Published in final edited form as:
Biomark Cancer. 2011 February 3; 2011(3): 1–13. doi:10.4137/BIC.S6111.

No association between variant *N*-acetyltransferase genes, cigarette smoking and Prostate Cancer susceptibility among men of African descent

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Abstract

Objective—We evaluated the individual and combination effects of *NAT1*, *NAT2* and tobacco smoking in a case-control study of 219 incident prostate cancer (PCa) cases and 555 disease-free men.

Methods—Allelic discriminations for 15 *NAT1* and *NAT2* loci were detected in germ-line DNA samples using TaqMan polymerase chain reaction (PCR) assays. Single gene, gene-gene and gene-smoking interactions were analyzed using logistic regression models and multi-factor dimensionality reduction (MDR) adjusted for age and subpopulation stratification. MDR involves a rigorous algorithm that has ample statistical power to assess and visualize gene-gene and gene-environment interactions using relatively small samples sizes (i.e., 200 cases and 200 controls).

Results—Despite the relatively high prevalence of *NAT1*10/*10* (40.1%), *NAT2* slow (30.6%), and *NAT2* very slow acetylator genotypes (10.1%) among our study participants, these putative risk factors did not individually or jointly increase PCa risk among all subjects or a subset analysis restricted to tobacco smokers.

Conclusion—Our data do not support the use of *N*-acetyltransferase genetic susceptibilities as PCa risk factors among men of African descent; however, subsequent studies in larger sample populations are needed to confirm this finding.

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Disclosures

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Keywords

N-acetyltransferase genes; Prostatic Neoplasms; African-Americans; smoking

Introduction

Prostate cancer (PCa) is the leading cause of cancer incidence and the second leading cause of cancer-related deaths among men in the United States.[1] These cancer statistics are even more alarming among men of African descent who have the highest incidence and mortality rates in the world. In 2009, over twenty-seven thousand African-American men were diagnosed with PCa and 3,690 men died from the disease. PCa incidence and mortality rates for African-American men are two-fold higher relative to European-Americans.[2] Other than race, the established etiological determinants of PCa include age and family history of disease. There is some evidence that environmental factors such as cigarette derived polycyclic aromatic hydrocarbons and meat-derived heterocyclic aromatic amines induce tumors in experimental animals, including prostate tumors in rodents.[3–5] The International Agency for Research on Cancer (IARC) regard heterocyclic amines as possible or probable human carcinogens. In addition, a recent meta-analysis, involving pooled data from 24 studies, revealed that smoking increased the chance of developing PCa and dying from the disease, while using crude smoking classifications as well as number of cigarettes smoked per day.[6] Consequently, inheritance of susceptibilities in genes responsible for the metabolism of environmental carcinogens may influence one's capacity to bioactivate or detoxify pro-carcinogens as well as alter individual propensity toward PCa. Unfortunately, the impact of genetic variations in two commonly studied biotransformation genes in relation to PCa still remains understudied, especially among men of African descent.

N-acetyltransferase (NAT) activity plays an important role in the activation and detoxification of meat-derived and cigarette derived pro-carcinogens (e.g., heterocyclic amines, aromatic amines), respectively.[7] Consequently, acetylator status may influence individual response to environmental carcinogens as well as susceptibility to various cancers.

NATs are encoded by two genes, *NAT1* and *NAT2*, located on chromosome 8p 21.3–23.1.[8] *NAT1* is highly polymorphic with over 25 variant alleles.[9] A common variant allele, *NAT1*10*, defined by two single nucleotide polymorphisms (SNPs) in the 3′ untranslated region ($T^{1088}A$ and $C^{1095}A$) may cause a shift in the position of the mRNA polyadenylation signal, resulting in a potential increase in mRNA stability.[10] Two studies show that the *NAT1*10* configuration may represent a rapid acetylator allele, since it was associated with increased *N*-acetylation activity in human bladder and colonic mucosa and higher DNA adduct formation in the urinary bladder.[10–11] The frequency of this putative rapid acetylator *NAT1*10* allele is fairly common among African-Americans (50%).[12] There is some evidence that inheritance of the *NAT1*10* variant allele is associated with an increase in colo-rectal, breast and PCa relative to carriers of the homozygous *NAT1*4/*4* genotype.[13–15] Rare variant *NAT1* alleles, such as *NAT1*14B*, **15*, **17*, **19*, and **22* alleles, exhibit negligible levels of *NAT1* protein expression and have lower catalytic activity toward *N*-hydroxylated heterocyclic. *NAT1*3* and *NAT1*11* are also rare variants. In particular, the *NAT1*11* allele appears to be associated with elevated breast cancer risk; however, the acetylator phenotype among individuals carrying this marker is not completely understood.[16–17]

Polymorphism in the *NAT2* gene has led to the identification of a *NAT2*4* (reference) and over 25 variant alleles.[9] Several SNPs alone or in combination (e.g., $C^{282}T$, $T^{341}C$, $C^{481}T$,

