Int. J. Cancer: **120,** 776–780 (2006)
© 2006 Wiley-Liss, Inc.

5α-Reductase type 2 gene variant associations with prostate cancer risk, circulating hormone levels and androgenetic alopecia

Vanessa M. Hayes[1,2*#], Gianluca Severi[3,4*], Emma J.D. Padilla[1], Howard A. Morris[5], Wayne D. Tilley[5,6], Melissa C. Southey[7,8], Dallas R. English[3,4], Robert L. Sutherland[1,2], John L. Hopper[4], Peter Boyle[8] and Graham G. Giles[3,4]

[1]*Cancer Research Program, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, Australia*
[2]*University of New South Wales, Sydney, Australia*
[3]*Cancer Epidemiology Centre, Cancer Council of Victoria, Melbourne, Australia*
[4]*Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Melbourne, Australia*
[5]*Hanson Institute, Adelaide, Australia*
[6]*Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide, Adelaide, Australia*
[7]*Department of Pathology, University of Melbourne, Melbourne, Australia*
[8]*International Agency for Research on Cancer, Lyon, France*

Controversy exists over the significance of associations between the *SRD5A2* (5α-reductase type 2) polymorphisms, A49T and V89L, and risk of prostate cancer. These potentially functional polymorphisms may alter life-long exposure to androgens with subsequent effects on male health and aging. The aim of this study was to examine the association of these variants with prostate cancer risk, plasma hormone levels and androgenetic alopecia. Subjects include 827 cases and 736 controls from an Australian population-based case–control study of prostate cancer. Information on prostate cancer risk factors and patterns of balding were collected. Plasma levels of testosterone, 3α-diol glucuronide (3α-diolG), dehydroepiandrosterone sulfate, androstenedione, sex hormone-binding globulin and estradiol were measured for controls. No associations with the V89L polymorphism were found. Carriers of the rarer A49T A allele were at a 60% higher risk of prostate cancer (OR = 1.60; 95% CI 1.09–2.36; $p = 0.02$) and 50% lower risk of vertex and frontal balding ($p = 0.03$) compared with men homozygous for the more common G allele. Although we found little evidence of association between this variant and plasma levels of 5 measured androgens, circulating 3α-diolG levels were 34% lower in A49T A allele carriers ($p < 0.0001$). Our study provides evidence that the *SRD5A2* A49T A variant is associated with an increased risk of prostate cancer, lower levels of circulating 3α-diolG and decreased risk of baldness. These findings raise important questions with respect to previous assumptions concerning hormonal influences on prostate cancer risk in ageing males.
© *2006 Wiley-Liss, Inc.*

Key words: *SRD5A2*; polymorphisms; prostate cancer risk; population-based case-control study; plasma androgens; androgenetic alopecia

Membrane-bound 5α-reductase type 2 (SRD5A2) is responsible for the irreversible conversion of testosterone into its more active metabolite, dihydrotestosterone (DHT) and is essential for the normal growth and development of the prostate gland.[1] This hormone-regulator has been implicated in male pathophysiology, including prostate cancer and balding (androgenetic alopecia). The ratio of DHT to testosterone has been reported to be highest for African–Americans, intermediate for Europeans and lowest for Asian–Americans, which corresponds to reported ethnic-based risk of prostate cancer for these groups, suggesting an association with prostate cancer risk.[2] Prostate cancer risk has been associated with vertex pattern balding.[3] Increased levels of DHT have been demonstrated in the male balding scalp,[4] while lack of balding is seen in pseudohermaphrodites (men with congenital 5α reductase deficiency) with a concomitant reduced ability to convert testosterone to DHT.[5] DHT in turn is metabolized to 5α-androstane-3α-17β-diol glucuronide (3α-diolG), a predictor of both cutaneous and intraprostatic serum 5α-reductase levels.[6] These observations support the "androgen hypothesis" that increases in active androgens (and their metabolites) are associated with increased prostate cancer risk and male patterned balding. However, only one prospective epidemiological study has been able to demonstrate such an association.[7] Two recent studies have challenged this hypothesis, reporting an association between increased risk of aggressive prostate cancer and reduced circulating levels of testosterone.[8,9] In addition, we have argued that the observed increase in prostate cancer incidence with increased age may be related to the androcline age associated decreasing levels of androgens.[9]

SRD5A2, is encoded by the *SRD5A2* gene located at band p23 on chromosome 2.[10] Two single nucleotide polymorphisms (SNPs) identified within the coding region of *SRD5A2*, rs9282858 (G>A) and rs523349 (G>C), have been implicated in influencing prostate cancer risk *via* altering androgen levels. The alanine to threonine amino acid change at codon 49 (A49T, GCC to ACC) increases 5α-reductase activity 5-fold *in vitro*,[11] while the valine to leucine substitution at codon 89 (V89L, GTA to CTA) results in an almost 30% reduction in enzyme activity both *in vitro* and *in vivo*.[12] The commonly used amino acid substitution nomenclature, A49T and V89L, will be used to identify these genetic markers in this study.

