Association of SRD5A2 Genotype and Pathological Characteristics of Prostate Tumors

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ABSTRACT

The enzyme product of SRD5A2, 5α-reductase type II, is responsible for converting testosterone to the more metabolically active dihydrotestosterone. Therefore, SRD5A2 may be involved in the development or growth of prostate tumors. To examine the effects of allelic variants in the gene SRD5A2 on the presentation of prostate tumors, we studied a sample, primarily Caucasian, of 265 men with incident prostate cancer who were treated by radical prostatectomy. We assessed the relationship of the A49T and V89L polymorphisms at SRD5A2 with clinical and pathological tumor characteristics of these patients. We found no association of V89L genotypes with any of the characteristics studied. The presence of the A49T variant was associated with a greater frequency of extracapsular disease [odds ratio (OR), 3.16; 95% confidence interval (CI), 1.03–9.68] and a higher pathological tumor-lymph node-metastasis (pTNM) stage (OR, 3.11; 95% CI, 1.01–9.65). In addition, the A49T variant was overrepresented in two poor prognostic groups, which have been correlated with reduced rates of biochemical disease-free survival. One group included men with at least two of the following poor prognostic variables: (a) stage T3 tumor; (b) PSA level >10; and/or (c) Gleason score, 7–10 (OR, 3.46; 95% CI, 1.04–11.49). The second group included men with positive margins and high Gleason score (OR, 6.28; 95% CI, 1.05–37.73). Our results suggest that the A49T mutation may influence the pathological characteristics of prostate cancers and, thus, may affect the prognosis of these patients.

INTRODUCTION

Genes involved in androgen metabolism have been implicated in the etiology of prostate cancer. Examples include the androgen receptor gene and members of the cytochrome P450 supergene family. Another candidate prostate cancer gene is SRD5A2, which encodes 5α-reductase type II. This enzyme is responsible for converting testosterone to DHT (1–3). DHT has greater androgen activity in the prostate than testosterone itself (4, 5) and, when bound to the androgen receptor, activates a number of genes involved in prostate development and growth (6, 7). Prior studies have provided a strong rationale for the role of 5α-reductase type II and the SRD5A2 gene in the etiology of prostate cancer. Several studies have assessed 5α-reductase activity levels through measurements of the enzyme metabolite AAG. These studies have reported decreased levels in races with a lower incidence of prostate cancer (Chinese and Japanese; Refs. 8, 9) and elevated levels in races with higher prostate cancer rates (for example, African Americans; Ref. 10). Subsequent studies were undertaken to determine whether genetic variants in SRD5A2 correlate with androgen metabolism and prostate cancer risk. Makridakis et al. (11) reported a missense mutation that resulted in the substitution of a leucine for a valine amino acid in position 89 in this enzyme (denoted V89L), which was associated with lower levels of AAG. They reported that the prevalence of this mutation in African-American, Asian, and Latino men parallels the frequency of prostate cancer in these races. However, the V89L mutation did not have substantial effects on the Vmax of 5α-reductase, which raised questions about the functional significance of this polymorphism (12). Reichardt et al. (13) reported another SRD5A2 variant that changes an alanine to a threonine residue at amino acid 49 (denoted A49T). The same authors correlated this variant with an increase in 5α-reductase activity. This variant was reported to be more common in African-American and Latino men with prostate cancer, as compared with healthy African-American and Latino controls, and was found to be most common in African-American and Latino men with advanced disease (13). More recently, Makridakis et al. (14) provided additional support for the hypothesis that the A49T variant was associated with prostate cancer risk in African-American and Hispanic men and also reported that the variant enzyme had a higher Vmax in vitro than the normal enzyme. The effects of this mutation in other races have not been previously reported.

