The Association between Polymorphisms in the *CYP17* and 5α-Reductase (*SRD5A2*) Genes and Serum Androgen Concentrations in Men[1]

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Abstract

Prospective studies suggest that prostate cancer risk may be increased in association with high serum concentrations of free testosterone and androstanediol glucuronide (A-diol-g). Polymorphisms have been identified in the 17-hydroxylase cytochrome P450 gene (*CYP17*) and the steroid 5α-reductase type II gene (*SRD5A2*), two genes that are involved in the biosynthesis and metabolism of androgens in men. The *CYP17 Msp*A1 I polymorphism has been associated with increased prostate cancer risk, and the *SRD5A2 V89L* polymorphism has been associated with low A-diol-g in Asian men, a serum marker of 5α-reductase activity. The purpose of this study was to investigate the association between these two polymorphisms and serum sex hormone concentrations in 621 British men. In particular, we wanted to test the hypotheses that the *A2* allele in the *CYP17* gene is associated with increased serum testosterone concentrations, and the *L* allele in the *SRD5A2* gene is associated with reduced A-diol-g concentrations. Mean hormone concentrations were evaluated in each genotype and adjusted for age and other relevant factors. We found no evidence that the *CYP17 Msp*A1 I polymorphism was associated with higher testosterone levels. The *L/L* genotype of the *SRD5A2* V89L polymorphism was associated with a 10% lower A-diol-g concentration, but this was not significant at the 5% level. However, the *L/L* genotype of the *V89L* polymorphism was associated with significantly lower concentrations of testosterone and free testosterone (by 12% and 16%, respectively) and an 8% higher sex hormone-binding globulin concentration. These results suggest that the *CYP17 Msp*A1 I polymorphism is not associated with testosterone concentrations and that the *SRD5A2 V89L* polymorphism is not a strong determinant of A-diol-g concentration in Caucasian men.

Introduction

Testosterone and its metabolite DHT[5] are crucial for the growth and development of the prostate gland (1). Prospective studies suggest that prostate cancer risk may be increased in association with high serum concentrations of bioavailable testosterone (2) and A-diol-g, a serum marker of 5α-reductase activity and intraprostatic DHT (3). Little is known about the determinants of circulating concentrations of sex hormones and their related proteins in men other than age, body mass index (4, 5), and ethnicity (6, 7). Genetic polymorphisms that encode for key enzymes involved in androgen biosynthesis and metabolism have been of much epidemiological interest in their relation to hormone-dependent cancer risk (8–11). However, their role in determining endogenous hormone concentrations has been little studied.

The cytochrome P450c17α (*CYP17*) gene, located on chromosome 10q24.3, codes for the cytochrome P450c17α enzyme, which catalyzes 17α-hydroxylase and 17,20-lyase activity at key points in steroid hormone biosynthesis in both sexes. A T to C transition has been described in the 5′ untranslated region that creates an additional Sp-1 type (CCACC) motif and a *Msp*A1 I restriction enzyme site (12). Although the effect of this base change on gene expression is unknown, the variant *C* allele (designated *A2*) might result in increased transcriptional activity and, hence, increased biosynthesis of testosterone (12). The *A2* allele has been associated with male pattern baldness in men and polycystic ovarian syndrome in women (12), conditions that are associated with high androgen concentrations. The *A2* allele has been associated with an increased prostate cancer risk in Caucasian men (9, 10), although not all of the studies have found this (11). The *A2* allele has also been associated with elevated levels of estradiol, an important conversion product of the P450c17α enzyme in women (13, 14), although the evidence that this polymorphism affects breast cancer risk is also conflicting (8, 14–16).

The 5α-reductase type II (*SRD5A2*) gene, located on chromosome 2p23, encodes the enzyme that catalyzes the irreversible conversion of testosterone to DHT within prostatic cells.