Molecular epidemiological studies of these dimorphic markers have reported inconsistent findings with respect to their role in prostate cancer risk and/or clinicopathological behavior. A meta-analysis has reported a modest effect of the A49T polymorphism on prostate cancer risk, but no role for V89L.[13] Reports of associations between the A49T and V89L polymorphisms and plasma androgen levels, including testosterone and 3α-diolG, have been limited.[14–17] A single study has reported a null association between the V89L variant and balding patterns in European men.[18]

To further examine associations between these two well-defined polymorphisms and prostate cancer risk, plasma hormone levels

Abbreviations: 3α-diolG, 3α-diol glucuronide; 3β-diolG, 3β-diol glucuronide; CI, confidence interval; DHEAS, dehydroepiandrosterone-sulfate; DHT, dihydrotestosterone; E2, estradiol; H–N, Hamilton–Norwood; H–W, Hardy–Weinberg; LD, linkage disequilibrium; OR, odds ratio; RFPC, Risk Factors for Prostate Cancer; SRD5A1, 5α-reductase type 1; SRD5A2, 5α-reductase type 2; SHBG, sex hormone-binding globulin.

Grant sponsors: National Health and Medical Research Council (grant numbers: 251533, 940394, 991129, 299955, 396407); Cancer Institute of New South Wales; Tattersall Family Foundation; Whitten Foundation; Armati Family Foundation, Australia; and BNP Paribas Foundation, Australia and France. Infrastructure was provided by the Australian Cancer Research Foundation Unit for the Molecular Genetics of Cancer and The Cancer Council Victoria.

*These authors contributed equally to the manuscript.

#**Correspondence to:** Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia. Fax: +61-2-92958321. E-mail: v.hayes@garvan.org.au

Received 1 August 2006; Accepted after revision 12 September 2006
DOI 10.1002/ijc.22408
Published online 29 November 2006 in Wiley InterScience (www.interscience.wiley.com).

and androgenetic alopecia, we genotyped the A49T and V89L variants in a large population-based case–control study of Australian men (827 cases, 736 controls).

Subject and methods

Study population

Subjects were participants in the Melbourne and Perth arms of the Risk Factors for Prostate Cancer (RFPC) study, an Australian population-based case–control study of prostate cancer conducted between 1994 and 1998 and described in detail elsewhere.[19,20] The study focus was the prevention of prostate cancers likely to contribute to premature mortality and, consequently, recruitment was restricted to men with tumors diagnosed at an early age and of more aggressive histopathology. To this end, tumors that were well-differentiated and those with Gleason scores less than 5 were excluded. Eligible cases with histopathologically-confirmed adenocarcinoma of the prostate diagnosed before age 70 years were ascertained from the Cancer Registries of Victoria and Western Australia. Random samples of 100%, 50% and 25%, respectively, of the cases diagnosed in the age groups younger than 60 years, 60–64 years and 65–69 years were asked to participate in the study. Eligible controls were randomly selected from males on the State Electoral Rolls (registration to vote is compulsory for adult Australian citizens) and were frequency-matched to the expected age distribution of the prostate cancer cases in a ratio of 1 control per case. A total of 1,047 cases and 1,058 controls participated in the study (65% and 50%, respectively, of those eligible).[3] A face-to-face interview was conducted using structured questionnaires to obtain information on potential risk factors including age, history of prostate cancer in first-degree relatives, country of birth, life-style (including diet) and other potential risk factors for prostate cancer. The interviewer also scored the subject's androgenetic alopecia according to an adapted Hamilton–Norwood (H–N) scale,[21] as no balding (H–N stages I and II), frontal balding (H–N stages II, III, IIIa and IVa), vertex balding (H–N stage III vertex-V) and frontal baldness concurrent with vertex baldness (H–N stage IV, V, Va, VI and VII).[3] Tumor stage (stage I to IV according to the American Joint Committee on Cancer 2002) and grade (moderate, Gleason 5–7 or moderately differentiated; high, Gleason 8–10 or poorly differentiated) was recorded from histopathology reports. Informed consent was obtained from all study participants. Blood samples were available from 831 cases (79% of participants) and 738 controls (70%). A detailed description of participant characteristics has been published.[22] The study was approved by the Human Research Ethics Committee of the Cancer Council of Victoria (HREC 9500).

