Deletion Polymorphism of UDP-Glucuronosyltransferase 2B17 and Risk of Prostate Cancer in African American and Caucasian Men

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Abstract

Purpose: **UDP-glucuronosyltransferases (UGT) are a family of enzymes that glucuronidate many endogenous chemicals, including androgens. This makes them more hydrophilic, alters biological activity, and facilitates their excretion. A deletion polymorphism in the *UGT2B17* gene was recently described that was associated with a reduced rate of glucuronidation *in vivo*. The purpose of this study was to determine if the deletion polymorphism is associated with susceptibility to prostate cancer.**

Materials and Methods: **UGT2B17 expression was determined by reverse transcription-PCR of pathologically normal prostate tissues (n = 5). In a case-control study with 420 patients with incident primary prostate cancer (127 African Americans and 293 Caucasians) and 487 controls (120 African Americans and 367 Caucasians), the frequency of *UGT2B17* deletion polymorphism in genomic DNA was compared between cases and controls with PCR analysis.**

Results: ***UGT2B17* mRNA was detected only in individuals with at least one *UGT2B17* allele. The frequency of the null genotype was present in 0.11 and 0.12 of Caucasian and African American controls, respectively. When all subjects were considered, a significant association was found between the *UGT2B17* deletion polymorphism and prostate cancer risk [odds ratio (OR), 1.7; 95% confidence interval (95% CI), 1.2-2.6]. There was an increase in prostate cancer risk among individuals with *UGT2B17* deletion polymorphism in Caucasians (OR, 1.9; 95% CI, 1.2-3.0) but not in African Americans (OR, 1.3; 95% CI, 0.6-2.7).**

Conclusions: **These results suggest that the UGT2B17 enzyme may play a role in the metabolism of androgens in prostate tissue and that the *UGT2B17* deletion polymorphism is associated with prostate cancer risk.** (Cancer Epidemiol Biomarkers Prev 2006;15(8):1473–8)

Introduction

Prostate cancer is the fourth most common cancer in men, comprising approximately one eighth of all male-specific cancers in the world (1). Prostate cancer is much more common in developed that in developing countries. Developed countries account for nearly 75% of all new prostate cancer cases. The incidence and mortality rates of prostate cancer among African American men are significantly higher than that of any other ethnic group in the United States and the world (1-3). The etiology of prostate cancer remains unknown and few risk factors for prostate cancer, such as race, age, family history, and steroid hormone levels, have been suggested (4). Variations in androgen levels have been suggested as a risk factor for prostate cancer but results are not consistent (5-9). The differences in androgen levels may be due to variation in activity or expression of the enzymes responsible for metabolism of androgens. Most epidemiologic studies have focused on enzymes involved in production of androgens, such as CYP3A4 (10-13), CYP17 (14-22), CYP3A43 (23), and SRD5A2 (22, 24). Although differences in androgen levels may reflect variation in catabolism, fewer studies (25-28) have evaluated this hypothesis.

UDP-glucuronosyltransferases (UGT) are a family of enzymes that glucuronidate many endogenous chemicals (e.g., bilirubin and steroid hormones) and xenobiotics (29, 30). Two groups of UGTs (UGT1 and UGT2) have been identified based on sequence homology and substrate specificity (31). Generally, UGT2B enzymes metabolize steroid hormones and xenobiotics (32, 33). Both UGT2B15 and UGT2B17 have been implicated in the metabolism of androgens, including androsterone, testosterone, androstane-3α,17β-diol, and dihydrotestosterone, which have been postulated to modify risk of prostate cancer (refs. 34-37; Fig. 1). *UGT2B17* shares 95% and 94% identity with *UGT2B15* cDNA and its amino acid sequence (36). The high level of cDNA and amino acid sequence homology between *UGT2B15* and *UGT2B17* suggests that these genes may have arisen from a gene duplication event (38).

We and others previously reported that *UGT2B15* Asp85Tyr polymorphism is significantly associated with prostate cancer risk (25, 27, 28). In addition, it has been suggested that a deletion polymorphism of *UGT2B17* is a risk factor for high transplant-related mortality because the UGT2B17 enzyme can be immunogenic in the recipient with homozygous *UGT2B17* deletion (39, 40). A recent study suggested that *UGT2B17* deletion polymorphism is significantly associated with reduced glucuronidation activities in human liver microsomes (41) and the bimodal distribution of the testosterone excretion (42).