To further evaluate whether genotypes at SRD5A2 are associated with clinical and pathological features of prostate cancers, we examined the effects of the V89L and A49T variants in a sample made up predominantly of Caucasian men with newly diagnosed prostate cancer. We hypothesize that genotypes associated with higher levels of 5α-reductase activity, and, hence, enhanced metabolism of testosterone to DHT, are correlated with more serious disease presentation and, thus, may provide information relevant to prostate cancer detection, prognosis, and treatment outcome.

MATERIALS AND METHODS

Study Subjects. We identified 275 incident prostate cancer cases who underwent radical prostatectomy between 1994 and 1999 in the Urological Oncology Clinics at the HUP. All of the men were considered incident prostate cancer cases, as defined by participation in this study within 12 months of the time of their diagnosis. Three surgeons who were members of the same clinical practice at HUP performed all of the radical prostatectomies. Men were excluded from analysis if they had prior exposure to finasteride (Proscar; n = 9) or testosterone injections (n = 1) before diagnosis. The remaining 265 men formed the sample used in our study. All of the men had pathological stage T1–T4, N0, M0 disease. The mean age at diagnosis was 61.9 years (SD, 6.7 years; range, 43–74 years). The men consisted of 227 Caucasians, 32 African Americans, three Asians, and three Hispanics. This sample is representative of the population of men treated by radical prostatectomy at HUP. Written informed consent was obtained from all of the participants under a protocol approved by the Committee for Studies Involving Human Subjects at the University of Pennsylvania.

Data Collection. Genomic DNA for this study was self-collected by each study subject using sterile cheek swabs (Cyto-Pak Cytosoft Brush; Medical Packaging Corporation, Camarillo, CA). We processed the genomic DNA by

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using a protocol modified from that of Richards et al. (15). Briefly, the swab brush was placed inside a 1.5-ml microcentrifuge tube, and 600 μl of 50 mm NaOH was added. The closed tube was vortexed for 5 min and then heated at 95°C for 10 min. Finally, 120 μl of 1 m Tris (pH 8.0) was added, after which the brush was removed and discarded.

Preoperative clinical and pathological characteristics were obtained by medical records review. All preoperative PSA levels were obtained at least 2 weeks before the radical prostatectomy. Gleason score was based on biopsy findings using a protocol modified from that of Richards et al. (15). Briefly, the swab containing the biopsied tissue was placed in a sterile vial, and the swab was removed at the time of biopsy. All biopsy specimens contained at least 70 mg of tissue for analysis. The swabs were placed in the vials, which contained 1 ml of Tween 80 solution (5% v/v) and 600 μl of 25 mm Tris (pH 8.0) after which the swabs were removed. PCR reaction mixture consisted of 25 μl of double-distilled H2O, 6 μl of M DMSO, 1 μl of the two PCR primers at 5 μM concentration, 10 μl of template DNA, 0.9 μl of 10X PCR buffer (The Perkin-Elmer Corp., Foster City, CA), 4 μl of 25 mm Mg2+, 2 μl of 10 mm deoxynucleotide triphosphate, 10 μl of each of the two PCR primers at 5 μM concentration, 10 μl of template DNA, 0.9 μl of Taq polymerase (AmpliTaq, Perkin-Elmer) in 22.1 μl of double-distilled H2O, for a total reaction volume of 100 μl. The temperature profile for the PCR reaction was one cycle each at 95°C for 5 min and 82°C for 1 min, at which time the Taq polymerase was added to the reaction mixture; this was followed by 20 cycles at 94°C for 1 min, 68°C for 1 min with a 0.5°C per cycle decrease, and 72°C for 1 min, and 15 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, with a final single 10-min cycle at 72°C completing the cycling profile.

The PCR product mixture was subjected to restriction enzyme fragment analysis. The V89L variant was identified with the restriction enzyme MwoI, which produced 90-, 70-, 46/47-, and 17/20/21/22-bp fragments corresponding to the A allele, and 107-, 70-, 46/47-, and 20/21/22-bp fragments corresponding to the T allele. Visualization of the fragments was accomplished on a 4% Metaphor (FMC Corp.) gel after staining with ethidium bromide (Fig. 1).

