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# Association of *Uridine Diphosphate-Glucuronosyltransferase 2B* Gene Variants with Serum Glucuronide Levels and Prostate Cancer Risk

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*Aims:* Uridine diphosphate-glucuronosyltransferase 2B (*UGT2B*) enzymes conjugate testosterone metabolites to enable their excretion in humans. The functional significance of the *UGT2B* genetic variants has never been described in humans. We evaluated *UGT2B* variants in relation to plasma androstane-3α,17β-diol-glucuronide (AAG) levels and the prostate cancer risk. *Results:* AAG levels were measured in sera from 150 controls and compared to the polymorphisms of *UGT2B17*, *UGT2B15*, and *UGT2B7*. Genomic DNA from controls (301) and cases (148) was genotyped for the polymorphisms, and odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using unconditional logistic regression analyses. Having two copies of *UGT2B17* was associated with higher AAG levels in controls among Whites ($p = 0.02$), but not Blacks ($p = 0.82$). Logistic regression models adjusting for age and race revealed that homozygosity for the G allele of the $UGT2B15^{D85Y}$ polymorphism was directly associated with the prostate cancer risk (OR = 2.70, 95% CI = 1.28, 5.55). *Conclusions:* While the small sample size limits inference, our findings suggest that an association between the *UGT2B17* copy number variant (CNV) and serum AAG levels in Whites, but unexpectedly not in Blacks. This novel observation suggests that genetic determinants of AAG levels in Blacks are unrelated to the *UGT2B17* CNV. This study replicates the results that show an association of $UGT215^{D85Y}$ with an increased prostate cancer risk.

## Introduction

Prostate cancer is the second leading cause of cancer death behind lung cancer in men in the United States (U.S. Cancer Statistics Working Group, 2010). In 2012, it is estimated that there will be 241,740 new cases and ~28,000 deaths from prostate cancer in the United States (Siegel *et al.*, 2012). The fact that androgen ablation is extremely effective at reducing tumor burden in men with prostate cancer has led to the hypothesis that androgens may play a role in prostate cancer etiology.

The uridine diphosphate-glucuronosyltransferase 2B (UGT2B) enzymes, UGT2B7, UGT2B15, and UGT2B17, catalyze the glucuronidation of multiple substrates. Through this process, endobiotic or xenobiotic substances, including hormones, are conjugated, and eliminated from the body through excretion in the urine. Of relevance, these enzymes exhibit specificity for androgen metabolites, such as testosterone, dihydrotestosterone, androsterone, and androstane-3α,17β-diol (3α-diol; Fig. 1) (Turgeon *et al.*, 2001). The UGT2B enzymes each target different androgen metabolites, and their conjugation of one metabolite in particular, androstane 3α,17β diol, contributes to the circulating serum levels of its glucuronide, androstanediol-glucuronide (also known as androstane-3α,17β-diol-glucuronide [AAG]). UGT2B15 and UGT2B17 conjugate AAG in the lumen and basal epithelial tissue of the prostate, respectively, and UGT2B7 also conjugates AAG in the skin (Fig. 1) (Turgeon *et al.*, 2001; Chouinard

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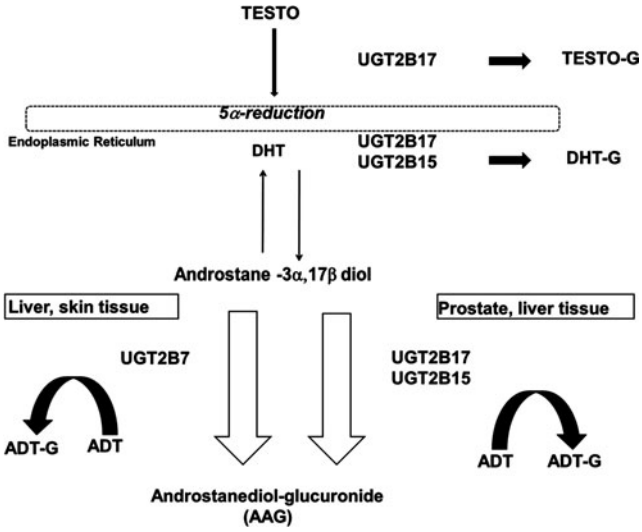


