No Association Between Variant N-acetyltransferase Genes, Cigarette Smoking and Prostate Cancer Susceptibility Among Men of African Descent

La Creis Renee Kidd\textsuperscript{1,2}, Tiva T. VanCleave\textsuperscript{1,2}, Mark A. Doll\textsuperscript{1,2}, Daya S. Srivastava\textsuperscript{3}, Brandon Thacker\textsuperscript{1,2}, Oyeyemi Komolafe\textsuperscript{4}, Vasyl Pihur\textsuperscript{4}, Guy N. Brock\textsuperscript{2,4,6} and David W. Hein\textsuperscript{1,2,5,6}

\textsuperscript{1}Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, Louisville, KY, USA. \textsuperscript{2}James Graham Brown Cancer Center, Cancer Prevention and Control Program, University of Louisville, Louisville, KY, USA. \textsuperscript{3}Division of Biochemistry, Central Drug Research Institute, M.G. Marg Lucknow, U.P., 226001, India. \textsuperscript{4}Department of Bioinformatics and Biostatistics, School of Public Health and Information Sciences, University of Louisville, Louisville, KY, USA. \textsuperscript{5}Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY, USA. \textsuperscript{6}Center for Environmental Genomics and Integrative Biology, University of Louisville, Louisville, KY, USA.

Corresponding author email: lrkidd01@louisville.edu

ABSTRACT

Objective: We evaluated the individual and combination effects of \textit{NAT1}, \textit{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 \textit{NAT1} and \textit{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 \textit{NAT1*10/*10} (40.1\%), \textit{NAT2} slow (30.6\%), and \textit{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 \textit{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.

Keywords: \textit{N}-acetyltransferase genes, Prostatic Neoplasms, African-Americans, smoking

\textit{Biomarkers in Cancer} 2011:3 1–13
doi: 10.4137/BIC.S6111

This article is available from http://www.la-press.com.

© the author(s), publisher and licensee Libertas Academica Ltd.

This is an open access article. Unrestricted non-commercial use is permitted provided the original work is properly cited.
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. 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. Other than race, the for African-American men are two-fold higher relative from the disease. PCa incidence and mortality rates for African-American men are two-fold higher relative to European-Americans. 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. 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. 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 (eg, heterocyclic amines, aromatic amines), respectively. 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. NAT1 is highly polymorphic with over 25 variant alleles. A common variant allele, NAT1*10, defined by two single nucleotide polymorphisms (SNPs) in the 3’ untranslated region (T1088A and C1095A) may cause a shift in the position of the mRNA polyadenylation signal, resulting in a potential increase in mRNA stability. 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. The frequency of this putative rapid acetylator NAT1*10 allele is fairly common among African-Americans (50%). There is some evidence that inheritance of the NAT1*10 variant allele is associated with an increase in colorectal, breast and PCa relative to carriers of the homozygous NAT1*4/*4 genotype. 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 amines. 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.

Polymorphism in the NAT2 gene has led to the identification of a NAT2*4 (reference) and over 25 variant alleles. Several SNPs alone or in combination (eg, C282T, T281C, C481T, G590A, A803G, and G857A) result in NAT2 alleles (eg, 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. Specifically, the T281C SNP targets the NAT2 protein for enhanced pro- teosomal degradation and is associated with the very slow NAT2 acetylator phenotype. 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. 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.

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 \( \text{NAT2} \) slow (OR = 5.08; 95% CI: 1.56–16.5; \( P = 0.008 \)) or very slow \( \text{NAT2} \) 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 \( \text{NAT1}^{*}10 \) and slow \( \text{NAT2} \) 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 \( \text{NAT2} \) SNPs (rs11120005, rs7832071, rs1801280, rs1799930) in relation to PCa susceptibility among participants of the current study.\(^{25} \) Two of these \( \text{NAT2} \) loci (rs1801280, rs1799930) have been documented to influence \( \text{NAT2} \) protein levels and \( N \)-acetyltransferase activity. However, there are thirteen other \( \text{NAT1} \) and \( \text{NAT2} \) 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 \( \text{NAT1} \) and \( \text{NAT2} \) 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 relation to a 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**

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 Cancer Center. 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 \( \leq 4.0 \) 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 DRE), 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 Boards.

