Association between Two Polymorphisms in the SRD5A2 Gene and Serum Androgen Concentrations in British Men

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Abstract
Androgens are essential for the growth of the prostate gland and have been implicated in the development of prostate cancer. Little is known about the determinants of androgen levels in men, although observed ethnic differences suggest they may have a genetic basis. Several polymorphisms have been identified in the steroid 5α-reductase type II gene (SRD5A2), which encodes an enzyme that catalyzes the conversion of testosterone to its more potent metabolite, dihydrotestosterone. Although some of these polymorphisms have been associated with increased prostate cancer risk, the association with circulating androgen levels remains unclear. The purpose of this study is to investigate the association between the (TA) n dinucleotide repeat polymorphism in the 3' untranslated region and the A49T polymorphism (which replaces the normal alanine with threonine at codon 49) in the SRD5A2 gene and serum androgen concentrations in 604 British men. In particular, we wanted to test the hypotheses that the variant alleles are associated with increased prostate cancer risk, the association with circulating androgen levels remains unclear. This work was supported by Cancer Research United Kingdom and by the Europe Against Cancer Program of the Commission of the European Communities. This work was also partially supported by a grant from the NIH (to J. K. V. R.; grant CA68581).

Introduction
It is well established that the steroid 5α-reductase type II enzyme, which irreversibly converts testosterone to its more potent metabolite DHT in prostatic cells, is required for the normal growth and development of the prostate gland (1). A meta-analysis of prospective studies suggests that the serum concentration of A-diol-g, a serum marker of the abundance of 5α-reductase type II and intraprostatic DHT, is slightly higher in men who subsequently develop prostate cancer compared with healthy individuals (2). The circulating A-diol-g concentration is believed to be a more accurate serum marker of 5α-reductase type II than serum DHT itself, because A-diol-g is the direct metabolite of DHT formed in the prostate gland. However, circulating DHT is largely derived from 5α-reductase type I activity in the skin and is therefore not an accurate marker of intraprostatic 5α-reductase type II activity. Other than age and body mass, little is known about the determinants of A-diol-g levels in men (3). The observation that young Japanese men have lower A-diol-g levels than Caucasian and African-American men (4) in parallel with a lower prostate cancer risk (5), suggests that differences in 5α-reductase type II activity may have a strong genetic basis.

The SRD5A2 gene is located in chromosome band 2p23. One polymorphism identified in the 3' untranslated region consists of a variable number of TA dinucleotide repeats (6). Three clusters of base-pair repeats have been identified, being assigned (TA) n comprising 96% of alleles, and the variants (TA) 16 and (TA) 18 alleles (6). Although this polymorphism does not affect the structure of the resulting protein, it may affect mRNA stability and, thus, alter enzymic activity. The observation that the rare (TA) 18 allele is limited to African-American populations (7), who have a higher rate of prostate cancer than Asian-American or Caucasian men (5), suggests that a long allele length may be associated with increased enzyme activity. However, subsequent case-control studies in Caucasian and Asian men have not found the (TA) 18 variant allele to be associated with an increased prostate cancer risk (8–11).

A second common SRD5A2 germ-line polymorphism is a single missense mutation that substitutes alanine with threonine at codon 49 (A49T; Ref. 12). This polymorphism results in a variant protein of which the in vitro enzymic activity is ~5-fold higher than normal (12). Although the A49T polymorphism is rare in all populations, the variant T allele is more prevalent among Caucasian men (5–6%; Ref. 13) than African-American (1%) or Hispanic men (2%; Ref. 12) and has thus far not been

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1 The abbreviations used are: DHT, dihydrotestosterone; A-diol-g, androstanediol glucuronide; SRD5A2, steroid 5α-reductase type II; TA, thymine-adenine; FT, free-testosterone; SHBG, sex-hormone binding globulin; EPIC, European Prospective Investigation into Cancer and Nutrition.
identified in Asian men (11, 14). Studies in Caucasian men have generally not found this polymorphism to be associated with an increased prostate cancer risk in Caucasian men (9, 13, 15), although the T allele has been associated with an increased prostate cancer risk in African-American and Hispanic men (12).