Received 2/23/06; revised 4/13/06; accepted 5/24/06.

Grant support: H. Lee Moffitt Cancer Center Faculty Support Fund and NIH/National Institute on Aging grant 1R01AG15722 (N. Lang).

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doi:10.1158/1055-9965.EPI-06-0141

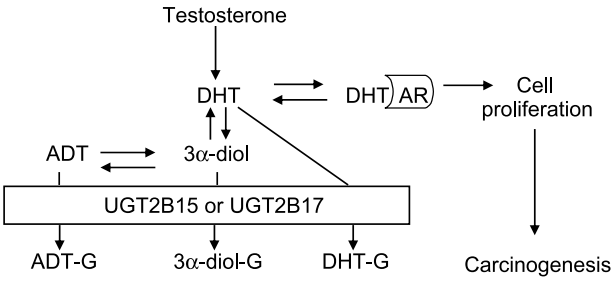

Figure 1. Elimination of dihydrotestosterone in prostate cells (modified from ref. 67). Dihydrotestosterone is conjugated and inactivated either by the UGT2B15 or UGT2B17 enzyme and then excreted in urine. DHT, dihydrotestosterone; ADT, androsterone; 3α-diol, androstane-3α,17β-diol; AR, androgen receptor.

Smoking is suggested as a contributing factor in prostate cancer mortality (43) and a putative risk factor for prostate cancer (44, 45). However, this association has been weak and inconsistent (46). One of many hypothesized biological mechanisms between smoking and prostate cancer is that smoking may be associated with higher levels of serum testosterone, which acts as a promoter of prostate cancer in men (47). Therefore, deletion of the UGT2B17 enzyme activity may be associated with prostate cancer risk because individuals with defective UGT2B17 activity may have a higher serum androgen level than subjects with normal activity. In the present study, we examined the potential association of the *UGT2B17* deletion polymorphism with prostate cancer risk by assessing the expression pattern of the UGT2B17 enzyme in prostate tissues and by comparing *UGT2B17* genotypes in prostate cancer patients and noncancer controls in African Americans and Caucasians.

Materials and Methods

Tissue Samples and *UGT2B17* Expression Analysis. Normal human prostate tissues were obtained from the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center and Research Institute (Tampa, FL). All specimens came from subjects undergoing cancer surgery and were quick-frozen at −70°C within 30 minutes of surgery. Total RNA was isolated from 0.5g samples of normal prostate tissues by the guanidinium isothiocyanate/cesium chloride method (48), followed by treatment with DNase I (Invitrogen, Carlsbad, CA).

Equimolar amounts of total RNA was reverse transcribed with the SuperScript reverse transcriptase amplification kit (Invitrogen) and 0.5 mg of oligo(dT)$_{12\text{-}18}$ primer as outlined in the protocol of the manufacturer. Sense (5′-gtgttgggaatattctgcactataatata-3′) and antisense (5′-caggtacataggaaggagggaa-3′) primers homologous to *UGT2B17* sequences were used in reverse transcription-PCR (RT-PCR; expected size, 242 bp). β-Actin sense (5′-tgatggtgggcatgggtcag-3′) and antisense (5′-gtgttggcgtacaggtcttt-3′) primers were added as an internal positive control in each RT-PCR assay (expected size, 756 bp). The primers were designed to amplify mRNA only from proposed genes by selecting primers that cross exon boundaries. Reactions without RNA were included as negative controls in all RT-PCR experiments. Ten-μl aliquots were used from each PCR and resolved by electrophoresis in 8% polyacrylamide gels. PCR products were detected after staining the gels with 1 μg/mL ethidium bromide and photography over UV light. The sequences of PCR products were confirmed by dideoxy sequencing (49) done at the Molecular Biology Core Facility.

Study Populations and Sample Processing. All cancer cases were histologically confirmed by the department of pathology in each institute. A total of 307 incident cases (293 Caucasian and 14 African American) with primary adenocarcinoma of the prostate were recruited between 2002 and 2004 at the H. Lee Moffitt Cancer Center. Ninety-five percent of case subjects who were asked to participate in the study consented. Controls consisted of 377 subjects (367 Caucasians and 10 African Americans) who were visiting the Lifetime Cancer Screening Center, which is affiliated with the H. Lee Moffitt Cancer Center. At this center, routine screenings are offered to men for cancers of the prostate, colorectum, and skin. All control subjects were male and had no previous diagnosis of cancer. The control subjects were matched to cases on age at diagnosis (±5 years) and race. Eighty-three percent of the control subjects who were asked to participate in the study consented.