Plasma hormone levels

Plasma samples of controls only were sent to the laboratory of one of us (HAM) in randomly assigned batches of around 80 samples each. The laboratory was blinded to the status of the samples (genotype). One scientist performed all measurements with 10% sample pooling per batch for quality control purposes. Plasma dehydroepiandrosterone-sulfate (DHEAS) and sex hormone-binding globulin (SHBG) levels were measured using a competitive immunometric assay (IMMULITE analyzer, DPC, CA). The inter-assay coefficient of variation (CV) was 12.4% at 2.1 μmol/l for DHEAS and 6% at 26 nmol/l for SHBG. Testosterone and estradiol (E2) levels were measured using electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). The CV was 1.6% at 36 nmol/l for testosterone and 11.1% at 93 pmol/l for E2. Plasma androstenedione and 3α-diolG levels were measured using a radioimmunoassay (DSL-4200 and DSL-6000 respectively, TX). The CV was 10.7% at 3.3 nmol/l for A and 4.3% at 21.1 nmol/l for 3α-diolG.

Genotyping

Genomic DNA was extracted from whole blood and genotyped in a blinded manner for the A49T (rs9282858) and V89L (rs523349) *SRD5A2* variants. Genotyping was performed using the Sequenom MassARRAY™ Compact system (Sequenom, San Diego, CA), using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. PCR and extension primer sequences were designed using Sequenom RealSNP (www.RealSNP.com). PCR was performed in duplex in 384-well plates with a final volume of 5 μl containing 2.5 ng of DNA, 10× Qiagen HotStar Taq PCR buffer, 25 mM $MgCl_2$, 25 mM dNTPs, 200 nM of each of the 4 PCR primers (primer sequences available upon request) and 0.15 U Qiagen HotStar Taq Polymerase. PCR cycling was executed using an Eppendorf Mastercycler, under the following conditions: 95°C for 15 min, 45 cycles of 95°C for 20 sec, 56°C for 30 sec and 72°C for 1 min, followed by a final extension step of 3 min at 72°C. The MassEXTEND reaction was performed using the 1× ACT termination mix (ddATP, ddCTP, ddTTP, dGTP), 600 nM of each extension primer (primer sequences available upon request) and 0.063 U of Thermosequenase. The cycling conditions were as follows: 94°C for 2 min, 40 cycles of 94°C for 5 sec, 52°C for 5 sec and 72°C for 5 sec. To assess reliability of the genotyping, 31% of the study samples were randomly regenotyped for both A49T and V89L with a concordance rate of 100% and 99.4%, respectively.

Statistical analysis

Estimates of allele/genotype frequencies and tests of deviation from Hardy–Weinberg (H–W) equilibrium were carried out using standard procedures based on asymptotic likelihood theory.[23] Linkage disequilibrium (LD) between the 2 variants was assessed by using Lewontin's D', and tests for significance were based on asymptotic likelihood theory.[24] Fisher's exact test was used to test for independence between the SNPs and categorized risk factors, namely, age (<55, 55–64, 65–69), country of birth (Australia, others), family history of prostate cancer (affected first-degree relatives, no affected relatives) and tumor stage (stage I to IV) and grade (moderate and high). Tests for association between genotypes and the various outcome of interest (*i.e.*, prostate cancer risk, circulating hormone levels and androgenetic alopecia) were performed under codominant, dominant and recessive models. Case–control analyses were conducted using unconditional logistic regression and odds ratio (OR) estimates and their 95% confidence intervals (CI) were derived under likelihood theory.[25] Adjustment for country of birth, age, history of smoking, history of prostate cancer in first-degree relatives (family history), body mass index (BMI) and alcohol consumption did not materially change the OR estimates from the logistic models. Polytomous logistic regression models were used to estimate ORs by tumor stage (dependent variable with 3 categories: 0, 1 and 2 for controls, stage I–II and stage III–IV tumors, respectively) and grade (dependent variable with 3 categories: 0, 1 and 2 for controls, moderate-grade and high-grade tumors, respectively).

As plasma levels of testosterone, 3α-diolG, DHEAS, androstenedione, SHBG and E2 were skewed, linear regression of the transformed levels to test the possible association with genotypes was used. Levels of 3α-diolG and E2 were log-10 transformed, while the others were square-root transformed. The linear regression models were adjusted for age and laboratory assay and were fitted using all the controls. Results are presented as adjusted back-transformed means and their corresponding 95% CI derived from the fitted regression models. These statistical analyses were performed using Stata/SE 8.2 (Stata Corporation, College Station, TX).

The association between genotype and androgenetic alopecia was tested using a maximum-likelihood multinomial (polytomous) logistic regression model (mlogit function in Stata/SE 8.2). The model, with alopecia as outcome, was fitted adjusting for age and including an indicator variable to adjust for the case/control status, because we previously found in this study an association between alopecia and prostate cancer.[3] Finally, an interaction term was added to test whether the effect of genotype in cases differed from the effect in controls. Two separate models were fitted for the 2 variants.