**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 (\( \text{NAT1} \) and \( \text{NAT2} \)) were determined using 10 \( \mu \)l Taqman Polymerase Chain Reaction (Taqman PCR) allelic discrimination assays, as described elsewhere.\(^{29,30} \) Briefly, approximately 40 ng/\( \mu \)l 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 (10 µl) 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 $P < 0.005$.

### Table 1. Patient and tumor characteristics.

| Characteristics                      | Total | Cases | Controls | $\chi^2$ P-value | Fisher’s exact P-value |
|--------------------------------------|-------|-------|----------|------------------|------------------------|
| Number of participants               | 774   | 219   | 555      | –                | –                      |
| **Age (yrs)**                        |       |       |          |                  |                        |
| Median (range)                       | 58.0  | 65.5  | 52.0     | 0.0001<sup>a</sup> | 0.7729 0.8472          |
| 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                              | 578 (74.7)| 82 (37.4)| 496 (89.4)|                  |                        |
| **Tobacco use, n (%)**<sup>c</sup>  |       |       |          |                  |                        |
| 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 (%)**<sup>c</sup>  |       |       |          |                  |                        |
| 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<sup>b</sup> | 2.2 × 10<sup>-16</sup> |
| 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)|       |          |                  |                        |
| 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 (range)                       | 0.718 (0.168)| 0.713 (0.255–0.946)| 0.729 (0.253–0.937)| 0.0227<sup>b</sup> |
| Missing, n (%)                       | 0 (0.0)| 0 (0.0) | 0 (0.0)  |                  |                        |

Notes: <sup>a</sup>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); <sup>b</sup>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; <sup>c</sup>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.
The aforementioned assay was designed to differentiate between SNPs in \textit{NAT1} (n = 8) and \textit{NAT2} (n = 7) to minimize \textit{NAT1} and \textit{NAT2} genotype misclassification. The SNPs detected in \textit{NAT1} were: C\textsuperscript{97}T (R\textsuperscript{33}Stop), C\textsuperscript{190}T (R\textsuperscript{64}W), G\textsuperscript{445}A (V\textsuperscript{149}I), C\textsuperscript{559}T (R\textsuperscript{187}Stop), G\textsuperscript{560}A (R\textsuperscript{187}Q), A\textsuperscript{752}T (D\textsuperscript{251}V), T\textsuperscript{1088}A (3'UTR), and C\textsuperscript{1095}A (3'UTR). SNPs detected in the \textit{NAT2} gene were: G\textsuperscript{191}A (R\textsuperscript{64}Q), C\textsuperscript{282}T (silent), T\textsuperscript{341}C (I\textsuperscript{114}T), C\textsuperscript{481}T (silent), G\textsuperscript{500}A (R\textsuperscript{197}Q), A\textsuperscript{803}G (K\textsuperscript{268}R), and G\textsuperscript{557}A (G\textsuperscript{286}E). \textit{NAT1} and \textit{NAT2} alleles, genotypes, and deduced phenotypes were determined as previously described\textsuperscript{31} and summarized in Table 2.

