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Associations between Smoking, Polymorphisms in Polycyclic Aromatic Hydrocarbon (PAH) Metabolism and Conjugation Genes and PAH-DNA Adducts in Prostate Tumors Differ by Race

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Abstract

Polycyclic aromatic hydrocarbon (PAH)-DNA adducts may induce mutations that contribute to carcinogenesis. We evaluated potential associations between smoking and polymorphisms in PAH metabolism [CYP1A1 Ile^{462}Val, CYP1B1 Ala^{119}Ser and Leu^{432}Val, microsomal epoxide hydrolase (mEH) Tyr^{113}His and His^{139}Arg, CYP3A4 A(−392)G] and conjugation [glutathione *S*-transferase (GST) M1 null deletion, GSTP1 Ile^{105}Val] genes and PAH-DNA adduct levels (measured by immunohistochemistry) in tumor and nontumor prostate cells in 400 prostate cancer cases. Although no statistically significant associations were observed in the total sample, stratification by ethnicity revealed that Caucasian ever smokers compared with nonsmokers had higher adduct levels in tumor cells (mean staining intensity in absorbance units ± SE, 0.1748 ± 0.0052 versus 0.1507 ± 0.0070; $P = 0.006$), and Caucasians carrying two mEH ^{139}Arg compared with two ^{139}His alleles had lower adducts in tumor (0.1320 ± 0.0129 versus 0.1714 ± 0.0059; $P = 0.006$) and nontumor (0.1856 ± 0.0184 versus 0.2291 ± 0.0085; $P = 0.03$) cells. African Americans with two CYP1B1 ^{432}Val compared with two ^{432}Ile alleles had lower adducts in tumor cells (0.1600 ± 0.0060 versus 0.1970 ± 0.0153; $P = 0.03$). After adjusting for smoking status, carrying the putative “high-risk” genotype combination, the faster metabolism of PAH-epoxides to PAH-diol-epoxides (CYP1B1 ^{432}Val/Val and mEH ^{139}Arg/Arg) with lower PAH-diol-epoxide conjugation (GSTP1 ^{105}Ile/Ile), was associated with increased adducts only in Caucasian nontumor cells (0.2363 ± 0.0132 versus 0.1920 ± 0.0157; $P = 0.05$). We present evidence, for the first time in human prostate that the association between smoking and PAH-DNA adducts differs by race and is modified by common genetic variants.

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Introduction

Although prostate cancer is the most commonly diagnosed nonskin cancer and the third leading cause of cancer death among men in the United States (1), increasing age, ethnicity, and family history are the only established risk factors for this disease (2,3). African Americans, in particular, present at an earlier age and with more advanced disease and have higher mortality rates compared with Caucasians (4). Having a strong family history suggests the presence of a highly penetrant gene, but, to date, no single gene which can account for the majority of prostate cancers has been identified. Thus, the pathogenesis of prostate cancer likely involves a complex interplay between multiple low penetrant genetic and environmental factors.

Polycyclic aromatic hydrocarbon (PAH) exposure from cigarette smoke (5-7), grilled meats (8), and various petroleum-related occupations (9-11) may play a role in prostate cancer. Although associations between smoking and these other PAH sources and prostate cancer have been equivocal, PAH require metabolic activation and subsequent binding to DNA (forming bulky “PAH-DNA adducts”) to exert their carcinogenic action (12). Therefore, functional polymorphisms in genes that metabolize PAHs and detoxify their reactive derivatives should be considered when evaluating potential effects of PAH exposure sources. Furthermore, many prior studies have relied upon self-reported measures from a single source; however, PAH-DNA adducts serve as a biological marker of the effective PAH dose from all sources, particularly when quantified in the target tissue. We previously observed that PAH-DNA adducts are present in human prostate cancer cells and vary with tumor characteristics (13).

In terms of PAH metabolism, parent compounds, such as benzo(*a*)pyrene, are initially metabolized by CYP1A1 or CYP1B1 (14,15) to an epoxide [benzo(*a*)pyrene-7,8-epoxide] and subsequently hydrolyzed by microsomal epoxide hydrolase (mEH) to a dihydrodiol [benzo (*a*)pyrene-7,8-dihydrodiol]. CYP1A1, CYP1B1, or CYP3A4 (16) can then transform the dihydrodiol to a highly reactive diol-epoxide [benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide, BPDE] that can covalently bind to DNA, creating a PAH [BPDE]-DNA adduct which may, in turn, induce mutation(s), predominantly in the form of G to T transversions (17). Although mEH (18) and CYP1B1 (19) are expressed in the prostate, CYP1A1 may only be induced under androgen dependency (20) and CYP3A4 may require vitamin D receptor mediation (21). Interestingly, CYP1B1 is highly expressed in the peripheral zone where most prostate cancers arise (22). The CYP1A1 Ile^{462}Val and CYP3A4 A(−392)G polymorphisms have variant alleles with higher enzymatic activity compared with their respective wild-type alleles (23,24), and the activity of the CYP1B1 Ala^{119}Ser and Leu^{432}Val variants is substrate dependent with ^{432}Val/^{119}Ala, having slightly higher activity in metabolizing benzo(*a*)pyrene-7,8-dihydrodiols but slightly lower activity in metabolizing parent benzo(*a*)pyrene than ^{432}Leu/^{119}Ala (15). Effects of the mEH Try^{113}His and mEH His^{139}Arg polymorphisms remain unclear; an earlier study reported that ^{113}Tyr/^{139}Arg had the most activity in hydrolyzing benzo(*a*)pyrene-epoxides to benzo(*a*)-pyrene-dihydrodiols (25), but recent work shows the ^{113}Tyr/^{139}His combination may be the most active (26). The CYP1A1 Ile^{462}Val, CYP1B1 Leu^{432}Val, CYP3A4 A(−392)G polymorphisms have been equivocally associated with prostate cancer (27-31), which may be attributed, in part, to heterogeneity in PAH exposure. In prostate cancer, only one (null) finding has been reported for the mEH His^{139}Arg polymorphism (32) and no studies have examined the mEH Tyr^{113}His polymorphism.

Before a PAH-diol-epoxide metabolite can adduct DNA, it may be detoxified by enzymes in the glutathione *S*-transferase (GST) family. In particular, GSTM1 and GSTP1 exhibit substrate specificity for PAH-diol-epoxides (33) and are expressed in the prostate (34-36). Although GSTT1 is highly expressed in the prostate (37), it does not seem to be involved in PAH metabolite conjugation (38). GSTP1 has two polymorphisms: Ile^{105}Val and Ala114Val (Ile^{105}Val is located near the hydrophobic binding site and has more influence on activity; ref.

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39). The effect of the GSTP1 Ile^{105}Val polymorphism is substrate dependent with the ^{105}Val allele having a higher affinity for conjugating the most reactive PAH-diol-epoxides (40,41). GSTM1 has a polymorphism that leads to complete loss of protein (GSTM1 null deletion), and this polymorphism, as well as GSTP1 Ile^{105}Val, has been associated with increased prostate cancer risk in some studies (42-44) but decreased risk in others (45-47), which may also be due, in part, to heterogeneity in PAH exposure.