TABLE I – SRD5A2 GENE POLYMORPHISMS IN CONTROLS AND PROSTATE CANCER CASES

	Controls (%) $n = 736$[1]	Cases (%) $n = 827$[1]	OR[2] (95% CI)	p-value[3]
A49T				
GG	691 (94)	752 (91)	Reference	
GA	43 (6)	75 (9)	1.60 (1.09–2.36)	0.02
	$P_{H\text{-}W} > 0.9$	$P_{H\text{-}W} = 0.4$		
V89L				
GG	360 (49)	393 (48)	Reference	0.5
GC	301 (41)	359 (43)	1.09 (0.89–1.35)	
CC	75 (10)	74 (9)	0.90 (0.64–1.28)	
	$P_{H\text{-}W} = 0.3$	$P_{H\text{-}W} = 0.6$		

[1]Number of subjects with at least one of the two variants measured. –[2]Odds ratios and 95% confidence intervals from unconditional logistic regression analysis.–[3]Test for association between genotype and prostate cancer risk (likelihood ratio test).

The likelihood ratio test was used to test nested hypotheses and the Wald test to assess statistical significance of individual parameters. All tests were two-sided. Following convention, nominal statistical significance was based on $p < 0.05$. No attempt was made to adjust for multiple comparisons.

Results

Genotyping of both A49T and V89L variants was successful in over 99% of the samples yielding 827 cases and 736 controls with at least 1 of the 2 variants determined (826 cases and 734 controls with both variants determined, Table I). Only 5 cases (<1%) and 7 controls (~1%) were not European in origin, with the great majority of subjects (98.5%) being born in Australia, the British Isles, or Western Europe. Median age at diagnosis for the cases was 62 years. The proportion of cases that were diagnosed at age <55, 55–64 and 65–69 was 14% ($n = 113$), 52% ($n = 435$) and 34% ($n = 279$), respectively. Two hundred and fifty-four cases (31%) had a stage III or stage IV tumor and 223 (27%) were poorly differentiated or had a Gleason score 8 or higher.

The distribution of the genotypes was consistent with H–W equilibrium for both loci in cases and controls, and for cases and controls combined (all $p > 0.2$). The 2 loci were in strong LD and D' was 0.74 for controls ($p = 0.001$), 0.99 for cases ($p < 0.0001$) and 0.92 for cases and controls combined ($p < 0.0001$). There was no association between either genotype and age, or country of birth or family history of prostate cancer (all $p > 0.05$).

Prostate cancer risk

The frequency of the A allele in A49T (G > A) was 4.5% for cases and 2.9% for controls, with no subjects homozygous AA (Table I). The proportion of carriers of the GA genotype was slightly higher in cases (9%) than for controls (6%, $p = 0.02$) and the corresponding OR was 1.60 (95% CI 1.09–2.36). The proportion of GA carriers was similar for stage I–II (47/569, 8%) and stage III–IV (28/254, 11%) tumors ($p = 0.2$; OR 1.38, 95% CI 0.84–2.25). There was no difference in the genotype distribution by tumor grade, with the portion of GA carriers being similar for both moderate 56/604 (9%) and high 19/223 (8.5%) Gleason grades ($p = 0.7$; OR 0.91, 95% CI 0.53–1.57).

The frequency of the C allele in V89L (G>C) was 31% for both cases and controls (Table I). There was no evidence of association between genotype and prostate cancer risk as the OR was very close to 1 ($p = 0.5$). There was no significant difference in the frequency of the C allele (both 31%) or genotype distribution for tumor stage I–II (8% CC, 45% GC) *versus* stage III–IV (10% CC, 41% GC) or for moderate (8% CC, 45% GC) *versus* high (12% CC, 39% GC) tumor grade (both $p > 0.1$).

Hormone levels

There was no association between the A49T variant and levels of testosterone, androstenedione, SHBG or E2 (all $p \geq 0.18$, Table II). Circulating levels of 3α-diolG for carriers of the GA genotype (adjusted mean = 9.2 nmol/l) were 34% lower than for carriers of the GG genotype (14.0 nmol/l, $p < 0.0001$). Similarly, DHEAS levels for carriers of the GA genotype (2.17 μmol/l) were lower than for carriers of the GG genotype (2.61 μmol/l), but the difference was only marginally significant ($p = 0.05$). We found no evidence of association between V89L and the levels of any analyte measured (all $p \geq 0.1$).

