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Longer $(TA)_n$ Repeat but Not A49T and V89L Polymorphisms in *SRD5A2* Gene May Confer Prostate Cancer Risk in South Indian Men

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ABSTRACT: Testosterone is converted to 5 α-dihydrotestosterone (DHT) by 5 α-reductase enzyme, which is encoded by the *SRD5A2* gene. DHT is the main androgen responsible for prostate growth. We have analyzed the complete coding region of the *SRD5A2* gene in 87 histologically confirmed prostate cancer (PC) patients, 40 benign prostatic hyperplasia (BPH) cases, and 96 control samples from southern parts of India. The study revealed the A49T site to be monomorphic, the V89L site to be highly polymorphic, and the $(TA)_n$ repeat site to be polymorphic with only 2 alleles in our populations. The distribution of V89L alleles between PC cases and controls was not significantly different; however, $(TA)_9$ alleles distributed differently between the 2 groups. BPH cases exhibited alleles similar to controls at all polymorphic sites. The sequencing of the whole coding region did not reveal any other known or novel polymorphism in this gene. Our study emphasizes that the $(TA)_9$ allele might confer certain PC risk but that A49T and V89L polymorphisms do not confer PC risk in South Indian men.

Key words: South Indian population, 5 α reductase, testosterone, dihydrotestosterone, benign prostatic hyperplasia.

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Prostate cancer (PC) is the most common form of cancer in males and the second most common cause of cancer leading to death (Jemal et al, 2008). Benign prostatic hyperplasia (BPH) is the most common urological condition in elderly men. BPH is not associated with PC; however, men can have both PC and BPH (Ziada et al, 1999). The incidence of PC increased in the American population in the late 1980s and early 1990s. A similar increase has also been observed in low-risk countries like India (Srinivas et al, 1995). The occurrence of PC demonstrates familial aggregation, with a 2- to 4-fold increased risk among men reporting PC in a father or brother after adjustment for age and dietary factors (Heinlein and Chang, 2004). Androgens (testosterone and 5 α-dihydrotestosterone) are actively involved in prostate development (Heinlein and Chang, 2004). Approximately 80%–90% of prostate cancers are dependent on androgens at initial diagnosis, and endocrine therapy of PC is directed toward the reduction of serum androgens and inhibition of androgen receptor (AR) (van der Kwast et al, 1991). Therefore, the *AR* gene has been studied extensively in PC, and polymorphisms in the *AR* gene have been associated with PC risk (for review see Rajender et al, 2007).

Testosterone, after entering into the target cells, is converted to the more potent androgen dihydrotestosterone (DHT) by the action of the enzyme, 5 α-reductase (Wilbert et al, 1983; Coffey, 1993). DHT is mainly responsible for prostate growth, and it has been demonstrated that the tissue DHT level is a useful marker in predicting the clinical response of prostate cancer to anti-androgen therapy (Geller et al, 1984). The levels of DHT and resulting androgen action vary among different individuals depending on the activity of 5 α-reductase. Ross et al (1992) demonstrated that young Japanese men have lower 5 α-reductase activity than young Caucasian American and African American men. Similarly, Wu et al (1995) reported that the DHT:testosterone ratio was highest in African Americans, intermediate in Caucasians, and lowest in Asian Americans, corresponding to the respective risk of developing PC in these groups. Taking the above into consideration, it has been hypothesized in several studies that the polymorphisms in the *SRD5A2* gene could

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Table 1. *Few earlier studies analyzing polymorphisms in the SRD5A2 gene in relation to prostate cancer (PC) risk*

Study	Ethnicity	Association With PC Risk		
		A49T	V89L	$(TA)_n$
Kantoff et al, 1997	American	Not analyzed	Not analyzed	No association
Lunn et al, 1999	American	Not analyzed	No association	Not analyzed
Febbo et al, 1999	American	Not analyzed	No association	Not analyzed
Yamada et al, 2001	Japanese	No T allele	No association	Not analyzed
Hsing et al, 2001	Chinese	No T allele	No association	No association
Cussenot et al, 2001	French	Not analyzed	Leu/Leu genotype associated	Not analyzed
Latil et al, 2001	French	No association	No association	No association
Mononen et al, 2001	Finnish	No association	Not analyzed	Not analyzed
Soderstrom et al, 2002	Swedish	No association	No association	Not analyzed
Giwercman et al, 2005	Sweden	T variant associated	Leu/Leu genotype associated	Not analyzed
Salam et al, 2005	Asian, Hispanic, Caucasian, African American	No association	Val/Val, Val/Leu genotypes associated, association stronger in Hispanics	Not analyzed
Hayes et al, 2007	Australian	A variant associated	No association	Not analyzed
Sobti et al, 2006	Indian	Not analyzed	No association	Not analyzed
Onen et al, 2007	Turkey	No T allele	No association	Not analyzed
Present study	Indian	No T allele	No association	Longer alleles associated

Abbreviations: Leu, leucine; Val, valine.

affect PC risk (Kantoff et al, 1997; Lunn et al, 1999; Hsing et al, 2001; Latil et al, 2001).

