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Advances in Brief

Genetic Variability of the Human *SRD5A2* Gene: Implications for Prostate Cancer Risk[1]

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Abstract

Elevated dihydrotestosterone levels have been suggested to increase the risk of prostate cancer. The human *SRD5A2* gene encodes the type II steroid 5α-reductase, which converts testosterone to the more bioactive compound dihydrotestosterone. We have determined the distribution of a dinucleotide repeat in low-risk Asian-Americans, high-risk African-Americans, and intermediate-risk non-Hispanic Whites. We found this marker to be more polymorphic than previously reported, with some alleles being specific to African-Americans. Genetic variants of the *SRD5A2* gene may play a role in predisposition to prostate cancer and in explaining the substantial racial/ethnic variability in risk.

Introduction

Prostate cancer in the United States will be diagnosed in some 240,000 men during this year, and more than 45,000 will die of this disease (1). This cancer is characterized by marked variations in incidence among U.S. population groups: African-American men have a 70% increase in risk, while Asian-American men (of either Chinese or Japanese ancestry) have an approximately 60% decrease in risk when compared to non-Hispanic White males (2). We have previously suggested that elevated testosterone levels and, more particularly, intraprostatic DHT levels may be responsible for some of these variations in risk (3). The molecular basis for these striking differences is presently unknown. Furthermore, molecular studies thus far have revealed few mutations responsible for predisposition to prostate cancer and early tumor progression (4, 5).

Testosterone is converted to the more active intracellular metabolite, DHT, by steroid 5α-reductase with NADPH as the cofactor (6). DHT binds to the AR, and the DHT-AR complex transactivates a number of genes with AR-responsive elements (6). Two isozyme forms of steroid 5α-reductase have been reported: the type I enzyme encoded by the *SRD5A1* gene, which is expressed mostly in newborn scalp and in skin and liver (7). The type II enzyme is primarily expressed in genital skin and the prostate, and is encoded by the *SRD5A2* gene (7). 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.

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 (8, 9). Mutations in the *SRD5A2* gene cause a rare human disorder, male pseudohermaphroditism. Males with this disorder are phenotypically female at birth, but develop male musculature and other secondary sex characteristics at puberty (7). The prostate, however, remains highly underdeveloped, and DHT levels are low despite a rise in testosterone at puberty (7). Thigpen *et al.* (9) and others have described mutations in the *SRD5A2* gene responsible for male pseudohermaphroditism. These mutations, however, do not appear to be involved in prostatic diseases in adults.

Davis and Russell (10) reported a polymorphic three-allele system for the *SRD5A2* gene that can be detected by PCR. The variable region is found in the transcribed 3′ untranslated region of the type II steroid 5α-reductase gene and consists primarily of variable numbers of TA dinucleotide repeats. The most common allele, accounting for 96% of the chromosomes examined, was reported as a $(TA)_0$ allele, *i.e.*, a chromosome lacking TA dinucleotide repeats in the amplified region (10). Two other alleles, $(TA)_9$ and $(TA)_{18}$ repeats, were also described on the few remaining chromosomes (10).

We examined the hypothesis that *SRD5A2* is a candidate gene for prostate cancer by genotyping males from three population groups at different risks for prostate cancer: high-risk African-American, intermediate-risk non-Hispanic White, and low-risk Asian men. We report here significant polymorphism in the *SRD5A2* gene, with some alleles restricted to African-Americans.

Materials and Methods

Samples. We have genotyped 199 healthy controls to date: 94 African-Americans, 37 Asian-Americans (16 of Chinese and 21 of Japanese ancestry), and 68 non-Hispanic Whites ("Whites"). The control subjects in this report are derived from three sources. Sixty-one subjects (45 African-Americans and 16 Whites) are participants of an ongoing multiethnic cohort study of diet and cancer involving populations in Hawaii and Los Angeles. In the Los Angeles component, approximately 29,000 healthy African-American and 12,300 White men and women between the ages of 45 and 74 years have been recruited during the past 2 years from lists of residents of Los Angeles County. Participants completed a detailed health and dietary questionnaire and are now being traced primarily through a population-based cancer registry of Los Angeles for occurence of all incident cancers. We also routinely collect blood and urine specimens from a 3% random sample of cohort members and all incident cancer cases of selected sites (including prostate). Another 117 control subjects (44 African-Americans, 36 Whites, and 37 Asian-Americans) were participants of a cross-sectional survey conducted during 1990–1991 among male residents of Los Angeles County who were ages 35–74 years (11). The remaining 21 subjects (5 African-Americans and 16 Whites) were detected from a pool of male subjects who had been recruited as control subjects for various case-control studies of cancer conducted by University of Southern California epidemiologists. These controls were all residents of Los Angeles County and were ages 50–74 years.