### Table 2. Functional consequences of N-acetyltransferase alleles.

| Gene | Allele | Nucleotide change | Amino acid change | Deduced acetylator status |
|------|--------|-------------------|-------------------|--------------------------|
| \textit{NAT1}\textsuperscript{a} | *4     | Reference         | Reference         | Reference                |
|      | *3     | C\textsuperscript{1095}A (rs15561) | Reference         | Reference                |
|      | *10    | T\textsuperscript{1088}A (rs1057126), C\textsuperscript{1095}A | Reference         | Reference                |
|      | *11    | G\textsuperscript{445}A (rs4987076), C\textsuperscript{1095}A | Reference         | Reference                |
|      | *14A   | G\textsuperscript{960}A (rs4986782), T\textsuperscript{1088}A, C\textsuperscript{1095}A | Reference         | Reference                |
|      | *14B   | G\textsuperscript{560}A              | Reference         | Reference                |
|      | *15    | C\textsuperscript{559}T (rs5030839)  | Reference         | Reference                |
|      | *17    | C\textsuperscript{590}T (rs56379106) | Reference         | Reference                |
|      | *19    | C\textsuperscript{590}A (rs56318881) | Reference         | Reference                |
|      | *22    | A\textsuperscript{752}T (rs56172717) | Reference         | Reference                |
| \textit{NAT2}\textsuperscript{b} | *4     | None              | Reference         | Reference                |
|      | *5A    | T\textsuperscript{191}C (rs1801280), C\textsuperscript{481}T (rs1799929) | Reference         | Reference                |
|      | *5B    | T\textsuperscript{191}C, C\textsuperscript{481}T, A\textsuperscript{803}G (rs1208) | Reference         | Reference                |
|      | *5C    | T\textsuperscript{191}C, A\textsuperscript{803}G | Reference         | Reference                |
|      | *5D    | T\textsuperscript{191}C | Reference         | Reference                |
|      | *5E    | T\textsuperscript{191}C, G\textsuperscript{590}A (rs1799930) | Reference         | Reference                |
|      | *5G    | C\textsuperscript{282}T (rs1041983), T\textsuperscript{341}C, C\textsuperscript{481}T, A\textsuperscript{803}G | Reference         | Reference                |
|      | *5J    | C\textsuperscript{282}T, T\textsuperscript{341}C, G\textsuperscript{590}A | Reference         | Reference                |
|      | *5A    | C\textsuperscript{282}T, G\textsuperscript{590}A | Reference         | Reference                |
|      | *6B    | G\textsuperscript{590}A | Reference         | Reference                |
|      | *6C    | C\textsuperscript{282}T, G\textsuperscript{590}A, A\textsuperscript{803}G | Reference         | Reference                |
|      | *6E    | C\textsuperscript{282}T, G\textsuperscript{590}A | Reference         | Reference                |
|      | *7A    | G\textsuperscript{557}A (rs1799931) | Reference         | Reference                |
|      | *7B    | C\textsuperscript{282}T, G\textsuperscript{557}A | Reference         | Reference                |
|      | *7C    | C\textsuperscript{282}T, A\textsuperscript{803}G, G\textsuperscript{557}A | Reference         | Reference                |
|      | *11A   | C\textsuperscript{282}T | Reference         | Reference                |
|      | *12A   | A\textsuperscript{803}G | Reference         | Reference                |
|      | *12B   | C\textsuperscript{282}T, A\textsuperscript{803}G | Reference         | Reference                |
|      | *12C   | C\textsuperscript{282}T, A\textsuperscript{803}G | Reference         | Reference                |
|      | *13A   | C\textsuperscript{282}T | Reference         | Reference                |
|      | *14A   | G\textsuperscript{191}A (rs1801279) | Reference         | Reference                |
|      | *14B   | G\textsuperscript{191}A, C\textsuperscript{282}T | Reference         | Reference                |
|      | *14C   | G\textsuperscript{191}A, T\textsuperscript{341}C, C\textsuperscript{481}T, A\textsuperscript{803}G | Reference         | Reference                |
|      | *14D   | G\textsuperscript{191}A, C\textsuperscript{282}T, T\textsuperscript{341}C, G\textsuperscript{590}A | Reference         | Reference                |
|      | *14E   | G\textsuperscript{191}A, A\textsuperscript{803}G | Reference         | Reference                |
|      | *14F   | G\textsuperscript{191}A, T\textsuperscript{341}C, A\textsuperscript{803}G | Reference         | Reference                |
|      | *14G   | G\textsuperscript{191}A, C\textsuperscript{282}T, A\textsuperscript{803}G | Reference         | Reference                |

Notes: \textsuperscript{a}NAT1, N-acetyltransferase 1, http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT1_alleles.pdf; \textsuperscript{b}NAT2, N-acetyltransferase 2, http://louisville.edu/medschool/pharmacology/consensus-human-arylamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT2_alleles.pdf.
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. 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. Individuals with a West African ancestry (WAA) score ≥25% and available NAT genotype data (178 cases, 492 controls for NAT1 and 190 cases, 493 controls for NAT2) were included in the final analysis.