The human *SRD5A2* gene, mapped on chromosome 2 (2p23), has 5 exons and 4 introns and encodes the enzyme 5 α-reductase, expressed in androgen-dependent tissues (Thigpen et al, 1993). A number of mutations/polymorphisms have been identified in the *SRD5A2* gene; however, A49T, V89L, and $(TA)_n$ repeat polymorphisms are the most frequent. Most of the study till date have analyzed only A49T, V89L single-nucleotide polymorphisms, and $(TA)_n$ polymorphic repeat regions. Controversies exist regarding the association of these polymorphisms with PC risk (Ntais et al, 2003). The meta-analyses are of immense use in such situations to resolve controversies; however, the bias in the publication of the studies showing positive correlation rather than no correlation might make the meta-analysis less transparent. To assess the correlation more judiciously, the studies finding no significant correlation of the polymorphism(s) with the disorder should also be brought forward, and an effort should be made to generate data on various populations of the world. This study is such an effort. We analyzed the *SRD5A2* gene for mutations/polymorphisms in Indian PC patients. To the best of our knowledge, no previous study has analyzed the full coding region of the *SRD5A2* gene among Indian prostate cancer patients. We have undertaken sequencing of the full coding region and a part of the 3′ UTR region encompassing the $(TA)_n$ repeat of the *SRD5A2* gene to understand the role of *SRD5A2* polymorphisms in disease risk and to assess the racial and ethnic differences from other populations of the world (Table 1).

Materials and Methods

Subjects

The study included 87 histologically confirmed PC cases, 40 BPH patients, and 96 male control subjects from the southern parts of India. The control comprised healthy individuals with normal serum PSA levels of 4 ng/mL of blood or less (reference level, <4 ng/mL), with no previous history of cancer, which was confirmed by digital rectal examination. BPH patients had normal serum PSA levels (≤4 ng/mL) and were examined by digital rectal examination to confirm enlarged prostate. However, no histology could be done for the BPH group because of the lack of biopsy. All cases and controls were enrolled under informed written consent. The patients and controls were enrolled from a population with the same ethnic origin. Relevant clinical and pathological data were collected for all the patients. In PC patients age ranged from 45 to 98 years, with a mean age of 67.5 years; 55–77 years with a mean of 65.5 years in BPH patients, and 50–81 years with a mean of 66.5 years in normal healthy controls. Pathological grading of the tumors by Gleason scores was obtained, and the patients were classified as low grade if their Gleason scores were less than 7 and high grade if their Gleason scores were greater than or equal to 7. The Gleason score was less than 7 in 48 patients and greater than or equal to 7 in 39 patients. The study was approved by the ethical committee of the Institute.

Table 2. *Polymerase chain reaction (PCR) primers used for amplification of the coding regions of the SRD5A2 gene*

Primer Symbol	Primer Sequences, 5′–3′	PCR Conditions	Product Size, bp
SRD 1	GGGGGCGGACACGGGTGGC GGCAGAAGAGGCCCAGAAGTAC	94°C, 12 min; (94°C, 45 s; 58°C, 45 s; 72°C, 1 min) × 35 cycles; 72°C, 10 min	363
SRD 2	CCGGCGGCTACCCGCCTGCCAG CTAGAGAGTTGACAAGCGGCTGCGGC	94°C, 12 min; (94°C, 45 s; 58°C, 45 s; 72°C, 1 min) × 30 cycles; 72°C, 10 min	361
SRD 3	TGGGTTAAGGCGAAATGGCAGAG GGTGCAGGGAGCCAGGAAAAGA	94°C, 12 min; (94°C, 45 s; 60°C, 45 s; 72°C, 1 min) × 30 cycles; 72°C, 10 min	607
SRD 4	CCCCATTCAGGACCAGGACTCA TGGCCAAGCATTCAGCATCATTT	94°C, 12 min; (94°C, 45 s; 58°C, 45 s; 72°C, 1 min) × 30 cycles; 72°C, 10 min	644
SRD 5	GCCCCACATTTGTTCTTGCTG CAAGCCCAGCAAGTCAGAATATG	94°C, 12 min; (94°C, 45 s; 58°C, 45 s; 72°C, 1 min) × 30 cycles; 72°C, 10 min	420
SRD6	GGCTGTGGGAAGGAGAAATAAGA GCTTCAGTTGGGGATCAGGATAG	94°C, 12 min; (94°C, 1 min; 58°C, 45 s; 72°C, 1: 30 min) × 30 cycles; 72°C, 10 min	439