G^{590}A, A^{803}G, and G^{857}A) result in *NAT2* alleles (e.g., *NAT2**5, **6, *7, *14*) with reduced activity and protein. However, polymorphism found in the *NAT2*5* gene cluster resulted in the greatest reduction in *N*- and *O*-acetylation activity when compared to the reference genotype.[18] Specifically, the T^{341}C SNP targets the NAT2 protein for enhanced proteosomal degradation and is associated with the very slow NAT2 acetylator phenotype.[19–20] Compared to rapid/intermediate acetylators, *NAT2* slow acetylators had a 1.4-fold increase in bladder and prostate cancer risk that was stronger for cigarette smokers than for never smokers.[21–22] This increased risk is attributed to reduced capacity to detoxify *N*-hydroxylated aromatic amines in the liver and extrahepatic tissue, including the small intestine, bladder and prostate.[23–24]

The *NAT1*10* and the slow *NAT2* genotypes (individually and jointly) are suspected to increase PCa risk due to their affect on the metabolic activation of heterocyclic aromatic amines (via *O*-acetylation) in the prostate and/or decreased detoxification of aromatic amines (via *N*-acetylation) in the liver and prostate. In fact, Hein and co-workers (2002) observed a 5- and 7.5-fold increase in PCa susceptibility among individuals who possess the putative rapid *NAT1*10* combined with the *NAT2* slow (OR = 5.08; 95% CI: 1.56–16.5; p = 0.008) or very slow *NAT2* genotypes (OR = 7.50; 95% CI: 1.55–15.4; p = 0.016), respectively.[15] However, additional studies are still warranted to clarify their role in susceptibility to PCa among men of African descent.

Despite the striking prevalence of PCa and the high frequency of *NAT1*10* and slow *NAT2* slow alleles among men of African descent, the phenotypic ramifications of polymorphic *N*-acetyltransferases remain understudied in this underserved subgroup. Hooker and co-workers (2008) evaluated 4 *NAT2* SNPs (rs11120005, rs7832071, rs1801280, rs1799930) in relation to PCa susceptibility among participants of the current study.[25] Two of these *NAT2* loci (rs1801280, rs1799930) have been documented to influence *NAT2* protein levels and *N*-acetyltransferase activity. However, there are thirteen other *NAT1* and *NAT2* SNPs that are used to correctly identify individuals as slow, intermediate and rapid acetylators that were not included in Hooker's analysis. The current study addresses this issue by evaluating the individual and joint effects of 15 functional *NAT1* and *NAT2* sequence variants on PCa risk among men of African descent using a statistically rigorous statistical tool, namely multi-factor dimensionality reduction (MDR). This data-mining tool has excellent statistical power (i.e., >80%) to evaluate main effects and complex interactions in relationship to discrete outcome, even with a relatively small sample size (i.e., >200 cases and >200 controls). This approach was also applied to explore whether susceptibilities detected in xenobiotic metabolizing genes combined with environmental factors (i.e., tobacco smoking) can significantly modify prostate cancer risk.

Materials and Methods

Study Population

Study population—Between 2001 and 2005, 774 unrelated male residents were recruited from the Washington, D.C. and Columbia, SC areas through the Howard University Hospital (HUH) Division of Urology or PCa screening programs. The study population of men of African descent (i.e., self-reported African Americans, East African Americans, West African Americans, and Afro-Caribbean Americans) consisted of 219 incident PCa cases and 555 unrelated controls. PCa patients between the ages of 41 and 91 were diagnosed within one year of enrollment. Following a visit to the HUH Division of Urology for an annual PCa screening exam or urinary symptoms, incident PCa cases were identified by a urologist using a transrectal ultrasound-guided biopsy.[26] Biopsy cores were reviewed by members of the Department of Pathology at the Howard University College of Medicine. PCa cases were classified according to a well-established Gleason scoring system.[27]

Inclusion criteria of controls included men older than 45 with a low prostate specific antigen (PSA) level ≤ 4.0 ng/ml and normal digital rectal exams (DREs) or biopsies. Individuals were excluded as controls if: they failed at least one diagnostic test (i.e., PSA > 4.0 and/or irregular PSA), even though they had a normal biopsy; or were ever diagnosed with benign prostatic hyperplasia (BPH). Clinical characteristics including age at diagnosis/enrollment, family history of PCa, PSA level (ng/ml), and Gleason score for PCa patients, were obtained from medical records, as summarized in Table 1. Histopathological grade was recorded as the Gleason score. Information on smoking history was also collected at the time of recruitment using a short questionnaire. Male residents from D.C. were classified as current (n = 37), former (n = 73) and never cigarette smokers (n = 104). Never smokers smoked less than 100 cigarettes over their lifetime; whereas ever/former cigarette smokers had at least 1 cigarette per day. All study participants had DNA extracted from whole blood and provided written informed consent for participation in genetic analysis studies under a protocol approved by Howard University, the HUH Division of Urology, and the University of Louisville Institutional Review Board.

