Advances in Brief

The V89L Polymorphism in the 5α-Reductase Type 2 Gene and Risk of Prostate Cancer


Lank Center for Genitourinary Oncology, Department of Adult Oncology, Dana Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts 02115 [P. G. F., P. W. K., D. C., S. B.]; Division of Preventive Medicine, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Physician’s Health Study, Harvard School of Public Health, Boston, Massachusetts 02115 [C. H. H.]; Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Physician’s Health Study, Harvard School of Public Health, Boston, Massachusetts 02115 [E. G.]; and Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115 [E. G., C. H. H., M. J. S.]

Abstract

5α-Reductase type 2, the predominant prostatic isozyme of this protein, converts testosterone to dihydrotestosterone. It has been hypothesized that individuals with greater 5α-reductase activity are at increased risk for prostate cancer (CaP). A single nucleotide polymorphism of the 5α-reductase type 2 gene (SRD5A2) gives rise to a substitution of leucine (leu) for valine (val) at codon 89 (V89L), the presence of which may affect serum androstanediol glucuronide (AAG) levels. We studied the effect of this polymorphism on the risk of prostate cancer in a prospective, nested, case-control design within the Physicians’ Health Study. In all controls (n = 799), the leu allele frequency was 0.30. Among the 386 controls with plasma AAG levels available, there was no significant association between AAG levels and V89L genotype. We also detected no significant association between risk for CaP and genotype [odds ratio: val/val = 1.0 (reference), leu/val = 0.96 (95% confidence interval, 0.76–1.20), and leu/leu = 0.84 (95% confidence interval, 0.57–1.24)]. These data do not support a moderate to large effect of the SRD5A2 V89L polymorphism on plasma AAG levels or CaP risk in this predominantly Caucasian cohort, although a small effect cannot be excluded completely.

Introduction

5α-Reductase converts testosterone, the most abundant androgen in the serum, to dihydrotestosterone (1). Two forms of 5α-reductase exist (SRD5A1 and SRD5A2; Ref. 2); SRD5A2 is the form exclusively expressed in the prostate (3, 4). Because SRD5A2 is critical to prostate growth and development (5, 6), altered prostatic SRD5A2 activity attributable to genetic polymorphisms within the SRD5A2 gene could affect risk for CaP.3 A TA-dinucleotide repeat polymorphism is present in the 3’ untranslated region of SRD5A2 (7, 8). Although this polymorphism has no known effect on protein function, longer alleles are associated with a modest reduction in risk for CaP (9).

More recently, several other polymorphisms in SRD5A2 have been reported (10). The most common is a G to C transition, resulting in a valine (val) to leucine (leu) variation at codon 89 (V89L; Ref. 10). In 102 Asian men, the val/val genotype was associated with 39% higher AAG levels (a surrogate marker of 5α-reductase activity) compared with leu/leu individuals; heterozygote, leu/val individuals had intermediate AAG levels (10). This polymorphism may also confer a 33% reduction in the Vmax of SRD5A2 as compared with the wild type (11). These preliminary findings prompted our study to determine whether the risk of CaP was associated with the V89L polymorphism using a nested case-control design within the Physicians’ Health Study cohort.

Materials and Methods

Study Population. The source population for this analysis was the Physicians’ Health Study, a randomized, double-blinded, placebo-controlled trial of aspirin and β-carotene in the prevention of heart disease and cancer (12, 13). The 22,071 men were 40–84 years of age at the start of the trial in 1982, and 97% were Caucasian. Men with a diagnosis of myocardial infarction, stroke, transient ischemic attacks, unstable angina, cancer (except nonmelanoma skin cancer), current renal or liver disease, peptic ulcer or gout, or who had a contraindication for aspirin, other platelet-active agents, or vitamin A supplement use were excluded from the trial. Updated medical history was obtained at 6 months, 1 year, and annually thereafter. Follow-up questionnaires were completed by 99% of the men through 1995, and vital status was known for all randomized men. A blood specimen, which was requested from participants before randomization, was returned by two-thirds of the men (n = 14,916; Ref. 9). CaP cases and controls were drawn from among the participants who supplied a blood specimen.