Genetic Analysis of SRD5A2 Gene

Genomic DNA was isolated from peripheral blood lymphocytes by the method described in our earlier study (Thangaraj et al, 2002). The primers used for polymerase chain reaction (PCR) amplification of the *SRD5A2* gene, including intron/exon junctions, were designed with GeneTool software and synthesized with the use of a 394 DNA/RNA oligosynthesizer (Applied Biosystems, Foster City, California). Conditions for PCR amplification along with primer sequences for each exon are detailed in Table 2. The PCR products were sequenced directly by the dideoxy cycle sequencing chain termination method (Big Dye V3.1, Applied Biosystems) on an ABI 3730 DNA analyzer (Thangaraj et al, 2003). Sequence editing and multiple alignments were carried out with AutoAssembler software (Applied Biosystems). The sequencing signal for the V89L polymorphism was not clear at the polymorphic site despite repeated efforts; therefore, this polymorphism was typed by PCR restriction fragment length polymorphism (PCR-RFLP). The restriction reaction included a 10-μL PCR product, 2 units of *Rsa*I restriction enzyme, 10 μL of restriction buffer, and incubation at 37°C for 1 hour. After restriction digestion, the reaction mixture was run on 10% PAGE to see the banding pattern. For distinguishing the homozygous and heterozygous genotypes at the $(TA)_9$ repeat, we amplified this region with the FAM (carboxyfluorescein)-labeled forward primer (Table 2) and genotyped it with 3730 DNA analyzer. For genotyping, 3.0 μL of PCR product was mixed with 0.3 μL of LIZ size standard (Applied Biosystems) and 6.7 μL of Hi-Di formamide (Applied Biosystems). Before running on the DNA analyzer, the mixture was heated to 95°C for 5 minutes, followed by cooling on ice for 5 minutes.

Statistical Analyses

We calculated the allelic percentages for *SRD5A2* polymorphisms and analyzed them for differences between cases and controls. Because it is known that the *SRD5A2* allele with valine (Val) at the V89L polymorphic site confers high activity to the enzyme compared with the allele with leucine (Leu) (Makridakis et al, 2000), the genotypes at this site were named high-activity (Val/Val) and low-activity (Val/Leu and Leu/Leu) genotypes. The high- and low-activity genotypes were analyzed for differences between cases and controls. The test was performed for PC vs controls and BPH vs controls, independently. We used the chi-square (χ^2) test to calculate the significance of differences between cases and controls. Chi-square values were calculated with the online Vassar Stats Calculator (http://faculty.vassar.edu/lowry/VassarStats.html). Two-sided P values of less than .05 were taken to be significant. We analyzed the frequency distribution of various genotypes at the V89L polymorphic site with respect to various clinical parameters using SPSS software (version 11; SPSS Inc, Chicago, Illinois). Furthermore, different alleles at the V89L and $(TA)_n$ polymorphic sites were examined for linkage disequilibrium in both the cases and the controls.

Results

We did not observe any polymorphism at the A49T polymorphic site (Table 3). PC, BPH, and control samples invariably had the wild-type allele (A) at this site. The V89L site was highly polymorphic (Figure 1); however, the differences in the frequency distribution of various alleles/genotypes at this site were not significant between PC cases and controls or BPH and controls

Table 3. *Comparison of the percentage of various SRD5A2 alleles between cases and controls*

Polymorphic Site	Allele	PC Cases, %	BPH Patients, %	Controls, %
A49T	A	100	100	100
	T	0	0	0
V89L	V	51.15	51.30	52.08
	L	48.85	48.70	47.92
$(TA)_n$ repeat	$(TA)_0$	97.7	100	100
	$(TA)_9$	2.3	0	0
	$(TA)_{18}$	0	0	0

Abbreviations: A, alanine (wild-type alleles); BPH, benign prostatic hyperplasia; L, leucine; PC, prostate cancer; T, threonine; V, valine (wild-type allele).