Genotyping

The genomic DNA was obtained from isolated lymphocytes using cell lysis, proteinase K-treatment, protein precipitation, and DNA precipitation.[28] SNPs in two carcinogen metabolism genes (*NAT1* and *NAT2*) were determined using a 10μl Taqman Polymerase Chain Reaction (Taqman PCR) allelic discrimination assay, as described elsewhere.[29–30] Briefly, approximately 40 ng/μl of germ line DNA was added to a reaction consisting of 1X Universal Master Mix and assay specific concentrations of primers (forward and reverse) and probes (FAM and VIC). All reactions (10μl) were performed in a 384 well plate and sealed using optical covers. Reaction plates were thermocycled on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The polymerase chain reaction (PCR) amplification conditions consisted of the following previously validated conditions: an initial 2 step hold (50°C for 2 minutes, followed by 95°C for 10 minutes) and 40 cycles of a two step PCR (92 °C for 15 seconds, 60 °C for 1 minute). To minimize misclassification bias, laboratory technicians were blinded to the case status of subjects. To ascertain percent concordance rates, 72 samples were subjected to repeat genotyping, resulting in concordance rates > 95%. Based on 24 non-DNA template controls per batch analysis, the percent cross-contamination during sample handling was minimal (< 3%). In addition, deviations from the Hardy-Weinberg equilibrium were tested among controls using a chi-square test (or Fisher's Exact test) and significance level of $P < 0.005$.

The aforementioned assay was designed to differentiate between SNPs in *NAT1* (n = 8) and *NAT2* (n = 7) to minimize *NAT1* and *NAT2* genotype misclassification. The SNPs detected in *NAT1* were: $C^{97}T$ (R^{33}Stop), $C^{190}T$ ($R^{64}W$), $G^{445}A$ ($V^{149}I$), and $C^{559}T$ (R^{187}Stop), $G^{560}A$ ($R^{187}Q$), $A^{752}T$ ($D^{251}V$), $T^{1088}A$ (3′UTR), and $C^{1095}A$ (3′UTR). SNPs detected in the *NAT2* gene were: $G^{191}A$ ($R^{64}Q$), $C^{282}T$ (silent), $T^{341}C$ ($I^{114}T$), $C^{481}T$ (silent), $G^{590}A$ ($R^{197}Q$), $A^{803}G$ ($K^{268}R$), and $G^{857}A$ ($G^{286}E$). *NAT1* and *NAT2* alleles, genotypes, and deduced phenotypes were determined as previously described [31] and summarized in Table 2.

Ancestry markers

Cases and controls were also genotyped with a set of 100 genome-wide ancestry informative markers to correct for potential population stratification among our admixed population of self-reported African-Americans, West African-Americans, East African-Americans, or Afro-Caribbean-Americans, as previously described.[32–33] Study participants were grouped from lowest to highest genetic West African Ancestry, with scores ranging from 0–100%. These 100 markers were assembled using DNA from self-identified African-Americans (Coriell Institute for Medical Research, n = 96), Yoruban West Africans (HapMap, n = 60),

West Africans (Bantu and Nilo Saharan speakers, n = 72), Europeans (New York City, n = 24), and CEPH Europeans (HapMap Panel, n = 60), as previously reported.[33] Individuals with a West African ancestry (WAA) score and available *NAT* genotype data (178 cases, 492 controls for *NAT1* and 190 cases, 493 controls for *NAT2*) ≥ 25% were included in the final analysis.

Statistical Analysis

Evaluation of Patient & Tumor characteristics—Differences in continuous [ie., age (yrs) and percent West African Ancestry] and categorical [i.e., PSA (ng/ml); tobacco smoking status (current/former versus never)] variables between PCa cases and controls were tested using the Wilcoxon Sign Rank test and the chi-square test for homogeneity (or Fisher's Exact test), respectively.

Evaluation of individual NAT loci and PCa risk using LR analysis—To assess whether individuals possessing at least one high-risk factor (e.g., *NAT1*10; NAT2* slow, *NAT2* very slow, *NAT2* rapid genotypes) have an elevated risk of developing PCa, we tested for significant differences in the distribution of *NAT1* and *NAT2* genotypes using the chi-square test of homogeneity, respectively. The associations between polymorphic carcinogen metabolism genes, expressed as odds ratios (ORs) and corresponding 95% confidence intervals were determined using unconditional multivariate logistic regression analysis models adjusting for potential confounders, namely age and West African ancestry, modeled as continuous variables. The estimated odds ratios were not adjusted for family history of PCa due to the high missing rate associated with this variable. For main effects, we compared the odds of developing disease for carriers of one or more *NAT1*10*, two *NAT2* slow *(*5ABC, *6AC, *7AB, *14ABE*) and two *NAT2* rapid *(*11A, *12ABC *13*) genotypes to the non-*NAT1*10*, *NAT2* rapid and *NAT2* slow referent categories, respectively. We estimated the odds ratios for the joint effects of *NAT1* and *NAT2* by comparing individuals possessing one or more risk factors to those who possessed homozygous *NAT1*4* and *NAT2*4* referent alleles, respectively. To evaluate combined and interacting effects of the genetic markers on PCa risk, we used conventional logistic regression (LR) modeling to build multi-locus models predictive of PCa status in a stepwise fashion.