African Americans (n = 113) with prostate cancer were recruited within 6 months of diagnosis between 1998 and 2003 at the University of Arkansas for Medical Sciences, University Hospital, the Central Arkansas Veterans Healthcare System (Little Rock, AR) and the Jefferson Regional Medical Center (Pine Bluff, AR). Controls (n = 110), frequency matched to cases on age at diagnosis (±5 years) and race, were identified from Arkansas State Driver's License records, Centers for Medicare and Medicaid Services records, and a mass-mailing database that covers ~80% of Arkansas residents. Exclusion criteria for the case-control study included a history of cancer. The recruitment rate for both cases and controls among African Americans was 55%. The appropriate Institutional Review Board approvals were obtained for the study protocol at each institute. Signed informed consent was obtained from all study subjects.

Nongenetic risk factor data for the current study were obtained through in-person interviews with cases and controls at the time of enrollment. The questionnaire covered (*i*) demographic information; (*ii*) family history of cancer; (*iii*) medical history; and (*iv*) detailed tobacco consumption. Smoking intensity (pack-years) was defined as the number of packs of cigarettes smoked per day multiplied by the number of years smoked. Study subjects who smoked 100 or fewer cigarettes in their lifetime were categorized as never smokers. Racial information was based on self-report and was broadly defined as Caucasian, African American, or other. Due to low numbers, individuals who reported other races were excluded (n = 8). For cases, data on cancer stage, Gleason score, and prostate-specific antigen were abstracted from medical records.

Subjects were asked to provide a blood or buccal sample after the interview as a source of genomic DNA. DNA was extracted according to standardized protocols (50).

Genotyping Assays. The presence of the *UGT2B17* deletion polymorphism was screened in all subjects using a multiplex-PCR assay. Because *β-actin* should be present in all cells, the amplification of this gene provides a positive control for each reaction. Therefore, samples in which only the *β-actin* gene is amplified were considered a deletion for *UGT2B17*. The multiplex PCR was done to amplify the part of *UGT2B17* gene using *UGT2B17*-specific primers (sense, ggagttgtggaaaggtgctg; antisense, cacagagctttatattatagtcag) with human *β-actin* primers (sense, ggcggcaccaccatgtaccct; antisense, aggggccggactcgtcatact) as an internal positive control with 60°C annealing temperature. Negative controls were included in each PCR experiment. Products of the PCR reaction (expected size: *UGT2B17*, 350 bp; *β-actin*, 207 bp) were detected by electrophoresis on 8% PAGE gels and visualized using ethidium bromide stain and UV transillumination.

To ensure quality control of genotyping results, randomly selected PCR-amplified DNA samples (n = 3) from subjects possessing *UGT2B17* [+] genotype were examined by dideoxy DNA sequencing (49).

Statistical Analysis. Number (%) of subjects and mean values (SDs) were generated for all variables by case-control status. χ^2 test for categorical variables and two sample *t* test (or Wilcoxon rank-sum test) for continuous variables were used to test the statistical significance of any observed differences in demographic and clinical characteristics between cases and controls.

The risk for prostate cancer in relation to *UGT2B17* genotypes (positive versus null) was estimated with logistic regression modeling approaches. Potential confounding and gene-environment interaction effects were tested in the logistic regression model. Logistic regression model was also fitted separately among light smokers (≤26.5 pack-years) and heavy smokers (>26.5 pack-years) for each race, with the cut point being the median among smoking controls. Final multivariable models for the *UGT2B17* were constructed using an empirical stepwise approach and they included the covariates of age (for both races). Odds ratios (OR) and their 95% confidence intervals (95% CI) were reported.

$P \leq 0.05$ (two sided) was considered to be statistically significant. The SAS software (v. 9.2, SAS, Cary, NC) was used for all analyses.

Results

Table 1 provides descriptive characteristics of the cases and controls. Despite the frequency matching on age, cases tended to be older than controls ($P < 0.0001$). Cases tended to smoke less than controls, but no differences were apparent among the African Americans. More men with prostate cancer (22%) than without (9%) reported having a first-degree family member with prostate cancer ($P < 0.0001$). As expected, prostate-specific antigen levels of most patients are >4 ng/ml and Gleason scores range between 5 and 9.