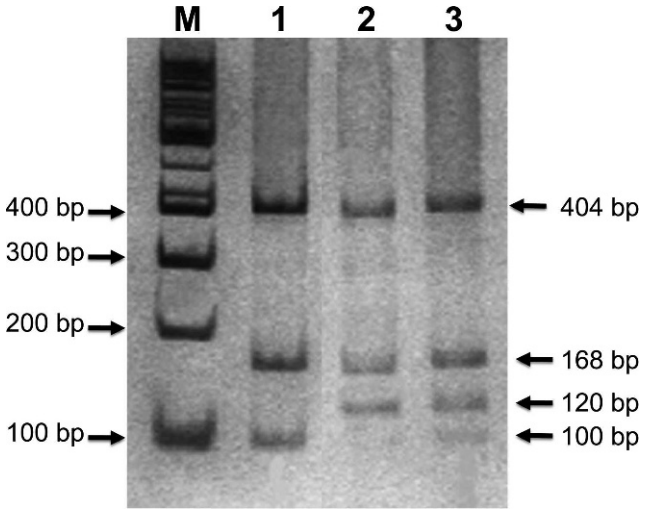

Figure 1. Restriction fragment length polymorphism (RFLP) analysis for V89L polymorphism. Lane 1 (marked M): 100-bp DNA ladder; lane 2 (marked 1): Val/Val; lane 3 (marked 2): Leu/Leu; lane 4 (marked 3): Val/Leu. The presence or absence of 120- and 100-bp fragments discriminates among the 3 possible genotypes at this site, whereas the fragments of 404 and 168 bp are common in the 3 samples.

(Table 3). For genotype analyses, the Val/Val genotype was considered to be a high-activity genotype, whereas Val/Leu and Leu/Leu were considered to be low-activity genotypes. We did not find any difference between cases and controls in the distribution of high- and low-activity genotypes (Table 4). At the $(TA)_n$ repeat site, we observed the $(TA)_0$ allele to be the most common in both the cases (97.7%) and the controls (100%), whereas $(TA)_9$ occurred at a very low frequency (2.3%) among the PC cases only (Table 3). PC cases exhibited all 3 possible genotypes at the $(TA)_n$ site, with a maximum frequency (96.55%) of $(TA)_0/(TA)_0$, a low frequency (2.3%) of $(TA)_0/(TA)_9$, and an even lower frequency (1.15%) of $(TA)_9/(TA)_9$ (Table 4). BPH and control samples invariably presented with the $(TA)_0/(TA)_0$ genotype, with the other 2 genotypes being absent. Although the genotypes with longer $(TA)_n$ alleles were not so common in the cases, its absence from the control and BPH groups might indicate disease risk associated with longer alleles in a subpopulation of Indian men. Haplotype analyses indicated that the presence of the $(TA)_9$ repeat did not preferentially associate with Val/Val, Val/Leu, or Leu/Leu genotypes at the V89L site, indicating the absence of linkage disequilibrium between the 2 polymorphic sites. Correlation analyses showed that the presence of the Val/Leu and Leu/Leu genotypes at this site correlated with higher levels of PSA compared with the Val/Val genotype ($r = .301$, $P = .005$; Figure 2). No other parameter correlated positively or negatively with the V89L polymorphic site. BPH cases possessed alleles and genotypes similar to the control samples at all the 3 polymorphic sites.

Discussion

Testosterone and its metabolite DHT are crucial for growth and development of prostate gland. The research

Table 4. *Comparison of various SRD5A2 genotypes between cases and controls. The respective percentage of each genotype is indicated in parentheses*[a]

	A/T Polymorphism				
	A/A	A/T and T/T	TT	χ^2	*P* value
PC	87	0	0	...	...
BPH	40	0	0	...	...
Controls	96	0	0	...	...
	V/L Polymorphism				
	V/V	V/L	L/L	χ^2	*P* value
PC	25 (28.74)	39 (44.83)	23 (26.44)	0	1
BPH	12 (30)	16 (40)	12 (30)	.03	.86
Controls	29 (30.20)	42 (43.75)	25 (26.04)	...	...
	$(TA)_n$ Polymorphism				
	$(TA)_0/(TA)_0$	$(TA)_0/(TA)_9$	$TA)_9/(TA)_9$		
PC	84 (96.55)	2 (2.30)	1 (1.15)	...	...
BPH	40 (100)	0 (0)	0 (0)	...	...
Controls	96 (100)	0 (0)	0 (0)	...	...

Abbreviations: A, alanine (wild-type alleles); BPH, benign prostatic hyperplasia; L, leucine; PC, prostate cancer; T, threonine; V, valine (wild-type allele).

[a] The χ^2 and the respective *P* values for the V89L polymorphism were calculated for PC cases vs control and for BPH cases vs controls separately. For this purpose, the 3 genotypes at this site were classified into 2 groups for both the cases and the controls: high activity (Val/Val) and low activity (Val/Leu and Leu/Leu).

