0.9)	1.3 × 10 ⁻⁴	
				CT+TT	116/264	REF	38/54	1.7 (1.0, 2.7)	47/53	2.0 (1.3, 3.1)		
rs4646450	CYP3A5	0.1301	DDVP	TT	432/748	REF	31/59	0.9 (0.6, 1.5)	22/73	0.5 (0.3, 0.9)	1.6 × 10 ⁻⁴	
				CT+CC	170/373	REF	11/31	0.7 (0.4, 1.5)	22/19	2.5 (1.3, 4.8)		
rs7659526	GSTCD	0.0071	Cyanazine	AA	322/486	REF	115/237	0.7 (0.5, 1.0)	103/235	0.6 (0.5, 0.9)	2.3 × 10 ⁻⁴	
				AG+GG	69/210	REF	35/71	1.4 (0.8, 2.4)	38/70	1.6 (0.9, 2.6)		
RECEPTORS												
rs2301677	AHR	0.3914	Malathion	AA	106/241	REF	111/197	1.3 (0.9, 1.8)	90/166	1.2 (0.9, 1.7)	3.2 × 10 ⁻⁵	
				AG+GG	111/141	REF	54/138	0.5 (0.3, 0.7)	49/131	0.5 (0.3, 0.7)		
rs3802082	AHR	0.1992	Malathion	TT	133/272	REF	122/218	1.1 (0.8, 1.6)	97/193	1.0 (0.7, 1.4)	2.3 × 10 ⁻⁴	
				CT+CC	88/119	REF	42/116	0.5 (0.3, 0.8)	41/102	0.5 (0.3, 0.9)		
rs2501870	NR1I3	0.9349	Glyphosate	CC	93/207	REF	166/307	1.2 (0.9, 1.7)	157/275	1.3 (1.0, 1.8)	6.4 × 10 ⁻⁴	
				CT+TT	89/126	REF	119/254	0.6 (0.5, 0.9)	117/252	0.6 (0.4, 0.9)		
rs3802082	AHR	0.1992	Toxaphene	TT	371/691	REF	30/52	1.1 (0.7, 1.7)	34/51	1.2 (0.7, 1.9)	8.1 × 10 ⁻⁴	
				CT+CC	192/352	REF	6/23	0.5 (0.2, 1.2)	9/31	0.5 (0.2, 1.1)		
rs2066853	AHR	0.1154	Malathion	AA	171/332	REF	149/274	1.1 (0.8, 1.4)	123/247	1.0 (0.7, 1.3)	8.7 × 10 ⁻⁴	
				AG+GG	54/66	REF	24/76	0.4 (0.2, 0.7)	19/63	0.4 (0.2, 0.7)		
OXIDATIVE STRESS												
rs2237584	PONI	0.4856	Paraquat	AA	544/954	REF	33/96	0.6 (0.4, 0.9)	26/63	0.7 (0.4, 1.1)	4.2 × 10 ⁻⁵	
				AG+GG	48/128	REF	6/9	1.7 (0.6, 5.1)	8/4	4.8 (1.4, 17.1)		

					Pesticide Use							
PHASE I/PHASE II ENZYMES					None		Low		High			
SNP	Gene	SNP p-trend	Pesticide	Genotype	Ca/Co		Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	P-interaction	
rs3732533	TXNRD3	0.9289	Alachlor	CC	258/473	REF	191/354	1.0 (0.8, 1.2)	152/343	0.8 (0.6, 1.0)	8.9 × 10 ⁻⁵	
				CT+TT	15/72	REF	31/41	4.0 (1.9, 8.3)	22/32	3.6 (1.6, 8.0)		
rs1883633	GCLC	0.2716	Petroleum Oil	AA	376/687	REF	36/81	0.8 (0.5, 1.3)	34/77	0.8 (0.5, 1.3)	1.0 × 10 ⁻⁴	
				AG+GG	11/274	REF	20/32	1.6 (0.9, 2.9)	23/16	3.7 (1.9, 7.3)		
rs13054371	TXNRD2	0.5143	Aldrin	TT	153/227	REF	10/44	0.4 (0.2, 0.9)	20/58	0.6 (0.4, 1.1)	1.9 × 10 ⁻⁴	
				CT+CC	328/668	REF	52/104	1.0 (0.7, 1.4)	63/107	1.2 (0.8, 1.7)		
rs230819	SEPP1	0.0251	2,4,5-T	AA	136/360	REF	16/51	0.6 (0.3, 1.0)	11/42	0.5 (0.2, 1.0)	2.5 × 10 ⁻⁴	
				AG+GG	354/614	REF	76/104	1.3 (0.9, 1.8)	38/99	0.7 (0.5, 1.0)		

Table 5

Stratified odds ratios for the association between pesticides and prostate cancer stratified by oxidative stress and Phase I/Phase II enzyme genotype where p-interaction ≤ 0.01 .