**Statistical Analyses.** Analyses were undertaken comparing SRD5A2 genotype with clinical and pathological indices. These indices included: (a) PSA level (coded with three levels as 0.0–4.0, 4.1–10.0, and >10.0); (b) surgical Gleason sum (coded with four levels as 2–4, 5–6, 7, and 8–10); (c) tumor volume (coded with three levels: <5%, 5–50%, and >50% of total prostate tissue replaced by tumor); (d) capsular status (presence or absence of capsular invasion); and (e) surgical margin status (positive or negative). Because of the small numbers in some of these categories, we also performed analyses in which we dichotomized several of the variables, including PSA (0.0–10.0 versus >10.0), surgical Gleason sum (2–6 versus 7–10), and tumor volume (<5% versus >5%). TNM stage was also dichotomized by combining all of the stages that reflected capsular invasion, margins positive, and/or seminal vesicle invasion (pT2+, pT3+, pT3+, or pT3c, respectively), and by comparing this combined-stage category to the more favorable pT2.

We also considered a series of prognostic indices. The prognostic significance of each of these indices has been suggested in previous studies through measurements of bRFS. First, PSA level and Gleason score were combined to form the prognostic categories identified by Kupelian et al. (19). The low-risk group included patients with PSA levels of 0.0–10.0 and Gleason sum 2–6 (81% bRFS at 5 years), whereas high-risk patients had PSA levels of >10.0 and/or Gleason sum 7–10 (40% bRFS at 5 years). A measure adapted from a study of radiation-treated patients (20) was used to study the combined effect of PSA level, Gleason sum, and TNM stage. The favorable prognostic group included men with PSA levels 0.0–10.0, Gleason sum 2–6, stage T1-T2 (85% bRFS at 5 years) or with any one of these factors increased (65% bRFS at 5 years). An increase in two or three of these factors placed patients in the unfavorable prognostic group (35% bRFS at 5 years). Finally, we combined margins status and Gleason sum according to the prognostic categories of Walsh (21). The low-risk category included patients with negative margins and Gleason sum 2–6 (100% bRFS at a median of 4 years). Patients in the intermediate prognostic category had negative margins and Gleason sum 7–10, or had positive margins and Gleason sum 2–6 (50% bRFS at a median of 4 years). The remaining patients with positive margins and Gleason sum 7–10 formed the high-risk group (30% bRFS at a median of 4 years).

On the basis of previous data concerning the functional significance of the V89L and A497T polymorphisms and their allele frequencies (10, 11, 13), all of the analyses were undertaken comparing the following genotypes: (a) homozygotes for the V allele (VV) versus the combined heterozygotes (VL) and homozygotes (LL) for the L allele of V89L; and (b) homozygotes for the A allele (AA) versus the combined heterozygotes (AT) and homozygotes (TT) for the A allele of A497T.

Descriptive analyses for discrete traits were carried out using contingency table methods and the Fisher’s exact tests or χ² statistics. Means or medians were used to summarize continuously distributed traits, and nonparametric Kruskal-Wallis statistics were used to compare these values across groups. Genotype-disease associations were undertaken using unconditional logistic regression. Analyses considered the effect of a genotype adjusted for potential confounders that included one or more of the following: (a) age (at time of...
Table 2 Association of SRD5A2 genotype with PSA level, surgical Gleason score, and tumor volume