Although associations between a few of the aforementioned polymorphisms in metabolism and conjugation genes and PAH-DNA adduct levels have been examined in human lung (48) and breast (49,50) cancer tissues and differences in PAH-DNA adduct levels by race in mononuclear cells have been reported (51), no prior studies have evaluated effects of these polymorphisms on adduct levels in human prostate cancer tissues. Therefore, in this study, we extend our earlier work (13) by evaluating the potential association between smoking and polymorphisms in genes that metabolize PAHs (CYP1A1 Ile^{462}Val, CYP1B1 Ala^{119}Ser and Leu^{432}Val, mEH Tyr^{113}His and His^{139}Arg, CYP3A4 A(−392)G) and detoxify their reactive derivatives (GSTM1 null deletion, GSTP1 Ile^{105}Val) and PAH-DNA adduct levels in tumor and adjacent nontumor prostate cells in 400 men with prostate cancer.

Materials and Methods

Study Population

The study design and population have been described elsewhere (13). Briefly, the study population was composed of men from a larger case (n = 637) and control (n = 244) study who were diagnosed with prostate cancer and underwent radical prostatectomy (n = 395; 62.0%) or transure-thral resection (n = 5; 0.7%) for treatment within the Henry Ford Health System, a network of facilities comprising an 800-bed hospital in the City of Detroit, Michigan, three smaller hospitals in surrounding suburbs, and 31 medical clinics located throughout the Metropolitan Detroit area. Potential cases that indicated primary adenocarcinoma of the prostate were identified through the Henry Ford Health System pathology reports. Two of the 395 cases used in this study were initially enrolled as controls. Cases were eligible for the larger case-control study if they used the Henry Ford Health System as their primary source of health care, lived in the study area at time of recruitment (2001-2004), and had no previous history of prostate cancer.

Subjects who agreed to participate were also asked to complete a two-part interviewer-administered risk factor questionnaire (the first part was conducted over the phone; the second part was done in person) and donate a blood sample. All study protocols were approved by the Henry Ford Hospital Institutional Review Board. Clinical characteristics were obtained from medical records, and demographic, general health, and habit information (age, ethnicity, smoking) were determined from the questionnaire. Alcohol was estimated from a standardized food frequency questionnaire originally developed for two studies investigating the associations of dietary supplements and cancer risk: Vitamin and Lifestyle Cohort Study (52) and Selenium and Vitamin E Cancer Prevention Trial (53).

Genotyping

Standard venipuncture was used to collect blood samples from all study participants in tubes with EDTA as an anticoagulant. Genomic DNA was extracted from buffy coats using QIAmp DNA Blood kit (Qiagen Inc., Valencia, CA). All purified DNA samples were diluted to a constant DNA concentration in 10 mmol/L Tris, 1 mmol/L EDTA buffer (pH 8).

mEH His^{139}Arg (rs2234922) and CYP1B1 Leu^{432}Val (rs1056836) were assayed by RFLP using primer and assay conditions that have been previously described (26,54). Digestion

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products were separated on a 2% agarose gel. CYP1A1 Ile^{462}Val (rs1048943), CYP1B1 Ala^{119}Ser (rs1056827) and mEPHx (mEH) Tyr^{113}His (rs1051740) polymorphisms were assayed using the GenomeLab SNP-Primer Extension assay (Beckman Coulter, Fullerton, CA) and analyzed on a CEQ 8000 Genetic Analysis System (Beckman Coulter). PCR was done using the primers (Invitrogen, Carlsbad, CA) 5′-GAACTGCCACTTCAGCTG-3′ (forward) and 5′-CTGGCTGCCCAACCAGA-3′ (reverse) for CYP1A1, 5′-GTGCTGGCCACTGTGCATGT-3′ (forward) and 5′-ACACGGCACTCATGACGTTG-3′ (reverse) for CYP1B1, and 5′-GATCGATAAGTTCCGTTTCACC-3′ (forward) and 5′-TCATTGGACTGGATGGTGCATT-3′ (reverse) for mEH. PCR was done in a 20-μL reaction with 40 ng DNA, 20 pmol of forward and reverse primers, 1.5 mmol/L $MgCl_2$, 0.25 mmol/L deoxynucleotide triphosphate, and 1.5 units AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). For CYP1B1 amplification, 2 μL DMSO was added to each reaction. PCR conditions were 10 min at 95°C, followed by 30 cycles of 95°C for 30 s, 61°C (CYP1A1) or 60°C (CYP1B1^{119} and mEH^{113}) for 35 s, and 72°C for 1 min, followed by a 6-min extension at 72°C. PCR reactions (6 μL) were cleaned with 2 units of shrimp alkaline phosphatase (Promega, Madison, WI) and 1 unit of Exonuclease I (New England Biolabs, Ipswich, MA) for 2 h at 37°C, followed by heat inactivation at 75°C for 30 min. The SNP primer extension assay was done using the GenomeLab SNP-Primer Extension kit (Beckman Coulter) according to manufacturer's instructions. SNP interrogation primers used were 5′-ATGGGCAAGCGGAAGTGTATCGGTGAGACC-3′ (forward) and 5′-AAAGACCTCCCAGCGGGCAA-3′ (reverse) for CYP1A1 Ile^{462}Val, 5′-AAAAAGGCCCTGGTGCAGCAGGGCTCGGCCTTCGCCGACCGGCCG-3′ (forward) and 5′-AAAAAAAAAAGACACCACACGGAAGGAGGCGAAGG-3′ (reverse) for CYP1B1 Ala^{119}Ser, and 5′-AAAAAAGGTGGAGATTCTCAACAGA-3′ (forward) and 5′-AAAAAAAAAATCAATCTTAGTCTTGAAGTGAGGGT-3′ (reverse) for mEH Tyr^{113}His.

The GSTP1 Ile^{105}Val (rs947894) polymorphism was detected using the Invader assay with reagents developed by Third Wave Technologies, Inc. (Madison, WI) (55). Each plate contained the following controls for the GSTP1 codon 105: (*a*) Ile/Ile homozygous, (*b*) Ile/Val heterozygous, (*c*) Val/Val homozygous, and (*d*) a no-target blank. The GSTM1 polymorphism, which results in the presence (nondeleted) or absence (null deletion) of the enzyme, was detected by a PCR product coamplified with β-globin as a positive internal control within a multiplex PCR as previously described (42).

To ensure quality control of all genotyping results, 5% of the samples were randomly selected and genotyped by a second investigator and 1% of the samples were sequenced using a 377 ABI automated sequencer.

PAH-DNA Adduct Scoring

H&E stained slides of study cases were reviewed by the study pathologist (Adnan T. Savera) to confirm the diagnosis and to identify a paraffin block with sufficient prostate tumor and nontumor prostate tissue from the radical prostatectomy for staining. For each patient sample, we used a microtome to cut five consecutive sections (5-μmol/L thick) from the tissue block. One slide was H&E stained and examined by the study pathologist who circled separate areas of prostate tumor and nontumor prostate cell populations to be used for subsequent PAH-DNA adduct scoring. The immunohistochemical assay for PAH-DNA adducts was carried out as described previously (13,49,56). This chemical assay uses the monoclonal 5D11 antibody, which in cell culture studies has been shown to produce strongly correlated staining levels (r = 0.99; P = 0.011) with the treatment dose of benzo(*a*)pyrene diol epoxide (57,58). Consistent with our previous (13) and other prior studies (49,59) using immunohistochemical assays to measure PAH-DNA adducts, we report our results in absorbance units which provides a measure of the relative intensity of staining.