Evaluation of gene-gene and gene-tobacco smoking combination effects—The joint modifying effects of two or more loci on PCa risk will be evaluated by the significance of the coefficient of the product term $\beta_3 loci\ 1 * loci\ 2$ (i.e., *NAT1*NAT2* and *NAT2**smoking) in the following models: (1) $Logit = \beta_0 + \beta_1 gene\ 1 + \beta_2 gene\ 2 + \beta_3 gene\ 1 * gene\ 2$; (2) $Logit = \beta_0 + \beta_1 gene + \beta_2 smoking + \beta_3 gene * smoking$. All chi-square test, Fisher's Exact test and logistic regression analysis computations were carried out using SAS software 9.1.3 (SAS Institute, Inc, Cary, NC). Test for trend included genotypes as ordinal variables. Statistical significance was assessed using a P-value < 0.05.

In order to compliment LR, MDR was used to further evaluate gene-gene and gene-smoking interactions associated with sporadic PCa. MDR has been described and reviewed elsewhere. [34–35] Briefly, MDR is a rigorous statistical method for detecting and characterizing high-order interactions in case-control studies, even in the presence of relatively small sample sizes. With MDR, multi-locus variables (e.g., *NAT* alleles, tobacco smoking status) are pooled into high-risk and low-risk groups, reducing high-dimensional data to a single dimension, permitting an investigation of gene-gene and gene-smoking interactions (i.e., *NAT1*-*NAT2*, *NAT1*-smoking, *NAT2*-smoking interactions) after adjusting for potential confounders. One dimensional multi-locus variables were evaluated for their ability to classify and predict PCa susceptibility. Validation of multi-locus models as effective predictors of PCa susceptibility was performed using permutation testing. This

approach accounts for multiple testing issues as long as the entire model fitting procedure was repeated for each randomized dataset, which provided an opportunity to identify false-positives. These MDR permutation results were considered statistically significant at the 0.05 level.

Statistical Power

We conducted calculations to determine the power of our sample to detect significant relationships between *N*-acetyltransferase genotypes and PCa risk. The expected risk estimates of our study can be estimated by specifying values for a number of parameters, including a minor allele frequency of at least 47.6%, PCa disease prevalence of 1.86% for African-Americans, statistical power (80%), #cases (178–190), and #controls (492–493). We assumed the outcome was in complete linkage disequilibrium with an innate immunity-predisposing variant ($r^2 = 1.0$). With our anticipated sample size of 178 cases and 492 controls, we have >80% power to detect genetic markers with risk estimates of 1.4–2.0 and larger, for MAFs of at least 47.6%, assuming a codominant model with 1 degrees of freedom (df). Recessive (df = 1), dominant (df = 1) and codominant (df = 2) genetic models would have ample statistical power to observe risk estimates ranging from 1.7–2.0. Power calculations were performed using Power for Genetic Association Version 2 Software.[36]

Results

Patient & Tumor Characteristics

Descriptive information for the study participants is summarized in Table 1. Cases were significantly older and had higher PSA levels relative to controls. There was a modest difference in median West African genetic ancestry estimates comparing cases and controls ($p = 0.0227$).

Prevalence of *NAT1* and 2 genotypes

The *NAT1*4/*10* (data not shown) followed by *NAT1*4/*4* (data not shown) and *NAT1*10/*10* (Table 3) were the most common genotypes among controls with frequencies of 40.1%, 29.7% and 27.0%, respectively. Sixty-two *NAT2* genotypes were well distributed among cases and controls (data not shown). The most common *NAT2* genotypes among controls were *NAT2*5B/*6A* (9.5%), followed by *NAT2*4/*6A* (7.3%), *NAT2*4/*5B* (6.9%) and *NAT2*5B/*5B* (7.7%). Collectively, men of African descent in the current study population were more likely to inherit intermediate (45.4%) and slow (30.6%) *NAT2* acetylator genotypes. The *NAT1* and *NAT2* allele frequencies among controls did not deviate from the Hardy-Weinberg Equilibrium ($P \geq 0.018$), given a significance level of 0.005 (data not shown).

Individual and Multilocus Effects

No statistically significant association was observed between tobacco smoking use (current/former versus never) and PCa ($P_{hmoogeneity} = 0.5709$). There was no association with PCa risk among carriers of: one or two copies of *NAT1*10* compared with zero copes, *NAT2* intermediate, slow, or very slow compared with the rapid genotype; and one or two copies of *NAT2*rapid* alleles compared to the slow genotypes ($P_{homogeneity} = 0.1574$ for *NAT1*, 0.3298 for *NAT2*slow* and 0.3278 *NAT2*rapid*). The absence of gene combination effects were confirmed by MDR analysis supplemented with permutation testing ($p \leq 0.078$; data not shown).