If these genetic polymorphisms do influence prostate cancer risk, one might expect their effects to be mediated via changes in androgen levels, and in particular, A-diol-g. However, very little is known about the associations between SRD5A2 genetic variants and circulating androgen concentrations in men. We previously found the SRD5A2 V89L polymorphism to be associated with a 10% reduction in circulating concentrations of A-diol-g in a large group of British men (16). The aim of the present study is to examine the association between the SRD5A2 (TA)_n and A49T polymorphisms in relation to circulating concentrations of A-diol-g and its main precursor hormones, testosterone and calculated FT, and its associated binding protein, SHBG, in this same group of men.

Materials and Methods

Subjects. This study is part of a larger investigation, designed to investigate diet and hormonal function in men, described elsewhere (17). Briefly, 750 white male subjects were selected from the Oxford United Kingdom component of the EPIC study. These men were recruited from the United Kingdom between 1994 and 1997 through vegetarian and health food magazines, the Vegetarian Society and the Vegan Society, and from friends and relatives of the participants. Men were eligible for the current study if they had donated a blood sample before 1998 and had no diagnosis of cancer or any other serious conditions known to influence hormone concentrations.

Blood Collection and Hormone Assays. Blood samples (30 ml) were collected for each subject, sent in the mail to the EPIC laboratory in Norfolk, and aliquoted into 0.5-ml straws of plasma, serum, buffy coat, and erythrocytes. Samples were stored in liquid nitrogen tanks at −196°C until needed for analysis. Immunoassays were used to measure serum testosterone (ImmuNo 1; Bayer Corp., New York, NY), A-diol-g (Diagnostic Systems, Webster, TX), and SHBG (Oy Medix Biochemica Ab, Kauniainen, Finland) in the Clinical Biochemistry Laboratory at the John Radcliffe Hospital (Oxford, United Kingdom) in 1998. Samples were randomly assayed into batches. Coefficients of variation were 6.8% at 10.2 nmol/liter for testosterone, 2.6% at 9.7 nmol/liter for A-diol-g, and 9.5% at 31 nmol/liter for SHBG. An estimate of the concentration of FT was derived from the known concentrations of testosterone and SHBG, based on the assumption that the albumin concentration was constant between individuals, using the formula based on the law of mass action (18).

Molecular Analyses. DNA was purified from 0.5-ml buffy coat samples of peripheral blood using Nucleon BACC2 kits according to the manufacturer’s instructions (Nucleon ST; Glasgow, Scotland, United Kingdom).

SRD5A2 (TA)_n Repeat Polymorphism. The (TA)_n marker was genotyped using PCR reactions containing the reverse primer (5’-GGCGCAAACCGCCACGAGAC-3’) and the forward primer (5’-GAAAACGTGAGGCTGTCG-3’). Cycling conditions were as follows: 95°C for 2 min; 35 cycles of 94°C for 1 min; 55°C for 1 min; and 72°C for 2 min. Amplification was performed in a PE Applied Biosystems GeneAmp PCR System 9700, and the number of (TA) repeats was determined using a Perkin-Elmer ABI Prism 310 genetic analyzer and running in parallel with a molecular weight DNA marker.

SRD5A2 A49T Polymorphism. The A49T mutation was screened using PCR amplification of radiosabeled exon 1 of the SRD5A2 gene (with the primers oNM16 GCAGCGCCAC-GGCG and oNM32 GTGGAAGTAATGTACGCAAGA), followed by single-stranded conformational polymorphism analysis to determine genotypes at the A49T locus, as reported previously (12). All of the samples were submitted in coded format for genotyping and included 5% of masked repeats.

Statistical Methods. Testosterone, FT, and A-diol-g were square-root transformed, and SHBG was natural-logarithmically transformed to approximate normal distributions, to perform multivariate ANOVA; back-transformed means and their corresponding 95% confidence intervals are presented. Multivariate ANOVA was used to evaluate the association between genotype and circulating hormone concentrations after adjusting for age (20–29, 30–39, 40–49, 50–59, 60–69, and 70+ years), time of day at venipuncture (<10:00, 10:00–13:29, and 13:30+), and time since last meal at venipuncture (<1.5, 1.5–3, and 3+ h). Adjustments for lifestyle factors such as body mass index, smoking, education, dietary group, and physical exercise were examined but did not affect the point estimates and were not included in the final model. Differences in adjusted mean hormone levels between the genotypes were evaluated using the (TA)_n/(TA)_0 genotype as the reference group for the (TA)_n polymorphism and the A/A genotype as the reference group for the A49T polymorphism. All of the Ps are derived from parametric tests of heterogeneity derived from ANOVA models and are taken from the F statistic that the underlying group means are all equal, unless otherwise stated. A P < 0.05 was considered statistically significant. A test for linear trend was also performed where appropriate, to assess statistical significance across genotypes by incorporating the categorical term in the model as a linear term. All of the statistical analyses were performed using Stata 7.0 (19).