Androgenetic alopecia

For A49T the model including the interaction between genotype and case–control status showed that the ORs for baldness in controls were lower than the corresponding ORs for cases. The ORs for vertex baldness and for vertex and frontal baldness combined were significantly lower than one for controls but not for cases. However, the statistical evidence of an interaction between A49T and case/control status was only marginal ($p = 0.03$) and there is no obvious reason why the association between genotype and baldness would be dependent on case/control status. In Table III, we present the ORs for cases and controls combined. We found marginal evidence of an association between A49T and alopecia ($p = 0.04$) with carriers of the GA allele being at lower risk of vertex baldness (OR 0.56, 95% CI 0.31–1.01) and vertex and frontal baldness (OR 0.52, 95% CI 0.29–0.94).

Although we found little evidence of an overall association between V89L and alopecia ($p = 0.1$ and 0.3 from the codominant and recessive models, respectively), the ORs associated with the CC genotype compared with the GG genotype were all higher than 1 and were statistically significant for vertex baldness (OR 1.92, 95% CI 1.04–3.54) and vertex and frontal baldness combined (OR 1.98, 95% CI 1.08–3.64). We did not find statistical evidence of different ORs between cases and controls (P for interaction between V89L and case/control status = 0.05).

Discussion

Our study suggests that carriers of the *SRD5A2* A49T variant have a 34% lower circulating level of 3α-diolG, a 60% higher risk of developing prostate cancer and a 50% lower risk of alopecia than men homozygous for the more common variant. We found no association between V89L and risk of prostate cancer, baldness or circulating hormone levels.

Strengths of our study include its large sample size and detailed data on tumor stage and grade and risk factors for prostate cancer including the direct assessment of the baldness status of participants. Another strength of our study is the evaluation of hormone levels in controls to test whether the genetic variants in *SRD5A2* modulate circulating hormone levels, thus facilitating interpretation of associations between the *SRD5A2* gene variants and risk of prostate cancer, and androgenetic alopecia. A major limitation of our study is its retrospective nature (case–control design) precluding meaningful testing of associations between circulating hormone levels and prostate cancer.

Although a meta-analysis,[13] and a large ($n = 2,216$) multiethnic population case-control study,[26] failed to support an increased risk conferred by the V89L polymorphism, studies since 2003 continue to report associations between this variant and prostate cancer risk in European populations.[27–30] In our study, we confirm a lack of association between V89L and risk of prostate cancer. The meta-analysis could not exclude the possibility of an association between the A49T polymorphism and risk of prostate cancer, as confirmed in our study.[13] The discrepancies between studies may be due to the relatively low frequency of the A49T risk allele in European populations (3% allele frequency/6% genotype frequency, controls); thus, many small studies will have lacked adequate statistical power to address this question.

The effect of these polymorphic markers on prostate cancer incidence and clinicopathological behavior has been investigated. An increase in the risk of more aggressive disease[27] and metastatic

TABLE II – HORMONE LEVELS[1] BY GENOTYPE IN THE CONTROLS OF THE RFPC STUDY

	Controls $n = 736$[2]	T (nmol/l)	3α-diolG (nmol/l)	DHEAS (μmol/l)	A (nmol/l)	SHBG (nmol/l)	E2 (pmol/l)
A49T							
GG	691	12.6 (12.3–13.0)	14.0 (13.4–14.6)	2.61 (2.50–2.73)	2.31 (2.22–2.40)	29.3 (28.3–30.3)	90.2 (88.5–92.0)
GA	43	13.5 (12.0–15.0)	9.2 (7.8–10.8)	2.17 (1.78–2.61)	2.06 (1.74–2.42)	28.4 (24.5–32.6)	93.8 (86.7–101.4)
p-value[3]		0.29	<0.0001	0.05	0.18	0.66	0.35
V89L							
GG	360	12.5 (12.0–13.0)	13.7 (13.0–14.5)	2.58 (2.42–2.74)	2.27 (2.14–2.39)	28.7 (27.4–30.1)	90.8 (88.3–93.4)
GC	301	12.9 (12.4–13.5)	14.0 (13.2–14.9)	2.61 (2.44–2.79)	2.32 (2.19–2.46)	29.5 (27.9–31.0)	90.2 (87.5–92.9)
CC	75	12.9 (11.8–14.0)	12.1 (10.7–13.6)	2.50 (2.17–2.85)	2.27 (2.01–2.55)	30.8 (27.7–34.1)	88.3 (83.2–93.8)
p-value[3]		0.46	0.10	0.84	0.81	0.45	0.71

[1]Adjusted back-transformed means and their corresponding 95% CI derived from linear regression of the transformed levels. Levels of 3α-diol G and E2 were log 10 transformed, while the others were square-root transformed. The models were adjusted for age and laboratory assay. –[2]Two had missing genotype for A49T.–[3]Likelihood ratio test for association between genotype and circulating hormone levels. Abbreviations: T, testosterone; 3α-diolG, 3α-diol glucuronide; DHEAS, dehydroepiandrosterone-sulfate; A, androstenedione; SHBG, sex hormone-binding globulin; E2, estradiol.