<table>
<thead>
<tr>
<th>Variable</th>
<th>V89L</th>
<th>Adjusted OR (95% CI)</th>
<th>A49T</th>
<th>Adjusted OR (95% CI)</th>
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<tbody>
<tr>
<td>PSA (ng/ml)</td>
<td></td>
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<td></td>
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<tr>
<td>0.0–4.0</td>
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<td>4.1–10.0</td>
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<td>&gt;10.0</td>
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<tr>
<td>Surgical Gleason score</td>
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<td>≤6</td>
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<td>Tumor volume</td>
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<td>&lt;5%</td>
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<td>≥50%</td>
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<td>adjusted OR</td>
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<tr>
<td>OR adjusted by multiple logistic regression for age, race, prior treatment by leuprolide (Lupron), PSA, and Gleason score.</td>
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<td>Not estimable.</td>
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Table 3 Association of SRD5A2 genotype with pathological tumor characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>V89L</th>
<th>Adjusted OR (95% CI)</th>
<th>A49T</th>
<th>Adjusted OR (95% CI)</th>
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<tbody>
<tr>
<td>Extracapsular extension</td>
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<td>No</td>
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<td>Yes</td>
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<td>Positive margins</td>
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<td>Yes</td>
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<td>Seminal vesicle invasion</td>
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<td>No</td>
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<td>Yes</td>
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<td>Pathological tumor stage (pTNM)</td>
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<tr>
<td>Organ confined, margin negative (pT2−)</td>
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<tr>
<td>Either margin positive or capsule positive (pT2+, pT1−, pT1+, pT1−, pT1+)</td>
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* Reference group.
† OR adjusted by multiple logistic regression for age, race, prior treatment by leuprolide (Lupron), PSA, and Gleason score.
‡ Not estimable.
None of the 15 men with seminal vesicle invasion carried the A49T variant, and it was, therefore, impossible to evaluate the effect of genotype on this aspect of pathological staging. Although not statistically significant, positive margins were observed in 33.3% of men with the A49T variant and in 18.6% of men with the wild-type allele \( (\chi^2 = 1.95, df = 1, P = 0.163) \), which suggests that a larger study sample may be required to observe a statistically significant effect.

**Prognostic Indices.** As shown in Table 4, the A49T variant was overrepresented in multiple high-risk prognostic groups defined by PSA level, margin status, Gleason sum, and/or TNM stage. Specifically, the A49T variant had a statistically significant effect in the prognostic categories of Zelefsky et al. (20), which combined PSA, Gleason score, and TNM stage, as well as in the prognostic categories of Walsh (21), which combined margin status and Gleason score. The other prognostic grouping, a combination of PSA and Gleason score (19), was associated with the A49T genotype with an OR estimate greater than 2. However, this association did not reach statistical significance, presumably because of insufficient statistical power. Therefore, the A49T variant was associated with not only individual stage variables but also multiple indices of prognosis.

**DISCUSSION**

We report that men who carry the A49T variant in SRD5A2 have prostate tumors that are more likely to exhibit extracapsular extension and higher pTNM stage than men who do not carry this variant. In addition, the A49T variant is overrepresented in men with poor prognosis based on a combined analysis of their PSA level, Gleason score, and TNM stage (20), or on a combined analysis of their margin status and Gleason score (21). Our results complement those reported by Reichardt et al. (13) and Makridakis et al. (14), who found the A49T variant to be overrepresented in men with advanced (high grade and stage) disease in African-American and Hispanic men. We report that this variant has similar effects in a Caucasian population, and we suggest that it may be associated with poor prognosis. Therefore, there is mounting evidence that the A49T variant of SRD5A2 is associated with prostate cancer risk and prognosis.

We also report that the V89L variant of the SRD5A2 gene had no significant impact on the pathological or clinical manifestations of tumors in a predominantly Caucasian sample of prostate cancer patients who underwent radical prostatectomy. However, some very small and statistically nonsignificant magnitude effects were observed that might be associated with the V89L variant in a larger study with greater statistical power. A recent study by Makridakis et al. (12) reported that this genotype did not exhibit a substantial decrease in the \( V_{\text{max}} \) of 5α-reductase activity, in contrast to a previous study that suggested decreased formation of the metabolite AAG in men with this mutation (11). Our study, along with the study of Makridakis et al., provides evidence that the V89L variant may not be associated with prostate carcinogenesis, despite its greater prevalence in races with a lower incidence of this disease (e.g., Chinese and Japanese men).

