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***SRD5A2* and *HSD3B2* Polymorphisms are Associated With Prostate Cancer Risk and Aggressiveness**

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Abstract

BACKGROUND—Dihydrotestosterone (DHT) is believed to play an important role in prostate carcinogenesis. Five alpha reductase type II (*SRD5A2*) and 3 beta-hydroxysteroid dehydrogenase type II (*HSD3B2*) are responsible for the biosynthesis and degradation of DHT in the prostate. Two polymorphisms, a valine (V) for leucine (L) substitution at the 89 codon of the *SRD5A2* gene and a (TG)_n, (TA)_n, (CA)_n repeat polymorphism within the third intron of the *HSD3B2* gene were evaluated with regard to prostate cancer risk.

METHODS—Blood samples were collected for 637 prostate cancer cases and 244 age and race frequency matched controls. In analysis, the *SRD5A2* VL and LL genotypes were combined into one group and the *HSD3B2* repeat polymorphism was dichotomized into short (<283) and long (≥283) alleles.

RESULTS—The *SRD5A2* V89L polymorphism was not independently associated with prostate cancer risk. Carriage of at least one *HSD3B2* intron 3 short allele was associated with a significant increased risk for prostate cancer among all subjects (OR = 2.07, 95% CI = 1.08–3.95, *P* = 0.03) and Caucasians (OR = 2.80, CI = 2.80–7.43, *P* = 0.04), but not in African Americans (OR = 1.50, CI = 0.62–3.60, *P* = 0.37). Stratified analyses revealed that most of the prostate cancer risk associated with the intron 3 *HSD3B2* short allele was confined to the *SRD5A2* 89L variant subgroup and indicated that in combination these polymorphisms may be associated with increased risk of aggressive (Gleason >7) disease (Gleason >7).

CONCLUSIONS—In Caucasians, the *HSD3B2* (TG)_n, (TA)_n, (CA)_n intron 3 length polymorphism is associated with both prostate cancer risk and aggressiveness and the *SRD5A2* V89L polymorphism may modify the risk conferred by this polymorphism.

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Keywords

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INTRODUCTION

Prostate cancer incidence in African-American men is on average 60% higher than the incidence observed in Caucasian men and mortality from the disease is approximately 2.4 times higher in African-American men [1]. The androgen biosynthesis pathway has been implicated in prostate carcinogenesis and frequency differences in gene polymorphisms within this pathway may explain observed race disparities in prostate cancer [2,3]. Androgens are primarily produced in the testes and adrenal glands, but are also synthesized in the prostate and skin. Within the prostate, dihydrotestosterone (DHT) is the primary and most potent nuclear androgen. DHT promotes DNA synthesis and cell replication by binding to the intracellular androgen receptor and forming a complex which activates gene transcription and cell proliferation. Increased cell division is presumed to heighten the potential for somatic mutations, leading to a higher likelihood of carcinogenesis [4]. Two enzymes involved in the regulation of DHT are 5- α reductase type II (*SRD5A2*) and 3 β -hydroxysteroid dehydrogenase type II (*HSD3B2*). These enzymes are responsible for the biosynthesis and degradation of DHT.

The 5 α -reductase gene (*SRD5A2*), located on chromosome 2p23 [5], is involved in the conversion of testosterone to DHT in the prostate. *SRD5A2* is perhaps best known as the target for finasteride, a drug used to treat benign prostatic hypertrophy and which has shown potential for prostate cancer prevention [6,7]. Several polymorphisms within *SRD5A2* have been identified, including a leucine for valine substitution at codon 89 (*V89L*) [7]. Findings overall have been equivocal in regard to this polymorphism in terms of prostate cancer risk. A meta-analysis of the *SRD5A2* *V89L* polymorphism concluded that there was no increased risk associated with prostate cancer (Odds ratio (OR) for L vs. V: 1.03, 95% CI: 0.84–1.26) [8]. However, in vivo and in vitro studies have consistently shown reduced 5 α -reductase activity with the substitution of leucine [9–11] and studies published since the meta-analysis in 2003 have found that the *SRD5A2* *V89L* is associated with prostate volume [12] and prostate cancer risk and aggressiveness [13]. Race/ethnicity differences in the frequency of the L variant have also been reported [14].