Statistical analysis
Evaluation of patient and tumor characteristics
Differences in continuous [i.e., 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. 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 were evaluated by the significance of the coefficient of the product term $\beta_{loci 1}*loci 2$ (i.e., NAT1*NAT2 and NAT2*smoking) in the following models: 1) $\text{Logit} = \beta_0 + \beta_{\text{gene 1}} + \beta_{\text{gene 2}} + \beta_{\text{gene 1}* \text{gene 2}}$; 2) $\text{Logit} = \beta_0 + \beta_{\text{gene}} + \beta_{\text{smoking}} + \beta_{\text{gene}* \text{smoking}}$. 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 complement LR, MDR was used to further evaluate gene-gene and gene-smoking interactions associated with sporadic PCa. MDR has been described and reviewed elsewhere. 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%), number of cases (178-190), and number of 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.

Table 3. N-acetyltransferase genotypes and PCa among men of African descent.

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

Notes: *Associations were determined using univariate logistic regression models to estimate the risk of developing PCa; *Estimated odds ratios were adjusted for age (yrs) and West African Ancestry (WAA; continuous variable); *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; *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; *Inheritance of zero (*3/*3, *4/*4), one (*10/*3, 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; *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.

Results

Patient and 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
Individual and multilocus effects

No statistically significant association was observed between tobacco smoking use (current/former versus never) and PCa ($P_{\text{homogeneity}} = 0.5709$). There was no association with PCa risk among carriers of: one or two copies of $NAT1*10$ compared with zero copies, $NAT2$ intermediate, slow, or very slow compared with the rapid genotype; and one or two copies of $NAT2^{\text{rapid}}$ alleles compared to the slow genotypes ($P_{\text{homogeneity}} = 0.1574$ for $NAT1$, 0.3278 for $NAT2^{\text{slow}}$ and 0.3278 $NAT2^{\text{rapid}}$). The absence of gene combination effects were confirmed by MDR analysis supplemented with permutation testing ($P \geq 0.78$; 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 ($P_{\text{interaction}} \geq 0.2897$ for $NAT1*10$ and $NAT2^{\text{slow}}$ and 0.2156 for $NAT1*10$ and $NAT2^{\text{rapid}}$; Supplementary Table A) or $NAT2$ and tobacco smoking were not significant in the unadjusted and adjusted models ($P_{\text{interaction}} = 0.1445$; 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 (eg, 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 publicly 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). The CGEMS data portal contains genotype data for 6 \textit{NAT1} and 10 \textit{NAT2} SNPs; however, none of these markers were related to either PCa or aggressive disease. Upon closer inspection, only the \textit{NAT2} SNP (rs1208; A\textsuperscript{603}G, Lys\textsuperscript{268}Arg) matched one out of 15 \textit{NAT} SNPs analyzed in the current study. Since the rs1208 SNP is one of 7 \textit{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, \textit{NAT1} and \textit{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 \textit{NAT1} and \textit{NAT2} rapid, intermediate, 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 \textit{NAT1} and \textit{NAT2} SNPs to avoid errors in the deduced acetylator phenotypes. These methodological differences in \textit{NAT} SNPs assayed across studies, makes it impossible to make comparisons and draw any firm conclusions about the role of polymorphic \textit{NATs} in prostate cancer risk. In the current study, genotype misclassification was minimized due to the determination of the fifteen SNPs in \textit{NAT1} and \textit{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 \textit{NAT1} and \textit{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. 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 \textit{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.

In summary, we did not observe strong main or gene combination effects of \textit{NAT1} and \textit{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.

Disclosures
This manuscript has been read and approved by all authors. This paper is unique and not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

Acknowledgements
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.