To evaluate the potential physiologic importance of UGT2B17 in prostate tissue and the association between expression and genotype, we did RT-PCR and genotyping analysis to determine whether the UGT2B17 enzyme is expressed in normal human prostate tissues from individuals with different *UGT2B17* genotypes. As shown in Fig. 2A, RT-PCR amplification of a fragment of the expected size (242 bp) corresponding to that expected for *UGT2B17* mRNA was detected only in individuals with *UGT2B17* [+] genotype (Fig. 2B). These PCR products were confirmed by direct sequencing analysis (results not shown).

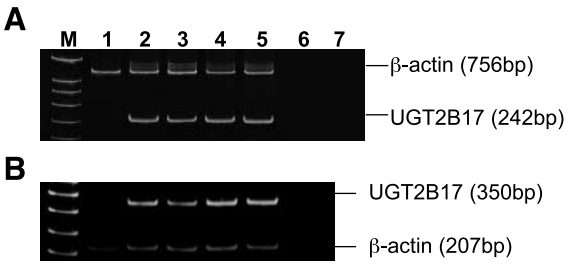

Figure 2. Expression and genotyping analysis of *UGT2B17* in normal prostate tissues. To investigate an association between *UGT2B17* genotypes and their expression, RNA and DNA samples extracted from same five individuals were used. **A.** *UGT2B17* mRNA was detected by RT-PCR of individual RNA samples. *M,* DNA marker; *lanes 1* to *5,* total prostate RNA from five different individuals. Only individuals with *UGT2B17* [+] genotype (subjects 2, 3, 4, and 5) show expression of *UGT2B17* mRNA. Upper bands indicate products from *β-actin* (756 bp) and lower bands are PCR products (242 bp) from *UGT2B17* mRNA. *Lanes 6* and *7,* negative controls for RT reaction and PCR, respectively. The primers were designed to amplify mRNA only from proposed genes by selecting primers that cross exon boundaries. **B.** The amplification of *β-actin* provides a positive internal control (expected size, 207 bp) for each PCR. A PCR amplification positive for *β-actin* but negative for *UGT2B17* (expected size: 350 bp) would indicate a null *UGT2B17* genotype, as shown in lane 1 (subject 1).

We obtained informative genotyping results of the *UGT2B17* null polymorphism from 97.9% of study population. Before beginning the analysis of the association of the polymorphisms with risk of prostate cancer, we first examined whether there were racial differences in genotype frequency. Racial differences in genotype frequency were not observed among the control groups ($P = 0.46$). The frequencies of the *UGT2B17* deletion genotype among the control subjects were 11% and 12% in Caucasians and African Americans, respectively. The frequency of this polymorphism among African Americans in this study is significantly different from the one reported in a previous study (51).

To determine whether this genetic variant was associated with increased risk for prostate cancer, we compared genotypes in prostate cancer cases and controls (Table 2). When all subjects were considered, a significantly increased risk for

Table 1. Selected characteristics of study subjects and comparison between cases and controls

Variables	Cases (n = 420)	Controls (n = 487)	P
Mean age (range), y	64 (42-85)	60 (37-82)	<0.0001
Ethnicity (%)			
Caucasians	293 (70)	367 (75)	>0.05
African Americans	127 (30)	120 (25)	
Smoking			
Mean pack-years (SD) in Caucasians	20.0 (24.1)	29.2 (20.1)	<0.0001
Mean pack-years (SD) in African Americans	18.1 (25.9)	16.2 (29.0)	0.58
Family history of prostate cancer (%)*	51 of 233 (22)	33 of 353 (9)	<0.0001
Prostate-specific antigen (%)			
<4 ng/ml	45 (16)		
4-10 ng/ml	199 (69)		
>10 ng/ml	43 (15)		
Gleason score (%)			
5-6	165 (71)		
7-9	69 (29)		
Stage at diagnosis (%)			
I	171 (70)		
II	64 (26)		
III	8 (3)		
IV	1 (0.4)		

*Men having a first-degree family member with prostate cancer.