***SRD5A2* Genotyping.** Genomic DNA was extracted from buffy coat by purifying high molecular weight DNA with proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation (12). Primers for PCR amplification were modified from Davis and Russell (10): 5s (GAAAACTGTCAAGCTGAA) and 3s (GGCAGAACGCCAGCAGAC). Syntheses of primers were performed in 30 nM scales on a Beckman Oligo1000 (Fullerton,

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[3] The abbreviations used are: DHT, dihydrotestosterone; AR, androgen receptor.

CA). One primer was radiolabeled by kinasing with $\gamma[^{32}P]$ATP (New England Nuclear, Boston, MA) and T4 polynucleotide kinase (Promega, Madison, WI: Ref. 12). PCR products were obtained by amplification in a Hybaid TR2 thermal cycler (Middlesex, United Kingdom) with 96-well dishes (Becton Dickinson, Oxnard, CA). The following cycle was repeated 30 times: denaturation at 94°C for 1 min, annealing for 5 s at 48°C, and extension at 72°C for 20 s. PCR reactions were fractionated on 6% denaturing polyacrylamide gels (sequencing gels; Ref. 12) to increase resolution. DNA sequencing reactions obtained with Sequenase 2.0 (USB, Cleveland, OH) were run in parallel as molecular weight markers (12). Gels were dried and exposed overnight to Kodak BIOMAX autoradiography film (Rochester, NY) at room temperature.

Statistical Analysis. The binomial test was used to compare the prevalence of alleles between African-Americans on one side and Asian-Americans and Whites on the other (13).

Results

We have increased the resolution of the TA repeat system of Davis and Russell (10) by using sequencing gels, allowing us to detect alleles differing by a single dinucleotide repeat unit (Fig. 1). Three major families of alleles can now be detected: the most common allele of *87 bp* probably corresponding to the $(TA)_0$ allele of Davis and Russell (Ref. 10; Fig. 1 and Table 1). A second group of alleles centers around *105 bp* and is probably the $(TA)_9$ originally described in Ref. 10. However, we have also identified rare alleles of *103* and *107 bp* (Fig. 1 and Table 1). Finally, we found six alleles ranging in size from *121* to *131 bp* with 2-bp increments, which probably include the $(TA)_{18}$ repeat (Ref. 10 and Table 1).

Table 1 presents genotypic data for the *SRD5A2* gene by race in 199 control subjects. The age distribution of the three racial/ethnic groups (African-Americans, Asian-American, and non-Hispanic Whites) are comparable. The mean ages for the three groups are 57.0, 58.2, and 60.9 years, respectively. The *87 bp* is the most common allele in all three groups of healthy men: it is homozygous (*87-bp/87-bp* genotype) in 67% of African-Americans, 78% of Asians, and 81% of Whites (Table 1). The *105-bp* allele is the second most common allele among Whites and Asians where it is found usually in the heterozygous state (*87-bp/105-bp* genotype) in 19% (Table 1). This allele is found in the heterozygous form in 14% of African-American men (Table 1). Most interestingly, 18% of African-American controls have at least one allele from the $(TA)_{18}$ family (*121–131 bp*), while no such alleles have been identified in any Asian-Americans or Whites. This difference in prevalence of *121–131-bp* alleles by race is statistically significant (two-sided $P < 0.00005$; 13; Table 1). Most individuals are homozygous for the *87-bp* allele while most others are heterozygous for the *87 bp* and another allele. Only two individuals of 199 examined bear two alleles other than the *87 bp*: one White man is homozygous for the *105-bp* allele and one African-American man has a *123-bp/125-bp* genotype (Fig. 1 and Table 1).