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For each prostate specimen, two technicians independently scored 50 epithelial cells (five fields with 10 cells per field scored) in the two areas (tumor and nontumor) circumscribed by the study pathologist. Scored cells were selected to be representative, in terms of intensity, of the cells in the field, and the mean of the two technicians' scores was used. The dual scoring technique has proved to yield a high test-retest reliability in prostate cells (13). PAH-DNA adduct data were standardized across experiments using a series of two prostate "control" slides (taken from two separate prostate specimens provided by men with prostate cancer who underwent radical prostatectomy but were not part of the study population) that were run across all batches.

Statistical Analysis

We tested the distribution of PAH-DNA adduct levels in prostatic epithelial tumor and adjacent nontumor cells for normality using the Shapiro-Wilk test statistic. Paired *t* tests were used to determine if PAH-DNA adduct levels between tumor and nontumor cells deviated significantly from zero. Correlations between explanatory variables and PAH-DNA adduct levels in tumor and nontumor cells were calculated using the parametric Pearson or nonparametric Spearman statistic if the variable deviated from normality. We calculated genotype frequencies and tested for Hardy Weinberg equilibrium within controls (prostate cancer–free men) in the larger study within major ethnic groups. We also calculated linkage disequilibrium between CYP1B1 Ala^{119}Ser and Leu^{432}Val and mEH Tyr^{113}His and His^{139}Arg alleles using epoxide hydrolase (60). We then used generalized linear regression models to estimate the association between genotypes and PAH-DNA adduct levels in prostate tumor cells and adjacent nontumor cells in the total study population and in Caucasians and African Americans, separately. Potential confounding by other factors including smoking, alcohol, and tumor characteristics [primary and total Gleason score, tumor volume, grade, prostate specific antigen (PSA) at diagnosis] was also evaluated. Models examining interactions included main effect terms (ethnicity; smoking; polymorphism under a dominant, recessive, or additive genetic model) and a multiplicative interaction term (e.g., ethnicity × genotype). All *P* values are from two-sided tests. All analyses were undertaken with SAS (version 8.2, SAS Institute Inc., Cary, NC).

Results

Characteristics of the study population are provided in Table 1. Approximately, 52.5% of the prostate cancer cases were Caucasian patients and 44.3% were African American patients. The mean age at diagnosis was 60.2 years with African American men diagnosed at a slightly younger age than Caucasian men. Approximately, 45.7% of the cases had a total Gleason score of 7 and 19.4% had a total Gleason score of >7. Although African Americans tended to present with a higher Gleason score and have greater tumor volume than Caucasians, these differences were not statistically significant. Similar to our earlier work that used 130 (13) of the 400 specimens in the present study, the distributions of PAH-DNA adduct levels in paired tumor and adjacent nontumor prostate specimens fell into two separate highly symmetrical normal distributions. Also consistent with our previous report (13), we observed a strong correlation between adduct levels in prostate tumor and nontumor prostate cells ($r = 0.51$; $P < 0.001$) and significantly higher levels of PAH-DNA adducts in nontumor cells compared with tumor cells (mean absorbance units ± SD, 0.23 ± 0.09 versus 0.16 ± 0.06; $P < 0.001$). No statistically significant differences in adduct levels between Caucasians and African Americans were observed in tumor or nontumor prostate cells. Among controls, genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium within major ethnic groups [Caucasians, $P = 0.33$ (GSTP1 Ile^{105}Val) to $P = 0.80$ (mEH His^{139}Arg); African Americans, $P = 0.12$ (GSTP1 Ile^{105}Val) to $P = 0.71$ (CYP3A4 A(−392)G)]. We also examined linkage disequilibrium between alleles in the CYP1B1 Ala^{119}Ser and Leu^{432}Val (Caucasians, D' ≤ 0.37; African Americans, D' ≤ 0.24) and the mEH Tyr^{113}His and His^{139}Arg (Caucasians, D'

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≤ 0.24; African Americans, D' ≤ 0.56) polymorphisms. Significant differences in genotype (and allele) frequencies between Caucasian and African American cases in this study were observed for several polymorphisms (Table 1).

Neither ever or current smoking nor any of the PAH metabolism or conjugation polymorphisms, when examined individually, were statistically significantly associated with PAH-DNA adduct levels in the total sample (Table 2). However, stratifying by race revealed that Caucasian ever smokers (Table 3) had significantly higher adducts than nonsmokers in tumor cells (0.1748 ± 0.0052 versus 0.1507 ± 0.0070; $P = 0.006$). Moreover, Caucasians carrying two copies of the mEH ^{139}Arg allele had decreased PAH-DNA adduct levels in tumor (0.1320 ± 0.0129 versus 0.1714 ± 0.0059; $P = 0.006$) and nontumor (0.1856 ± 0.0184 versus 0.2291 ± 0.0085; $P = 0.03$) cells. Having the A-G genotype compared with the A-A genotype of the CYP3A4(−392) promoter was also positively associated with adduct levels in Caucasian tumor (0.1970 ± 0.0148 versus 0.1648 ± 0.0044; $P = 0.04$) but not Caucasian nontumor cells. In African Americans, carrying one or two CYP1B1 ^{432}Val compared with two ^{432}Leu alleles significantly increased adduct levels in tumor cells (0.1970 ± 0.0153 versus 0.1621 ± 0.0076; $P = 0.04$ or 0.1600 ± 0.0060; $P = 0.03$). Carrying one copy of the GSTP1 ^{105}Val allele significantly decreased PAH-DNA adduct levels in Caucasian nontumor cells (0.2059 ± 0.0090 versus 0.2362 ± 0.0092; $P = 0.02$) and marginally increased adduct levels in African American nontumor cells (0.2461 ± 0.0088 versus 0.2176 ± 0.0124; $P = 0.06$).

We also tested for joint effects between ethnicity, smoking, and genotypes by including an ethnicity × smoking (or genotype) interaction term in the model (Table 3). Using Caucasians as the reference group and African Americans as the risk group, we observed significant interactions between ethnicity and ever smoking [P value for interaction term (P_{int}) = 0.02] and between ethnicity and the mEH His^{139}Arg (Arg/Arg versus His/His or His/Arg; $P_{int} = 0.02$) polymorphisms in tumor cells. In nontumor cells, we found significant interactions between ethnicity and the mEH His^{139}Arg (Arg/Arg versus His/His or His/Arg; $P_{int} = 0.05$) and GSTP1 Ile^{105}Val (Ile/Val or Val/Val versus Ile/Ile; $P_{int} = 0.004$) polymorphisms.