**FIG. 1.** Glucuronidation targets for UGT2B7, UGT2B17, and UGT2B15 enzymes in the liver, skin, and prostate tissue (Turgeon *et al.*, 2001; Chouinard *et al.*, 2007; Valentini *et al.*, 2007). Narrow black arrows indicate reversible catabolism of DHT; thick black arrows indicate irreversible production of testosterone- and DHT-G; gray arrows (on left and right) indicate irreversible production of ADT-G; and parallel black outlined arrows indicate irreversible production of AAG. Testo, testosterone; DHT, dihydrotestosterone; ADT, androsterone; G, glucuronide.

*et al.*, 2007; Valentini *et al.*, 2007). A common genetic polymorphism in the *UGT2B17* gene is gene deletion, and evidence shows that individuals who have two deleted *UGT2B17* alleles secrete little-to-no urinary testosterone compared to individuals with at least one copy of the gene (Jakobsson *et al.*, 2006). In addition, a polymorphism in the regulatory region of the *UGT2B17* gene has been associated with altered levels of AAG (Hu *et al.*, 2010). While both the *UGT2B17* and *UGT2B15* polymorphisms are significantly associated with AAG levels in European populations, no observations have been made in individuals of African descent (Hsing, 2001; Swanson *et al.*, 2007; Olsson *et al.*, 2011).

The *UGT2B17* and *UGT2B15* genes have variants that have been characterized and evaluated in population-based association studies. *UGT2B17* has a copy number variant (CNV) that is present in 0, 1, or 2 copies (Wilson *et al.*, 2004; Jakobsson *et al.*, 2006), while an *UGT2B15* variant, $UGT2B15^{D85Y}$, has a missense polymorphism at codon 85 that changes an aspartic acid residue (D allele) to a tyrosine residue (Y allele), resulting in an increased $V_{max}$. The resulting phenotype of the enzyme leads to quicker androgen metabolite clearance, while the wild type confers lower clearance, possibly raising the effective amount of steroids within the prostate (Levesque *et al.*, 1997; Chouinard *et al.*, 2008). While several *UGT2B7* variants have been described, they have not been examined in relation to prostate cancer (Turgeon *et al.*, 2001; Menard *et al.*, 2011). However, the expression of all three genes has been measured in prostate cancer cell lines (Valentini *et al.*, 2007). The association of the $UGT2B15^{D85Y}$ polymorphism with prostate cancer has been examined with conflicting results (MacLeod *et al.*, 2000; Gsur *et al.*, 2002; Hajdinjak and Zagradisnik, 2004; Park *et al.*, 2004; Cunningham *et al.*, 2007). Similarly, numerous studies have explored the association of the *UGT2B17* CNV and prostate cancer also with inconsistent conclusions (Park *et al.*, 2006, 2007; Gallagher *et al.*, 2007; Karypidis *et al.*, 2007; Olsson *et al.*, 2008; Setlur *et al.*, 2010). Some of the inconsistencies in these findings could be explained by differences in the composition of the study participants. To date, an assessment of the risk for prostate cancer by single-nucleotide polymorphisms (SNPs) in linkage with *UGT2B17* and *UGT2B7* has not been accomplished.

In this report, *UGT2B* genetic variants were assessed for their relationship with serum AAG levels in healthy men and for their association with the risk for prostate cancer in a case–control analysis.