Results

The mean age of the subjects was 47 years (range, 20–78 years), and the mean body mass index was 24.0 kg/m² (range, 17.5–48.0 kg/m²). Genotyping was successful for the (TA)_n polymorphism in 584 subjects and for the A49T polymorphism in 604 subjects. The genotype distribution of the (TA)_n polymorphism is shown in Table 1. The variant allele was either 103 bp or 105 bp (97%) in length and was, thus, considered to be a part of the (TA)_n family of alleles; no subject had repeat lengths >105 bp. The prevalence of the variant (TA)_n allele was 10.8%, and the proportion of homozygotes was 1.2%. The genotype distribution of the A49T polymorphism is shown in Table 2. The prevalence of the variant allele was 3.4%, and the proportion of homozygotes was 0.33%. Both genotype frequencies were in Hardy-Weinberg equilibrium (P = 0.997 and 0.979 for the (TA)_n and the A49T polymorphism, respectively).

The mean hormone concentrations in each (TA)_n genotype are also presented in Table 1. The (TA)_n allele was not significantly associated with testosterone, FT, A-diol-g, or SHBG concentrations. However, men who possessed the (TA)_n/(TA)_0 genotype had an 11% higher mean A-diol-g concentration than men who possessed the (TA)_n/(TA)_n genotype, although this was not statistically significant and was based on small numbers (P = 0.813). A test for linear trend of increasing A-diol-g levels across genotypes was also not statistically significant (P = 0.797).

The association between the A49T polymorphism and mean hormone concentrations is shown in Table 2. Individuals who possessed either one or two copies of the variant T allele...
had a 24% lower mean A-diol-g concentration than individuals who were homozygous for the wild-type A allele (test for heterogeneity; \( P = 0.0003 \)). Although based on very small numbers, there was also some evidence of a dose-response relationship; men who possessed the \( TT \) genotype had a 23% lower A-diol-g concentration than the \( AA \) genotype (\( P = 0.194 \)) and a 54% lower A-diol-g concentration than the \( A/A \) genotype (\( P = 0.034 \)). The \( T \) allele was not significantly associated with concentrations of any other hormone, although testosterone and SHBG concentration was 7.8% and 9.2% higher in men who had either one or two \( T \) alleles compared with men who had two \( A \) alleles (test for heterogeneity; \( P = 0.198 \) and 0.222 for testosterone and SHBG, respectively).

**Discussion**

This is the largest study to date to investigate the association between two common polymorphisms in the \( SRD5A2 \) gene and circulating androgen levels in men. To ensure the groups were as comparable as possible, hormone concentrations were adjusted for age and variables associated with blood collection and analysis. Furthermore, all of the hormone assays were conducted blind and in a randomly assorted order.

Our results suggest that the \( (TA)_n \) repeat polymorphism is not a strong determinant of circulating androgen levels. However, the proportion of men who are homozygous for the variant \( (TA)_n \) allele in our study and in other Caucasian populations is low, at between 1% and 2% (7, 8, 10), which limits the power to detect small effects. Nevertheless, our findings are consistent with a previous study in Asian men that also found no association between this polymorphism and circulating androgen levels (11) or an increased risk for prostate cancer in Caucasian men (8–10).

This is the first study to investigate the association between the \( A49T \) polymorphism and circulating androgen concentrations in men. Despite the low prevalence of the \( T \) allele in our population, which is consistent with other Caucasian populations (13), this polymorphism was a strong determinant of circulating A-diol-g levels. Men who possessed one or two copies of the variant \( T \) allele had a significant 24% lower A-diol-g concentration than men who were homozygous for the wild-type allele. These findings are unexpected because this polymorphism has been associated with a 5-fold increase in the \( V_{max} \) of the 5\( \alpha \)-reductase type II enzyme in vitro (12), suggesting this polymorphism might increase DHT production in vivo. Some epidemiological studies have also found the \( A49T \) polymorphism to be associated with an increased risk of advanced prostate cancer in African-American and Hispanic men (12), and with poor prognostic indicators in prostatic tumors (20). However, other studies have found no association between this polymorphism and prostate cancer risk in Caucasian men (9, 13, 15).