References
1. American Cancer Society. Cancer Facts and Figures 2010. Atlanta: American Cancer Society, 2010.
2. American Cancer Society. Cancer Facts and Figures for African Americans 2009–2010. Atlanta: American Cancer Society, 2009.
3. Sugimura T. Overview of carcinogenic heterocyclic amines. Mutat Res. 1997 May 12;376(1–2):211–9.
4. Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, Felton JS. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis. 1995 Jan;16(1):39–52.
5. Shirai T, Sano M, Tamano S, et al. The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) derived from cooked foods. Cancer Res. 1997 Jan 15;57(2):195–8.
6. Huncharek M, Haddock KS, Reid R, Kulpelnick B. Smoking as a risk factor for prostate cancer: a meta-analysis of 24 prospective cohort studies. Am J Public Health. 2010 Apr;100(4):693–701.
7. Hein DW, Doll MA, Rustan TD, et al. Metabolic activation and detoxication of aryamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis. 1993;14(8):1633–8.
8. Blum M, Grant DM, McBride W, Heim M, Meyer UA. Human arylamine N-acetyltransferase genotypes: isolation, chromosomal localization, and functional expression. DNA Cell Biol. 1990 Apr;9(3):193–203.
9. Hein DW, Grant DM, Sim E. Update on consensus arylamine N-acetyltransferase gene nomenclature. Pharmacogenomics. 2000;10(4):291–2.
10. Bell DA, Badawi AF, Lang NP, Ilett KF, Kadlubar FF, Hirvonen A. Polymorphism in the N-acetyltransferase 1 (NAT1) polyadenylation signal: association of NAT1*10 allele with higher N-acetylation activity in bladder and colon tissue. Cancer Res. 1995;55(22):5226–9.
11. Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in the human urinary bladder. Cancer Res. 1995;55(22):5230–7.
12. Millikan RC, Pittman GS, Newman B, et al. Cigarette smoking, N-acetyltransferases 1 and 2, and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 1998;7(5):371–8.
13. Bell DA, Stephens EA, Castranio T, et al. Polyadenylation polymorphism in the acetyltransferase 1 gene (NAT1) increases risk of colorectal cancer. Cancer Res. 1995;55(16):3357–42.
14. Fukumoto K, Watanabe M, Shiraiishi T, et al. N-acetyltransferase 1 genetic polymorphism influences the risk of prostate cancer development. Cancer Lett. 1999;136(1):83–7.
15. Hein DW, Left MA, Ishibe N, et al. Association of prostate cancer with rapid N-acetyltransferase 1 (NAT1*10) in combination with slow N-acetyltransferase 2 acylator genotypes in a pilot case-control study. Environ Mol Mutagen. 2002;40(3):161–7.
16. Doll MA, Jiang W, Deitz AC, Rustan TD, Hein DW. Identification of a novel allele at the human NAT1 acetyltransferase locus. Biochem Biophys Res Commun. 1997;233(3):584–91.
17. Hughes NC, Janiecz SA, McQueen KL, et al. Identification and characterization of variant alleles of human acetyltransferase NAT1 with defective function using p-gaminosaliclate as an in vivo and in vitro probe. Pharmacogenetics. 1998 Feb;8(1):55–66.
18. Frettland AJ, Left MA, Doll MA, Hein DW. Functional characterization of human N-acetyltransferase 2 (NAT2) single nucleotide polymorphisms. Pharmacogenomics. 2001;11(3):207–15.
19. Zang Y, Zhao S, Doll MA, States JC, Hein DW. The T341C (Ile114Thr) polymorphism of N-acetyltransferase 2 yields slow acetylator phenotype by enhanced protein degradation. Pharmacogenetics. 2004 Nov;14(11):717–23.
20. Hein DW, Milliner LM, Leggett CS, Doll MA. Relationship between N-acetyltransferase 2 single nucleotide polymorphisms and phenotype. Carcinogenesis. 2009 Nov 23.
21. Green J, Banks E, Berrington A, Darby S, Deo H, Newton R. N-acetyltransferase 2 and bladder cancer: an overview and consideration of the evidence for gene–environment interaction. Br J Cancer. 2000 Aug;83(3):412–7.
22. Harasaki T, Inatomi H, Katoh T, et al. N-acetyltransferase-2 gene polymorphism as a possible biomarker for prostate cancer in Japanese men. Int J Urol. 2003;10(3):167–73.
23. Hein DW, Doll MA, Xiao GH, Feng Y. Prostate expression of N-acetyltransferase 2 and bladder cancer: an overview and consideration of the evidence for gene–environment interaction. Br J Cancer. 2006 Feb;94(4):549–56.
24. Hooker S, Bonilla C, Akereyeni F, Ahaghotu C, Kittles RA. NAT2 and NER genetic variants and sporadic prostate cancer susceptibility in African Americans. Prostate Cancer Prostatic Dis. 2008;11(4):379–84.
25. Raja J, Ramachandran N, Munneke G, Patel U. Current status of transrectal ultrasound-guided prostate biopsy in the diagnosis of prostate cancer. Clin Radiol. 2006 Feb;61(2):142–53.
26. Gleason DF. Classification of prostatic carcinomas. Cancer Chemother Rep. 1966 Mar;50(3):125–9.
Polymorphic N-acetyltransferase genes and PCa risk

29. Doll MA, Hein DW. Comprehensive human NAT2 genotype method using single nucleotide polymorphism-specific polymerase chain reaction primers and fluorogenic probes. *Anal Biochem*. 2001;288(1):106–8.