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[5] The abbreviations used are: DHT, dihydrotestosterone; A-diol-g, androstanediol glucuronide; EPIC, European Prospective Investigation into Cancer and Nutrition; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; FT, free testosterone; ANCOVA, analysis of covariance.

The *SRD5A2 V89L* polymorphism is caused by a G to C transversion that results in the substitution of valine for leucine at codon 89 (denoted the *L* allele). The distribution of the polymorphism appears to parallel prostate cancer risk between different ethnic groups, with Caucasian and African-American men having a low prevalence of the *L* allele (24% and 22%, respectively), compared with a prevalence of 46% among Asian men (17). The *L/L* genotype was associated with a significantly lower mean serum A-diol-g concentration within the Asian men, suggesting that the *L* allele may reduce 5α-reductase activity (17). However, this polymorphism has not been found to be significantly associated with prostate cancer risk among Caucasian men (9, 18).

This study aimed to investigate the association between these two genetic polymorphisms and serum concentrations of sex hormones and their related proteins in a large group of Caucasian men. In particular, we sought to test the hypotheses that the *CYP17 A2* allele is associated with elevated serum testosterone concentrations and that the *SRD5A2 L* allele is associated with decreased A-diol-g concentrations.

Materials and Methods

Subjects

This study is part of a larger investigation designed to investigate diet and hormonal function in men, described elsewhere (19). Briefly, 750 white male subjects were selected from the Oxford, United Kingdom component of the EPIC. These men were recruited throughout 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. Immunoassays were used to measure serum testosterone (Immuno 1; BayerCorp, New York, NY), A-diol-g (Diagnostic Systems, Webster, Texas), SHBG (Oy Medix Biochemica Ab, Kauniainen, Finland), and LH (Technicon Immuno 1; BayerCorp) in the Clinical Biochemistry Laboratory at the John Radcliffe Hospital, Oxford, United Kingdom in 1998. Samples were randomly assorted 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, 9.5% at 31 nmol/liter for SHBG, and 1.7% at 3.7 IU/liter for LH. An estimate of the concentration of FT was derived from the known concentrations of testosterone and SHBG and the assumption that albumin is constant between individuals, using the formula based on the law of mass action (20, 21).

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).

***CYP17* Assay.** PCR amplification of the polymorphic fragment was performed using the forward primer 5′-CATTCGCACCTCTGGAGTC-3′ and the reverse primer 5′-GGCTCTTGGGGTACTTG-3′(8). PCR reactions were carried out in 15-μl aliquots containing 50 ng of genomic DNA, 1 μM of each primer, 1 × Perkin-Elmer Buffer II (PE Biosystems) reaction buffer, 1.5 mM $MgCl_2$, 0.4 mM deoxynucleotide triphosphates, and 0.75 units of AmpliTaq Gold polymerase (PE Biosystems). Amplification conditions were 95°C for 10 min, followed by 30 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 1 min, and a final step at 72°C for 10 min on PE 9700 thermocyclers (PE Biosystems). Products were digested using 1 unit of *Msp*A1 I (New England Biolabs) in 20 μl of 1 × NEBuffer 4 (New England Biolabs) and 1 × BSA at 37°C for 3 h. Products were separated by electrophoresis on 1.5% agarose gels and stained with ethidium bromide to detect the RFLP. Quality control samples were inserted to validate genotype identification procedures.

***SRD5A2* Assay.** PCR reactions were performed in 15-μl aliquots containing 50 ng of genomic DNA, 1 × PE Buffer II (Perkin-Elmer Biosystems), 1.5 mM $MgCl_2$, 0.8 mM deoxynucleotide triphosphate (total), 5% DMSO, and 0.2 μM of each primer in 15 μl of water. PCR forward primer *SRD5A2*415 (TCCAGAAGTTGCCGCATCAG) and reverse primer *SRD5A2*039 (CGGTGCGCGCTCCAC) were used (9). Cycle conditions were 95°C for 10 min, followed by 40 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 30 s, and a final step at 72°C for 10 min on PE 9700 thermocyclers. Products were then digested with 1 unit of *Rsa*I restriction enzyme (New England Biolabs) in 1 × NEBuffer 1 and 1 × BSA in 17-μl volumes overnight at 37°C. Products were separated by electrophoresis on 2% agarose gels and stained with ethidium bromide to detect the RFLPs. Quality control samples were inserted to validate genotype identification procedures.