In an exploratory analysis, we assessed whether *NAT1-NAT2* or *NAT2*-smoking modified PCa risk. The two-way interactions of *NAT1* and *NAT2* combined ($P_{interaction} \geq 0.2897$ for *NAT1*10* and *NAT2*slow* and 0.2156 for *NAT1*10* and *NAT2*rapid*; Supplementary Table

A) or *NAT2* and tobacco smoking were not significant in the unadjusted and adjusted models ($P_{interaction}$ = 0.1445; Supplementary Table B).

Discussion

Inter-individual differences in PCa susceptibility may be mediated in part through polymorphic variability in genes encoding enzymes that activate and deactivate chemical carcinogens. The current study sought to determine whether genetic polymorphisms in the bio-activation and deactivation enzymes for meat-and tobacco-derived carcinogens (e.g., heterocyclic amines, aromatic amines) may contribute to increased risk for PCa. To our knowledge, the impact of *NAT1* and *NAT2* genes on PCa susceptibility among men of African descent remains under-reported. The current study addressed this deficiency by evaluating the single gene, gene-gene, and gene-environmental effects among men of African descent using logistic regression modeling and a data-mining tool (i.e., MDR) designed to handle single and multi-locus analyses. However, even in the presence of a 10-fold cross validation scheme afforded by MDR, we did not generate evidence supporting the role of individual or joint modifying effects for *NAT1* or *NAT2* in relation to PCa risk among men of African descent.

To date, there are twelve published reports and one publically available database with inconsistent findings on the relationship between genetic polymorphisms in *NAT1* and/or *NAT2* and the risk of developing PCa. With the exception of two studies,[25, 37] ten of these PCa epidemiology studies have not included men of African descent. For instance, two independent studies revealed a 2-fold increase in PCa risk among Japanese (OR = 2.4; 95%CI = 1.0–5.6) or European (OR = 2.17; 95%CI = 1.08–4.33) men who possessed one or more of the putative "high-risk" *NAT1*10* alleles.[14–15] Similar risk estimates were observed for carriers of the *NAT2* slow or very slow acetylator genotypes in relation to PCa susceptibility among Japanese.[22] However, nine subsequent published reports, as well as a paper in press (Kidd, L.R., "unpublished data", August 2010), did not substantiate the aforementioned marginal main effects for either *NAT1* and/or *NAT2* in relation to PCa.[25, 37–44]

Failure to observe significant relationships between genetic polymorphisms and PCa may be partially attributed to small samples sizes, failure to consider gene combination effects or methodological differences. Two out of the twelve previously mentioned studies evaluated *NAT1-NAT2*, *NAT*-heterocyclic aromatic amines, and/or *NAT*-tobacco smoking interactions.[15, 37] However, these two studies, like many genetic epidemiology studies, failed to implement MDR, a rigorous statistical tool with the capacity to detect and validate higher-order interactions that would remain undetected by conventional methods, such as logistic regression modeling. As a consequence, in the absence of studies with adequate statistical power or rigor, it is challenging to conclude with certainty whether these biomarkers are important in relation to prostate cancer. The current study attempted to overcome statistical issues that often plague genetic epidemiology studies by evaluating both main and joint effects using MDR.

In light of the genome wide association era, in a post-hoc analysis, we attempted to evaluate our findings in the context of those found in the Cancer Genetic Markers of Susceptibility (CGEMS) data portal that houses over a half million SNPs collected from 2277 Caucasian participants (1176 PCa cases, 1101 controls).[45] The CGEMS data portal contains genotype data for 6 *NAT1* and 10 *NAT2* SNPs; however, none of these markers were related to either PCa or aggressive disease. Upon closer inspection, only the *NAT2* SNP (rs1208; $A^{803}G$, $Lys^{268}Arg$) matched one out of 7 SNPs analyzed in the current study. Since the rs1208 SNP is one of 8 *NAT2* SNPs that are used to generate various haplotypes to properly classify

individuals as slow, intermediate and rapid acetylators, it was not feasible to compare our data to the CGEMS database. Unfortunately, *NAT1* and *NAT2* SNP data in relation to prostate cancer risk among men of African descent has not been collected within the context of genome wide association studies, to our knowledge.

Failure to consider all NAT sequence variants necessary to properly classify individuals as *NAT1* and *NAT2* rapid, intermedicate, and slow acetylators is not unique to the CGEMS database. Surprisingly, only 3 out of twelve of the previously mentioned studies considered all 15 essential *NAT1* and *NAT2* SNPs to avoid errors in the deduced acetylator phenotypes. These methodological differences in *NAT* SNPs assayed across studies, makes it impossible to make comparisons across studies and draw any firm conclusions about the role of polymorphic *NAT*s in prostate cancer risk. In the current study, genotype misclassification was minimized in the current study due to the determination of the fifteen SNPs in *NAT1* and *NAT2* pertinent to classifying genotypes accurately. In addition, the quality of the genotype data is strengthened by lack of departures from Hardy-Weinberg equilibrium, stringent quality control standards, and the observation of *NAT1* and *NAT2* genotype frequencies commensurate with published reports involving men of African descent.