The association between the \( T \) allele and a lower mean A-diol-g concentration was highly statistically significant, and is therefore unlikely to be because of chance. Furthermore, a sub-sample of genotypes was confirmed via direct sequencing, and the mean hormone concentration in each genotype was adjusted for potential confounders. However, the use of serum A-diol-g concentration as a marker of the abundance of 5\( \alpha \)-reductase type II has limitations. This is because 5\( \alpha \)-reductase exists in two isoenzymes: 5\( \alpha \)-reductase type I, encoded by the \( SRD5A1 \) gene and which regulates DHT production in the skin, and 5\( \alpha \)-reductase type II, encoded by the \( SRD5A2 \) gene and which regulates DHT production in prostatic tissue (21). Therefore, the serum A-diol-g concentration is a marker of both cutaneous and intraprostatic DHT production. Little is known about the contribution of 5\( \alpha \)-reductase type I activity to the overall circulating level of A-diol-g, although the observation that specific inhibition of either 5\( \alpha \)-reductase type I (22) or type II (23) lowers serum A-diol-g concentrations suggests that both isoenzymes are important. Therefore, individual variations in type I activity will introduce error when using A-diol-g as an index of 5\( \alpha \)-reductase type II activity. However, it is unlikely that the reduction in A-diol-g concentration associated with the \( T \) allele is because of a larger reduction in type I activity relative to type II, as the two isoenzymes are encoded on separate chromosomes and, thus, contribute independent genetic sources of variation on A-diol-g levels. Of course, one cannot discount
the possibility that the T allele might be in linkage disequilibrium with a nearby SRD5A2 locus, that is itself related to 5 α-reductase type II activity and A-diol-g concentration.

In addition, interindividual variation in the activity of other enzymes involved in the production of A-diol-g may effect circulating A-diol-g levels, such as 3 α-hydroxysteroid dehydrogenase and 17 β-hydroxysteroid dehydrogenase, which convert DHT and dehydroepiandrosterone to A-diol-g, respectively. Furthermore, local concentrations of growth factors (24) and other androgens (24, 25) are also involved in the regulation of 5 α-reductase type II activity in vivo. Finally, circulating androgen levels are likely to be only weakly correlated with androgen levels within the prostate gland and can only provide a limited view of the complexity of physiological events that regulate 5 α-reductase type II activity. Future studies that measure DHT concentration within the prostate tissue itself are, therefore, warranted to investigate the effect of SRD5A2 polymorphisms on 5 α-reductase type II levels. However, despite such limitations, epidemiological data have shown A-diol-g levels to parallel ethnic differences in prostate cancer risk (4) and to be the only androgen to be consistently associated with increased prostate cancer risk (2). These observations strengthen the hypothesis that A-diol-g may reflect intraprostatic androgen activity and that increased 5 α-reductase type II activity is important in prostate cancer development.

In addition to genetic factors, it has been hypothesized that environmental factors such as diet may influence androgen levels, which may be partly mediated through changes in enzyme activity. Although this study population included men with different dietary habits, a previous analysis conducted in these men reported that serum concentrations of testosterone, FT, A-diol-g, SHBG, and luteinising hormone were similar between the meat-eaters, vegetarians, and vegans (17). In this present analysis, additional adjustment for diet group made no appreciable difference to the findings and is, therefore, unlikely to influence the association between genotype and hormone levels. Similarly, although the population was, on average, younger than the typical patient population with prostate cancer, there was no evidence that the effect of genotype on hormone levels varied with age, despite the age-related decline in androgen levels.

The results from this present study indicate that the SRD5A2 (TA)n repeat polymorphism is not significantly associated with circulating androgen levels in British men. The SRD5A2 A49T polymorphism is associated with a significantly lower A-diol-g concentration and, in the light of previous results from in vitro studies, additional large studies are needed to substantiate this finding.

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References

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