30. Doll MA, Hein DW. Rapid genotype method to distinguish frequent and/or functional polymorphisms in human N-acetyltransferase-1. *Anal Biochem*. 2002;301(2):328–32.

31. Hein DW. N-acetyltransferase SNPs: emerging concepts serve as a paradigm for understanding complexities of personalized medicine. *Expert Opin Drug Metab Toxicol*. 2009 Apr;5(4):353–66.

32. Giri VN, Egleston B, Ruth K, et al. Race, genetic West African ancestry, and prostate cancer prediction by prostate-specific antigen in prospectively screened high-risk men. *Cancer Prev Res (Phila Pa)*. 2009;2(3):244–50.

33. Tian C, Hinds DA, Shigeta R, Kittles R, Ballinger DG, Seldin MF. A genomewide single-nucleotide-polymorphism panel with high ancestry information for African American admixture mapping. *Am J Hum Genet*. 2006;79(4):640–9.

34. Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. *Bioinformatics*. 2003;19(3):376–82.

35. multi-factor dimensionality reduction, http://www.epistasis.org

36. Menashe I, Rosenberg PS, Chen BE. PGA: power calculator for case-control genetic association analyses. *BMC Genet*. 2008;9:36.

37. Sharma S, Cao X, Wilkens LR, et al. Well-done meat consumption, NAT1 and NAT2 acetylator genotypes and prostate cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev*. 2010 Jul;19(7):1866–70.

38. Wang CY, Jones RF, Debiec-Rychter M, Soos G, Haas GP. Correlation of the genotypes for N-acetyltransferases 1 and 2 with double bladder and prostate cancers in a case-comparison study. *Anticancer Res*. 2002 Nov–Dec;22(6B):3529–35.

39. Gao JP, Huang YD, Yang GZ, Yang YQ. Relationship between genetic polymorphisms of metabolizing enzymes and prostate cancer. *Zhonghua Nan Ke Xue*. 2003;9(1):32–5.

40. Rovito PM Jr, Morse PD, Spinek K, et al. Heterocyclic amines and genotype of N-acetyltransferases as risk factors for prostate cancer. *Prostate Cancer Prostatic Dis*. 2005;8(1):69–74.

41. Costa S, Pinto D, Morais A, et al. Acetylation genotype and the genetic susceptibility to prostate cancer in a southern European population. *Prostate*. 2005;64(3):246–52.

42. Agundez JA, Martinez C, Olivera M, et al. Expression in human prostate of drug- and carcinogen-metabolizing enzymes: association with prostate cancer risk. *Br J Cancer*. 1998;78(10):1361–7.

43. Wadelius M, Autrup JL, Stubbins MJ, et al. Polymorphisms in NAT2, CYP2D6, CYP2C19 and GSTP1 and their association with prostate cancer. *Pharmacogenetics*. 1999 Jun;9(3):333–40.

44. Srivastava DS, Mittal RD. Genetic polymorphism of the N-acetyltransferase 2 gene, and susceptibility to prostate cancer: a pilot study in north Indian population. *BMC Urol*. 2005;5:12.

45. Cancer Genetic Markers of Susceptibility (CGEMS), http://cgems.cancer.gov
### Table SA. Effect modification of NAT1 and NAT2 in relation to PCa susceptibility.

