

## A Prevalent Missense Substitution That Modulates Activity of Prostatic Steroid 5 $\alpha$ -Reductase<sup>1</sup>

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### Abstract

Prostate cancer is the most common serious cancer diagnosed in men in the United States. This disease is also characterized by a striking racial/ethnic variation in incidence: highest in African-Americans, intermediate in Caucasians, slightly lower in Latinos, and lowest in Asians. Ample biochemical and epidemiological evidence suggests a role for androgens, particularly testosterone and dihydrotestosterone, in prostate cancer etiology. We have analyzed a candidate gene for prostate cancer, *SRD5A2*, encoding prostatic steroid 5 $\alpha$ -reductase type II, which converts testosterone into the more bioactive dihydrotestosterone, for mutations. We report here one amino acid substitution, V89L, which replaces valine at codon 89 with leucine. This substitution is a “germline” (constitutional) DNA polymorphism, and it is common, panethnic, and reduces *in vivo* steroid 5 $\alpha$ -reductase activity. This substitution is particularly common among Asians and may explain the low risk for prostate cancer in this population.

### Introduction

Prostate cancer in the United States will be diagnosed in some 317,000 men during this year and over 41,400 will die of the disease (1). Prostate cancer is characterized by a marked variation in incidence among racial/ethnic populations in this country: African-American men have a 70% increase in risk, whereas Asian-American men (of either Chinese or Japanese ancestry) have an approximately 60% decrease in risk when compared to (non-Hispanic) White (*i.e.*, Caucasian) males (2). We have suggested previously that circulating TT<sup>3</sup> and intraprostatic DHT levels may be responsible for some of these variations in risk (3). We showed that Japanese men had reduced androgen metabolism when compared to African-American and Caucasian controls (3). Others have found some support for the hypothesis that steroid 5 $\alpha$ -reductase activity is important in determining prostate cancer risk (4). Molecular genetic studies thus far have revealed few mutations responsible for predisposition to prostate cancer and early tumor progression (5, 6).

TT is converted to the more active intracellular metabolite, DHT, by the enzyme steroid 5 $\alpha$ -reductase with NADPH (nicotine adenine dinucleotide phosphate; reduced form) as the cofactor (7). DHT binds to the AR, and the DHT-AR complex transactivates a number of genes with AR-responsive elements (7). These events ultimately result in cell division in the prostate (7). Two isozyme forms of steroid 5 $\alpha$ -

reductase have been reported: type I enzyme encoded by the *SRD5A1* gene, which is expressed mostly in newborn scalp and in skin and liver; and type II enzyme, which is primarily expressed in genital skin and the prostate is encoded by the *SRD5A2* gene (8). These data suggest that the type I enzyme is primarily responsible for virilization and male pattern baldness, whereas the type II enzyme is involved in prostate development and growth (8).

The human *SRD5A2* gene was cloned independently by two groups and shown to map to a single band on the short arm of chromosome 2 (2p23) spanning over 40 kb of genomic DNA (9, 10). Mutations in the *SRD5A2* gene cause a rare human disorder, male pseudohermaphroditism (8). Males with this disorder are phenotypically female at birth but develop male musculature and other secondary sex characteristics at puberty (8). The prostate, however, remains highly underdeveloped, and DHT levels are low despite a rise in TT at puberty (8). Thigpen *et al.* (10) and others have described mutations in the *SRD5A2* gene responsible for male pseudohermaphroditism. However, these mutations have not been implicated in prostatic diseases.

Our group (11) has recently reported extensive genetic polymorphism in the *SRD5A2* gene, expanding the data of Davis and Russell (12) substantially. We found that certain alleles of a (TA)<sub>n</sub> dinucleotide repeat were unique to the highest prostate cancer risk population: African-Americans (11). In this study, we expand our investigations of the *SRD5A2* gene as a candidate gene for predisposition to prostate cancer. We demonstrate that one common polymorphism, V89L, is differentially distributed among racial/ethnic groups and appears to determine *in vivo* steroid 5 $\alpha$ -reductase activity.