HSD3B2 functions upstream as well as downstream of *SRD5A2* in the androgen pathway and the coding region for the *HSD3B2* gene is located on chromosome 1p13 [15], a region that has shown evidence for linkage to prostate cancer [16–19]. *HSD3B2* is one of two enzymes responsible for the degradation of DHT to 3 β -androstenediol [20]. In addition, *HSD3B2* is involved in the production of testosterone (T) via its role in converting DHEA to androstenedione, a precursor to testosterone. A complex (TG)_n(TA)_n(CA)_n repeat polymorphism within the third intron of the *HSD3B2* gene has been identified [21], and allelic frequency differences have been observed between African Americans and Caucasians [22]. It has been suggested that the repeat complex may influence the formation of hairpin-like structures that could modify the rate of *HSD3B2* transcription [23] or may promote alternative spliced forms of mRNA resulting in truncated or unstable proteins [24]. Altering intracellular levels of *HSD3B2* enzyme could potentially change the rate at which testosterone is produced and/or DHT is degraded. Higher ratios of serum testosterone to DHT have been associated more consistently with prostate cancer risk than either steroid alone [25–27]. To the best of our knowledge no previous reports have assessed the intron 3 repeat *HSD3B2* polymorphism in regard to prostate cancer risk or aggressiveness.

Therefore, in this study, we evaluated the potential associations between the *SRD5A2 V89L* and *HSD3B2* (TG)n(TA)n(CA)n repeat polymorphisms on prostate cancer risk and aggressiveness by race/ethnicity. We also evaluated these polymorphisms in combination since altered activity or expression of both may ultimately have a stronger impact on the availability of DHT and/or testosterone in the prostate potentially leading to increased cell division and prostate carcinogenesis.

METHODS

Study Population and Data Collection

The study population included men that received their primary health care at the Henry Ford Health System (HFHS), a large, vertically integrated, health system. HFHS provides care for an ethnically diverse population that is representative of the geographic region it serves. All study procedures and processes were approved by the HFHS institutional review board. Cases were identified through the centralized Department of Pathology and had histological confirmation of adenocarcinoma of the prostate between January 1999 and December 2004 with no prior history of prostate cancer. The primary objective of the study was to evaluate gene-environment interactions using a case-only analytic approach [28], therefore, the study sampling frame was focused on cases, but controls were also sampled in a stratified random manner from the health system's electronic data stores based on 5-year age group and race, such that the final enrolled sample would include approximately 3 cases per 1 control. Cases and controls were also required to be 75 years of age or younger at diagnosis/enrolment and have at least one primary care contact in the preceding 5 years. The younger age criterion was used in this gene-environment study to enrich the potential genetic contribution to disease among our subjects.

A study introduction letter was sent to all potential subjects and trained study interviewers made initial contact with patients via telephone. Between July 2001 and December 2004, 77% of potential cases and 68% of potential controls enrolled in the study (total N = 881) and provided a blood sample for DNA analysis. Blood samples for controls were also used for prostate specific antigen testing (PSA) at the time of enrolment. Family history of prostate cancer was assessed as part of the patient interview and was considered positive if either a brother or father had been diagnosed with prostate cancer. Cases' cancer stage and Gleason grade were abstracted from the medical record and were verified using the institutions certified tumor registry.