## Materials and Methods

### *Study populations*

The details of the case–control study have been previously reported (Antonelli *et al.*, 2009). In brief, male patients from the Durham Veterans Affairs Medical Center (DVAMC) in Durham, North Carolina, who were undergoing a prostate needle biopsy between January 2007 and July 2010, were recruited to enroll in a hospital-based, prostate cancer case–control study. Eligibility criteria for cases included age >18 years, undergoing a prostate biopsy for concerns of potential prostate cancer after presentation with elevated prostate specific antigen (PSA), and/or abnormal digital rectal examination and diagnosed with prostate cancer after pathological review of biopsy tissue. A control population was recruited from the VA Internal Medicine Clinic who fit the criteria of age >18 and had a PSA test performed, but were not recommended to undergo biopsy. Of the 768 eligible control men, 377 signed consent forms (768/377 = 49% participation rate) by July 2010. Questionnaires were administered to prostate cancer cases before biopsy and to controls to assess risk factors, including race and age. Of the 759 men with a biopsy indication and who were screened for eligibility, 539 (759/539 = 71% participation rate) provided written consent to participate by July 2010. Of these 539, 517 underwent a biopsy, of which 202 were biopsy positive (cases). This report is limited to the first 150 cases for CNV analysis and 100 cases for genotyping based on available funding. The healthy controls were selected for the genotype ($n = 297$), CNV ($n = 201$), and ($n = 150$) AAG analyses. Institutional Review Board approval was obtained at the Duke University, North Carolina Central University, and the DVAMC, and all patients signed an informed consent at the DVAMC before enrollment.

### *AAG measurements*

AAG was measured on only the first 150 healthy control serum samples due to availability of funding. An enzyme immunoassay (EIA; ALPCO Diagnostics) at the Children's Hospital Boston, Department of Laboratory Medicine, Clinical and Epidemiologic Research Laboratory, was employed for determination of concentration. The EIA follows the basic principle of competitive binding assays where there is competition between an unlabeled antigen and an enzyme-labeled antigen for a fixed number of antibody-binding sites. A 96-well microtiter plate is coated with a polyclonal antibody to AAG. AAG in the samples competes with an AAG/horseradish peroxidase conjugate for binding sites. After

incubation, unbound materials are removed by aspirating and washing the wells. The substrate tetramethylbenzidine is added, and a color is generated that is indirectly proportional to the amount of AAG in the sample. An acidic stopping solution is added, and the degree of enzymatic turnover of the substrate is determined by dual-wavelength absorbance measurement. The assay possesses a sensitivity of 0.1 ng/mL and a run-to-run imprecision at AAG concentrations of 0.98, 7.05, and 20.92 ng/mL of 10.4%, 6.5%, and 10.8%, respectively. The samples were from healthy controls with available sera and completed questionnaires. Only samples from Whites and Blacks were included in the final analysis, which reduced the number to 147.

### *Genotyping*

Major *UGT2B* variants, *UGT2B17* CNV and *UGT2B15*$^{D85Y}$ (rs1902023), were selected for genotyping as well as SNP Tags for *UGT2B17* (rs7434408) and *UGT2B7* (7435335). Candidate SNP Tags were selected from the *UGT2B17* and *UGT2B7* genes that were in high linkage disequilibrium ($R^2 \geq 0.8$), and had minor alleles at frequencies that were at least 50% different in the CEU (European/White) and YRI (African/Black) HapMap populations. DNA was isolated from peripheral blood by standard DNA isolation (Qiagen, Inc.) and quantified by ultraviolet spectrophotometry. Before genotyping, DNA concentration was determined using PicoGreen assay (Life Technologies) and measured using the fluorescence intensity measurements plotted against a standard curve that was generated from the average fluorescence intensity of standards run in the replicate. Based on the PicoGreen quantification, 10 ng of genomic DNA from each sample was used in the iPlex assay for Sequenom-iPlex Genotyping (Sequenom, Inc.). The Sequenom MassArray (Sequenom, Inc.) was used, and the assays for *UGT2B17* (rs7434408), *UGT2B15*$^{D85Y}$ (rs1902023), and *UGT2B7* (rs7435335) were designed by the Sequenom online assay tools (Assay Designer 4.0) at the David H. Murdock Research Institute (DHMRI) Genomics Laboratory. The data were analyzed by Sequenom-Typer 4.0. The Sequenom-iPlex genotyping and analysis were validated with CEPH gDNA controls by performing the iPlex assay and scanning on the MALDI-TOF mass spectrometer. At the time of this analysis, 364 samples were submitted, and 358 successfully produced good spectra for genotyping at a failure rate of <2.2%. The post-QC (call rate) of *UGT2B17* (rs7434408), *UGT2B15*$^{D85Y}$ (rs1902023), and *UGT2B7* (rs7435335) was 100%. The assays included DHMRI control DNA (CEPH) on each plate in replicates that were checked for concordance for each SNP. Samples that were analyzed for the *UGT2B17* CNV were quantified with PicoGreen as described above and genotyped using the ABI 7900HT (Applied Biosystems) platform and commercially available TaqMan CNV assays. Each sample was assayed in quadruplicate and compared with controls previously genotyped (Wilson *et al.*, 2004). Data analysis was accomplished using SDS v2.4 and CopyCaller v1.0 (Applied Biosystems), and the confidence level was ≥0.99.