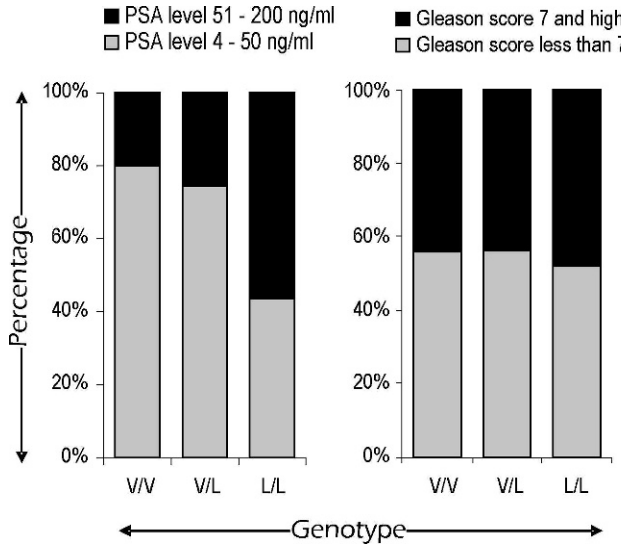

Figure 2. Bar graph showing association of various parameters (PSA level and Gleason score) with different genotypes at V89L polymorphic site. *Y*-axis shows the percentage of PC cases.

on PC has focused mainly on the genes related to androgen action. In this study, we have sequenced the entire coding region and 3′ UTR region encompassing the $(TA)_n$ repeat of the *SRD5A2* gene in 87 PC cases, 40 BPH cases, and 96 control samples. Most of the studies on the *SRD5A2* gene in PC have focused only on these 3 polymorphisms (A49T, V89L, and $[TA]_n$ repeat). We have for the first time analyzed the entire coding sequence. The study revealed no polymorphism at the A49T locus. Differences in the distribution of $(TA)_n$ alleles but not V89L alleles were observed between the cases and the controls. Earlier studies on these polymorphisms in *SRD5A2* have shown contradictory results. However, a meta-analysis has shown no significant association of any of these polymorphisms with prostate cancer susceptibility (Ntais et al, 2003). More studies might help to resolve the controversy regarding the association of genetic variations in the *SRD5A2* gene with PC risk.

We observed no polymorphism at the A49T locus. All PC, BPH, and control samples had A at this site. A49T has been one of the most commonly studied polymorphisms in the *SRD5A2* gene. The A49T variant (Ala 49 Thr substitution) has been linked to pathological characteristics of PC (Jaffe et al, 2000). It increases the activity of the steroid 5-α-reductase enzyme by 5-fold. The T variant has been found to be more prevalent in Caucasians (3.5%) than African Americans, Asians, or Hispanics (Jaffe et al, 2000). An A49T amino acid substitution increased the risk of clinically significant disease by 7.2-fold in African American men (95% confidence interval [CI], 2.17–27.91; $P = .001$) and 3.6-fold in Hispanic men (CI, 1.09–12.27; $P = .04$). The polymorphism was reported to increase PC risk, and the mutant enzyme had a higher in vitro V_{max} than the normal enzyme (9.9 compared with 1.9 nmol · min^{-1} mg^{-1}; Makridakis et al, 1999). This polymorphism has been reported among Caucasian men and Asians but showed no association with PC risk (Latil et al, 2001; Mononen et al, 2001; Soderstrom et al, 2002). Earlier studies on Japanese (Yamada et al, 2001) and Chinese men (Hsing et al, 2001) also demonstrated monomorphism at the A49T locus. Taking our results into account, it appears that Asian or, more narrowly, South Asians have similar alleles (A) at this locus, which might signify their genetic affinities. Indian men thus carry a low-risk allele at this locus compared with Caucasians, African Americans, and Hispanics. Nevertheless, it is very important to generate similar data for other Indian and South Asian populations to strengthen or refute the above statement.

We observed the V89L site to be highly polymorphic, but no significant difference was observed between PC cases and controls or BPH and control samples. The V89L substitution occurs at a frequency of 8.5% in Caucasians, 2.5% in African Americans, and 28% in Taiwanese men (Nwosu et al, 2001). The V89L polymorphism is more common than the A49T, at around 30% frequency (Febbo et al, 1999; Jaffe et al, 2000; Makridakis et al, 2000). It has been shown that the distribution of *V89L* genotypes parallels the patterns of PC incidence in high- and low-risk populations, with an Leu/Leu genotype prevalence of 22%–25% among Asians and of only about 4% among Caucasian and African Americans, and a Val/Val genotype prevalence of 27%–29% among Asians and about 58% among African and Caucasian Americans (Makridakis et al, 1999; Ross et al, 1992; Yamada et al, 2001). Similarly, Nam et al (2001) have claimed a 3.3-fold increased risk of disease recurrence with the Val/Val and Val/Leu genotypes in Canadian populations. The results of the above studies are supported by in vitro studies showing that, compared with the Val/Val genotype, the Leu/Leu genotype confers a 42% reduction in 5 α-reductase enzymatic activity (Makridakis et al, 2000). Conversely, other studies on French (Cussenot et al, 2007) and Swedish populations (Giwercman et al, 2005) reported that the Leu/Leu genotype is associated with PC risk. This polymorphism has not found significant association with PC risk among Caucasian men (Febbo et al, 1999; Lunn et al, 1999) and in Japanese men (Yamada et al, 2001). Similar to the above studies, we did not observe any difference in the distribution of V89L alleles/genotypes between cases and controls. An earlier study of a North Indian population also reported no

association of the V89L polymorphism with PC risk (Sobti et al, 2006). In all the studies on South Asian populations, no association was detected with the V89L polymorphism; however, contradicting results are evident from studies on other populations (Table 1).