Genotyping

Genotyping of the *SRD5A2 V89L* polymorphism was performed using the Invader assay (biplex format). Each plate contained the following controls: valine/valine homozygous, valine/leucine heterozygous, leucine/leucine homozygous and a no target blank. All components of the assay were provided by Third Wave Technologies, Inc. Ten microliters of genomic DNA samples were aliquoted into individual wells of a 96-well microtitre plate and denatured at 95°C for 5 min. Ten microliters of a reaction mix containing the appropriate probes/Invader oligo/MgCl₂ mix were added, and reactions were overlaid with 20 µl of mineral oil. Each 20 µl reaction contained 40 ng of Cleavase enzyme, 3.5% PEG 8000, 2% glycerol, 0.06% NP40, 0.06% Tween-20, 12 µg/ml BSA, 0.25 µmol/L each of F (FAM) dye and R (Redmond Red) dye FRET cassettes, 8 mmol/L MgCl₂, 0.5 µmol/L of each allele-specific probe, and 0.05 µmol/L Invader oligo. The sequences of the oligos and probes are available upon request. The plates were incubated at 63°C for 4 hr in a PE 9700 thermal cycler. Fluorescence was measured using a CytoFluor 4000 fluorescence plate reader (Applied Biosystems, Foster City, CA). The settings used were: 485/20 nm excitation/bandwidth and 530/25 nm emission/bandwidth for F dye detection, and 560/20 nm excitation/bandwidth and 620/40 nm emission/bandwidth for

R dye detection. Any sample that yielded inconclusive results using the Invader assay was re-quantified and repeated. If the second attempt did not yield acceptable results, the sample was genotyped using a PCR-RFLP assay previously described [29].

The *HSD3B2* intron 3 dinucleotide repeat was genotyped by first amplifying genomic DNA using a primer pair previously described [30]. The forward primer was fluorescently tagged with 6-carboxyfluorescein (FAM). Amplification was performed in a 20 µl final volume containing 200 µM of dCTP, dATP, dGTP, and dTTP, 2 mM MgCl₂, 20 pmol each primer, 2.5 U Taq Gold DNA polymerase (Perkin-Elmer), and 1 × Taq Gold buffer. After an initial denaturation step at 95°C for 10 min, 30 cycles of 92°C 2 min, 65°C 1 min and 72°C 2 min were followed by a 45 min final extension at 72°C.

The final products were analyzed by electrophoresis on a 3100 Genetic Analyzer (Applied Biosystems) using GeneScan and Genotyper software. The size of the PCR products for each specimen was determined by the size of the predominant PCR product(s) according to peak area, in relation to the GeneScan-500 ROX size standard (Applied Biosystems). GeneScan results for a sample of homozygous subjects were checked against the actual size of the PCR product as determined through sequence analysis and were determined to be similar.

Statistical Analyses

Hardy-Weinberg equilibrium of genotypic frequencies for both polymorphisms was verified in controls using chi-squared tests. For the multi-allele *HSD3B2* polymorphism, an empirical *P* value was estimated using a simplified Monte Carlo significance test [31]. Case-control differences in categorical variables were assessed using chi-squared tests as well. *SRD5A2* 89 valine/leucine (VL) and leucine/leucine (LL) genotypes were combined for stratified analyses due to low frequencies of the LL genotype. Polynomial regression was used to test the linear association of the *HSD3B2* (TG)_n(TA)_n(CA)_n allele lengths with prostate cancer risk. Family history and benign prostatic hyperplasia (BPH) were coded as present or absent and were included in multivariable regression models as potential confounders because of previous reports indicating potential associations with the *SRD5A2* V89L polymorphism [12, 13,32,33]. Body mass index (continuous) and smoking status (ever/never) were also included in models as potential confounders since each has been shown to influence circulating androgen levels [34]. Age (continuous) was also included in each model and race was adjusted for in analyses of all subjects combined. Aggressive prostate cancer was defined as having Gleason grade of 7–10. Statistical analysis was performed using SPSS (version 11.5). Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between genotypes and prostate cancer risk and aggressiveness, respectively. An alpha level of *P* < 0.05 was considered significant.