### *Statistical analyses*

All analyses were done using Stata Software. Quartiles of AAG levels in White and Black healthy controls were determined for *UGT2B* CNV, and genotypes and *p*-values were calculated by Kruskal-Wallis equality-of-population rank test. Chi-square tests were used to compare risk factors such as age and race with tumor grade based on low (Gleason sum <7) or high grade (Gleason sum >7). Unconditional logistic regression analyses were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer risk. Models were adjusted for age and race separately for CNV and SNPs, and analyses were completed assuming an ordinal, recessive, or dominant model. We estimated ORs for the association between the minor allele for SNPs, rs7434408 and rs7435335, and prostate cancer risk, adjusting for age and race. These analyses were repeated stratified by race and tumor grade. All *p*-values <0.05 were considered significant.

## Results

The distribution of the *UGT2B17* CNV genotypes was significantly associated with median serum AAG levels in Whites ($p=0.02$), but unexpectedly not in Blacks (Table 1). Specifically, a higher number of CNVs was associated with higher AAG levels. Conversely, there was a weak suggestion that the *UGT2B15*$^{D85Y}$ genotypes may be associated with AAG levels in Whites, although the association did not reach significance ($p=0.15$). No other significant associations between SNPs and AAG levels were observed in Whites. Notably, no significant association of genotypes and AAG levels was observed in Blacks. We did not observe any significant racial differences in AAG levels in the present study.

Demographic characteristics of the study population were compared among controls, men with low-grade tumors (Gleason <7), and those with high-grade tumors (Gleason ≥7) (Table 2). Cases were significantly older than controls ($p=0.004$). Black men comprised over half of the cases (61.6% vs. 38.4%) when compared to Whites and had the majority of both high-grade tumors (60.3% vs. 39.7%) and low-grade tumors (63% vs. 37%).

Dominant, heterozygous, and recessive genotypes for the *UGT2B17* CNV, *UGT2B17* SNP rs7434408, *UGT2B15*$^{D85Y}$, and the *UGT2B7* SNP 7435335 were evaluated in relation to the prostate cancer risk (Table 3). Crude analyses of the homozygous dominant allele (G) of the *UGT2B15* missense polymorphism, *UGT2B15*$^{85D}$ (rs1902023), showed a significant association with increased risk for prostate cancer (OR=3.22, 95% CI=1.59, 6.67). Adjusting for age and race did not alter these findings (OR=2.70, 95% CI=1.28, 5.55). No other significant associations between SNPs and prostate cancers were observed. Analyses stratified by race were done to determine if the association of the *UGT2B17* CNV and other *UGT2B* SNPs with prostate cancer differed between Whites and Blacks, though when this was done, the power to detect associations was reduced compared to the whole-population analyses (data not shown).