The $(TA)_n$ repeat site has been relatively less studied. Since identification of the $(TA)_n$ repeat polymorphism, 3 major alleles have been reported—$(TA)_0$, $(TA)_9$, and $(TA)_{18}$—with $(TA)_0$ being the most common in most of the populations (Akalu et al, 1999). The observation that the rare $(TA)_{18}$ allele is limited to African American populations (Reichardt et al, 1995), who have a higher rate of prostate cancer than Asian American or Caucasian men (Parkin et al, 1997), suggests that a longer allele might be associated with increased enzyme activity. The relevance of the $(TA)_n$ marker in prostate cancer is further supported by the finding that 56% of 30 prostate tumors possessed somatic mutations at this locus (Akalu et al, 1999). Thus, polymorphism at this locus might increase the risk of prostate cancer. We observed the $(TA)_9$ allele in 2.3% of the cases, although the frequency was too low to lay a strong association with PC risk (Table 3). In contrast, certain case control studies in Caucasian and Asian men have not found the $(TA)_9$ variant to be associated with an increased prostate cancer risk (Kantoff et al, 1997; Hsing et al, 2001; Latil et al, 2001). In a few other studies, the longer $(TA)_n$ was not found to be associated with increased prostate cancer risk (Kantoff et al, 1997; Hsing et al, 2001; Latil et al, 2001). The longest allele, $(TA)_{18}$, was found exclusively in African Americans, who are at increased risk of prostate cancer (Reichardt et al, 1995). In accordance with the above observation, the $(TA)_{18}$ allele was absent from our cases as well controls.

A49T and V89L polymorphisms are present in the coding regions of the *SRD5A2* gene and hence affect the enzyme activity by amino acid substitutions. But the $(TA)_n$ repeat is located in the 3′ UTR of the *SRD5A2* gene; therefore, it is unlikely to affect the structure or function of the resulting protein. However, it has been proposed that this polymorphism might influence regulation of 5 α-reductase enzyme production (Reichardt et al, 1995; Kantoff et al, 1997), probably by altering mRNA stability (Zubiaga et al, 1995). As a result, this polymorphism might alter DHT levels and consequently the risk of developing PC (Reichardt et al, 1995). However, currently no study has addressed length of the $(TA)_n$ repeat in concert with intraprostatic levels of DHT. Out of 14 studies listed in Table 1, only 5 (35.71%) have analyzed the A49T polymorphism, 12 (85.71%) have analyzed the V89L polymorphism, and only 3 (21.43%) have analyzed the $(TA)_n$ repeat. Only 2 studies (14.28%) have analyzed all 3 polymorphisms discussed above. Contrasting results for the A49T and V89L polymorphisms in our populations and the PC risk associated with longer $(TA)_n$ alleles emphasize the need to analyze the $(TA)_n$ repeat polymorphism in addition to A49T and V89L polymorphisms in further studies on this gene. Nevertheless, no study to date has analyzed the effect of the environment, if any.

Our study revealed monomorphism at the A49T locus and polymorphisms at the V89L and $(TA)_n$ sites. Inference on the basis of other populations indicates that our population carries a low-risk allele at the A49T site. Different variants at the V89L locus were almost equally frequent and conferred no PC risk. We found that the presence of the $(TA)_9$ allele might confer a PC risk; however, the difference was small. As observed in other populations, the $(TA)_{18}$ allele was absent from our samples, which could be for ethnic reasons. Sequence analysis of the whole coding region of the *SRD5A2* gene did not reveal any other known or novel polymorphism. Therefore, it seems that V89L and $(TA)_n$ are the major polymorphic sites in the *SRD5A2* gene in our population. The allelic variations in BPH were similar to controls at the $(TA)_n$ site, but the absence of longer $(TA)_n$ alleles in BPH cases cannot be ascertained given the lesser number of these cases in this study. The limitation of the sample size and certain variations in the percentage of various alleles on increasing the sample size cannot be denied. Our study emphasizes that A49T and V89L are not useful markers to use to estimate PC risk in Indian men, but the $(TA)_9$ repeat might be associated with PC risk to a certain extent; however, the low frequency limits any diagnostic value of this polymorphism. Therefore, it is clear from our study that the polymorphic status of the *SRD5A2* gene is entirely different in Indian populations. We strongly suggest analysis of the *SRD5A2* gene in more populations to clearly understand the conflicting observations regarding the association of A49T, V89L, and the $(TA)_n$ repeat polymorphisms with PC risk.