Cigarette smoking may increase a man's risk for developing and dying from PCa, based on pooled data from 24 studies involving 21,600 men with the disease indicates.[6] This meta-analysis, conducted by Huncharek and colleagues (2010) pooled findings from numerous studies to better illuminate risks not clearly shown in previous individual studies. Pooled analysis revealed consistent evidence that both the chance of developing PCa and dying from PCa increases with smoking, even though many of the studies analyzed used crude smoking classifications (i.e., ever versus never smoking). In eight studies that provided more in-depth number of cigarettes smoked per day in nearly 8,700 men, Huncharek's team revealed a 1.3 fold increase in the risk of dying from PCa in the heaviest smokers versus nonsmokers. They also observed a 1.22 fold increase in PCa risk among the heaviest smokers, based on pooled information from four studies of about 2,100 men. In an exploratory analysis, we assessed whether a crude smoking classification (i.e., ever versus never smoking) combined with inheritance of slow *NAT2* (linked with a reduced capacity to detoxify cigarette-derived procarcinogens (i.e., aromatic amines) would increase one's chances of developing PCa. However, we were unable to observe significant gene-environment interactions. Nevertheless, we cannot ignore the possibility that the lack of available data on duration of smoking, tobacco smoking preferences (cigarette, pipe, cigars), and the extent of inhalation may lead to under-estimation of both exposure to cigarette-smoke derived agents as well as observed risk estimates. In addition, subsequent studies with adequate statistical power are necessary to effectively evaluate gene-environment interactions. Emphasis needs to be placed on studies that quantify the number of cigarette packs and smoking duration that influence PCa risk and progression.

We considered the strengths and challenges of the current study. Like many genetic epidemiology studies, the current study did not adjust risk estimates for potential cofounders such as family history of prostate cancer, body mass index, and socio-economic status. However, this study took advantage of a freely available data-mining tool to evaluate main and joint modifying effects in relation to prostate cancer risk among men of African descent. Utilization of bioinformatic tools designed to detect higher-order interactions even in the absence of main effects should become a standard practice within future prostate cancer epidemiology studies. This is a reasonable suggestion especially since MDR has been reported in more than 90 genetic epidemiology studies based on a recent pubmed search.

In summary, we did not observe strong main or gene combination effects of *NAT1* and *NAT2* polymorphisms in relation to PCa risk among men of African descent. However,

confirmation is required in culturally diverse studies with more detailed exposure assessments using publically available data-mining tools. Consequently, our laboratory will consider whether other biotransformation related genes alone or in combination with environmental exposures predict PCa risk among men of African descent using data collected from a multi-center study. Such findings will facilitate future studies focused on improving cancer prevention or detection strategies and ultimately reducing PCa health disparities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Rick Kittles for the contribution of DNA samples for this study.

This work was partially supported by JGBCC Pilot Project Initiative Grant to LRK, and the JGBCC Bucks for Brains “Our Highest Potential” in Cancer Research Endowment to LRK, National Cancer Institute/National Institute of Health grant (R03 CA128028, 3R01 CA034627-19S) to LRK, and the National Institute of Health/ National Institute of Environmental Health Sciences grants P30-ES014443 to GNB.

The authors appreciate access to the CGeMM DNA Core Facility at UofL, directed by Dr. Ron Gregg, for the use of their high-throughput genotyping facility.

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Table 1

Patient and Tumor Characteristics

Characteristics	Total	Cases	Controls	χ^2 P-value[a]	Fisher's Exact P-value
Number of Participants	774	219	555		
Age (yrs)					
Median (range)	58.0 (41–91)	65.5 (41–91)	52.0 (45–89)	< 0.0001	
Missing, n (%)	9 (1.1)	0 (0.0)	9 (4.1)		
Family History of PCa, n (%)					
Yes	39 (5.0)	28 (12.8)	11 (2.0)	0.7729	0.8472
No	157 (20.3)	109 (49.8)	48 (8.6)		
Missing, n (%)	578 (74.7)	82 (37.4)	496 (89.4)		
Tobacco Use, n (%)[b]					
Current	37 (4.8)	26 (12.0)	11 (2.0)	0.8249	0.8471
Former	73 (9.5)	53 (24.5)	20 (3.6)		
Never	104 (13.5)	71 (32.9)	33 (6.0)		
Missing	557 (72.2)	66 (30.6)	491 (88.4)		
Ever Smoker, n (%)[b]					
Yes	110 (14.3)	79 (36.5)	31 (5.6)	0.5709	0.6544
No	104 (13.5)	71 (32.9)	33 (6.0)		
Missing	557 (72.2)	66 (30.6)	491 (88.4)		
PSA in ng/ml, n (%)					
<2.0	493 (63.7)	30 (13.7)	463 (83.4)	<0.0001	$<2.2\times10^{-16}$
2.0–4.0	86 (11.1)	14 (6.4)	72 (13.0)		
>4.0	161 (20.8)	161 (73.5)	0 (0.0)		
Missing	34 (4.4)	14 (6.4)	20 (3.6)		
Gleason Score, n (%)					
4		18 (8.2)			
5		15 (6.9)			