We next examined joint PAH metabolism and conjugation genotype combinations on adduct levels based upon the function of the polymorphic alleles in key steps of the PAH metabolic pathway (Table 4). For example, carrying the mEH ^{113} Tyr/Tyr and mEH ^{139}His/His or His/Arg genotype combination, which may have increased PAH-epoxide to PAH-dihydrodiol hydrolysis compared with the mEH ^{113}Tyr/His or His/His and mEH ^{139}Arg/Arg genotype combination (26), increased adducts in Caucasian tumor (0.1692 ± 0.0050 versus 0.1252 ± 0.0220; $P = 0.05$) and nontumor (0.2349 ± 0.0082 versus 0.1603 ± 0.0317; $P = 0.02$) cells (data not shown). When pairing the higher metabolizing mEH ^{113}Tyr/Tyr and mEH His/His or His/Arg genotypes with the lower conjugating GSTP1 ^{105}Ile/Ile genotype compared with the mEH ^{113} Tyr/His or His/His and mEH ^{139} Arg/Arg and GSTP1 ^{105}Ile/Val or Val/Val genotype combination, we observed a significant increase in adduct levels in the nontumor cells of all study subjects (0.2472 ± 0.0130 versus 0.1537 ± 0.0331; $P = 0.01$), but when we stratified by race, this association only remained significant in Caucasian nontumor cells (0.2625 ± 0.0157 versus 0.1433 ± 0.0351; $P = 0.01$; data not shown). Similar effects were seen when combining the mEH His^{139}Arg and GSTP1 Ile^{105}Val polymorphisms (Table 4). Finally, carrying the "high-risk" genotype combination, the faster metabolism of PAH-diols to the most reactive PAH-diol-epoxide forms (CYP1B1 ^{432}Val/Val and mEH ^{139}His/His or His/Arg) with lower capacity to conjugate these PAH-diol-epoxides (GSTP1 ^{105}Ile/Ile), was associated with significantly increased adduct levels in the nontumor cells of Caucasians (0.2363 ± 0.0132 versus 0.1920 ± 0.0157; $P = 0.05$), but decreased adduct levels in non-tumor cells of African Americans (0.2121 ± 0.0175 versus 0.3060 ± 0.0408; $P = 0.05$). Cell sizes, however, continued to diminish with increasing genotype combination complexity and models with four or more polymorphisms were not estimable.

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We also examined the potential joint effects of ever (Table 5) and current smoking and PAH metabolism and conjugation genes on PAH-DNA adduct levels. Increased adducts were observed in tumor cells of Caucasian ever smokers carrying the potentially faster PAH-epoxide metabolizing mEH ^{139}His/His or His/Arg genotype compared with nonsmokers with the mEH ^{139}Arg/Arg genotype (0.1798 ± 0.0056 versus 0.1358 ± 0.0182; $P = 0.02$), and Caucasian ever smokers carrying the lower PAH-diol-epoxide conjugating GSTP1 ^{105}Ile/Ile genotype had higher adducts compared with nonsmokers with the GSTP1 ^{105}Ile/Val or Val/Val genotype (0.1783 ± 0.0071 versus 0.1538 ± 0.0088; $P = 0.03$). The mEH ^{139}His/Arg or His/His genotype association became more pronounced in Caucasian current smokers' tumor (0.1937 ± 0.0147 versus 0.1342 ± 0.0132; $P = 0.003$) and nontumor cells (0.2567 ± 0.0207 versus 0.1890 ± 0.0187; $P = 0.02$; data not shown) as did the effects of the GSTP1 ^{105}Ile/Ile genotype (0.2667 ± 0.0248 versus 0.2099 ± 0.0082; $P = 0.003$). In African Americans, nonsmokers who carried the potentially lower conjugating GSTP1 ^{105}Ile/Ile genotype compared with non-smokers with GSTP1 Ile/Val or Val/Val genotype had lower adducts in nontumor cells (0.1960 ± 0.0210 versus 0.2497 ± 0.0121; $P = 0.03$). Similar effects for GSTP1 ^{105}Ile/Ile (0.2137±0.0132 versus 0.2430±0.0082; P=0.06) were observed in African Americans when examining current smoking (data not shown).

Although cell sizes became even smaller when examining the joint effects of smoking and combinations of polymorphisms, several notable associations were observed (Table 5). Ever smokers with the faster PAH-epoxide metabolizing mEH ^{113}Tyr/Tyr and mEH ^{139}His/His or His/Arg genotype combination had higher adducts in tumor (0.1697 ± 0.0046 versus 0.1295 ± 0.0161; $P = 0.02$) and nontumor (0.2377 ± 0.0065 versus 0.1725 ± 0.0232; $P = 0.008$) cells, but when we stratified by race, this effect only remained significant in Caucasian tumor (0.1740 ± 0.0070 versus 0.1349 ± 0.0182; $P = 0.05$) and nontumor cells (0.2318 ± 0.0098 versus 0.1703 ± 0.0255; $P = 0.03$; data not shown). Caucasian ever smokers with the faster metabolizing mEH ^{139}His/His or His/Arg and lower conjugating GSTP1 Ile/Ile genotype compared with nonsmokers with the mEH ^{139}Arg/Arg and GSTP1 Ile/Val or Val/Val genotype had higher adduct levels in nontumor cells (Table 5), but this effect was only statistically significant in current smokers (0.2667 ± 0.0257 versus 0.1796 ± 0.0230; $P = 0.01$; data not shown). In Caucasians, after adjusting for smoking status carrying the putative high-risk genotype, the faster metabolism of PAH-epoxides to PAH-diol-epoxides (CYP1B1 ^{432}Val/Val and mEH ^{139}His/His or His/Arg) with lower PAH-diol-epoxide conjugation (GSTP1 ^{105}Ile/Ile), was associated with marginally increased adducts in nontumor cells compared with carriers of the CYP1B1 Leu/Leu or Leu/Val, mEH ^{139}Arg/Arg and GSTP1 Ile/Val or Val/Val genotype (0.2363 ± 0.0132 versus 0.1920 ± 0.0157; $P = 0.05$; data not shown).

Discussion

When stratifying by major ethnic group, we observed significant associations between smoking, polymorphisms in PAH metabolism [CYP1B1 Leu^{432}Val, CYP3A4 A(−392)G and mEH His^{139}Arg] and conjugation (GSTP1 Ile^{105}Val) genes and PAH-DNA adducts in tumor and adjacent nontumor prostate cells. Specifically, Caucasians who reported ever smoking had significantly increased PAH-DNA adduct levels compared with nonsmokers, but this effect was not observed in African Americans. The mEH ^{139}Arg/Arg genotype, which may potentially metabolize PAH-epoxides to PAH-dihydrodiols, more slowly (26) decreased adduct levels in both prostate tumor and nontumor prostate cells of Caucasians, but this effect was not found in African Americans. In addition, having one or two copies of the GSTP1 ^{105}Val allele, which may more effectively conjugate the most reactive PAH-diol epoxides (40,41), was inversely associated with adduct levels in the tumor cells of Caucasians. Finally, carrying the putative high-risk genotype combination, the faster metabolism of PAH-dihydrodiols to their most reactive PAH-diol-epoxide forms (CYP1B1 ^{432}Leu/Leu or Leu/Val and mEH ^{139}His/His or His/Arg) with lower capacity to conjugate these PAH-diol-epoxides

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(GSTP1 ^{105} Ile/Ile), was associated with increased adduct levels in nontumor cells of Caucasians, but was associated with decreased adduct levels in non-tumor cells of African Americans.