Prostate Cancer Cases and Controls. For men reporting a CaP diagnosis on a follow-up questionnaire, medical records and pathology reports were obtained and reviewed by study physicians to confirm the diagnosis. Through 1995, 592 CaP cancer cases were confirmed. One study physician reviewed the case records to determine stage at diagnosis [modified Whitmore-Jewett classification scheme (14)], tumor grade, and Gleason score (15). If pathological staging was not available, the case was considered to be of indeterminate stage, unless metastasis was clinically evident. We categorized cases as high stage grade if diagnosed at stages C or D and/or had a Gleason score of ≥7 or poor histological differentiation. We selected 1 or 2 controls/case at random from among those men who returned a blood specimen and met the matching criteria. Matched controls were men who had not undergone a radical prostatectomy, had not been diagnosed with CaP by the date of the case diagnosis, and who were within 1 year of age (±2 years for elderly cases) and of the same smoking status (current, former, or never) at baseline as the case. Smoking history was used as a matching variable because the same set of cases and controls was used for other serum-based analysis that could be affected by smoking status; tobacco use is unlikely to be related to the V89L polymorphism and, thus, is unlikely to affect the results.

Laboratory Analysis. Whole-blood samples from cases and matched controls were received from the Physicians’ Health Study, with the laboratory investigators blinded to the name and case/control status. Genomic DNA was obtained from 500 µl of the thawed whole blood using a commercially available kit (QIAamp DNA extraction kit; Qiagen, Chatsworth, CA). DNA concentration and purity were determined by UV absorbance on a Beckman DU640 spectrophotometer. Each sample was diluted to a final concentration of 20 ng/µl and stored at −20°C until analysis.

Adequate DNA quantities were available, and successful amplification was achieved for 584 of the 592 cases (98.6%) and 799 of the 807 controls (99.0%).
RFLP analysis after PCR amplification in combination with allele-specific PCR were used to identify the alleles for cases and controls. RFLP analysis was used to screen the entire cohort. The G to C transversion, leading to a valine to leucine substitution at codon 89, results in the loss of a Rsal restriction site (GTAC to CTAC). Amplification was performed with primers 5'-GGCCACCTGGGACGTTACTTCTTG-3' (sense) and 5'-AAACGCTACCTGTGGAAAGTAA-3' (antisense). The first primer leads to a single nucleotide substitution in the amplified product (G mutated to T) and loss of a second Rsal site. Thus, the polymorphic allele of interest leads to the loss of a restriction site unique within the amplified fragment, and Rsal digestion readily identifies the genotype for each sample. Examples of each allele were directly sequenced to verify the accuracy of the assay (Fig. 1).

The PCR reaction mixture for RFLP analysis included 80 ng of template DNA, 1.0 μM of each primer, 50 mM KCl, 1.5 mM MgCl₂, 500 μM each deoxynucleotide triphosphate, and 1.5 units of AmpliTaq (Perkin-Elmer, Norwalk, CT) in a final volume of 22 μl. All amplifications were performed using MicroAmp tubes (Perkin-Elmer).

A Perkin-Elmer GeneAmp PCR System 9600 thermocycler was programmed for three-step PCR. After 5 min at 94°C, samples underwent 35 cycles of melting at 94°C for 30 s, annealing at 55°C for 45 s, and elongation at 65°C for 20 s. There was a final elongation step for 8 min at 65°C, and samples were then cooled to 4°C.

After amplification, 12 μl of amplified product were digested with 5 units of Rsal according to the manufacturer’s recommendations (New England Biolabs, Beverly, MA). Digested product was separated using a 2.5% agarose gel containing ethidium bromide. Genotype was based on banding pattern (Fig. 1). A primer dimer, with a length slightly less than that of the undigested leu allele, would intermittently obscure results, and for those samples with unclear results, allele-specific PCR was designed. Allele-specific PCR was performed using two sense primers that were designed and that differed by 2 bp in the final 4 bp from the 3' end. These primers were: leu (sense), 5'-TACTTCTGGGCCTCTCTGCGC-3'; and val (sense), 5'-TACTTCTGGGCCTCTTTGGCGC-3'. The antisense primer was the second primer listed above. The PCR reaction mixture included 40 ng of template DNA, 0.5 μM either val (sense) or leu (sense), and 0.5 μM antisense primer, 50 mM KCl, 1.5 mM MgCl₂, 500 μM each deoxynucleotide triphosphate, and 1.8 units of AmpliTaq (Perkin-Elmer) in a final volume of 25 μl.

Three-step PCR was used to amplify product. After 5 min at 94°C, samples underwent 35 cycles of melting at 94°C for 30 s, annealing at 55°C for 60 s, and elongation at 65°C for 25 s. There was a final elongation step for 8 min at 72°C, and samples were then cooled to 4°C. Each sample was amplified in reaction mixtures containing val (sense) and leu (sense), and product was separated using a 2.5% agarose gel containing ethidium bromide. Amplification of product identified the presence of either allele (Fig. 1b).