TABLE III – SRD5A2 POLYMORPHISMS AND ANDROGENETIC ALOPECIA

	No balding ($n = 278$)	Frontal ($n = 516$)	Vertex ($n = 353$)	Frontal and vertex ($n = 389$)	*p*-value[1]
A49T					
GG	250 (90%)	468 (91%)	332 (94%)	368 (95%)	0.04
GA	28 (10%)	47 (9%)	21 (6%)	21 (5%)	
OR[2] (95% CI)	Reference	0.92 (0.56–1.51)	0.56 (0.31–1.01)	0.52 (0.29–0.94)	
p-value	–	0.7	0.05	0.03	
V89L					
GG	145 (52%)	252 (49%)	170 (48%)	173 (44%)	
GC	116 (42%)	218 (42%)	143 (41%)	172 (44%)	
CC	17 (6%)	46 (9%)	40 (11%)	44 (11%)	
OR (95% CI) GC *vs*. GG	Reference	1.09 (0.81–1.49)	1.06 (0.76–1.48)	1.26 (0.91–1.74)	0.3
OR (95% CI) CC *vs*. GG	Reference	1.48 (0.82–2.70)	1.92 (1.04–3.54)	1.98 (1.08–3.64)	
p-value[3]	–	0.4	0.1	0.05	
OR (95% CI) any C *vs*. GG	Reference	1.43 (0.80–2.55)	1.86 (1.03–3.38)	1.78 (0.99–3.21)	0.1
p-value	–	0.2	0.03	0.05	

[1]Test for association between genotype and baldness status (*i.e*., the three ORs for the different baldness categories are all 1); the likelihood ratio test was used.–[2]ORs are from a generalized logit model fitted through a log-linear model for multiway contingency tables. Two separate models were fitted for the two variants. The models were adjusted for age and case–control status because the prevalence of vertex baldness in cases was slightly higher than in controls ($p = 0.04$).–[3]Test for association between genotype and the specific category of baldness (*i.e*., both ORs for GC and CC are 1).

disease,[31] for patients carrying the V89L C allele or CC genotype, respectively, and a trend for more advanced stage disease for patients carrying the A49T A allele (GA genotype)[11,31,32] has been reported. In agreement with other studies, we found no association between either tumor stage or grade and the V89L or A49T variant.[27,32–34]

It has been suggested that genetic polymorphisms of *SRD5A2* mediate their effects via changes in androgen levels, including 3α-diolG, a major metabolite of DHT synthesized in the prostate. As DHT is largely derived from the SRD5A2 isoform SRD5A1 activity in the skin, circulating 3α-diolG concentration is believed to be a more accurate marker of SRD5A2 activity. A study of 604 British men participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study showed that V89L was not associated with serum androgen levels, while carriers of the A49T variant had 24% lower serum levels of 3α-diolG than individuals homozygous for the common allele ($p = 0.0003$).[14,15] This strong association between A49T and levels of 3α-diolG was confirmed in our study, in which we measured levels of this DHT metabolite in plasma. After adjusting for age and assay/batch, levels of 3α-diolG were 34% lower for carriers of the A49T variant than for men homozygous for the common variant. The latter finding is in contrast with the results from a study that showed that the A49T variant increases the activity of SRD5A2 *in vitro*.[11] However, one cannot exclude the possibility that the rarely studied 3β-diolG, an alternate metabolite of DHT,[35] together with other hormones, may not have influenced these findings thus providing an explanation for the apparent inconsistency between our results and the *in vitro* study.

The interpretation of these results in terms of prostate cancer risk is further complicated by the historically prevailing hypothesis that high levels of androgens would be associated with an increase in prostate cancer risk. In contrast, we found an association between the *SRD5A2* A49T variant and decreased 3α-diolG levels, as well as increased prostate cancer risk. More recently, 2 large prospective studies have shown that high circulating levels of androgens decrease the risk of aggressive prostate cancer.[8,9]