Statistical Methods

Testosterone, FT, and A-diol-g were square-root transformed, and SHBG and LH were natural-logarithmically transformed to approximate normal distributions to perform ANCOVA. None of the transformed variables was statistically significantly different from a normal distribution at the 5% level according to the Shapiro-Wilk test (*P*s were 0.237, 0.069, 0.179, 0.104, and 0.083 for testosterone, FT, A-diol-g, SHBG, and LH, respectively). Back-transformed means and their corresponding 95% confidence intervals are presented. ANCOVA was used to evaluate the association between genotype and circulating hormone concentrations after adjusting for age, time of day at venipuncture, time since last eaten at venipuncture, and time between venipuncture and blood processing. Adjustments for lifestyle factors such as body mass index, smoking, education, dietary group, and exercise were examined but did not effect the point estimates and were not included in the final model. Differences in adjusted mean hormone levels between the genotypes were evaluated using *A1/A1* genotype as the reference group for *CYP17* and *V/V* as the reference group for *SRD5A2*. All of the *P*s are derived from parametric tests of heterogeneity derived from ANCOVA models and are taken from the F statistic that all of the underlying group means are equal, unless otherwise stated. A *P* of less than 0.05 was considered statistically significant, and all of the significance tests were 2-sided. 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 5.0 (22).

Table 1 Adjusted[a] mean hormone concentrations by CYP17 *Msp*A1 I and SRD5A2 V89L polymorphisms in 622 British men

	CYP17 gene				*SRD5A2* gene			
	A1/A1 n = 266	*A1/A2* *n* = 273	*A2/A2* *n* = 83	*P*[b]	*V/V* *n* = 317	*V/L* *n* = 245	*L/L* *n* = 49	*P*[b]
Testosterone (nmol/liter)	20.2 (19.4–21.1)	21.0 (20.1–21.9)	20.6 (19.1–22.2)	0.446	21.2 (20.4–22.0)[c]	20.2 (19.4–21.1)	18.7 (16.8–20.6)[c]	0.037
FT (nmol/liter)	0.43 (0.41–0.44)	0.43 (0.42–0.45)	0.42 (0.39–0.44)	0.477	0.44 (0.42–0.45)[d]	0.43 (0.42–0.45)[c]	0.37 (0.34–0.40)[d,e]	0.001
A-diol-g (nmol/liter)	8.90 (8.46–9.36)	8.74 (8.31–9.19)	8.13 (7.36–8.93)	0.256	8.82 (8.41–9.24)	8.74 (8.27–9.21)	7.96 (6.99–9.00)	0.313
SHBG (nmol/liter)	44.3 (42.1–46.7)	46.3 (44.0–48.7)	49.3 (45.0–54.2)	0.128	46.6 (44.5–48.9)	44.3 (42.0–46.7)[c]	50.7 (45.0–57.1)[e]	0.093
LH (IU/liter)	5.34 (5.04–5.66)	5.06 (4.79–5.36)	5.50 (4.96–6.10)	0.266	5.39 (5.11–5.68)	5.29 (4.98–5.62)	5.16 (4.51–5.91)	0.809

[a] Values are adjusted for age (in categories of 20–29, 30–39, 40–49, 50–59, 60–69, 70+), time of day at venipuncture (<10, 10–13.29, 13.30+ h), time since last meal at venipuncture (<1.5, 1.5–<3, 3+ h), and time between blood draw and processing (1, 2, 3, 4+ days).
[b] *P* is test for heterogeneity across the mean hormone concentrations in each genotype (see text).
[c] Test of heterogeneity between two means; where two superscripts are the same, *V/V* genotype is different from *V/L* genotype at $P < 0.05$.
[d] Test of heterogeneity between two means; where two superscripts are the same, *V/V* genotype is different from *L/L* genotype at $P < 0.05$.
[e] Test of heterogeneity between two means; where two superscripts are the same, *V/L* genotype is different from *L/L* genotype at $P < 0.05$.