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References

Akalu A, Dlmajian DA, Highshaw RA, Nichols PW, Reichardt JK. Somatic mutations at the *SRD5A2* locus encoding prostatic steroid 5α-reductase during prostate cancer progression. *J Urol*. 1999;161: 1355–1358.

Coffey DS. In: Lepor H, Lawson RK eds. *The Molecular Biology of the Prostate*. Philadelphia, PA: Saunders; 1993;28–56.

Cussenot O, Azzouzi AR, Nicolaiew N, Mangin P, Cormier L, Fournier G, Valeri A, Cancel-Tassin G. Low-activity V89L variant in SRD5A2 is associated with aggressive prostate cancer risk: an

explanation for the adverse effects observed in chemoprevention trials using 5-alpha-reductase inhibitors. *Eur Urol*. 2007;52: 1082–1087.

Febbo PG, Kantoff PW, Platz EA, Casey D, Batter S, Giovannucci E, Hennekens CH, Stampfer MJ. The *V89L* polymorphism in the 5-reductase type 2 gene and risk of prostate cancer. *Cancer Res*. 1999;59:5878–5881.

Geller J, de la Vega DJ, Albert JD, Nachtsheim DA. Tissue dihydrotestosterone levels and clinical response to hormonal therapy in patients with advanced prostate cancer. *J Clin Endocrinol Metab*. 1984;58:36–40.

Giwercman YL, Abrahamsson PA, Giwercman A, Gadaleanu V, Ahlgren G. The 5alpha-reductase type II A49T and V89L high-activity allelic variants are more common in men with prostate cancer compared with the general population. *Eur Urol*. 2005;48:679–685.

Hayes VM, Severi G, Padilla EJ, Morris HA, Tilley WD, Southey MC, English DR, Sutherland RL, Hopper JL, Boyle P, Giles GG. 5Alpha-reductase type 2 gene variant associations with prostate cancer risk, circulating hormone levels and androgenetic alopecia. *Int J Cancer*. 2007;120:776–780.

Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev*. 2004;25:276–308.

Hsing AW, Chen C, Chokkalingam AP, Gao YT, Dightman DA, Nguyen HT, Deng J, Cheng J, Sesterhenn IA, Mostofi K, Stanczyk FZ, Reichardt JKV. Polymorphic markers in the *SRD5A2* gene and prostate cancer risk: a population-based case-control study. *Cancer Epidemiol Biomark Prev*. 2001;10:1077–1082.

Jaffe JM, Malkowicz SB, Walker AH, MacBride S, Peschel R, Tomaszewski J, Van Arsdalen K, Wein AJ, Rebbeck TR. Association of SRD5A2 genotype and pathological characteristics of prostate tumors. *Cancer Res*. 2000;60:1626–1630.

Jemal A, Thun MJ, Ries LA, Howe HL, Weir HK, Center MM, Ward E, Wu XC, Eheman C, Anderson R, Ajani UA, Kohler B, Edwards BK. Annual report to the nation on the status of cancer, 1975–2005, featuring trends in lung cancer, tobacco use, and tobacco control. *J Natl Cancer Inst*. 2008;100:1672–1694.

Kantoff PW, Febbo PG, Giovannucci E, Krithivas K, Dahl DM, Chang G, Hennekens CH, Brown M, Stampfer MJ. A polymorphism of the 5-reductase gene and its association with prostate cancer: a case-control analysis. *Cancer Epidemiol Biomark Prev*. 1997;6:189–192.

Latil AG, Azzouzi R, Cancel GS, Guillaume EC, Cochan-Priollet B, Berthon PL, Cussenot O. Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. *Cancer*. 2001;92:1130–1137.

Lunn RM, Bell DA, Mohler JL, Taylor JA. Prostate cancer risk and polymorphism in 17 hydroxylase (CYP17) and steroid reductase (SRD5A2). *Carcinogenesis*. 1999;20:1727–1731.

Makridakis NM, di Salle E, Reichardt JK. Biochemical and pharmacogenetic dissection of human steroid 5alpha-reductase type II. *Pharmacogenetics*. 2000;10:407–413.

Makridakis NM, Ross RK, Pike MC, Crocitto LE, Kolonel LN, Pearce CL, Henderson BE, Reichardt JK. Association of mis-sense substitution in *SRD5A2* gene with prostate cancer in African-American and Hispanic men in Los Angeles, USA. *Lancet*. 1999;354:975–978.

Mononen N, Ikonen T, Syrjakoski K, Matikainen M, Schleutker J, Tammela TLJ, Koivisto PA, Kallioniemi OP. A missense substitution *A49T* in the steroid 5-reductase gene (*SRD5A2*) is not associated with prostate cancer in Finland. *Br J Cancer*. 2001;84:1344–1347.