## Discussion

*UGT2B* enzymes are important in the biotransformation of steroid hormones, though their associations with serum steroid levels remain unclear. Moreover, though some studies have suggested their association with prostate cancer risk (MacLeod *et al.*, 2000; Hajdinjak and Zagradisnik, 2004; Park *et al.*, 2004, 2006, 2007; Karypidis *et al.*, 2007), others have not (Gsur *et al.*, 2002; Cunningham *et al.*, 2007; Gallagher *et al.*, 2007; Olsson *et al.*, 2008; Setlur *et al.*, 2010), making it difficult

Table 1. Median Androstane-3α,17β-Diol-Glucuronide Levels Among Healthy Controls and Quartiles and Associated *UGT2B* Sequence Variants

| | *Whites* | | *Blacks* | |
|---|---|---|---|---|
| *Genotype* | *Individuals (%)* | *Median (25th, 75th percentile)* | *Individuals (%)* | *Median (25th, 75th percentile)* |
| *UGT2B17* | | | | |
| CNV 0 | 6 (6.4) | 4.34 (3.3, 6.19) | 2 (4.2) | 8.8 (5.64, 12) |
| 1 | 41 (44.1) | 6.51 (4.08, 9.24) | 22 (45.8) | 6.9 (3.91, 10.57) |
| 2 | 46 (49.5) | 7.68 (5.69, 13.69) | 24 (50.0) | 6.75 (4.14, 8.8) |
| *p* Value | | 0.02 | | 0.82 |
| *UGT2B17* (rs7434408) | | | | |
| A | 28 (30.8) | 6.71 (4.63, 9.33) | 36 (76.6) | 6.71 (4.28, 10.78) |
| A/G | 41 (45.0) | 6.84 (4.47, 12.14) | 2 (4.2) | 7.39 (4.07, 10.16) |
| G | 22 (24.2) | 7.64 (5.44, 13.71) | 9 (19.1) | 5.07 (3.38, 6.77) |
| *p* Value | | 0.77 | | 0.67 |
| $UGT2B15^{D85Y}$(rs1902023) | | | | |
| G | 17 (18.7) | 8.75 (5.36, 14.14) | 17 (36.2) | 5.52 (3.74, 10.61) |
| G/T | 46 (50.5) | 6.85 (4.83, 11.44) | 16 (34.0) | 7.62 (4.41, 11.12) |
| T | 28 (30.8) | 6.49 (3.54, 9.33) | 14 (29.8) | 7.21 (4.62, 10.37) |
| *p* Value | | 0.15 | | 0.44 |
| *UGT2B7* (rs7435335) | | | | |
| G | 65 (72.2) | 6.71 (4.63, 11.52) | 26 (55.3) | 6.01 (3.75, 9.85) |
| G/A | 24 (26.7) | 7.12 (4.65, 11.02) | 19 (40.4) | 7.39 (4.26, 11.86) |
| A | 1 (1.1) | 10.07 (10.07) | 2 (4.2) | 5.55 (4.71, 6.4) |
| *p* Value | | *0.73* | | *0.59* |

UGT2B, uridine diphosphate-glucuronosyltransferase 2B; CNV, copy number variant.

to draw any firm conclusions. In the current study, among U.S. veterans, the *UGT2B17* CNV genotypes were significantly correlated with AAG levels in White healthy controls, but showed no association with AAG levels in Black healthy controls. The results suggested the novel observation that AAG levels in Blacks may not be associated with *UGT2B17* CNV and may be determined by other *UGT2B* variants. This study also confirmed the results of some previous studies that showed the association of the $UGT2B15^{D85Y}$ polymorphism with prostate cancer risk. Individuals homozygous for the $UGT2B15^{85G}$ (G) allele had a significantly increased risk for prostate cancer on both crude and adjusted analyses.