Results

The mean age of the subjects was 47 years (range, 20 to 78 years), and the mean body mass index was 24.0 (range, 17.5 to 48.0). One subject was identified as having a nonsense mutation in the *SRD5A2* gene c.309-319delGGGACGGTACT, and further examination revealed substantially lower testosterone, FT, and A-diol-g concentration compared with mean values. Therefore, this subject was excluded from both analyses. Genotypes were determined for the *CYP17 Msp*A1 I polymorphism in 621 subjects and for the *SRD5A2 V89L* polymorphism in 611 subjects. The distributions of the *CYP17* and *SRD5A2* genotypes are shown in Table 1. Both genotype frequencies were in Hardy-Weinberg equilibrium, with expected frequencies for the *CYP17* genotypes being *A1/A1* = 259, *A1/A2* = 284, and *A2/A2* = 78 ($P = 0.80$); and for the *SRD5A2* genotypes, *V/V* = 316, *V/L* = 247, and *L/L* = 48 ($P = 0.99$). The prevalences of the *CYP17 A2* allele and *SRD5A2 L* allele were 35% and 28%, respectively, and the proportions of homozygotes were 13% for the variant *A2* allele in the *CYP17* polymorphism and 8% for the variant *L* allele in the *SRD5A2* polymorphism.

The *CYP17* polymorphism was not significantly associated with testosterone concentration (test for heterogeneity; $P = 0.461$), and possession of this polymorphism had no significant effect on the other hormones studied (Table 1). A-diol-g concentration was not significantly associated with the *SRD5A2* polymorphism (test for heterogeneity; $P = 0.313$), although the mean A-diol-g concentration in the *L/L* genotype was 10% lower than in the *V/V* genotype ($P = 0.129$). A test for linear trend of decreasing A-diol-g levels across genotypes was also not statistically significant ($P = 0.230$). However, the *L/L* genotype was associated with a significant 12% reduction in testosterone concentration and a 16% reduction in FT concentration compared with the *V/V* genotype (test for heterogeneity; $P = 0.037$ and 0.001, respectively). The mean SHBG concentrations were not significantly different between genotypes (test for heterogeneity; $P = 0.093$), although the *L/L* genotype had a significant 13% higher mean SHBG concentration compared with the *V/L* genotype ($P = 0.044$). However, the 8% difference in mean SHBG concentration between the *V/V* and the *L/L* genotypes was not significant.

Discussion

Testosterone and its metabolite DHT play a critical role in promoting prostate growth, and the identification of genetically determined differences in androgen metabolism may be important in explaining some of the observed differences in prostate cancer risk between individuals (9) and populations (6, 17).

The sample size was large, but the prevalence of the homozygotes for the variant alleles was low (13% and 8%), which may have led to insufficient power to detect a significant difference in mean hormone concentrations between genotypes. Hormone measurements were taken from a single sample for each man, and although a single measure of testosterone has been shown to reliably reflect mean annual testosterone concentrations in middle-aged men (23), little is known about the long-term reliability of a single serum measurement for other hormones. To ensure that the groups were as comparable as possible, hormone concentrations were adjusted for potential confounding variables including age, time of day at venipuncture, time since last eaten at venipuncture, and time between venipuncture and blood processing. Furthermore, all of the hormone assays were conducted blinded and in randomly assorted order.