Our results are generally biologically plausible, given the expected activity of the alleles in key steps of the PAH metabolic pathway; however, functional studies are not entirely consistent and have focused on variation in one enzyme at a time, making it difficult to anticipate how variation in multiple enzymes affects PAH-DNA adduct levels. An initial *in vitro* study reported that the mEH ^{113}Tyr/^{139}Arg combination exhibits the highest protein expression (25); however, a recent study concluded the rate of hydrolysis by mEH ^{113}Tyr/^{139}His was ~2-fold greater than that measured in the other allelic combinations (26). In our observational study, we found that prostate cancer cases, particularly Caucasians, who smoke and carry two copies of the mEH ^{113}Tyr and/or one or more ^{139}His alleles have significantly higher PAH-DNA adducts in their tumor and nontumor cells, which is consistent with the most recent functional study. Although CYP1B1 ^{432}Val (with ^{119}Ala) has slightly higher activity in metabolizing benzo(*a*)pyrene-7,8-diols and other PAH metabolites [e.g., dibenzo(*a*,*l*)pyrene-diols], it has lower activity in metabolizing parent benzo(*a*)pyrene than ^{432}Leu (with ^{119}Ala), and the presence of the CYP1B1 ^{119}Ser variant seems to enhance activity in several substrates (15). We did not find any statistically significant associations with CYP1B1 Ala^{119}Ser polymorphism alone or in combination with Leu^{432}Val. Thus, additional functional studies in prostate cells, ideally with multiple polymorphic enzyme combinations, are needed to confirm our findings.

Consistent with our prior smaller study (13), PAH-DNA adducts were higher in adjacent nontumor prostate cells compared with prostate tumor cells, irregardless of ethnicity. Similar effects have not been observed in other organs. For example, Rundle et al. (49) reported that tumor cells of breast cancer cases had slightly higher PAH-DNA adducts compared with adjacent nontumor cells. As we reported previously (13), our results suggest that adducts diminish as prostate cancer foci grow and become more poorly differentiated, which may be due, in part, to loss of estrogen receptor expression in tumor cells.

Differences between ethnic groups were not totally unexpected, because African Americans, compared with Caucasians generally present with prostate cancer at an earlier age and with more advanced disease, have higher prostate cancer mortality rates (4) and higher PAH-DNA adduct levels in mononuclear cells (51). Associations between genetic polymorphisms and PAH-DNA adducts may differ by race due to differences in PAH exposure. Although we did not find a significant difference in smoking frequency between Caucasians and African Americans, PAH-DNA adduct levels, which measure the biologically effective PAH dose, were significantly increased in tumor cells of Caucasian, but not African American, ever smokers. Further stratification by genotype after adjusting for smoking status revealed that a putative high-risk genotype combination (CYP1B1 ^{432}Val/Val and mEH ^{139}His/His or His/Arg and GSTP1 Ile/Ile) significantly increased adducts in Caucasians, but not African Americans who carried this genotype combination, suggesting that other PAH-exposure sources (e.g., diet and/or occupation) may contribute to adducts in the prostate. Furthermore, why some associations varied by cell type within an ethnic group is not entirely clear. Tumor characteristics may contribute to these varying results, and in our prior (13) and current work, PAH-DNA adduct levels tended to be lower in malignant cells, but those less-differentiated measures of tumor differentiation (primary and total Gleason score), tumor volume, tumor stage, and PSA at diagnosis into the multivariable models did not materially alter results. Alternatively, differences in the cellular microenvironment, such as aberrant methylation, may lead to differential expression of these enzymes, which are within tumor and nontumor cells and between Caucasians and African Americans, potentially affecting the importance of a polymorphism. For example, silencing of GSTP1 through hypermethylation has been observed

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in prostate cancer cells but not normal cells (61), and GSTP1 hypermethylation has been observed to differ significantly between Caucasians and African Americans (62). Interestingly, smoking, which contains substantial quantities of carcinogenic PAHs (63), modifies GSTP1 methylation in prostate cancer cells with current smokers having a significantly higher frequency than former or nonsmokers (64). GSTP1 hypermethylation has been shown to modify 2-amino-1-methyl-6-phenylimi-dazo[4,5-*b*]pyridine–DNA adduct levels in the prostate (65), although in a recent study involving tumor and adjacent nontumor cells from hepatic carcinoma patients, no association between GSTP1 hypermethylation and PAH-DNA adducts was found (66). Hypomethylation of the CYP1B1 promoter has been shown to increase its expression in prostate cancer but not normal cells (67), although reports on how CYP1B1 hypomethylation affects PAH-DNA adduct levels are lacking.

No prior studies have examined the effects of polymorphisms in PAH metabolism and conjugation genes and PAH-DNA adducts in prostate cancer cells; therefore, we can only compare our results to those from other tissues. Because immunoassay methods are more specific to PAH [BPDE and other similar structured]-DNA adducts whereas ^{32}P-postlabeling methods measure all hydrophobic DNA adducts (68) and results differ considerably between these two methods (69), we restrict our comparison to those studies using immunoassay methods similar to the one we used. In lung tumor tissue, the variant alleles of the CYP1A1 1*/2* (includes CYP1A1 Ile^{462}Val) and the GSTM1 null deletion polymorphisms have been positively associated with (+)*anti* –BPDE–DNA adduct levels, and the effect was more pronounced in individuals with both polymorphisms (48). In addition, carrying the GSTM1 null deletion has been shown to be positively associated with PAH-DNA adducts in tumor and adjacent nontumor cells obtained from breast cancer cases (49), but this effect was not found in another study (50). We did not find an effect with the GSTM1 null deletion polymorphism; however, we did observe significant individual and joint gene associations with the GSTP1 ^{105}Val allele which may be more efficient than GSTM1 in conjugating the most reactive PAH-diol-epoxides (33).

Strengths of our study include its large sample size for this type of molecular evaluation and nearly equal distribution of Caucasians and African Americans. However, even larger samples are needed for effectively evaluating joint effects, particularly those involving two or more polymorphisms. Furthermore, we have treated ethnicity as a dichotomous variable when, in fact, it is really a continuous trait given the large degree of racial admixture in the United States. Incorporating and adjusting for ancestry informative markers in the analyses would help minimize any error induced by dichotomizing and help clarify interpretation of results. To obtain a more complete understanding of the underlying mechanisms of PAH-induced DNA damage in prostate cells, future work should include prostate specimens from “healthy” men without prostate cancer and should evaluate the influence of other sources of PAH exposure and DNA methylation and repair mechanisms on PAH-DNA adduct formation.

In summary, this is the first report describing the individual and joint associations between smoking and polymorphisms in PAH metabolism and conjugation genes and PAH-DNA adduct levels in tumor and nontumor prostate cells. Our results suggest that the association between smoking and PAH-DNA adducts differs by race and is modified by common genetic variants lending further insight to potential gene-environment interactions in prostate carcinogenesis.