A study investigating the relationship of the *UGT2B* genes and hormones showed that both the $UGT2B15^{D85Y}$ polymorphism and the *UGT2B17* CNV were associated with serum levels of AAG ($p < 0.001$), but not with levels of another steroid glucuronide, 3α-diol-3-glucuronide, in a population-based cohort of young adult ($n = 1068$; mean age = 18.9 years) and elderly ($n = 1001$; mean age = 75.3 years) Swedish men (Swanson *et al.*, 2007). We did not determine 3α-diol-3-glucuronide levels in our study as AAG represents 80% of the combined isoforms of 3α-diol, and thus we felt measuring AAG, which gave a good overall measure of glucorinidation activity. Other studies showed that *UGT2B17* CNV was associated with AAG levels in subjects of European descent. Olsson *et al.* (2011) showed that AAG levels were significantly associated with the *UGT2B17* CNV, suggesting that the *UGT2B17* CNV genotypes are significantly associated with AAG levels. Thus, the relationship we observed between *UGT2B17* CNV and AAG levels in Whites is consistent with the two previous reports. Importantly, the levels of AAG measured in this study are similar to levels of AAG previously reported by an NHANES III study, suggesting that the measurements of AAG are stable and reliable (Rohrmann *et al.*, 2007). A key surprising finding from our study, which has not been previously examined, was the absence of an association between *UGT2B17* CNV and AAG among Black healthy controls. If confirmed, this suggests that the clearance of intraprostatic steroids may be influenced by different genetic determinants among Black men.

Table 2. Characteristics of Study Participants

| *Age* | *Controls (%)* | *Cases 1 (low grade)* | *Cases 2 (high grade)* | *Significance* p *value* |
|---|---|---|---|---|
| <50 | 18 (6) | 2 (2.7) | 1 (1.4) | |
| 50–59 | 80 (26.6) | 21 (28.8) | 14 (19.2) | |
| 60–69 | 162 (53.8) | 38 (52.0) | 43 (59) | |
| ≥70 | 41 (13.6) | 12 (16.4) | 15 (20.5) | |
| Mean age [SD] | 61.4 [7.5] | 62.2 [7] | 64.6 [6.4] | 0.004 |
| Race | | | | |
| Whites | 192 (63.8) | 27 (37) | 29 (39.7) | |
| Blacks | 109 (36.2) | 46 (63.0) | 44 (60.3) | <0.001 |

We replicated the findings of previous studies that have shown an association of the $UGT2B15^{D85Y}$ polymorphism and prostate cancer risk (Table 4). However, in the literature, both significant and null associations have been observed. The results from this study showed a significant increase in the risk for prostate cancer in individuals homozygous for the G allele, consistent with previous reports (Table 4) (MacLeod *et al.*, 2000; Hajdinjak and Zagradisnik, 2004; Park *et al.*, 2004). However, null associations of the $UGT2B15^{D85Y}$ polymorphism have also been described. The study by Gsur *et al.*

Table 3. Odds Ratios for the Association Between Genetic Variants of *UGT2B17* CNV, *UGT2B17* A/G (rs7434408), *UGT2B15*$^{D85Y}$ G/T (rs1902023), and *UGT2B7* A/G (rs7435335) and prostate cancer risk