| #NAT1*10 alleles | #Slow NAT2 alleles | Cases (%) | Estimated OR (95% CI)a | Estimated OR (95% CI)\(b\) | \(P\)-value for Interaction |
|------------------|-------------------|-----------|------------------------|-----------------------------|-----------------------------|
| 0 or 1           | NAT2 Slow         | 12 (7.1) || 43 (9.4) | 1.00 (Referent)         | 1.00 (Referent)             | 0.2897                     |
| 0 or 1           | 1 NAT2 Slow       | 58 (34.3) || 158 (34.8) | 1.32 (0.65–2.67)        | 1.01 (0.46–2.22)            |                            |
| 0 or 1           | 2 NAT2 Slow       | 63 (37.3) || 132 (29.1) | 1.71 (0.84–3.47)        | 1.63 (0.74–3.58)            |                            |
| 2                | 0 NAT2 Slow       | 3 (1.8)   || 18 (4.0)    | 0.60 (0.15–2.37)        | 0.73 (0.17–3.13)            |                            |
| 2                | 1 NAT2 Slow       | 22 (13.0) || 54 (11.9)  | 1.46 (0.65–3.28)        | 1.18 (0.48–2.90)            |                            |
| 2                | 2 NAT2 Slow       | 11 (6.5)  || 49 (10.8)   | 0.80 (0.32–2.01)        | 0.75 (0.28–2.04)            |                            |
| 0 or 1           | 0 NAT2 Rapid      | 63 (37.3) || 132 (29.1) | 1.00 (Referent)         | 1.00 (Referent)             | 0.2156                     |
| 0 or 1           | 1 NAT2 Rapid      | 58 (34.3) || 158 (34.8) | 0.77 (0.50–1.18)        | 0.62 (0.38–1.02)            |                            |
| 0 or 1           | 2 NAT2 Rapid      | 12 (7.1)  || 43 (9.4)    | 0.58 (0.29–1.18)        | 0.61 (0.28–1.34)            |                            |
| 2                | 0 NAT2 Rapid      | 11 (6.5)  || 49 (10.8)   | 0.47 (0.23–0.97)        | 0.46 (0.21–1.01)            |                            |
| 2                | 1 NAT2 Rapid      | 22 (13.0) || 54 (11.9)  | 0.85 (0.48–1.52)        | 0.72 (0.38–1.40)            |                            |
| 2                | 2 NAT2 Rapid      | 3 (1.8)   || 18 (4.0)    | 0.35 (0.10–1.23)        | 0.45 (0.12–1.68)            |                            |

**Notes:** aAssociations were determined using univariate logistic regression models to estimate the risk of developing PCa. 151 subjects had missing genotype data for NAT1 and/or NAT2; bRisk estimates adjusted for age (continuous variable) and West African Ancestry (WAA; continuous variable).
Polymorphic N-acetyltransferase genes and PCa risk

Table SB. Combined effects of N-acetyltransferase polymorphisms and cigarette smoking on PCa risk.

| N-acetyltransferase status | Unadjusted OR (95% CI)a | Adjusted OR (95% CI)b |
|----------------------------|--------------------------|-----------------------|
|                            | #Cases||#Ctrlsc | Non-smokers | Ever-smokers | Non-smokers | Ever-smokers |
| NAT2 Rapid alleles         | 1.00 (Reference) | 2.04 (0.54–7.62) | 1.00 (Reference) | 1.46 (0.33–6.45) |
| 5||5 | 57||28 |
| ≥1 NAT2 Slow allele(s)     | 0.71 (0.13–3.87) | 3.19 (0.84–12.10) | 0.33 (0.05–2.24) | 2.06 (0.45–9.42) |
| 5||7 | 67||21 |

P-value for interaction = 0.1445

Notes: *Associations were determined using multivariate logistic regression models to estimate the risk of developing PCa using Inheritance of two NAT2 rapid alleles and non-smokers as the referent category; "Risk estimates adjusted for age and West African Ancestry, modeled as continuous variables; "The analysis was restricted to study participants who had available smoking and N-acetyltransferase genotype status analysis.

Publish with Libertas Academica and every scientist working in your field can read your article

"I would like to say that this is the most author-friendly editing process I have experienced in over 150 publications. Thank you most sincerely.”

“The communication between your staff and me has been terrific. Whenever progress is made with the manuscript, I receive notice. Quite honestly, I’ve never had such complete communication with a journal.”

“LA is different, and hopefully represents a kind of scientific publication machinery that removes the hurdles from free flow of scientific thought.”

Your paper will be:
- Available to your entire community free of charge
- Fairly and quickly peer reviewed
- Yours! You retain copyright

http://www.la-press.com