The involvement of androgens in androgenetic alopecia is well accepted, implicating hormone regulatory genes in predisposing men to this predominantly male condition.[36] In addition, a moderate association between prostate cancer and vertex type baldness compared with no balding has been described.[3] Two independent studies have shown an association between the presence of a non-functional androgen receptor (AR) polymorphism (*AR*-E211 G>A) and reduced risk of alopecia,[22,37] while no association between the *SRD5A2* V89L variant has been observed.[18] We found little evidence of an increased risk of vertex or vertex and frontal balding combined for carriers of the V89L variant, but we did observe a marginally significant association between the presence of the A49T variant and decreased risk of vertex or vertex plus frontal balding combined. This association with decreased balding is surprising as balding is usually associated with increased DHT levels, which in turn is associated with increased SRD5A2 activity in the presence of the A49T variant. Our findings that the A49T variant is associated with lower 3α-diolG plasma levels and, thus, decreased DHT (assuming 3α-diolG is a true indicator of circulating DHT levels) supports our observed association between A49T and decreased balding. Thus, contrary to current understanding, our findings suggest an alternative functional effect of the A49T variant on enzymatic activity. Once again we cannot exclude the possibility that circulating 3α-diolG plasma levels are not being

influenced by the enzymatic activity of SRD5A1 (known to be active in the skin), by the hydroxysteroid dehydrogenases which convert DHT and dehydroepiandrosterone to 3α-diolG, or by the production of 3β-diolG. The interplay between hormones in the skin (*i.e.*, scalp and hair follicles), in the circulation and in the prostate is not well understood. Thus, further studies are needed to investigate whether androgen levels in the circulation are truly markers of androgen levels within tissues like the prostate and skin.

In this large population-based, case–control study we confirm previous observations that the *SRD5A2* A49T variant is associated with increased prostate cancer risk, as well as a decrease in the circulating hormone 3α-diolG in European men. This is also the first study to suggest an association between this polymorphism and decreased male patterned balding. These findings raise a number of questions; including the perceived functional consequences of the A49T variant and whether 3α-diolG is a true indicator of circulating DHT levels. It readdresses issues surrounding limitations of assessing circulating androgens as a true reflection of tissue-specific androgen levels and lends weight to the historically less accepted "androcline hypothesis" of prostate cancer risk.

Acknowledgements

We would like to thank the study participants and the many urologists, nurses, and histopathologists who kindly facilitated the recruitment and collection of patient information and pathologist reports. V.M.H. is a Fellow of the Cancer Institute of New South Wales.