### Materials and Methods

**Samples.** In 1993, we initiated a population-based cohort study among African-American, Latino, Caucasian, and Japanese individuals aged 45–74 years in Los Angeles and Hawaii. This volunteer cohort was recruited by mail with a projected cohort size of approximately 210,000.

The initial mailing consisted of a 26-page questionnaire that collected information on diet, physical activity, prior history of specific medical illnesses, use of vitamins and selected drugs, as well as family history of cancer. All respondents are being followed for incident cancers by matching with Surveillance, Epidemiology, and End Results (SEER) registries in the two locations, as well as active follow-up.

A similar population-based cohort was initiated among Chinese residents of Singapore in 1993 with the aim of accruing 60,000 respondents. Asians in this study are drawn from both the Singapore and the Hawaii/Los Angeles cohorts.

In all three locations, an attempt has been made to collect biological samples (blood and urine) in the cohorts. Participation rate for the sample collection component in all populations has exceeded 70% thus far. Blood samples are processed within 4 h of collection and separated into components (lymphocytes, plasma, serum, and erythrocytes), aliquoted into plastic “straws” using an automated cryo-bio system, and stored in liquid nitrogen until analysis.

**Androgen Measurements.** Serum AAG levels were quantitated by a specific RIA, as described (13).

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<sup>3</sup> The abbreviations used are: TT, testosterone; DHT, dihydrotestosterone; AR, androgen receptor; AAG, 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol-17 $\beta$ -glucuronide; SSCP, single-strand conformational polymorphism.

**Molecular Analyses.** Genomic DNA was extracted from WBCs by standard procedures (14). The protein-coding region of the *SRD5A2* gene from 50 individuals was PCR amplified (10, 15) in the presence of one radiolabeled primer and analyzed on nondenaturing polyacrylamide gels in the presence or absence of 10% glycerol (Ref. 14; SSCP). Primers were radiolabeled with [ $\gamma$ -<sup>32</sup>P]ATP (DuPont NEN, Boston, MA) as described (11). Positive samples were sequenced either by a PCR-based kit (Life Technologies, Inc., Gaithersburg, MD) or directly with Sequenase 2.0 (Amersham Corp., Cleveland, OH). Substitutions, once identified, were screened by SSCP in the 286 men accumulated in our cohort thus far.

**Statistical Analyses.** Biochemical, epidemiological, and molecular data were analyzed by standard methods (16). Hormone levels were log-transformed for statistical analysis. All *P*s reported here are two-sided.

**Results**

We identified 50 individuals drawn from three U. S. populations at very different risk for developing prostate cancer: African-Americans, Asians, and Caucasians. Genomic DNA from these samples was PCR-amplified and subjected to SSCP analysis for mutation identification in the *SRD5A2* gene (10, 15). Sequencing of aberrant SSCP patterns identified seven missense substitutions (C5R, P30L, A49T, V89L, T187M, R227Q, and F234L) and six nucleotide substitutions (C905T, C950T, T1039C, G1047T, A1395C, and T2038C). The latter were either intronic and removed from the conserved splice junctions, or they were silent (*i.e.*, third base pair) changes. We screened for all seven missense substitutions and found that the V89L substitution was the most common. This polymorphism results in the substitution of valine at codon 89 with leucine due to a G to C transversion (Fig. 1).

We next examined the distribution of the V89L substitution in 286 randomly chosen control men from four racial/ethnic groups in the two cohorts (Table 1). The valine 89 homozygote genotype (*VV*) was the most common genotype found in African-Americans, Caucasians, and Latinos. The highest frequency was identified among African-Americans, accounting for 58.9% of men, and lowest in Asians, with a valine (*VV*) homozygote frequency of 29.4% (Table 1; *P* = 0.00001). The leucine 89 homozygote genotype (*LL*) was most common in Asians, with a prevalence of 21.6% (Table 1). Caucasians have a slightly lower frequency of the valine allele (Table 1). The allele frequency for the V89L polymorphism was also statistically different between intermediate risk Caucasians and low risk Asians (Table 1; *P* = 0.0002). Latino men have intermediate frequencies of the three V89L genotypes (*VV*, *VL*, and *LL*; Table 1).