RESULTS

Demographics and Clinical Characterization

A total of 637 cases and 244 controls met all criteria and were enrolled in the study (Table I). By design, cases and controls did not differ significantly by race or age. African-American (AA) men represented 43.2% of all participants and were slightly younger than Caucasian (W) subjects (61.8 vs. 62.6 years, *P* = 0.06). Family history of prostate cancer (21.0% vs. 13.1%, *P* = 0.01) and BPH (32.3% vs. 19.7%, *P* < 0.0001) were more prevalent in cases than in controls but did not differ by race (family history: AA 19.4% vs. W 18.6%, *P* = 0.76; BPH: AA 26.8% vs. W 30.6%, *P* = 0.34). Among cases, 55% had a Gleason grade of seven or greater and one in five cases undergoing definitive surgical treatment had a pathological tumor stage of T3 or higher. Gleason grade (AA 57.1% vs. W 53.5%, *P* = 0.37) and pathological tumor stage (AA

18.1% vs. W 20.1%, $P = 0.61$) were not significantly different between African-American and Caucasian cases.

SRD5A2.V 89L Polymorphism

Among all study subjects, the *SRD5A2* VV genotype was most common (48.4%), followed by the VL (42.2%), and LL (9.4%) genotypes. The overall difference in the frequency of the L allele by race approached significance (AA 48.3% vs. W 54.3%, $P = 0.08$) and African-American men were significantly less likely to carry the LL genotype as compared to Caucasian men (6.8% vs. 11.5%, $P = 0.02$). Among controls, the *SRD5A2* V89L polymorphism was in Hardy–Weinberg equilibrium. Overall and by race, as Table II indicates, there were no statistically important differences in risk of prostate cancer between cases and controls for the *SRD5A2* V89L genotype alone.

HSD3B2 Length Polymorphism

A total of 48 *HSD3B2* alleles ranging in length from 213 to 375 were genotyped in our study subjects. Genotype frequencies in controls were in Hardy–Weinberg equilibrium. The 283 bp length allele accounted for 35.3% of all alleles typed. Alleles 286 bp (20.8%) and 333 bp (16.0%) were the next most common alleles with all other alleles occurring at frequencies of less than 10%. Stratification by race showed that in African American and Caucasian controls, the most common alleles occurred at very different frequencies (*HSD3B2* 283 bp: African American 12.4% vs. Caucasian 52.6%; *HSD3B2* 286 bp: African American 36.7% vs. Caucasian 9.6%, *HSD3B2* 333 bp: 11.0% vs. Caucasian 19.9%; Fig. 1). Polynomial regression in African Americans and Caucasians as well as in combined analyses indicated that the relationship between this polymorphism and outcome was not linear. A sensitivity analysis at various binary cut points showed that dichotomizing *HSD3B2* allele lengths into long and short risk alleles at the 283 bp cut point resulted in the formation of risk groups that best differentiated cases from controls. Therefore, we chose to simplify the analysis of this polymorphism by subdividing alleles into two groups of “short” (<283 bp) and “long” (≥283 bp) alleles.

Race-stratified and overall results for the *HSD3B2* polymorphism are shown in Table II. Using the long/long genotype as the referent, the genotypes including short alleles (long/short and short/short) were associated with an elevated risk for prostate cancer in Caucasians (OR 2.80, CI 1.05–7.43, $P = 0.04$) and among all subjects (OR 2.07, CI 1.08–3.95, $P = 0.03$). Risk was also elevated but not significant in African Americans carrying at least one *HSD3B2* (TG)n, (TA)n, (CA)n short allele (OR 1.50, CI .62–3.6, $P = 0.37$).