References

1. di Salle E, Giudici D, Radice A, Zaccheo T, Ornati G, Nesi M, Panzeri A, Delos S, Martin P. PNU 157706, a novel dual type I and II 5α-reductase inhibitor. J Steroid Biochem Mol Biol 1998;64:179–86.
2. Wu AH, Whittemore AS, Kolonel LN, John EM, Gallagher RP, West DW, Hankin J, Teh 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 Biomarkers Prev 1995;4:735–41.
3. Giles GG, Severi G, Sinclair R, English DR, McCredie MR, Johnson W, Boyle P, Hopper JL. Androgenetic alopecia and prostate cancer: findings from an Australian case-control study. Cancer Epidemiol Biomarkers Prev 2002;11:549–53.
4. Schweikert HU, Wilson JD. Regulation of human hair growth by steroid hormones. II. Androstenedione metabolism in isolated hairs. J Clin Endocrinol 1974;39:1012–19.
5. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE. Steroid 5α-reductase deficiency in a man: an inherited form of male pseudohermaphroditism. Science 1974;186:1213–15.
6. Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5 α-reductase isozyme expression. J Clin Invest 1993;92:903–10.
7. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. J Natl Cancer Inst 1996;88:1118–26.
8. Platz EA, Leitzmann MF, Rifai N, Kantoff PW, Chen YC, Stampfer MJ, Willett WC, Giovannucci E. Sex steroid hormones and the androgen receptor gene *CAG* repeat and subsequent risk of prostate cancer in the prostate-specific antigen era. Cancer Epidemiol Biomarkers Prev 2005; 14:1262–9.
9. Severi G, Morris HA, MacInnis RJ, English DR, Tilley W, Hopper JL, Boyle P, Giles GG. Circulating steroid hormones and the risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:86–91.
10. Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, Griffin JE, Francke U, Wilson JD, Russell DW. Molecular genetics of steroid 5α-reductase 2 deficiency. J Clin Invest 1992;90: 799–809.
11. 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-Americans and Hispanic men in Los Angeles, USA. Lancet 1999;354:975–8.
12. Makridakis N, Ross RK, Pike MC, Chang L, Stanczyk FZ, Kolonel LN, Shi CY, Yu MC, Henderson BE, Reichardt JK. A prevalent missense substitution that modulates activity of prostatic steroid 5α-reductase. Cancer Res 1997;57:1020–2.
13. Ntais C, Polycarpou A, Ioannidis JPA. *SRD5A2* gene polymorphisms and risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2003;12:618–24.
14. Allen NE, Forrest MS, Key TJ. The association between polymorphisms in the CYP17 and 5 α-reductase type II genes and serum androgen concentrations in men. Cancer Epidemiol Biomarkers Prev 2001;10:185–9.
15. Allen NE, Reichardt JKV, Nguyen H, Key TJ. Association between two polymorphisms in the *SRD5A2* gene and serum androgen concentrations in British men. Cancer Epidemiol Biomarkers Prev 2003;12:578–81.
16. Kakinuma H, Tsuchiya N, Habuchi T, Ohyama C, Matsuura S, Wang L, Nakamura A, Kato T. Serum sex steroid hormone levels and polymorphisms of CYP17 and SRD5A2: implication for prostate cancer risk. Prostate Cancer Prostatic Dis 2004;7:333–7.
17. Ellis JA, Panagiotopoulos S, Akdeniz A, Jerums G, Harrap SB. Androgenic correlates of genetic variation in the gene encoding 5α-reductase type 1. J Hum Genet 2005;50:534–7.
18. Ellis JA, Stebbing M, Harrap SB. Genetic analysis of male pattern baldness and the 5α-reductase genes. J Invest Dermatol 1998;110: 849–53.
19. Giles GG, Severi G, McCredie MR, English DR, Johnson W, Hopper JL, Boyle P. Smoking and prostate cancer: findings from an Australian case-control study. Ann Oncol 2001;12:761–5.
20. Severi G, Giles GG, Southey MC, Tesoriero A, Tilley W, Neufing P, Morris H, English DR, McCredie MR, Boyle P, Hopper JL. ELAC2/HPC2 polymorphisms, prostate-specific antigen levels, and prostate cancer. J Natl Cancer Inst 2003;95:818–24.
21. Norwood OT. Male pattern baldness: classification and incidence. South Med J 1975;68:1359–65.
22. Hayes VM, Severi G, Eggleton SA, Padilla EJ, Southey MC, Sutherland RL, Hopper JL, Giles GG. The E211 G>A androgen receptor polymorphism is associated with decreased risk of metastatic prostate cancer and androgenetic alopecia. Cancer Epidemiol Biomarkers Prev 2005;14:993–6.
23. Sham P. Statistics in human genetics. London: Arnold, 1998.
24. Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 1995;29:311–22.
25. Breslow NE, Day NE. Statistical methods in cancer research. Vol. 1: The analysis of case-control studies. Lyon: IARC, 1980. IARC publication no. 32.
26. Pearce CL, Makridakis NM, Ross RK, Pike MC, Kolonel LN, Henderson BE, Reichardt JK. Steroid 5-α reductase type II V89L substitution is not associated with risk of prostate cancer in a multiethnic population study. Cancer Epidemiol Biomarkers Prev 2002;11:417–18.
27. Cicek MS, Conti DV, Curran A, Neville PJ, Paris PL, Casey G, Witte JS. Association of prostate cancer risk and aggressiveness to androgen pathway genes; *SRD5A2*, *CYP17* and the *AR*. Prostate 2004;59:69–76.
28. Forrest MS, Edwards SM, Houlston R, Kote-Jarai Z, Key T, Allen N, Knowles MA, Turner F, Ardern-Jones A, Murkin A, Williams S, Oram R, et al. Association between hormonal genetic polymorphisms and early-onset prostate cancer. Prostate Cancer Prostatic Dis 2005;8: 95–102.
29. Giwercman YL, Abrahamsson PA, Giwercman A, Gadaleanu V, Ahlgren G. The 5α-reductase type II A49T and V89L high-activity alleleic variants are more common in men with prostate cancer compared with the general population. Eur Urol 2005;48:679–85.
30. Salam MT, Ursin G, Skinner EC, Dessissa T, Reichardt JKV. Associations between polymorphisms in the steroid 5-α reductase type II (*SRD5A2*) gene and benign prostattic hyperplasia and prostate cancer. Urol Oncol 2005;23:246–53.
31. Soderstrom T, Wadelius M, Andersson SO, Johansson JE, Johansson S, Granath F, Rane A. 5α-reductase 2 polymorphisms as risk factors in prostate cancer. Pharmacogenetics 2002;12:307–12.
32. 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;60:1626–30.
33. 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;92:1130–7.
34. Mononen N, Ikonen T, Syrjakoski K, Matikainen M, Schleutker J, Tammela TL, Koivisto PA, Kallioniemi OP. A missense substitution A49T in the steroid 5-α-reductase gene (*SRD5A2*) is not associated with prostate cancer in Finland. Br J Cancer 2001;84:1344–7.
35. Stanczyk FZ, Bretsky P. Biosynthesis, transport, and metabolism of steroid hormones. In: Henderson BE, Ponder B, Ross RK, eds. Hormones, genes, and cancer. New York: Oxford University Press, 2003. 12–37.
36. Ellis JA, Harrap SB. The genetics of androgenetic alopecia. Clin Dermatol 2001;19:149–54.
37. Ellis JA, Stebbing M, Harrap SB. Polymorphism of the androgen receptor gene is associated with male pattern baldness. J Invest Dermatol 2001;116:452–5.