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Table 1

Prostate cancer study population characteristics and genotype frequencies for polymorphisms in PAH metabolism and conjugation genes

Characteristic or genotype	All subjects (*N* = 400)	Caucasians (*n* = 210)	African Americans (*n* = 177)	*P*[*]
Age (y)	60.2 (6.7)[†]	61.0 (6.4)[†]	59.2 (7.0)[†]	0.01
Total Gleason score				
<7	138 (34.9%)	71 (34.1%)	64 (36.6%)	0.76
7	181 (45.7%)	98 (47.1%)	76 (43.4%)	—
>7	77 (19.4%)	39 (18.8%)	35 (20.0%)	—
Tumor volume[‡]	21.1 (16.3)[†]	20.2 (15.8)[†]	21.5 (16.7)[†]	0.44
Tumor stage				
≤T2b	299 (75.5%)	154 (74.0%)	138 (78.9%)	0.27
≥T2c	97 (24.5%)	54 (26.0%)	37 (21.1%)	—
PSA at diagnosis	7.05 (6.26)[†]	7.02 (6.81)[†]	6.98 (4.97)[†]	0.94
Ever smoker	252 (63.0%)	136 (64.7%)	109 (61.6%)	0.51
Current smoker	43 (10.8%)	21 (10.0%)	20 (11.3%)	0.68
Alcohol (g)	11.91 (24.78)[†]	13.41 (24.74)[†]	10.69 (25.56)[†]	0.29
PAH-DNA adducts levels[§]				
Prostate tumor cells	0.16 (0.06)[†]	0.17 (0.06)[†]	0.16 (0.06)[†]	0.59
Prostate nontumor cells	0.23 (0.09)[†]	0.22 (0.09)[†]	0.23 (0.09)[†]	0.15
PAH metabolism genes				
CYP1A1 ^{462}Ile/Ile	358 (95.7%)	183 (94.3%)	163 (97.0%)	0.21
CYP1A1 ^{462}Ile/Val	16 (4.3%)	11 (5.7%)	5 (3.0%)	—
CYP1A1 ^{462}Val/Val	0	0	0	—
CYP1B1 ^{119}Ala/Ala	159 (42.7%)	109 (55.9%)	44 (26.8%)	<0.01
CYP1B1 ^{119}Ala/Ser	158 (42.5%)	68 (34.8%)	84 (51.2%)	—
CYP1B1 ^{119}Ser/Ser	55 (14.8%)	18 (9.2%)	36 (22.0%)	—
CYP1B1 ^{432}Leu/Leu	87 (21.9%)	66 (31.7%)	16 (9.1%)	<0.01
CYP1B1 ^{432}Leu/Val	164 (41.3%)	98 (47.1%)	61 (34.7%)	—
CYP1B1 ^{432}Val/Val	146 (36.8%)	44 (21.2%)	99 (56.2%)	—
CYP3A4 (-392)A/A	225 (57.7%)	186 (89.9%)	32 (18.8%)	<0.01
CYP3A4 (-392)A/G	94 (24.1%)	16 (7.7%)	75 (44.1%)	—
CYP3A4 (-392)G/G	71 (18.2%)	5 (2.4%)	63 (18.8%)	—
mEH ^{113}Tyr/Tyr	194 (55.3%)	88 (48.9%)	99 (62.2%)	0.02
mEH ^{113}Tyr/His	136 (38.7%)	77 (42.8%)	54 (40.0%)	—
mEH ^{113}His/His	21 (6.0%)	15 (8.3%)	6 (3.8%)	—
mEH ^{139}His/His	188 (49.6%)	106 (53.3%)	72 (43.1%)	0.11
mEH ^{139}His/Arg	150 (39.6%)	71 (35.7%)	77 (46.1%)	—
mEH ^{139}Arg/Arg	41 (10.8%)	22 (11.1%)	18 (10.8%)	—
PAH conjugation genes				
GSTM1 nondeleted[‖]	214 (61.0%)	104 (49.5%)	133 (75.6%)	<0.01
GSTM1 null	154 (39.0%)	102 (50.5%)	43 (24.4%)	—
GSTP1 ^{105}Ile/Ile	145 (36.3%)	91 (43.3%)	49 (27.7%)	0.01
GSTP1 ^{105}Ile/Val	196 (49.0%)	92 (43.8%)	98 (55.4%)	—
GSTP1 ^{105}Val/Val	59 (14.7%)	27 (12.9%)	30 (16.9%)	—

[*] *P* value comparing Caucasians to African Americans from *t* test or χ^2 test as applicable.

[†] Mean and SD (values in parentheses) of the mean.

[‡] Expressed as a percentage of the gland with tumor.

[§] Expressed as absorbance units. Adduct levels in prostate tumor and nontumor prostate cells were strongly correlated ($r = 0.51$; $P < 0.0001$).

[‖] Includes subjects with at least one copy of the nondeleted (+) allele.

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Table 2

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells for smoking and genotypes in PAH metabolism and conjugation genes

Variable/genotype	Tumor cells		Nontumor cells	
	Mean ± SE*	*P*†	Mean ± SE*	*P*†
Ever smoker				
No	0.1585 ± 0.0049	—	0.2244 ± 0.0072	—
Yes	0.1680 ± 0.0038	0.13	0.2284 ± 0.0055	0.66
Current smoker				
No	0.1632 ± 0.0033	—	0.2256 ± 0.0048	—
Yes	0.1749 ± 0.0094	0.25	0.2407 ± 0.0131	0.30
CYP1B1 Ala^{119}Ser				
Ala/Ala	0.1671 ± 0.0048	—	0.2294 ± 0.0069	—
Ala/Ser	0.1638 ± 0.0049	0.62	0.2252 ± 0.0070	0.67
Ser/Ser	0.1671 ± 0.0083	0.99	0.2383 ± 0.0121	0.53
CYP1B1 Leu^{432}Val				
Leu/Leu	0.1663 ± 0.0064	—	0.2136 ± 0.0094	—
Leu/Val	0.1686 ± 0.0046	0.51	0.2300 ± 0.0068	0.16
Val/Val	0.1602 ± 0.0049	0.69	0.2340 ± 0.0072	0.09
CYP1A1 Ile^{462}Val				
Ile/Ile	0.1651 ± 0.0032	—	0.2284 ± 0.0047	—
Ile/Val	0.1746 ± 0.0149	0.54	0.2287 ± 0.0219	0.98
Val/Val	—‡	—	—‡	—
CYP3A4 A(-392)G				
A/A	0.1624 ± 0.0040	—	0.2227 ± 0.0059	—
A/G	0.1737 ± 0.0062	0.12	0.2347 ± 0.0091	0.27
G/G	0.1598 ± 0.0072	0.76	0.2305 ± 0.0105	0.52
mEH Tyr^{113}His				
Tyr/Tyr	0.1699 ± 0.0043	—	0.2383 ± 0.0063	—
Tyr/His	0.1695 ± 0.0051	0.96	0.2246 ± 0.0075	0.16
His/His	0.1786 ± 0.0129	0.52	0.2500 ± 0.0189	0.56
mEH His^{139}Arg				
His/His	0.1687 ± 0.0045	—	0.2272 ± 0.0064	—
His/Arg	0.1660 ± 0.0049	0.68	0.2227 ± 0.0071	0.57
Arg/Arg	0.1521 ± 0.0096	0.12	0.2220 ± 0.0138	0.73
GSTM1 null deletion				
+/− or +/+	0.1631 ± 0.0039	—	0.2283 ± 0.0057	—
−/−	0.1694 ± 0.0048	0.31	0.2275 ± 0.0071	0.94
GSTP1 Ile^{105}Val				
Ile/Ile	0.1613 ± 0.0050	—	0.2283 ± 0.0073	—
Ile/Val	0.1650 ± 0.0043	0.57	0.2264 ± 0.0063	0.84
Val/Val	0.1709 ± 0.0078	0.30	0.2250 ± 0.0114	0.81

NOTE: All analyses were adjusted for age, smoking, alcohol, ethnicity, Gleason score, tumor stage, and PSA at diagnosis.

*Mean absorbance units and SE of mean.