Nam RK, Toi A, Vesprini D, Ho M, Chu W, Harvie S, Sweet J, Trachtenberg J, Jewett MA, Narod SA. V89L polymorphism of type-2, 5-reductase enzyme gene predicts prostate cancer presence and progression. *Urology*. 2001;57:199–205.

Ntais C, Polycarpou A, Ioannidis JP. *SRD5A2* gene polymorphisms and the risk of prostate cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2003;12:618–624.

Nwosu V, Carpten J, Trent JM, Sheridan R. Heterogeneity of genetic alterations in prostate cancer: evidence of the complex nature of the disease. *Hum Mol Genet*. 2001;10:2313–2318.

Onen IH, Ekmekci A, Eroglu M, Polat F, Biri H. The association of 5alpha-reductase II (SRD5A2) and 17 hydroxylase (CYP17) gene polymorphisms with prostate cancer patients in the Turkish population. *DNA Cell Biol*. 2007;26:100–107.

Parkin DM, Whelan SL, Ferlaym J, Raymond L, Young J. *Cancer Incidence in Five Continents*. Vol 2. Lyon, France: IARC; 1997.

Rajender S, Singh L, Thangaraj K. Phenotypic heterogeneity of mutations in androgen receptor gene. *Asian J Androl*. 2007;9: 147–179.

Reichardt JK, Makridakis N, Henderson BE, Yu MC, Pike MC, Ross RK. Genetic variability of the human *SRD5A2* gene: implications for prostate cancer risk. *Cancer Res*. 1995;55:3973–3975.

Ross RK, Bernstein L, Lobo RA, Shimizu FZ, Stanczyk FZ, Pike MC, Henderson BE. 5-Reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet*. 1992;339:887–889.

Salam MT, Ursin G, Skinner EC, Dessissa T, Reichardt JK. Associations between polymorphisms in the steroid 5-alpha reductase type II (SRD5A2) gene and benign prostatic hyperplasia and prostate cancer. *Urol Oncol*. 2005;23:246–253.

Sobti RC, Onsory K, Al-Badran AI, Kaur P, Watanabe M, Krishan A, Mohan H. CYP17, SRD5A2, CYP1B1, and CYP2D6 gene polymorphisms with prostate cancer risk in North Indian population. *DNA Cell Biol*. 2006;25:287–294.

Soderstrom T, Wadelius M, Andersson SO, Johansson JE, Johansson S, Granath F, Rane A. 5Alpha-reductase 2 polymorphisms as risk factors in prostate cancer. *Pharmacogenetics*. 2002;12: 307–312.

Srinivas V, Mehta H, Amin A, Choudary R, Gadgil N, Ravishanker D, Phadke AG. Carcinoma of the prostate—state at initial presentation. *Int Urol Nephrol*. 1995;27:419–422.

Thangaraj K, Joshi MB, Reddy AG, Gupta NJ, Chakravarty B, Singh L. CAG repeat expansion in the androgen receptor gene is not associated with male infertility in Indian populations. *J Androl*. 2002;23:815–818.

Thangaraj K, Singh L, Reddy AG, Rao VR, Sehgal SC, Underhill PA, Pierson M, Frame IG, Hagelberg E. Genetic affinities of the Andaman Islanders, a vanishing human population. *Curr Biol*. 2003;13:86–93.

Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5-reductase isozyme expression. *J Clin Invest*. 1993;92:903–910.

van der Kwast TH, Schalken J, Ruizeveld de Winter JA, van Vroonhoven CC, Mulder E, Boersma W, Trapman J. Androgen receptors in endocrine-therapy-resistant human prostate cancer. *Int J Cancer*. 1991;48:189–193.

Wilbert DM, Griffin JE, Wilson JD. Characterization of the cytosol androgen receptor of the human prostate. *J Clin Endocrinol Metab*. 1983;56:113–120.

Wu AH, Whittemore AS, Kolonel LN, John EM, Gallagher RP, West DW, Hankin J, The CZ, Dreon DM, Paffenbarger RS Jr. Serum androgens and sex-hormone binding globulins in relation to lifestyle factors in older African-American, white, and Asian men in the United States and Canada. *Cancer Epidemiol Biomark Prev*. 1995;4:735–741.

Yamada Y, Watanabe M, Murata M, Yamanaka M, Kubota Y, Ito H, Katoh T, Kawamura J, Yatani R, Shiraishi T. Impact of genetic polymorphisms of 17-hydroxylase cytochrome P-450 (CYP17) and steroid 5α-reductase type II (*SRD5A2*) genes on prostate cancer risk among the Japanese population. *Int J Cancer*. 2001;92:683–686.

Ziada A, Rosenblum M, Crawford ED. Benign prostatic hyperplasia: an overview. *Urology*. 1999;53:1–6.

Zubiaga AM, Belasco JG, Greenberg ME. The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol Cell Biol*. 1995;15:2219–2230.