Serum AAG levels are a commonly used *in vivo* measure of steroid 5 $\alpha$ -reductase activity because they are derived from DHT (3, 4, 7, 13).

Table 1 Frequency of the V89L missense substitution

Genotype <sup>a</sup>	Racial/Ethnic group (Subjects)				
	Total (286)	African-American (95)	Caucasian (49)	Latino (40)	Asian (102)
<i>VV</i> (%)	46.5	58.9	57.1	47.5	29.4
<i>VL</i> (%)	42.0	37.9	38.8	37.5	49.0
<i>LL</i> (%)	11.5	3.2	4.1	15.0	21.6
Leucine allele (%)	32.5	22.1	23.5	33.7	46.1

<sup>a</sup> *VV* are valine 89 homozygotes for the V89L substitution, *LL* are leucine 89 homozygotes, and *VL* are heterozygote individuals. The allele frequency of the V89L polymorphism is significantly different in Asians from that in African-Americans (*P* = 0.00001), Caucasians (*P* = 0.0002), and Latinos (*P* = 0.041).

Table 2 Steroid 5 $\alpha$ -reductase activity and genotype<sup>a</sup>

Genotype	AAG (median in ng/ml) (Asian individuals)
<i>VV</i>	4.72 (30)
<i>VL</i>	4.05 (50)
<i>LL</i>	3.40 (22)

<sup>a</sup> *In vivo* steroid 5 $\alpha$ -reductase activity was measured by assaying serum AAG levels (3, 13). The number of Asian individuals is given in parentheses after each median AAG level. The age-adjusted *P* for *VV* versus *LL* in Asians is 0.04 and 0.02 in all controls studied (data not shown).

Therefore, we measured serum AAG levels in 102 Asian individuals (Table 2) because they were the only racial/ethnic group with a sufficiently large number of *LL* homozygotes (Table 1). When steroid 5 $\alpha$ -reductase enzyme activity was correlated with molecular genotype in our sample, we found that the *VV* homozygotes also have the highest AAG levels (4.72 ng/ml median among Asian controls; Table 2), and *LL* homozygotes have the lowest AAG levels (median of 3.40 ng/ml; Table 2; *P* = 0.04), whereas *VL* heterozygotes possess intermediate serum AAG levels (4.05 ng/ml median; Table 2). A similar finding was made when all four racial/ethnic groups were analyzed together for this genotype/activity correlation (data not shown). Overall, the V89L substitution results in almost 30% reduced activity (Table 2).

**Discussion**

Prostate cancer is a significant public health problem in the United States, which is characterized by a substantial racial/ethnic variation in risk (1, 2). It is noteworthy in this context that Asian (*i.e.*, Japanese and Chinese) men have the lowest risk and low indices of steroid 5 $\alpha$ -reductase activity (2, 3). Unfortunately, molecular analyses of prostate cancer have not yet identified major genes contributing to it (5, 6). We showed recently that the human *SRD5A2* gene may play a role in explaining the substantial racial/ethnic variation in risk (11). This study reports a missense substitution, V89L, which we show to be distributed differentially among races, paralleling risk for prostate cancer, and an alteration of the *in vivo* steroid 5 $\alpha$ -reductase enzyme.

The V89L substitution was identified by SSCP screening and sequencing (Fig. 1). It is noteworthy that the two groups who cloned the *SRD5A2* gene reported different amino acids for codon 89 (9, 10). Because the V89L substitution is common (Table 1), it is plausible that this discrepancy in the literature is a result of genetic differences between the two different subjects that the two groups of investigators analyzed.