†*P* value for tests comparing wild-type/variant to wild-type/wild-type and variant/variant to wild-type/wild-type. *P* value shown is not corrected for multiple tests.

‡Not estimated because no subjects had this genotype.

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Table 3

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells by major ethnic group for smoking and genotypes in PAH metabolism and conjugation genes

Variable/genotype	Caucasians				African Americans				Tumor cells, P_{int}*	Nontumor cells, P_{int}*
	Tumor cells		Nontumor cells		Tumor cells		Nontumor cells			
	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*		
Ever smoker										
No	0.1507 ± 0.0070	—	0.2138 ± 0.0102	—	0.1690 ± 0.0073	—	0.2363 ± 0.0106	—	—	—
Yes	0.1748 ± 0.0052	0.006	0.2243 ± 0.0075	0.41	0.1607 ± 0.0058	0.37	0.2349 ± 0.0085	0.92	0.02	0.67
Current smoker										
No	0.1648 ± 0.0045	—	0.2201 ± 0.0065	—	0.1603 ± 0.0062	—	0.2341 ± 0.0070	—	—	—
Yes	0.1848 ± 0.0140	0.16	0.2367 ± 0.0191	0.42	0.1628 ± 0.0130	0.86	0.2395 ± 0.0190	0.80	0.22	0.53
CYP1B1 $Ala^{119}Ser$										
Ala/Ala	0.1681 ± 0.0058	—	0.2258 ± 0.0084	—	0.1657 ± 0.0091	—	0.2357 ± 0.0131	—	—	—
Ala/Ser	0.1665 ± 0.0073	0.86	0.2198 ± 0.0106	0.66	0.1644 ± 0.0066	0.91	0.2332 ± 0.0095	0.88	—	—
Ser/Ser	0.1763 ± 0.0146	0.61	0.2298 ± 0.0213	0.86	0.1625 ± 0.0103	0.81	0.2425 ± 0.0149	0.74	0.72†	0.58†
CYP1B1 $Leu^{432}Val$										
Leu/Leu	0.1578 ± 0.0074	—	0.2121 ± 0.0108	—	0.1970 ± 0.0153	—	0.2167 ± 0.0227	—	—	—
Leu/Val	0.1736 ± 0.0060	0.10	0.2332 ± 0.0088	0.13	0.1621 ± 0.0076	0.04	0.2264 ± 0.0113	0.70	—	—
Val/Val	0.1608 ± 0.0091	0.80	0.2078 ± 0.0133	0.80	0.1600 ± 0.0060	0.03	0.2434 ± 0.0089	0.27	0.81†	0.18†
CYP1A1 $Ile^{462}Val$										
Ile/Ile	0.1674 ± 0.0045	—	0.2232 ± 0.0066	—	0.1644 ± 0.0047	—	0.2354 ± 0.0069	—	—	—
Ile/Val‡	0.1761 ± 0.0182	0.64	0.2068 ± 0.0267	0.55	0.1713 ± 0.0268	0.80	0.2769 ± 0.0389	0.29	0.94§	0.22§
CYP3A4 A(−392)G										
A/A	0.1648 ± 0.0044	—	0.2196 ± 0.0065	—	0.1589 ± 0.0108	—	0.2534 ± 0.0157	—	—	—
A/G	0.1970 ± 0.0148	0.04	0.2367 ± 0.0219	0.45	0.1683 ± 0.0069	0.46	0.2327 ± 0.0101	0.27	—	—
G/G	0.1246 ± 0.0266	0.14	0.1898 ± 0.0392	0.45	0.1622 ± 0.0076	0.81	0.2326 ± 0.0111	0.28	0.15§	0.16§
mEH $Tyr^{113}His$										
Tyr/Tyr	0.1711 ± 0.0063	—	0.2258 ± 0.0094	—	0.1698 ± 0.0061	—	0.2493 ± 0.0086	—	—	—
Tyr/His	0.1730 ± 0.0067	0.84	0.2256 ± 0.0101	0.98	0.1672 ± 0.0082	0.80	0.2266 ± 0.0117	0.12	—	—
His/His	0.1917 ± 0.0152	0.21	0.2496 ± 0.0229	0.34	0.1457 ± 0.0246	0.34	0.2510 ± 0.0351	0.96	0.33§	0.16§
mEH $His^{139}Arg$										
His/His	0.1714 ± 0.0059	—	0.2291 ± 0.0085	—	0.1675 ± 0.0071	—	0.2260 ± 0.0103	—	—	—
His/Arg	0.1724 ± 0.0072	0.92	0.2292 ± 0.0103	0.99	0.1615 ± 0.0069	0.54	0.2372 ± 0.0100	0.44	—	—
Arg/Arg	0.1320 ± 0.0129	0.006	0.1856 ± 0.0184	0.03	0.1782 ± 0.0146	0.51	0.2692 ± 0.0212	0.07	0.02†	0.05†
GSTM1 Null Deletion										
+/− or +/+	0.1622 ± 0.0059	—	0.2149 ± 0.0087	—	0.1646 ± 0.0052	—	0.2394 ± 0.0076	—	—	—
−/−	0.1737 ± 0.0059	0.16	0.2292 ± 0.0087	0.27	0.1641 ± 0.0093	0.96	0.2257 ± 0.0135	0.38	0.44	0.22
GSTP1 $Ile^{105}Val$										
Ile/Ile	0.1633 ± 0.0064	—	0.2362 ± 0.0092	—	0.1608 ± 0.0086	—	0.2176 ± 0.0124	—	—	—
Ile/Val	0.1693 ± 0.0063	0.50	0.2059 ± 0.0090	0.02	0.1615 ± 0.0061	0.95	0.2461 ± 0.0088	0.06	—	—
Val/Val	0.1665 ± 0.0116	0.81	0.2193 ± 0.0166	0.37	0.1775 ± 0.0111	0.24	0.2296 ± 0.0161	0.56	0.96§	0.004§

NOTE: All analyses were adjusted for age, smoking, alcohol, Gleason score, tumor stage, and PSA at diagnosis.

* *P* value for multiplicative interaction term: smoking or genotype × ethnic group.

† *P* value for interaction term (gene × race) under a recessive genetic model.

‡ Val/Val not estimated because no subjects had this genotype.

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§ P value for interaction term (gene × race) under a dominant genetic model.