Table 2. Prostate cancer risk for the *UGT2B17* genotypes alone and by race and smoking level

UGT2B17 genotypes	Cases (%)	Controls (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	*P*
All					
UGT2B17 [+]*	331 (82)	426 (88)	1.0 (reference)	1.0 (reference)	
UGT2B17 null	75 (18)	56 (12)	1.7 (1.2-2.5)	1.7 (1.2-2.6)†	0.004
Caucasians					
UGT2B17 [+]	237 (81)	325 (89)	1.0 (reference)	1.0 (reference)	
UGT2B17 null	56 (19)	42 (11)	1.8 (1.2-2.8)	1.9 (1.2-3.0)‡	0.006
African Americans					
UGT2B17 [+]	94 (83)	101 (88)	1.0 (reference)	1.0 (reference)	
UGT2B17 null	19 (17)	14 (12)	1.5 (0.7-3.1)	1.3 (0.6-2.7)‡	0.32
Light smokers (≤26.5 pack-years)					
UGT2B17 [+]	99 (78)	171 (89)	1.0 (reference)	1.0 (reference)	
UGT2B17 null	28 (22)	22 (11)	2.2 (1.2-4.0)	2.4 (1.2-4.8)†	0.011
Heavy smokers (>26.5 pack-years)					
UGT2B17 [+]	99 (82)	168 (86)	1.0 (reference)	1.0 (reference)	
UGT2B17 null	21 (18)	27 (14)	1.3 (0.7-2.5)	1.2 (0.6-2.3)†	0.382

**UGT2B17* [+] = *UGT2B17* (+/+) homozygous wild and (+/0) heterozygous genotypes.
†Adjusted for race, age, and pack-years.
‡Adjusted for age and pack-years.

prostate cancer was observed for subjects with at least one *UGT2B17* allele. The crude OR was 1.7 (95% CI, 1.2-2.5). The association remained (OR, 1.7; 95% CI, 1.2-2.6) after additional adjustment for age, race, and pack-years of smoking. Stratified analysis by race revealed an elevated prostate cancer risk among Caucasian individuals with *UGT2B17* deletion polymorphism (OR, 1.9; 95% CI, 1.2-3.0). However, the main effect of the *UGT2B17* deletion polymorphism on prostate cancer risk among African Americans was not statistically significant (OR, 1.3; 95% CI, 0.6-2.7). When tests for interaction were done, interaction between *UGT2B17* polymorphism and race was not significant ($P = 0.61$).

To examine whether the association between genotypes and prostate cancer risk varied by smoking history, we did a stratified analysis on genotype and smoking level (pack-years). There was a statistically significant association between *UGT2B17* null genotype and prostate cancer risk among light smokers (≤26.5 pack-years; OR, 2.4; 95% CI, 1.2-4.8) but not among heavy smokers (>26.5 pack-years; OR, 1.2; 95% CI, 0.6-2.3). Interaction between *UGT2B17* polymorphism and smoking was not significant ($P = 0.53$).

Discussion

In the present study, we showed that the UGT2B17 enzyme is expressed in normal prostate tissues of individuals with *UGT2B17* [+] genotype, consistent with a previous study (34). These data are consistent with the hypothesis that the UGT2B17 enzyme may play a role in degradation of dihydrotestosterone (52) and that an excessive amount of dihydrotestosterone may be associated with carcinogenesis in the prostate tissue (53). A significant association was found between the *UGT2B17* deletion polymorphism and prostate cancer risk in Caucasians. However, we did not observe a similar association in African Americans. Potential explanations can be either biological differences between races in the androgen metabolism pathway or a small sample size. One hypothesis to explain the variation in prostate cancer incidence between racial groups is reference range differences among men of different races in androgens (54). For example, adult African American men have higher mean circulating concentrations of testosterone or other androgens than similarly aged white men (6). Therefore, against this background of high androgen levels in African Americans, the *UGT2B17* deletion polymorphism, which is responsible for degradation of androgens, may have a diminished effect.

We previously reported a significant association between *UGT2B15* polymorphism and prostate cancer risk (28). To the best of our knowledge, no previous epidemiologic studies for *UGT2B17* deletion polymorphism have examined an association with human cancer.