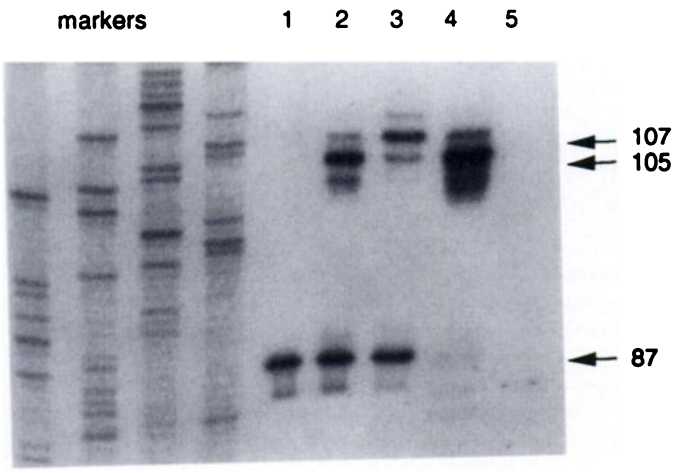

Fig. 1. Representative TA repeat alleles of the *SRD5A2* gene. *Lane 1*, an *87-bp* homozygote; *Lane 2*, an *87/105-bp* heterozygote, *Lane 3*, an *87/107-bp* heterozygote; *Lane 4*, a *105-bp* homozygote; *Lane 5*, a negative PCR amplification control ("water blank"). The molecular weight markers are derived from the human GALE sequence (14). Alleles commonly found in all populations tested and excluded alleles that are restricted to a single group such as the high molecular weight alleles, which we have only seen in African-Americans, are shown.

Table 1 *Distribution of SRD5A2 alleles in three U.S. population groups*[a]

Population	Allele (bp)									
	87	*103*	*105*	*107*	*121*	*123*	*125*	*127*	*129*	*131*
African-American (n = 94)	63	1	13	0	4	1	4	3	2	3
Non-Hispanic White (n = 68)	55	0	13	0	0	0	0	0	0	0
Asian-American (n = 37)	29	0	7	1	0	0	0	0	0	0

[a] Only the rare allele is given for people who are heterozygous. Two control individuals were homozygous for alleles other than the common *87 bp* allele. The three families of alleles are *87 bp*, *103–107 bp*, and *121–131 bp*. The prevalence of the *121–131 bp* family of alleles is statistically different in African-Americans from that in Asian-Americans and Whites (two-sided, $P < 0.00005$; Ref. 13).

Discussion

We report here an improved method for genotyping the previously reported TA dinucleotide repeat polymorphism in the 3′ untranslated region of the human *SRD5A2* gene (10). Our data indicate (*a*) that this gene is more polymorphic than previously reported, and (*b*) that certain polymorphisms are restricted to African-Americans (Table 1). There appear to be three families of alleles as described by Davis and Russell (10). However, our data indicate that two of the families are much more diverse than previously reported. The *87-bp* allele, which probably corresponds to the $(TA)_0$ chromosome described earlier (10), is the predominant allele in all three populations examined: African-Americans, Asian-Americans, and non-Hispanic Whites (Fig. 1 and Table 1). The $(TA)_9$ family (10) appears to be heterogeneous: it is comprised of three alleles of *103*, *105*, and *107 bp* (Fig. 1 and Table 1). The *121–131-bp* allele family, which probably includes the previously described $(TA)_{18}$ repeat (10), is very diverse and apparently is found exclusively in African-Americans (Table 1).

We have previously proposed that the difference in racial/ethnic incidence of prostate cancer (2) may be determined by different levels of testosterone and more specifically its intraprostatic metabolite DHT (3). This difference was particularly noticeable between African-Americans who are at very high risk for prostate cancer and lower risk Whites and Japanese (3). The present study provides a possible molecular genetic basis for our hypothesis. The statistically significant finding of *SRD5A2* TA repeat alleles that are present only in high-risk African-Americans and not in lower risk Whites and Asians (Table 1) supports our initial study (2). Thus, we propose that certain steroid 5α-reductase enzyme variants encoded by *SRD5A2* genes marked by particular TA repeat alleles may result in an elevation of enzyme activity, resulting in an increased prostatic level of DHT. Elevated levels of this hormone would then increase the risk for developing prostate cancer.

We have begun genotyping incident prostate cancer cases occurring among African-American participants of the multiethnic cohort study described in "Materials and Methods." A preliminary analysis of the first 54 cancer cases with race- and age-matched control subjects revealed an excess of *121–131-bp* alleles among cases relative to controls (relative risk, 1.8 with respect to the *87-bp* allele). In another preliminary study of 59 White subjects with prostate cancer and an equal number of race- and age-matched controls, we have detected a

relative risk of 1.9 for prostate cancer associated with the *103–107-bp* family of alleles as compared to the *87-bp* allele (data not shown). None of these associations achieves statistical significance due to the relatively small number of cases and controls studied. We recognize that these preliminary findings require confirmation with adequate sample size. Nonetheless, our data are compatible with the hypothesis that the *SRD5A2* gene plays a role in the racial/ethnic variation in prostate cancer risk.

The present study has provided a plausible molecular rationale for the difference in prostate cancer risk linked to androgen metabolism, and in particular DHT levels (3), in African-Americans on the one hand and Asian-Americans and Whites on the other.

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