SRD5A2 and HSD3B2: Prostate Cancer Risk and Aggressiveness

Table III shows the risk of prostate cancer associated with the *HSD3B2* polymorphism after stratification by *SRD5A2* V89L status. Elevated risk for prostate cancer associated with the long/short or short/short *HSD3B2* genotype were observed for both *SRD5A2* genotypic strata, but within the stratum defined by individuals with either the *SRD5A2* VL or LL genotypes prostate cancer risk associated with the long/short or short/short *HSD3B2* genotype in Caucasians was elevated (Table II). Risk estimates for Caucasians associated with the *HSD3B2* polymorphism were greater than African Americans even after adjusting for age, family history of prostate cancer, BPH, BMI, and smoking.

We also dichotomized cases based on aggressive disease (Gleason sum ≥7) to assess whether clinical heterogeneity among cases revealed any additional genetic risk (Table III). ORs were significantly increased for aggressive disease within the subset of Caucasians with the *SRD5A2* 89L allele (OR 10.2, CI 1.29–80.5, $P = 0.03$) and among all subjects (OR 3.82, CI 1.27–11.50, $P = 0.02$) even after adjustment for covariates. Overall, stratifying by either the *SRD5A2*

V89L polymorphism or aggressive disease did not appear to affect prostate cancer risk estimates for the *HSD3B2* intron 3 short allele in African Americans.

DISCUSSION

This study sought to elucidate potential independent and joint effects of two polymorphisms in the androgen metabolism pathway, *SRD5A2 V89L* and a (TG)_n,(TA)_n,(CA)_n repeat polymorphism in the third intron of the *HSD3B2* gene, with regard to prostate cancer risk and aggressiveness. Our findings suggest that both of these polymorphisms differ in genotype frequency by race, and that the *HSD3B2* length polymorphism independently and in conjunction with the *SRD5A2 V89L* polymorphism is associated with elevated prostate cancer risk and potentially prostate cancer aggressiveness in all subjects and among Caucasians. The latter result, in particular, must be viewed cautiously since this *HSD3B2* polymorphism has not been previously reported in terms of prostate cancer risk and subject numbers upon which this result was based were very small.

Lachance et al. [21] first reported the *HSD3B2* intron 3 (TG)_n,(TA)_n,(CA)_n repeat and noted hairpin-like structures associated with the polymorphism. These secondary structures have the potential to vary or terminate the rate of transcription of the enzyme [23] or potentially cause alternative spliced forms of mRNA resulting in truncated or unstable proteins [24]. In fact, three alternative spliced forms of the transcribed pre-mRNA of *HSD3B2* are known to exist (<http://www.ebi.ac.uk/asd/>), with intron 3 excluded from the two shorter transcribed mRNAs that also contain different coding sequences that might alter *HSD3B2* activity and therefore may degrade DHT at varying rates. In terms of which alleles are more likely to form hairpins, previous studies have shown that longer allele length is associated with hairpin structures that are more stable, but this stability is achieved at a certain maximum length such that alleles longer than this threshold offer no further increased stability for the hairpin structure [35,36]. This would support the conservative decision we made to divide the *HSD3B2* length polymorphism into “long” and “short” categories. In addition alternative splicing of pre-mRNA increases genetic diversity [37] and therefore it follows that an allele length that is both long enough to promote alternative spliced forms of the *HSD3B2* protein while at the same time short enough to ensure sufficient copies of the full functional version of the protein are made may be highly selected for.