This is the first study to report on the association between the *CYP17 Msp*A1 I polymorphism and testosterone concentrations in men. As yet, there is no knowledge whether the *A2* allele confers a higher expression of enzyme activity *in vivo*, although the fact that it does not appear to bind to the transcription site, Sp-1, in an *in vitro* assay (24) suggests that this polymorphism may not have any functional effect on enzyme activity. Nevertheless, the variant *A2* allele has been associated with elevated concentrations of estradiol among premenopausal (13) and postmenopausal women (16). To date, two case-control studies have found a small but significant increased risk of prostate cancer associated with the *A2* allele among Caucasian men (9, 10), although a study in Sweden found an increased risk associated with the *A1* allele (11). The results from this present study suggest that this polymorphism is not associated with serum testosterone concentrations in men.

Our findings suggest that the *SRD5A2 V89L* polymorphism is not a strong determinant of serum 5α-reductase activity. However, subjects with the *L/L* genotype had a nonsignificant 10% lower mean A-diol-g concentration compared with men with the *V/V* genotype. This finding is very similar to that reported among 386 Caucasian men (18) and is consistent with the hypothesis that the variant allele may reduce 5α-reductase activity. However, there was limited power to detect a small effect on A-diol-g levels as the prevalence of the *L* allele in this study and other Caucasian populations is low (9, 17, 18). The effect of this polymorphism on A-diol-g levels, therefore, needs to be investigated with a larger sample size or in a population where the prevalence of the *L* allele is higher, such as among Asian men (17). Indeed, small differences in serum A-diol-g of 5% (ratio, 1.05; 95% confidence interval, 1.00–

1.11) have been found between 644 men who subsequently developed prostate cancer and 1048 healthy individuals in a meta-analysis of prospective studies on sex hormones and prostate cancer (3). If small differences in serum A-diol-g levels reflect larger differences in intraprostatic androgen activity, then these genetic differences may be of biological relevance to prostate cancer risk. A large prospective study found the *L/L* genotype to be associated with a 16% nonsignificant reduction in prostate cancer risk (18), although a case-control study found no association with the *L/L* genotype and a small increase in prostate cancer risk with the *L* allele alone among Caucasian men (9).

The reason why a stronger genetic effect has been observed among Asian men compared with Caucasian men is not clear; it may be attributable to chance, or the *V89L* polymorphism may be in linkage disequilibrium with a locus involved in androgen metabolism that only exists among Asian populations. Because the *V89L*, $(TA)_n$, and *A49T* polymorphisms identified in the *SRD5A2* have been shown to vary across racial/ethnic groups (17, 25, 26), this may be a possibility. However, recent data suggest a significant increased risk of early-onset prostate cancer for individuals homozygous for the *V89L* leucine variant and no association with the $(TA)_n$ or the *A49T* polymorphisms.[6] Additional work is needed to establish whether these and other polymorphisms in the *SRD5A2* gene are associated with circulating sex hormone concentrations.

Our finding that the *SRD5A2 L/L* genotype was associated with significantly lower testosterone and FT concentrations was unexpected and not an *a priori* hypothesis. Indeed, one might expect serum testosterone concentration to increase with the *V89L* variant because trials of finasteride, a chemical inhibitor of 5α-reductase, have generally found increased serum concentrations of testosterone (27, 28), together with substantial reductions in serum A-diol-g concentrations (29). Interestingly, Makridakis *et al.* (17) found no association between the *V89L* polymorphism and testosterone concentration among Asian men. These unexpected findings should, therefore, be interpreted with caution, especially considering that the effects were small, the *P*s for the tests of heterogeneity were of marginal significance, and there is no clear biological mechanism through which a genotype associated with decreased DHT production within the prostate would lead to decreased serum levels of total testosterone and FT. These findings should, therefore, be interpreted with extreme caution and need to be confirmed in other studies.

In summary, these findings suggest that the *Msp*A1 I polymorphism in the *CYP17* gene is not associated with testosterone concentrations and that the *V89L* polymorphism in the *SRD5A2* gene is not a strong determinant of A-diol-g concentration in Caucasian men.

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