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Table 4

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells among all study subject and within major ethnic group for genotype combinations of PAH metabolism and conjugation genes

Genotype combination	All subjects					Caucasians					African Americans				
	n	Tumor		Nontumor		*n*	Tumor		Nontumor		*n*	Tumor		Nontumor	
		Mean ± SE	*P*	Mean ± SE	*P*		Mean ± SE	*P*	Mean ± SE	*P*		Mean ± SE	*P*	Mean ± SE	*P*
CYP1B1 Leu^{432}Val and GSTP1 Ile^{105}Val:															
Lower metabolism and higher conjugation:* CYP1B1 Leu/Leu or Leu/Val and GSTP1 Ile/Val or Val/Val	159	0.1679 ± 0.0047	—	0.2179 ± 0.0065	—	96	0.1688 ± 0.0063	—	0.1980 ± 0.0408	—	57	0.1695 ± 0.0080	—	0.2275 ± 0.0112	—
Faster metabolism and lower conjugation:* CYP1B1 Val/Val and GSTP1 Ile/Ile	52	0.1570 ± 0.0083	0.26	0.2218 ± 0.0115	0.77	22	0.1589 ± 0.0134	0.51	0.2474 ± 0.0078	0.66	29	0.1560 ± 0.0111	0.33	0.2187 ± 0.0155	0.64
mEH His^{139}Arg and GSTP1 Ile^{105}Val:															
Lower metabolism and higher conjugation:* mEH Arg/Arg and GSTP1 Ile/Val or Val/Val	31	0.1537 ± 0.0108	—	0.2316 ± 0.0080	—	16	0.1332 ± 0.0135	—	0.1754 ± 0.0222	—	15	0.1626 ± 0.0105	—	0.2107 ± 0.1338	—
Faster metabolism and lower conjugation:* mEH His/His or His/Arg and GSTP1 Ile/Ile	126	0.1630 ± 0.0053	0.08	0.2150 ± 0.0162	0.36	81	0.1651 ± 0.0061	0.04	0.2451 ± 0.0100	0.005	42	0.1771 ± 0.0182	0.49	0.2603 ± 0.0238	0.08
CYP1B1 Leu^{432}Val and mEH His^{139}Arg and GSTP1 Ile^{105}Val:															
Lower metabolism and higher conjugation:* mEH Arg/Arg and CYP1B1 Leu/Leu or Leu/Val & GSTP1 Ile/Val or Val/Val	18	0.1497 ± 0.0166	—	0.2256 ± 0.0198	—	18	0.1354 ± 0.0133	—	0.1920 ± 0.0157	—	6	0.1840 ± 0.0321	—	0.3060 ± 0.0408	—
Faster metabolism and lower conjugation:* mEH His/His or His/Arg and CYP1B1 Val/Val & GSTP1 Ile/Ile	46	0.1587 ± 0.0102	0.65	0.2236 ± 0.0122	0.93	18	0.1658 ± 0.0111	0.10	0.2363 ± 0.0132	0.05	27	0.1547 ± 0.0138	0.41	0.2121 ± 0.0175	0.05

NOTE: All analyses were adjusted for age, smoking, alcohol, Gleason score, tumor stage, and PSA at diagnosis. Analyses in the total sample were also adjusted for ethnicity.

* Putative metabolism and conjugation combinations (see text for details).

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Table 5

Mean PAH-DNA adduct levels in prostate tumor and adjacent nontumor prostate cells in major ethnic groups for select genotypes and genotype combinations of PAH metabolism and conjugation genes by ever smoker status

Genotype	Caucasians										African Americans									
	Tumor						Nontumor				Tumor						Nontumor			
	Nonsmoker			Smoker			Nonsmoker		Smoker		Smoker			Nonsmoker			Smoker		Nonsmoker	
	*n**	Mean ± SE	*P*	*n**	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*	*n**	Mean ± SE	*P*	*n**	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*
CYP1B1 Leu/Leu or Leu/Val	145	0.1506 ± 0.0079	—	42	0.1763 ± 0.0058	0.01	0.2192 ± 0.0116	—	0.2278 ± 0.0085	0.55	70	0.1718 ± 0.0075	—	86	0.1597 ± 0.0059	0.43	0.2432 ± 0.0114	—	0.2393 ± 0.0090	0.77
CYP1B1 Val/Val	19	0.1522 ± 0.0154	0.93	2	0.1654 ± 0.0113	0.28	0.1977 ± 0.0225	0.40	0.2132 ± 0.0165	0.77	7	0.2285 ± 0.0286	0.42	13	0.2026 ± 0.0202	0.17	0.2306 ± 0.0433	0.24	0.2242 ± 0.0306	0.31
mEH ^{139}Arg/Arg	11	0.1358 ± 0.0182	—	11	0.1281 ± 0.0181	0.77	0.1971 ± 0.0261	—	0.1739 ± 0.0261	0.53	7	0.1859 ± 0.0228	—	11	0.1729 ± 0.0191	0.66	0.2790 ± 0.0331	—	0.2624 ± 0.0277	0.71
mEH ^{139}His/His or His/Arg	59	0.1557 ± 0.0079	0.31	118	0.1798 ± 0.0056	0.02	0.2235 ± 0.0114	0.36	0.2320 ± 0.0080	0.20	58	0.1704 ± 0.0079	0.52	91	0.1606 ± 0.0064	0.29	0.2331 ± 0.0115	0.19	0.2309 ± 0.0092	0.16
GSTP1 Ile/Val or Val/Val	47	0.1538 ± 0.0088	—	72	0.1783 ± 0.0071	0.03	0.2128 ± 0.0126	—	0.2064 ± 0.0102	0.69	51	0.1756 ± 0.0083	—	77	0.1581 ± 0.0068	0.10	0.2497 ± 0.0121	—	0.2373 ± 0.0100	0.43
GSTP1 Ile/Ile	27	0.1450 ± 0.0118	0.55	64	0.1708 ± 0.0076	0.14	0.2157 ± 0.0169	0.89	0.2447 ± 0.0109	0.06	17	0.1492 ± 0.0145	0.12	32	0.1669 ± 0.0105	0.52	0.1960 ± 0.0210	0.03	0.2292 ± 0.0153	0.30
CYP1B1 Leu/Leu or Leu/Val and GSTP1 Ile/Val or Val/Val	87	0.1540 ± 0.0099	—	20	0.1790 ± 0.0082	0.05	0.2220 ± 0.0133	—	0.2063 ± 0.0110	0.36	52	0.1811 ± 0.0136	—	25	0.1635 ± 0.0098	0.30	0.2381 ± 0.0193	—	0.2220 ± 0.0139	0.50
CYP1B1 Val/Val and GSTP1 Ile/Ile	9	0.1518 ± 0.0233	0.93	2	0.1625 ± 0.0165	0.66	0.2316 ± 0.0313	0.78	0.2165 ± 0.0221	0.83	5	0.1388 ± 0.0188	0.07	4	0.1651 ± 0.0136	0.41	0.2127 ± 0.0267	0.44	0.2218 ± 0.0193	0.55
mEH ^{139} Arg/Arg and GSTP1 Ile/Val or Val/Val	8	0.1391 ± 0.0192	—	8	0.1274 ± 0.0193	0.67	0.2033 ± 0.0317	—	0.1476 ± 0.0318	0.22	6	0.1885 ± 0.0280	—	9	0.1686 ± 0.0243	0.59	0.2754 ± 0.0355	—	0.2490 ± 0.0307	0.58
mEH ^{139}His/His or His/Arg and GSTP1 Ile/Ile	22	0.1490 ± 0.0119	0.66	59	0.1709 ± 0.0071	0.12	0.2364 ± 0.0196	0.38	0.2482 ± 0.0118	0.19	15	0.1688 ± 0.0132	0.53	27	0.1514 ± 0.0177	0.27	0.1898 ± 0.0224	0.05	0.2224 ± 0.0167	0.18

NOTE: All analyses were adjusted for age, smoking, alcohol, Gleason score, tumor stage, and PSA at diagnosis. Reference group includes nonsmokers with the putative lower metabolizing, higher conjugating or lower metabolizing and higher conjugating genotype combination.

*Sample size is the same for tumor and nontumor cells within the same major ethnic genotype by smoking group; thus, sample size is only listed once under the tumor cell heading.