| *Genotype* | *Cases (%)* | *Controls (%)* | *Crude OR (95% CI)* | *Adjusted OR (age, race)* |
|---|---|---|---|---|
| *UGT2B17 CNV* | | | | |
| 2 | 79 (53.4) | 101 (50.2) | Reference | Reference |
| 1 | 58 (39.2) | 91 (45.3) | 0.81 (0.52, 1.27) | 0.83 (0.53, 1.31) |
| 0 | 11 (7.4) | 9 (4.5) | 1.56 (0.62, 3.95) | 1.64 (0.63, 4.28) |
| *UGT2B17* (rs7434408) | | | | |
| A | 60 (66.7) | 136 (45.8) | Reference | Reference |
| A/G | 15 (16.7) | 66 (22.2) | 0.58 (0.32, 1.07) | 0.85 (0.44, 1.67) |
| G | 17 (18.9) | 95 (32.0) | 0.36 (0.19, 0.67) | 0.64 (0.32, 1.31) |
| A/G+G vs. A | 32 (34.8) | 136 (45.8) | 0.45 (0.28, 0.73) | 0.75 (0.42, 1.31) |
| *UGT2B15*$^{D85Y}$ (rs1902023) | | | | |
| T | 13 (14.1) | 90 (30.3) | Reference | Reference |
| G/T | 47 (51.1) | 139 (46.8) | 1.39 (0.81, 2.38) | 1.19 (0.68, 2.13) |
| G | 32 (34.8) | 68 (22.9) | 3.22 (1.59, 6.67) | 2.70 (1.28, 5.55) |
| G/T+G vs. T | 60 (65.2) | 229 (77.1) | 1.78 (1.09, 3.03) | 1.51 (0.89, 2.56) |
| *UGT2B7* (rs7435335) | | | | |
| G | 67 (72.8) | 208 (70.2) | Reference | Reference |
| G/A | 20 (21.7) | 83 (28.0) | 0.75 (0.43, 1.31) | 0.59 (0.32, 1.06) |
| A | 5 (5.4) | 5 (1.7) | 3.10 (0.87, 11.05) | 2.16 (0.56, 8.30) |
| G/A+A vs. G | 25 (27.1) | 88 (29.7) | 0.88 (0.52, 1.49) | 0.68 (0.39, 1.12) |

OR, odds ratio; CI, confidence interval.

(2002) showed that the homozygous *UGT2B15*$^{85Y}$ (T) allele was not associated (OR = 1.2; 95% CI = 0.65, 2.22) with prostate cancer. The authors of that study stated that a potential limitation to their study could be the use of a control population of men with benign prostate hyperplasia. In addition, a case–control study by Cunningham *et al.* also found no significant association with prostate cancer risk for the *UGT2B15*$^{D85}$ (G) allele, although the association with familial prostate cancer showed borderline significance (OR = 1.4, 95% CI = 1.0, 1.9) (Cunningham *et al.*, 2007). The results from previous reports assessing the association of the *UGT2B17* CNV and prostate cancer risk have been inconsistent (Table 4). We showed no association with prostate cancer risk consistent with some previous reports (Gallagher *et al.*, 2007; Olsson *et al.*, 2008; Setlur *et al.*, 2010). However, other studies reported that individuals homozygous for the *UGT2B17* deletion were at increased risk for prostate cancer, while a report showed that only carriers of the *UGT2B17* deletion had increased risk (Park *et al.*, 2006, 2007; Karypidis *et al.*, 2007). These differences across studies could be the result of interindividual variability in the *UGT2B* gene expression and haplotype structure of the *UGT2B* genes within each study population (Izukawa *et al.*, 2009; Menard *et al.*, 2009). This variability may be more apparent in individuals that have the *UGT2B17* gene deleted. Recent evidence however points to the relevance of UGT2B15 to prostate cancer etiology. Expression analysis of *UGT2B15* showed that decreased levels of UGT2B15 mRNA and proteins were associated with hormone-naïve and castration-

Table 4. Summary of Studies Evaluating the Association Between *UGT2B* Variants and Prostate Cancer