In our subjects, we found that the short allele (<283 bp) of the *HSD3B2* intron 3 polymorphism was associated with increased risk of prostate cancer and potentially aggressiveness of disease. Carriage of *HSD3B2* short alleles may predispose toward having more copies of the full length *HSD3B2* protein that is more active, indirectly producing larger quantities of testosterone and degrading DHT more rapidly resulting in an imbalance of serum testosterone to DHT. Higher serum testosterone to DHT ratios have been associated with increased risk of prostate cancer in some studies [25-27]. But in primary prostate cancer cells DHT levels have been reported to be higher in tumor than adjacent normal cells [38]. If the *HSD3B2* (TG)_n,(TA)_n,(CA)_n polymorphism does have an impact on gene expression, the resulting impact on testosterone and DHT levels may or may not be equivalent. First, DHT is metabolized through multiple pathways most of which are reversible reactions, including the conversion of DHT to 3 β-androstanediol by *HSD3B2*. It is possible that over expression of *HSD3B2* could have a greater effect on the rate of anabolism of DHT than the rate of catabolism of DHT. Secondly, Ji et al. [38] have reported on two proteins also involved in the metabolism of DHT, AKR1C1 and AKR1C2. Although AKR1C1, which is associated with the *HSD3B* pathway of DHT metabolism, is expressed at higher levels than AKR1C2, AKR1C2 had more influence on DHT-dependent androgen receptor reporter activity. In prostate cancer, the *HSD3B2* metabolic pathway of DHT, therefore, may be out competed by other metabolic pathways. The higher expression yet lower affect of the AK1C1 protein would suggest this.

In vivo and in vitro studies of the *SRD5A2* V89L polymorphism have indicated reduced activity of the *SRD5A2* enzyme with the leucine substitution [9-11] indicating a slower conversion rate of testosterone to DHT. In terms of prostate cancer risk, this polymorphism has shown mixed results. A meta analysis that included the review of 12 studies was conducted by Ntais et al. [8] and indicated no overall change in risk with the *L* variant. Since this analysis Forrest et al. [39] in a case-control analysis using controls from the EPIC study reported increased risk for prostate cancer associated with the *LL* genotype and Cicek et al. [13] found increased risk was primarily driven by men diagnosed at younger age or with more aggressive disease. This polymorphism has also been associated with progression of disease [40]. Stanbrough et al. [41] found increased levels of *HSD3B2* activity and decreased levels of *SRD5A2* (approximately 50%) in metastatic androgen-independent prostate cancer tumors. Others have also shown lower levels of *SRD5A2* in prostate tissue [42-44]. The increased prostate cancer risk we found associated with Caucasian carriers of the *HSD3B2* intron 3 short allele and *SRD5A2* 89L allele that may predispose to higher levels of *HSD3B2* but less active *SRD5A2* enzyme are in line with these findings. Among African Americans, risk associated with the *HSD3B2* intron 3 short allele was elevated but not significant and stratification by the *SRD5A2* V89L polymorphism appeared not to impact risk. Interestingly, the most common *HSD3B2* intron 3 repeat allele length was different in Caucasians (283 bp) and African Americans (286 bp) suggesting that some evolutionary advantage selecting for the longer allele length in African populations may exist. In addition, the frequency of the *LL* genotype in our African-American sample was at the lower end of previously reported ranges [13,14,45] limiting the statistical power for testing associations of this polymorphism. The V89L polymorphism in exon 1 of the *SRD5A2* gene lies at the far 3' end of the gene and therefore is in linkage equilibrium with several other polymorphisms within the *SRD5A2* gene that have been described by Reichardt et al. [46] and others [47,48]. In terms of prostate cancer risk, Loukola et al. [47] interrogated the entire *SRD5A2* gene and found 25 SNPs, but only the V89L variant and another SNP in an untranslated region of the gene in strong linkage disequilibrium with the V89L variant were associated with prostate cancer. Polymorphism in *HSD3B2* may be limited, with a recent sequencing study [49] finding only six SNPs. The few coding SNPs in *HSD3B2* that have been reported are not polymorphic enough to be of any practical utility for testing associations with disease risk in moderately sized samples. Interestingly, while the *HSD3B2* intron 3 repeat polymorphism was reported to vary significantly with race 10 years ago [22], until the current study it has never been investigated in the context of prostate cancer risk.