Allele-specific PCR was performed on samples with unclear genotype after restriction digestion (n = 341), and all samples were identified by the restriction digestion assay as leu/leu (n = 82) to assure results were not attributable to poor restriction digestion. Of the samples determined to be leu/leu with the restriction digestion, 96% (79 of 82) were confirmed by the allele-specific assay to be leu/leu. The three discordant samples were found to be heterozygous by the allele-specific assay and were analyzed as heterozygotes. Fifty-three samples were analyzed with both methods to test correlation, and only one sample was discordant (98% concordant, n = 52 of 53). The discordant sample was identified as leu/leu by the restriction assay but was subsequently matched with the allele-specific PCR result.

Fig. 1. Restriction-site (A) and allele-specific (B) PCR-based assays and confirmation by direct sequencing (C). Three samples (Sample 1, homozygote leu/leu; Sample 2, homozygote val/val; and Sample 3, heterozygote val/leu) were subjected to both PCR-based assays and sequenced. The more common val allele contains a Rsal site (GTAC) that is lost with the leu polymorphism (CTAC). Allele-specific primers and parameters are described in the text. A primer-dimer band (**), with a size detectably smaller but very close to the uncut (leu) allele, is present in the water lane under optimal conditions. This primer dimer is occasionally present in samples lanes, confounding accurate genotyping. For samples with unclear restriction analysis, allele-specific PCR was used for genotyping. *, 100-bp ladder (NE BioLabs).
found to be heterozygous (leu/val) using the allele-specific assay, underscoring the importance of confirming all samples that were determined to be leu/leu by the restriction digestion. Plasma AAG levels were determined previously by RIA (16).

**Statistical Analysis.** We used the chi-squared test to evaluate whether the distribution of genotype varied significantly between cases and controls (17). Using unconditional maximum regression models controlling for the matching variables baseline age (5-year intervals) and smoking status (never, former, or current), we estimated ORs and 95% CIs for the relation of 5\alpha-reductase type 2 (SRD5A2) genotype and total and high stage/grade CaP (18). The OR is an estimation of the relative risk. We included all controls irrespective of whether the matched case was omitted to increase the power to estimate the relation between 5\alpha-reductase genotype and high stage/grade CaP. Similar results were obtained from conditional logistic regression models to account for the matching.

We used the Kruskal-Wallis test (17) to assess whether mean concentration of plasma AAG varied by genotype among the controls and, to test for trend, we used the Wald test where genotype was entered as a categorical variable in a linear regression model. We used SAS to conduct all analyses (SAS Institute Inc., Cary, NC).

**Results**

**Genotyping.** Genotypes were determined for 1383 individuals (Table 1). There was no difference in the distribution of genotype between controls and all cases (P = 0.7) or high stage/grade cases (P = 0.9).

**Relationship between Genotype and CaP Risk.** The ORs for total CaP and aggressive CaP, in relation to genotype, are shown in Table 2. Although the likelihood of developing CaP and aggressive CaP was slightly lower in individuals with leu alleles compared with those homozygote for the val allele, the magnitudes were small and not statistically significant (P\(_{\text{trend}}\) = 0.4 and 0.6, respectively).

**Hormone Analysis.** Plasma steroid hormone levels were available for 386 (48%) of the controls. Mean AAG levels by genotype are shown in Table 3. Although controls with the val/val genotype had a higher mean AAG level than individuals with the leu/leu genotype, the differences among the means were not statistically significant (P = 0.21). The trend for decreasing levels across genotypes was also not statistically significant (P\(_{\text{trend}}\) = 0.18).

**Discussion**

Androgens are necessary for prostate development and critical in the pathogenesis of CaP (19–21). Variability in the plasma level of androgens may account for some of the observed differences in risk of CaP between racial/ethnic groups (1, 16, 22). In addition, genetic polymorphisms affecting an individual’s androgen metabolism and signal transduction may also influence the incidence or behavior of CaP. In this study, we found no significant association between the V89L polymorphism within the SRD5A2 gene and a measure of 5\alpha-reductase activity or risk of total CaP and aggressive CaP.

There are several possible explanations for the lack of association observed between the V89L polymorphism and plasma AAG levels: (a) plasma AAG levels may be poor surrogates for intraprostatic 5\alpha-reductase, type 2 activity. 5\alpha-Reductase type 1 (expressed in the liver) likely contributes to plasma AAG levels and may decrease or eliminate the association between the V89L polymorphism and serum AAG levels. Our study may not have had sufficient statistical power to detect a small to modest effect of the V89L polymorphism on AAG levels, if present.

In the initial report of the V89L polymorphism, Makridakis et al. (10) found a statistically significant 39% higher mean AAG level in val/val individuals than in leu/leu in an Asian cohort. In our cohort of predominately Caucasian men, we found only a 10% higher mean AAG level in men with val/val compared with men having the leu/leu genotype.