Characteristics	Total	Cases	Controls	χ^2 P-value[a]	Fisher's Exact P-value
6		33 (15.1)			
7		40 (18.3)			
8		6 (2.7)			
9		14 (6.4)			
10		4 (1.8)			
Missing		89 (40.6)			
West African Ancestry					
Median	0.718	0.713	0.729	0.0227	
(range)	(0.168)	(0.255–0.946)	(0.253–0.937)		
Missing, n (%)	0 (0.0)	0 (0.0)	0 (0.0)		

[a]Differences in frequencies between cases and controls were tested using a Chi-square test of homogeneity or Fisher's Exact test (i.e., PSA ng/ml, tobacco use, and ever smoker); Differences in continuous variables (i.e., age and Global West African Ancestry) between cases and controls were tested using the Wilcoxon rank sum test. Calculation of all p-values excluded missing values from the analysis.

[b]Five cases were removed since they did not accurately report their tobacco smoking history when we compared the tobacco use and ever tobacco smoking status.

Table 2

Functional Consequences of *N*-acetyltransferase Alleles.

Gene	Allele	Nucleotide Change	Amino Acid Change	Deduced Acetylator Status
NAT1[a]	*4	**Reference**	**Reference**	**Reference**
	*3	$C^{1095}A$ (rs15561)		
	*10	$T^{1088}A$ (rs1057126), $C^{1095}A$		Rapid
	*11	$G^{445}A$ (rs4987076), $C^{1095}A$	$Val^{149}Ile$	
	*14A	$G^{560}A$ (rs4986782), $T^{1088}A$, $C^{1095}A$	$Arg^{187}Gln$	Slow
	*14B	$G^{560}A$	$Arg^{187}Gln$	Slow
	*15	$C^{559}T$ (rs5030839)	Arg ^{187}Stop	Slow
	*17	$C^{190}T$ (rs56379106)	Arg ^{64}Trp	Slow
	*19	$C^{97}T$ (rs56318881)	Arg ^{33}Stop	Slow
	*22	$A^{752}T$ (rs56172717)	$Asp^{251}Val$	Slow
NAT2[b]				
	* 4	None	**Reference**	**Rapid**
	* 5A	$T^{341}C$ (rs1801280), $C^{481}T$ (rs1799929)	Ile ^{114} Thr, $Leu^{161}Leu$	Slow
	*5B	$T^{341}C$, $C^{481}T$, $A^{803}G$ (rs1208)	Ile ^{114} Thr, $Leu^{161}Leu$, $Lys^{268}Arg$	Slow
	*5C	$T^{341}C$, $A^{803}G$	Ile ^{114} Thr, $Lys^{268}Arg$	Slow
	*5D	$T^{341}C$	$Ile^{114}Thr$	Slow
	*5E	$T^{341}C$, $G^{590}A$ (rs1799930)	$Ile^{114}Thr$, $Arg^{197}Gln$	Slow
	*5G	$C^{282}T$ (rs1041983), $T^{341}C$, $C^{481}T$, $A^{803}G$	$Tyr^{94}Tyr$, $Leu^{161}Leu$	Slow
	*5J	$C^{282}T$, $T^{341}C$, $G^{590}A$	$Tyr^{94}Tyr$, $Ile^{114}Thr$,	Slow
	*6A	$C^{282}T$, $G^{590}A$	$Tyr^{94}Tyr$, $Arg^{197}Gln$	Slow
	*6B	$G^{590}A$	$Arg^{197}Gln$	Slow
	*6C	$C^{282}T$, $G^{590}A$, $A^{803}G$	$Tyr^{94}Tyr$, $Arg^{197}Gln$, $Lys^{268}Gln$	Slow
	*6E	$C^{481}T$, $G^{590}A$	$Leu^{161}Leu$, $Arg^{197}Gln$	Slow
	*7A	$G^{857}A$ (rs1799931)	$Gly^{286}Arg$	Slow
	*7B	$C^{282}T$, $G^{857}A$	$Tyr^{94}Tyr$, $Gly^{286}Glu$	Slow
	*7C	$C^{282}T$, $A^{803}G$, $G^{857}A$	$Tyr^{94}Tyr$, $Gly^{286}Glu$, $Lys^{268}Gln$	Slow
	*11A	$C^{481}T$	Leu161Leu	Rapid