| UGT2B *variant* | *Genotype* | *Study* | *Controls* | *Cases* | *Blacks* | *OR* | *95% CI* | p | *References* |
|---|---|---|---|---|---|---|---|---|---|
| *UGT2B17* CNV | Del/Del | Incident Pca | 487 | 420 | 247 | Cau 1.9 | (1.2–3.0) | 0.006 | Park *et al.* (2006) |
| | | | | | | AA 1.3 | (0.6–2.7) | 0.32 | |
| *UGT2B17* CNV | Del/Del | Incident Pca | 363 | 356 | 34 | 1.7 | (1.03–2.9) | n.d. | Park *et al.* (2007) |
| *UGT2B17* CNV | Del/Del | Incident Pca | 161 | 176 | 0 | 1.63 | (0.37–7.15) | n.d. | Karypidis *et al.* (2007) |
| | Del/Del+ Del/Ins | | | | | 2.07 | (1.32–3.25) | | |
| *UGT2B17* CNV | Del/Del | Incident Pca | 397 | 411 | 0 | 0.89 | (0.55–1.45) | n.d. | Gallagher *et al.* (2007) |
| *UGt2B17* CNV | Del/Del | Incident Pca | 1722 | 2779 | | 1.01 | (0.83–1.23) | 0.91 | Olsson *et al.* (2008) |
| *UGT2B17* CNV | Del/Del | Incident Pca | 205 | 221 | 0 | 0.88 | (0.51–1.16) | 0.71 | Setlur *et al.* (2010) |
| *UGTB15*$^{D85Y}$ | D85/D85 | Incident Pca | 64 | 64 | 6 | 2.96 | ($\chi^2$ = 7.34) | < 0.01 | Macleod *et al.* (2000) |
| *UGTB15*$^{D85Y}$ | Y85/Y85 | Incident Pca | 190 | 190 | 0 | 1.20 | (0.65–2.22) | 0.54 | Gsur *et al.* (2002) |
| *UGTB15*$^{D85Y}$ | D85/D85 | Incident Pca | 155 | 155 | 0 | 2.70 | (1.1–6.6) | n.d. | Park *et al.* (2004) |
| *UGTB15*$^{D85Y}$ | D85/D85 | Sporadic Gleason scores | 178 | 206 | | 2.04 | | 0.032 | Hajdinjak *and* Zagradisnik (2004) |
| *UGTB15*$^{D85Y}$ | D85/Y85 | Familial and sporadic | 493 | 438 | 0 | 1.4 | (1.0, 1.9) | n.d. | Cunningham *et al.* (2007) |
| | | | 493 | 499 | | 1.1 | (0.8, 1.4) | n.d. | |

Pca, prostate cancer; n.d., no data.

resistant prostate tumors when compared to benign prostatic hyperplasia (Paquet *et al.*, 2011). It would be important in future association studies to investigate the impact of functional polymorphisms in high linkage with the $UGT2B15^{D85Y}$ polymorphism, given its effect on enzyme kinetics.

The strengths of this study are that while hospital based, the setting is the DVAMC, where there is equal access to care regardless of race, providing a pool of Blacks in the dataset compared to other studies. Also, the DVAMC more readily provides access to health care for diverse populations, allowing enrollment of a large number of Black men. As with any case–control study, especially those studies with patients with prostate cancer, it is possible that some controls may have actually had prostate cancer. However, this misclassification would tend to bias the results toward the null, and thus may underestimate the strength of the true significant associations between the genotype and prostate cancer risk. The main limitation of our study is the small sample size for prostate cancer cases and controls, which limited our inability to detect modest, but potentially important, associations. However, the results which show differences in the relationships between the *UGT2B17* CNV genotypes and AAG levels in White and Black healthy controls point to a need to include diverse populations in future studies to determine the association of molecular markers of steroid metabolism with serum metabolites across racial groups. The importance of including diverse racial groups is further accentuated by results from a recent study that found that *UGT2B* CNVs had different associations with a risk for biochemical recurrence after surgery and levels of androgen glucuronides in Whites compared to Asians who underwent radical prostatectomies (Nadeau *et al.*, 2011).

In summary, the inclusion of Blacks in this study population, although limited by sample size, revealed the novel observation that the *UGT2B17* genotype was associated with AAG levels, but that the association is limited to Whites. This observation points to a need to develop hypotheses about the genetic determinants of AAG clearance in Black populations given the disparity in prostate cancer incidence and mortality. We also found a direct association between the $UGT2B15^{D85}$ polymorphism and prostate cancer, an association that persisted after adjustment for age and race. Future research that evaluates the association of the *UGT2B* sequence variants with survival and prostate cancer risk should assess the importance of these genes as future biomarkers and therapeutic targets.

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## Author Disclosure Statement

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