Although our study sample size was relatively large and included a large proportion of African-American subjects, the original study design included only one age-race frequency matched control to every three cases limiting statistical power for stratified case-control comparisons. The controls were representative of the population from which the cases were sampled and controlling for family history and BPH, two factors which differed by case-control status, showed no important difference from crude odds ratios. We also compared mean rank PSA levels for all genotypes and haplotypes (Tables II and III) using the Kruskal-Wallis test, as genetic variations in genes that control androgens may affect PSA levels and detection of disease. We found no significant differences in PSA levels between groups, reducing the possibility that detection bias lead to our observations. In addition, the frequency of high Gleason scores in our subjects may have contributed to our findings on aggressive disease, as nearly 65% of our case subjects underwent prostatectomy. Gleason scores have been shown to increase from biopsy in prostatectomy cases [50] and the higher use of radical prostatectomy at our institution may have provided higher than expected frequencies of aggressive cases. None of the gene-gene interactions we tested were statistically significant, nonetheless interaction odds ratios in Caucasians were greatly elevated suggesting a possible synergy of effect between *HSD3B2* and *SRD5A2* in the prostate carcinogens is pathway.

To our knowledge, our study represents the first report of an association between the *HSD3B2* (TG)_n,(TA)_n,(CA)_n polymorphism with risk of prostate cancer. Risk and aggressiveness of disease among all subjects and in Caucasians was associated with short allele genotypes of the *HSD3B2* (TG)_n,(TA)_n,(CA)_n and the *SRD5A2* V89L polymorphism may modify this risk. Further study of this repeat polymorphism, independently and in conjunction with other genetic polymorphism within the androgen pathway is clearly warranted.

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Fig. 1.
HSD3B2 repeat polymorphism allele distribution among controls by race.

TABLE I

Patient Characteristics

Characteristic	Controls N =244 n (%)	Cases N =637 n (%)	P-value [*]
Race			
African American	104 (42.7)	274 (43.0)	0.92
White	140 (57.4)	363 (57.0)	
Age			
<60	76 (31.2)	228 (35.8)	0.39
60–69	130 (53.3)	323 (50.7)	
70+	38 (15.6)	86 (13.5)	
Smoking			
Never	82 (33.6)	219 (34.4)	0.83
Ever	162 (66.4)	418 (65.6)	
BMI			
<25	45 (18.4)	127 (19.9)	0.43
25–29	108 (44.3)	302 (47.4)	
≥30	91 (37.3)	208 (32.7)	
Family history			
Positive	32 (13.1)	134 (21.0)	0.01
Negative	212 (86.9)	503 (79.0)	
BPH history			
Positive	48 (19.7)	206 (32.3)	<0.0001
Negative	193 (79.1)	430 (67.5)	
Unknown	3 (1.2)	1 (0.2)	
PSA level			
<4	223 (91.4)	108 (17.0)	<0.0001
4–10	17 (7.0)	424 (66.6)	
>10	4 (1.6)	105 (16.5)	
Path tumor stage ^a			
<T3		339 (79.8)	
≥T3		86 (20.2)	
Gleason 4–6	—	279 (43.9)	
7		254 (39.9)	
8–10		97 (15.3)	

^a Surgical cases only.

* P-value significant at <0.05.

TABLE II
Association Between *SRD5A2* and *HSD3B2* Genotype Frequencies and Risk of Prostate Cancer