Gene	Allele	Nucleotide Change	Amino Acid Change	Deduced Acetylator Status
	*12A	$A^{803}G$	$Lys^{268}Arg$	Rapid
	*12B	$C^{282}T$, $A^{803}G$	$Tyr^{94}Tyr$, $Lys^{268}Arg$	Rapid
	*12C	$C^{481}T$, $A^{803}G$	$Leu^{161}Leu$, $Lys^{268}Arg$	Rapid
	*13A	$C^{282}T$	$Tyr^{94}Tyr$	Rapid
	*14A	$G^{191}A$ (rs1801279)	$Arg^{64}Gln$, $Tyr^{94}Tyr$	Slow
	*14B	$G^{191}A$, $C^{282}T$	$Arg^{64}Gln$	Slow
	*14C	$G^{191}A$, $T^{341}C$, $C^{481}T$, $A^{803}G$	$Arg^{64}Gln$, $Ile^{114}Thr$, $Leu^{161}Leu$, $Lys^{268}Arg$	Slow
	*14D	$G^{191}A$, $C^{282}T$, $T^{341}C$, $G^{590}A$	$Arg^{64}Gln$, $Tyr^{94}Tyr$, $Arg^{197}Gln$	Slow
	*14E	$G^{191}A$, $A^{803}G$	$Arg^{64}Gln$, $Lys^{268}Arg$	Slow
	*14F	$G^{191}A$, $T^{341}C$, A^{803G}	$Arg^{64}Gln$, $Ile^{114}Thr$ $Lys^{268}Arg$	Slow
	*14G	$G^{191}A$, $C^{282}T$, $A^{803}G$	$Arg^{64}Gln$, $Tyr^{94}Tyr$, $Lys^{268}Arg$	Slow

[a] *NAT1*, *N*-acetyltransferase 1, http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT1_alleles.pdf

[b] *NAT2*, *N*-acetyltransferase 2, http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT2_alleles.pdf

Table 3

N-Acetyltransferase Genotypes and PCa Among Men of African Descent.

N-acetyltransferase alleles	Case n (%)	Control n (%)	Estimated OR (95% CI)[a]	Estimated OR (95% CI)[b]	P-value	P for Trend
NAT1 (178 cases, 492 controls)[e]						
No *NAT1*10 alleles	53 (29.8)	157 (31.9)	1.00 (Reference)	1.00 (Reference)	0.1574[c]	0.5895
1 *NAT1*10* allele	87 (48.9)	202 (41.1)	1.28 (0.86–1.90)	1.29 (0.81–2.05)		
2 *NAT1*10* alleles	38 (21.3)	133 (27.0)	0.85 (0.53–1.36)	0.86 (0.50–1.48)		
≥1 NAT1*10 allele(s)	125 (70.2)	335 (68.1)	1.10 (0.76–1.60)	1.12 (0.72–1.72)		
NAT2 (190 cases, 493 controls)[f]						
Rapid	19 (10.0)	68 (13.8)	1.0 (Reference)	1.00 (Reference)	0.3278[d]	0.8529
Intermediate	93 (49.0)	224 (45.4)	1.49 (0.85–2.61)	1.21 (0.65–2.26)		
Slow	64 (33.7)	151 (30.6)	1.52 (0.84–2.73)	1.42 (0.74–2.72)		
Very Slow	14 (7.3)	50 (10.1)	1.0(0.46–2.19)	0.99 (0.42–2.34)		
Very Slow	14 (7.3)	50 (10.1)	1.00 (Reference)	1.00 (Reference)		
Slow	64 (33.7)	151 (30.6)	1.51 (0.78–2.93)	1.43 (0.69–2.97)		
Intermediate	93 (49.0)	224 (45.4)	1.48 (0.78–2.81)	1.22 (0.60–2.47)		
Rapid	19 (10.0)	68 (13.8)	1.00 (0.46–2.18)	1.01 (1.10–2.37)		

[a] Associations were determined using univariate logistic regression models to estimate the risk of developing PCa.

[b] Estimated odds ratios were adjusted for age (yrs) and West African Ancestry (WAA; continuous variable).

[c] Overall p-value comparing differences in the frequency zero, one, and two *NAT1*10* genotypes between cases and controls using the chi-square test of homogeneity with two degrees of freedom and a significance level of 0.05.

[d] Overall p-value comparing differences in the frequency rapid, intermediate, slow and very slow *NAT2* genotypes between cases and controls using the chi-square test of homogeneity with three degrees of freedom and a significance level of 0.05.

[e] Inheritance of zero (*3/*3, *4/*4), one (*10/*3, 10*/*4, *10/*17) or two (*10/*10) *NAT1* alleles was collected for 178 cases and 492 controls. One hundred-four subjects had missing *NAT1* genotype data.

[f] Inheritance of two *NAT2* rapid (*11A, *12ABC *13), intermediate (one slow and one rapid), slow (one of *5ABC, *6AC, *7AB, *14ABE), or very slow (two *5) alleles were collected for 190 cases and 493 controls. Ninety-one subjects had missing *NAT2* genotype data.