The clinical syndromes resulting from mutations or deletion of UGT2B members have not been described, although mutations of UGT1A family members have been well investigated and are responsible for the Crigler-Najjar and Gilbert syndromes (55-57). The deletion of *UGT2B17* may be not clinically significant because results from various tests, such as normal physical exam, complete blood count, and serum chemistry, are normal. Notably, a higher prevalence (67-85%) of *UGT2B17* deletion polymorphism has been reported in Asian individuals (40, 42) but the incidence rate of prostate cancer among Asian men is much lower than rates among Caucasian and African American men (58). These data suggest that other steroid-metabolizing enzymes may also have a role in prostate cancer risk.

The other UGT2Bs, especially UGT2B15, can glucuronidate similar androgens and are expressed in the prostate gland (34-36). However, *in vitro* studies suggest that UGT2B17 is more labile than UGT2B15, suggesting that regulation of UGT2B17 expression would lead to a more rapid change in the level of glucuronidated androgens (59). Furthermore, a recent study reported a different expression pattern between UGT2B17 and UGT2B15 in prostate tissues. UGT2B17 is expressed in basal cells where dehydroepiandrosterone is converted into androstane-3α,17β-diol and androsterone. However, expression of UGT2B15 was observed only in luminal cells, where dihydrotestosterone is formed from testosterone. These results suggest that UGT2B17 and UGT2B15 may have complementary roles and are expressed in cells where their specific substrates are synthesized (60). Therefore, comprehensive analyses of the UGT2B family and risk for prostate cancer are warranted. The proposed mechanism of carcinogenesis in prostate tissue involves the androgen receptor in the presence of dihydrotestosterone interacting with DNA in the proliferating prostate epithelial cells to produce permanent genomic mutations (Fig. 1).

Smoking has a positive correlation with level of serum testosterone, which acts as a promoter of prostate cancer and is degraded by the UGT2B17 enzyme (47). Dai et al. (61) found significant correlations between smoking level and serum androstenedione as well as testosterone in men. Therefore, excessive amount of dihydrotestosterone due to smoking and/or defective catabolism of androgens may increase prostate cancer risk. Another potential biological mechanism between smoking and prostate cancer is that *UGT2B17* deletion polymorphism may enhance the effect of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol exposure from smoking

on the risk of prostate cancer because the UGT2B17 enzyme not only metabolizes androgens but also detoxifies 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, which is a lung carcinogen in rodents (41, 62, 63).

We observed a stronger association between *UGT2B17* null polymorphism and prostate cancer among light smokers. This pattern is not unusual in previous studies examining polymorphism genotypes and cancer risk (64). It has been suggested that genetic variations in the ability to respond to tobacco carcinogens are most important in determining cancer risk at low levels of exposure and may be less relevant at higher smoking doses where high levels of carcinogen exposure overwhelm polymorphism-induced differences in enzyme activity and/or expression (65).

The prevalence of the *UGT2B17* null polymorphism in Caucasians was similar to those reported from previous studies (39, 51) but significantly lower than that among Asian men (40). We also observed a higher prevalence in African Americans than previously reported (51). Although the reason for the different prevalence was not clear, these data suggest that variability in *UGT2B17* genotype prevalence exists in African Americans from different regions of the United States, as we previously observed in *GSTM1* null polymorphism (66).

Results from this study must be interpreted in light of limitations. We are aware of potential limitations of this study. Due to the nature of the genotyping analysis, we cannot distinguish between heterozygous (0/+) and homozygous (+/+) *UGT2B17* genotypes. However, a recent study reported that a significant difference was observed when phenotypes from subjects who have at least one *UGT2B17* allele were compared with those from *UGT2B17* homozygous deletion subjects (41, 42). Second, although we had adequate power to detect main effects, the sample size is not large enough to adequately detect small interactions, especially among African Americans. Third, we observed significant age differences between cases and controls despite matching on age (±5 years). To address this, we adjusted for age when ORs were estimated. Finally, our control groups were not screened for prostate cancer by either prostate-specific antigen levels or digital rectal examination. Therefore, because of their ages, we estimate that some of them may have benign prostatic hyperplasia or even early stage of prostate cancer. This would result in misclassification and would bias the ORs to the null.

In conclusion, this study suggests that the *UGT2B17* null polymorphism may play a role in prostate cancer risk. It will be important to replicate these observations in additional studies involving larger numbers of subjects.

Acknowledgments

We thank the Molecular Biology Core facility and Tissue Procurement facilities at H. Lee Moffitt Cancer Center for excellent technical assistance.

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Cancer Epidemiol Biomarkers Prev 2006;15:1473-1478.