African-Americans, the group at highest risk for prostate cancer in the United States, have also the highest frequency for valine 89 allele of the V89L amino acid substitution (Table 1). Asians, on the other hand, are the lowest risk population, and these men have the lowest frequency of the valine allele and the highest frequency of the leucine allele (Table 1). Latinos, the intermediate risk population, have intermediate frequencies of the V89L substitution. Thus, a gradient of the

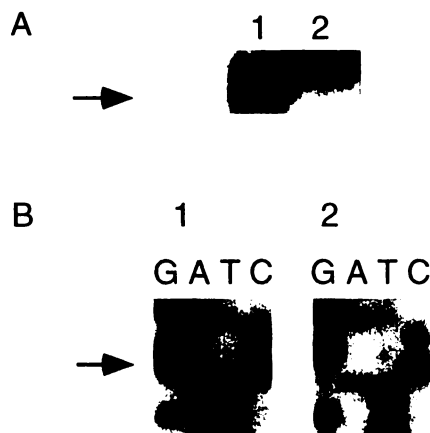


Fig. 1. Molecular identification of the V89L amino acid substitution. A, an SSCP gel of a heterozygote in Lane 1 (*VL*) and a *VV* homozygote in Lane 2. B, the relevant sequencing information on the antisense strand of the same two individuals.

V89L missense substitution exists among racial/ethnic groups that parallels prostate cancer risk.

We investigated whether the V89L amino acid substitution has any functional consequences. Therefore, we measured *in vivo* steroid 5 $\alpha$ -reductase enzyme activity by assaying serum AAG levels, a DHT metabolite (3, 4, 13, 17). We found that the common V89L polymorphism correlates well with altered *in vivo* steroid 5 $\alpha$ -reductase enzyme activity: the valine 89 allele appears to encode substantially higher enzyme activity than leucine 89, because valine homozygotes (VV) have about 30% higher AAG levels than leucine homozygotes (LL; Table 2). Heterozygote individuals (VL) have, as expected, intermediate AAG levels (Table 2). The serum TT and DHT levels for VV and LL homozygotes are identical (data not shown). Therefore, AAG levels are correlated with the V89L polymorphism of the steroid 5 $\alpha$ -reductase, suggesting that particular *SRD5A2* alleles encode enzyme variants that can be monitored by measuring AAG levels *in vivo*.

Increased DHT levels are predicted to increase the risk for prostate cancer (3, 4) because it binds to the AR and then transactivates a number of hormone-responsive genes and modulates cell division in the prostate (7). Therefore, we examined in a preliminary study whether the V89L substitution increases risk for prostate cancer. We found that the valine allele, as predicted by its increased activity (Table 2), modestly increases risk in African-Americans, where we had accumulated the most cases to date (data not shown). We plan to confirm this finding with larger numbers of cases and controls in this and other racial/ethnic groups. Furthermore, we will examine the *in vitro* properties of the V89L substitution by careful enzymological studies (15).

In summary, we report here a missense substitution in the *SRD5A2* gene, V89L, which substitutes valine 89 with leucine, that is differentially distributed in four racial/ethnic groups and alters enzyme activity. The valine 89 allele of the V89L substitution is most common in high-risk African-Americans and also appears to encode higher steroid 5 $\alpha$ -reductase activity. The leucine 89 allele, conversely, is most common in low-risk Asians and seems to encode reduced enzyme activity. We reported earlier that Asians are at lower risk for prostate cancer (2), perhaps because they have reduced steroid 5 $\alpha$ -reductase activity, as measured by AAG and other DHT metabolite levels (3). Reduced enzyme activity would produce lower levels of intraprostatic DHT, perhaps reducing a man's risk for developing prostate cancer. Asian men have the highest frequency of the leucine 89 allele of the V89L polymorphism in the *SRD5A2* gene (Table 1).

This allele apparently reduces *in vivo* enzyme activity measured as serum AAG levels (Table 2). Thus, our molecular data reported here provide a plausible genetic explanation for the observation of Ross *et al.* (3) that Asians have reduced indices of steroid 5 $\alpha$ -reductase activity, which may result in a lower risk for prostate cancer by generating less DHT, the most active androgen in the human prostate.

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