						Pesticide Use						
OXIDATIVE STRESS						None		Low		High		
SNP	Gene	SNP p-trend	Pesticide	Genotype	Ca/Co		Ca/Co	OR* (95% CI)	Ca/Co	OR* (95% CI)	P-interaction	
rs1883633	GCLC	0.2716	Petroleum Oil	AA	376/687	REF	36/81	0.8 (0.5, 1.3)	34/77	0.8 (0.5, 1.3)	1.0 × 10 ⁻⁴	
				AG+GG	111/274	REF	20/32	1.6 (0.9, 2.9)	23/16	3.7 (1.9, 7.3)		
rs10040697	GLRX	0.1111	Petroleum Oil	CC	368/663	REF	39/86	0.8 (0.6, 1.2)	33/63	1.0 (0.6, 1.5)	9.0 × 10 ⁻³	
				CT+TT	117/294	REF	17/26	1.7 (0.8, 3.4)	24/28	2.2 (1.2, 4.1)		
rs17299478	NQO1	0.4819	Petroleum Oil	CC	347/690	REF	39/91	0.9 (0.6, 1.3)	38/75	1.1 (0.7, 1.6)	5.0 × 10 ⁻³	
				CT+TT	141/274	REF	17/22	1.6 (0.8, 3.0)	19/18	2.2 (1.1, 4.3)		
rs4485648	TXNRD2	0.0065	Petroleum Oil	TT	301/634	REF	31/86	0.8 (0.5, 1.2)	26/61	0.9 (0.6, 1.5)	0.010	
				CT+CC	184/326	REF	25/27	1.7 (0.9, 3.0)	31/31	1.9 (1.1, 3.2)		
rs11079344	MPO	0.0007	Terbufos	AA	35/655	REF	133/211	1.2 (0.9, 1.5)	99/204	0.9 (0.7, 1.2)	2.0 × 10 ⁻³	
				AG+GG	32/124	REF	17/36	1.8 (0.9, 3.7)	22/30	3.0 (1.5, 6.0)		
rs9332950	MGST1	0.288	Methyl Bromide	GG	484/911	REF	41/88	0.8 (0.6, 1.3)	38/84	0.8 (0.5, 1.3)	4.0 × 10 ⁻³	
				GC+CC	145/283	REF	11/19	1.1 (0.5, 2.4)	17/10	3.1 (1.3, 7.5)		
rs17309872	GSS	0.6370	Atrazine	AA	170/309	REF	242/430	1.0 (0.8, 1.3)	235/480	0.9 (0.7, 1.2)	9.0 × 10 ⁻³	
				AT+TT	18/66	REF	35/64	2.1 (1.1, 4.3)	40/35	2.3 (1.2, 4.6)		
PHASE I/PHASE II ENZYMES												
rs7291934	SULT4A1	0.4650	Fonofos	AA	399/726	REF	68/135	1.0 (0.7, 1.3)	51/108	0.9 (0.6, 1.3)	5.0 × 10 ⁻⁴	
				AG+GG	96/248	REF	33/42	1.9 (1.1, 3.3)	19/19	2.4 (1.2, 4.9)		
rs2331564	UGT2A3	0.5114	Fonofos	CC	361/674	REF	71/135	1.0 (0.7, 1.4)	41/99	0.8 (0.5, 1.2)	6.0 × 10 ⁻⁴	
				CG+GG	141/304	REF	33/41	1.7 (1.0, 2.9)	32/31	2.2 (1.3, 3.9)		
rs8192775	CYP2E1	0.8473	Chlorpyrifos	GG	393/713	REF	116/215	1.0 (0.8, 1.3)	131/278	0.9 (0.7, 1.1)	1.0 × 10 ⁻³	
				AG+AA	58/139	REF	20/33	1.5 (0.8, 2.9)	33/33	2.5 (1.4, 4.5)		
rs12676857	NAT2	0.1233	Carbofuran	TT	325/588	REF	92/157	1.1 (0.8, 1.4)	82/185	0.8 (0.6, 1.1)	3.0 × 10 ⁻³	
				CT+CC	108/268	REF	37/55	1.7 (1.0, 2.7)	38/52	1.8 (1.1, 3.0)		
rs5764318	SULT4A1	0.7212	Methyl Bromide	TT	449/815	REF	33/78	0.7 (0.5, 1.2)	37/79	0.8 (0.5, 1.3)	6.0 × 10 ⁻³	

					Pesticide Use							
OXIDATIVE STRESS					None		Low		High			
SNP	Gene	SNP p-trend	Pesticide	Genotype	Ca/Co		Ca/Co	OR* (95% CI)	Ca/Co	OR* (95% CI)	P-interaction	
				CT+CC	186/396	REF	19/30	1.3 (0.7, 2.5)	19/18	2.2 (1.0, 4.5)		
				GG	350/728	REF	41/85	1.0 (0.7, 1.5)	33/70	1.0 (0.7, 1.6)		
rs2292566	EPHX1	0.0057	Petroleum Oil	AG+AA	137/236	REF	15/27	1.0 (0.5, 2.0)	24/21	2.1 (1.1, 4.0)	0.010	

* Adjusted for age and state.