The hypothesis that SRD5A2 may be involved in modifying the presentation of prostate cancer is based on knowledge of testosterone metabolism. The enzyme product of SRD5A2, 5α-reductase type II, is responsible for the conversion of testosterone to DHT, the principal androgenic hormone that promotes cell proliferation in the prostate. Cell proliferation appears to be a necessary precursor to the genetic changes leading to prostate tumorigenesis, such as activation of proto-oncogenes or inactivation of tumor suppressor genes (22, 23). Therefore, it is biologically plausible that genetic variants that are associated with increased 5α-reductase activity may promote androgen-mediated prostate tumor growth and, thus, increase the severity of prostate tumors.

Previous studies support the role of 5α-reductase type II in the development and progression of prostate cancer. Higher levels of DHT have been found in prostate cancer tissue compared with noncancerous tissue (24). Inhibitors of 5α-reductase type II have been shown to reduce the growth of certain types of androgen-dependent prostate tumors in both rodents and humans, with an associated decrease in tissue DHT levels (25, 26). Finally, prostate cancer has not been reported in men with a constitutional 5α-reductase deficiency (27).

The small number of participants with the A49T mutation is the major analytical limitation of our study. This variant had an allele frequency of approximately 4%, and, hence, we may have had insufficient statistical power to detect significant effects for some of the variables of interest. This may likely explain the effects that were suggestive, as evidenced by ORs greater than 2, but had confidence intervals that overlapped with a value of 1. Margin positivity, as well as one of the prognostic indices presented in Table 3, is an example of this phenomenon. Future studies of the A49T variant and these traits will, therefore, require larger samples sizes. More prominent effects for margin status might also be seen if the degree of margin positivity (i.e., focal versus extensive) were assessed, because these distinctions represent significant differences in disease severity (28, 29). These data were not available in our data set. An additional limitation of the present study is that it was restricted to men who had undergone radical prostatectomy. As a result, the range of patient characteristics and clinical
phenotypes was limited to those men who were eligible to undergo this surgery. Studies of men with a wider range of clinical diagnoses or pathological types may be quite different from those reported here. Finally, our case-control study design does not allow us to directly address the role of the SRD5A2 allele in the etiology of prostate cancer. Case-control studies are currently being undertaken to address this issue.

Our findings have potential implications for prostate cancer detection, prognosis, and treatment. First, our capsular and pTNM stage findings imply that the A49T variant at SRD5A2 is associated with extraprostatic extension and/or positive margins. These are pathological features that are associated with a poorer prognosis. Once cancer extends beyond the prostate, it is associated with a 40%-10 year actuarial failure rate in the absence of adjunctive postoperative treatment (reviewed in Ref.30). Furthermore, extraprostatic extension is correlated with the presence of advanced pathological features such as seminal vesicle invasion and lymph node metastasis, which were variables not analyzed in our study (31, 32). Conversely, tumor that is pathologically confined to the prostate has an excellent prognosis, with most studies reporting 5–10 year progression rates of <10% in these patients (28, 33, 34). The potential prognostic significance of our findings is further supported by the overrepresentation of the A49T genotype in two of the high-risk prognostic categories (combined PSA-Gleason-TNM stage and combined margins-Gleason), that have been associated with reduced biochemical relapse-free survival (20, 21). In addition to potential prognostic value, knowledge of SRD5A2 genotypes may also have applications in prostate cancer screening. Patients at risk for pathologically significant tumors could potentially benefit from increased screening and early detection of disease. Finally, the 5α-reductase inhibitor finasteride Proscar has recently been tested as a treatment in men with metastatic prostate cancer (35), as well as in men with low-level postprostatectomy PSA elevation (36), with promising results. Its value as a chemopreventive agent is also being assessed (37). The efficacy of such interventions may be in part determined by SRD5A2 genotype.

REFERENCES


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