The AAG levels reported for Asian men by Makridakis et al. (10) are 35–48% lower than those observed in our study. The observed difference may be attributable to differences in specimen handling, age, and race/ethnicity of study subjects or interassay variation. Like Makridakis et al., we used a RIA-based test on serum that was frozen prior to use, but the Asian men in their study had, on average, lower levels of AAG than our population of mostly Caucasian men. This racial/ethnic difference agrees with a prior report demonstrating statistically significantly lower AAG levels in young Asian men compared with young Caucasian men, although the differences were less pronounced (23% between Japanese men and White men; Ref. 3).

Two other studies of serum AAG levels in Caucasians reported values similar to ours (3, 23), and in a fourth study, AAG levels were at an intermediate level in Japanese-American men (24).

The association found by Makridakis et al. (10) between the V89L polymorphism and plasma AAG levels, which is not supported by our study, suggests that the V89L polymorphism may be important in some racial/ethnic populations but not others. Alternatively, the V89L could be in linkage disequilibrium with a locus that alters AAG levels in some racial/ethnic groups but not others (such as another polymorphic locus within the same gene). It will be important to further investigate this polymorphism in Asian (higher prevalence of leu allele) and African American (lower prevalence of leu allele) men to understand how racial/ethnic differences affect the interaction of this polymorphism with plasma AAG levels and CaP risk or behavior.

We found no statistically significant association between genotype for the V89L polymorphism in SRD5A2 and CaP risk. We used two PCR-based assays, validated by direct sequencing, to determine genotype for the participants in this study. The overall frequency of the leu allele of 0.30 is similar to a prior published report (0.23 among Caucasians; Ref. 10). There was a weak suggestion of a trend toward

### Table 1 Distribution of the V89L polymorphism in the 5α-reductase type 2 gene by CaP status in the Physicians’ Health Study, 1982–1995

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n)</th>
<th>Total cases (n)</th>
<th>High stage/grade cases (n)</th>
<th>OR(_{\text{a}}) (95%) CI</th>
<th>(P_{\text{trend}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>leu/leu</td>
<td>78</td>
<td>50</td>
<td>24</td>
<td>0.88</td>
<td>0.9</td>
</tr>
<tr>
<td>leu/val</td>
<td>330</td>
<td>239</td>
<td>110</td>
<td>0.96</td>
<td>0.7</td>
</tr>
<tr>
<td>val/val</td>
<td>391</td>
<td>295</td>
<td>135</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* OR estimated from logistic regression model, controlling for age and smoking status.

### Table 2 Relationship between V89L polymorphism in the 5α-reductase type 2 gene and CaP in the Physicians’ Health Study, 1982–1995

<table>
<thead>
<tr>
<th>CaP</th>
<th>Controls (n)</th>
<th>Total cases (n)</th>
<th>High stage/grade cases (n)</th>
<th>OR(_{\text{a}}) (95%) CI</th>
<th>(P_{\text{trend}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR(_{\text{a}})</td>
<td>0.84</td>
<td>0.96</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR(_{\text{b}})</td>
<td>0.57–1.24</td>
<td>0.76–1.20</td>
<td>(Reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High stage/grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR(_{\text{a}})</td>
<td>0.88</td>
<td>0.97</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR(_{\text{b}})</td>
<td>0.54–1.46</td>
<td>0.72–1.30</td>
<td>(Reference)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* OR estimated from logistic regression model, controlling for age and smoking status.

<table>
<thead>
<tr>
<th>(P_{\text{trend}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21</td>
</tr>
</tbody>
</table>

"\(\)a\) P for \(\chi^2\) test for independence.

### Table 3 Mean ± SD plasma AAG concentration (ng/ml) by the V89L polymorphism in the 5α-reductase type 2 gene in controls in the Physicians’ Health Study, 1982–1995

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Total</th>
<th>High stage/grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((n))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leu/leu</td>
<td>6.52 ± 2.76 (39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leu/val</td>
<td>6.91 ± 3.54 (164)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>val/val</td>
<td>7.22 ± 3.12 (183)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* OR for Kruskal-Wallis test for difference of means by genotype.

Downloaded from cancerres.aacrjournals.org on November 9, 2017. © 1999 American Association for Cancer Research.
the Leu allele being protective (Table 2), agreeing with the a priori hypothesis, but it was not statistically significant in this cohort. Thus, we cannot exclude a small association, but a moderate to large effect of this polymorphism on CaP is unlikely.

References

The V89L Polymorphism in the 5α-Reductase Type 2 Gene and Risk of Prostate Cancer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/23/5878

Cited articles
This article cites 18 articles, 7 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/23/5878.full#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/23/5878.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/59/23/5878.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.