<i>SRD5A2</i> genotype	Controls n (%)	Cases n (%)	OR (CI) ^a	P-value [*]
African Americans				
VV	54 (50.9)	143 (52.0)	Reference	
VL	45 (42.5)	113 (41.1)	0.89 (0.55–1.44)	0.64
LL	7 (6.6)	19 (6.9)	0.99 (0.62–1.59)	0.97
Total	106 (100.0)	275 (100.0)		
Caucasians				
VV	66 (48.5)	160 (44.7)	Reference	
VL	53 (39.0)	158 (44.1)	1.33 (0.86–2.06)	0.20
LL	17 (12.5)	40 (11.2)	1.01 (0.73–1.40)	0.96
Total	136 (100.0)	358 (100.0)		
All subjects				
VV	120 (49.6)	303 (47.9)	Reference	
VL	98 (40.5)	271 (42.8)	1.11 (0.81–1.53)	0.51
LL	24 (9.9)	59 (9.3)	0.98 (0.76–1.28)	0.92
Total	242 (100.0)	633 (100.0)		
<i>HSD3B2</i> genotype	Controls n (%)	Cases n (%)	OR (CI) ^a	P-value [*]
African Americans				
Long/long	98 (93.3)	247 (90.1)	Reference	
Long/short or short/short	7 (6.7)	27 (9.9)	1.50 (0.62–3.6)	0.37
Total ^b	105 (100.0)	274 (100.0)		
Caucasians				
Long/long	131 (96.3)	325 (90.8)	Reference	
Long/short or short/short	5 (3.7)	33 (9.2)	2.80 (1.05–7.43)	0.04
Total	136 (100.0)	358 (100.0)		
All subjects				
Long/long	229 (95.0)	572 (90.5)	Reference	
Long/short or short/short	12 (5.0)	60 (9.5)	2.07 (1.08–3.95)	0.03
Total	241 (100%)	632 (100%)		

^a Odds Ratios adjusted for race (all subjects), age, family history, BPH, BMI and smoking history, CI 95%.

^b After multiple attempts, two African Americans could not be genotyped for the *HSD3B2* polymorphism.

^{*} P value significant at <0.05.

TABLE III
Association Between *SRD5A2* and *HSD3B2* Haplotype and Prostate Cancer and Aggressiveness

<i>SRD5A2</i> genotype	<i>HSD3B2</i> genotype	Controls n (%)	All cases n (%)	OR (CI) ^a	<i>P</i> -value ^{**}	Case Gleason 4–6 ^d OR (CI)	<i>P</i> -value ^{**}	Case Gleason 8–10 ^d OR (CI)	<i>P</i> -value ^{**}
VV	African Americans	49 (92.5)	129 (90.8)						
	Long/long	4 (7.5)	13 (9.2)	Reference	0.43	Reference	0.36	Reference	0.54
	Long/short or short/short			1.62 (0.48–5.48)		1.99 (0.46–8.65)		1.50 (0.41–5.44)	
	Caucasians	62 (93.9)	146 (91.3)						
	Long/long	4 (6.1)	14 (8.8)	Reference	0.40	Reference	0.56	Reference	0.36
VL or LL	Long/short or short/short			1.66 (0.50–5.44)		1.49 (0.38–5.74)		1.86 (0.49–7.02)	
	All subjects	111 (93.3)	275 (91.1)						
	Long/long	8 (6.7)	27 (8.9)	Reference	0.26	Reference	0.33	Reference	0.29
	Long/short or short/short			1.62 (0.70–3.76)		1.62 (0.62–4.27)		1.65 (0.66–4.15)	
	African Americans	49 (94.2)	118 (89.4)						
	Long/long	3 (5.8)	14 (10.6)	Reference	0.48	Reference	0.37	Reference	0.55
	Long/short or short/short			1.64 (0.42–6.33)		2.05 (0.43–9.71)		1.57 (0.36–6.77)	
	Caucasians	69 (98.6)	179 (90.4)						
	Long/long	1 (1.4)	19 (9.6)	Reference	0.06	Reference	0.19	Reference	0.03
	Long/short or short/short			7.42 (0.96–57.4)		4.37 (0.48–39.7)		10.18 (1.29–80.48)	
	All subjects	118 (96.7)	297 (90.0)						
	Long/long	4 (3.3)	33 (10.0)	Reference	0.04	Reference	0.12	Reference	0.02
	Long/short or short/short			3.16 (1.08–9.25)		2.60 (0.78–8.69)		3.82 (1.27–11.50)	

^a Odds ratios adjusted for race (all subjects), age, family history, BPH, BMI, smoking, 95% CI.

^{**} *P*-value significant at <0.05.