Sex Steroid Hormones and the Androgen Receptor Gene CAG Repeat and Subsequent Risk of Prostate Cancer in the Prostate-Specific Antigen Era

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Abstract

Objective: **Sex steroid hormones are thought to contribute to the growth, differentiation, and progression of prostate cancer. We investigated plasma levels of sex steroid hormones and length of the androgen receptor gene CAG repeat in relation to incident prostate cancer diagnosed in the prostate-specific antigen (PSA) era.**

Methods: **Using a nested case-control design, we included 460 prostate cancer cases diagnosed after providing a blood specimen in 1993 but before February 1998 among men in the Health Professionals Follow-up Study. Controls were 460 age-matched men without prostate cancer who had a screening PSA test after the date of providing a blood specimen. We measured plasma concentrations of total testosterone, free testosterone, dihydrotestosterone, androstanediol glucuronide, estradiol, and sex hormone binding globulin (SHBG) and determined the length of the androgen receptor gene CAG repeat. Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) of prostate cancer.**

Results: **Mean concentrations of the sex steroids adjusted for SHBG, and mean CAG repeat length did not differ significantly between the prostate cancer cases and controls. No significant associations with total prostate cancer were detected for plasma total testosterone concentration (comparing highest versus lowest quartiles: OR, 0.78; 95% CI, 0.48-1.28; P_{trend} = 0.73) or the other sex hormones after adjusting for SHBG. However, plasma total testosterone concentration was positively associated with low-grade disease (Gleason sum < 7: OR, 1.91; 95% CI, 0.89-4.07; P_{trend} = 0.02) and inversely associated with high-grade disease (Gleason sum ≥ 7: OR, 0.26; 95% CI, 0.10-0.66; P_{trend} = 0.01). Similar patterns for grade were observed for free testosterone. Short CAG repeat length was not associated with total prostate cancer (≤ 19 versus ≥ 24: OR, 0.84; 95% CI, 0.57-1.23; P_{trend} = 0.22) or grade of disease. No clear associations with regionally invasive or metastatic (≥ T3b, N1, or M1) were found for any of the hormones or the CAG repeat, although the number of these cases was small.**

Conclusions: **The overall lack of association of prostate cancer diagnosed in the PSA era with sex steroid hormones and the androgen receptor gene CAG repeat length is consistent with the hypothesis that these factors do not substantially contribute to the development of early prostate cancer in the PSA era. The influence of plasma total and free testosterone concentrations on prostate cancer grade merits further evaluation.** (Cancer Epidemiol Biomarkers Prev 2005;14(5):1262–9)

Introduction

Androgens are clearly important in the development, maturation, and maintenance of the prostate, affecting both proliferation and differentiation of the luminal epithelium. More than a dozen epidemiologic studies have prospectively assessed the association of circulating sex steroid hormone concentrations with prostate cancer (1), but only the Physicians' Health Study observed what has been hypothesized: testosterone and androstanediol glucuronide (a metabolite of dihydrotestosterone) were positively associated and estradiol and sex hormone binding globulin (SHBG) were inversely associated with prostate cancer (2). In that prospective study, most of the cases were diagnosed between 1982 and 1992, before prostate-specific antigen (PSA) screening became a routine. In addition, these findings were apparent only after mutual statistical adjustment of the hormones and SHBG.

The effects of testosterone and dihydrotestosterone in androgen-responsive tissues are mediated by the androgen receptor (3). The gene encoding this receptor, located on the long arm of the X chromosome, contains a variable length CAG repeat (encodes polyglutamine) in exon 1. In experimental constructs, the fewer the number of CAG repeats, the greater the transactivational activity of the receptor (4, 5). The typical range of CAG repeats is 11 to 31 (6), with the mean being shorter in African Americans (~20 repeats) than in Whites (~22 repeats; refs. 6, 7). Several (8-14) but not all (15-24) epidemiologic studies found that shorter androgen receptor gene CAG repeats are associated with a higher risk of prostate cancer. Of these studies, two were prospective designs, one conducted largely in the pre-PSA era (11) and one that straddled the PSA era (22). In the former study, risk of advanced disease seemed to increase monotonically with decreasing CAG repeat number (11). Suggested explanations for the inconsistency in findings among these studies have

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included the heterogeneity in the stage case mix and differences in the age distributions of the cases (25). For example, the associations have tended to be stronger for advanced prostate cancer and for younger men; thus, studies of primarily early-stage disease or older men would be more likely null.

To address whether sex steroid hormones and length of the androgen receptor gene CAG repeat are associated with risk of prostate cancer in the PSA era, we conducted an analysis of 460 prostate cancer cases diagnosed since 1993 and 460 matched controls nested in the prospective Health Professionals Follow-up Study.

Materials and Methods

Study Population. We identified incident prostate cancer cases and controls from among members of the Health Professionals Follow-up Study, an ongoing prospective cohort study of 51,529 U.S. men ages 40 to 75 years enrollment in 1986. The men completed a mailed questionnaire on demographics, lifestyle, and medical history and a semiquantitative food frequency questionnaire at baseline. Every other year, the men provided updated information on exposures and diseases, and every 4 years they provided an updated diet questionnaire. Deaths were identified through reports by family members or by the postal system in response to the follow-up questionnaires, or were identified through a search of the National Death Index (26). Between 1993 and 1995, 18,018 of the men gave a blood specimen. The blood was collected in tubes containing sodium EDTA and was shipped by overnight courier while chilled. On receipt, the blood was centrifuged; aliquoted into plasma, erythrocytes, and buffy coat; and stored in liquid nitrogen freezers. Men who had a diagnosis of any cancer, except nonmelanoma skin cancer, before the date of blood draw were excluded from the analysis. Among the men who gave a blood specimen, 94.6% responded to the 1998 questionnaire and 3.5% died before the end of follow-up. This study was approved by the Human Subjects Committee at the Harvard School of Public Health.

Prostate Cancer Cases and Controls. We sought medical and pathology records for each man who reported a prostate cancer diagnosis on a follow-up questionnaire or when prostate cancer was mentioned on the death certificate. The records were reviewed by study investigators blinded to information from the questionnaires to confirm adenocarcinoma of the prostate and to abstract clinical presentation, stage, and Gleason sum of the tumor. We categorized clinical presentation as an elevated serum PSA concentration only, abnormal digital rectal examination with or without an elevated serum PSA concentration, or other. We categorized cases as regionally invasive or metastatic ($\geq$ T3b, N1, or M1), organ confined or minimal extraprostatic extension (T1b to T3a and N0M0), higher grade (Gleason sum ≥ 7), and lower grade (Gleason sum < 7). Because incidental microscopic focal tumors (T1a) are generally indolent and are most susceptible to detection bias due to differential rates of surgery for benign prostatic hyperplasia, we excluded them from the analysis. Between the date that a blood specimen was provided and January 31, 1998, we identified 463 eligible non-T1a prostate cancer cases. Among them, 92.4% were confirmed by medical record review and 4.8% were confirmed by other corroborating information. Because we found the reporting of a prostate cancer diagnosis by these health professionals accurate, we also included as cases the remaining self-reported but unconfirmed cases.

For each case, one control was selected who was alive and not diagnosed with cancer by the date of the case's diagnosis and who had a PSA test after the date of blood draw. This latter criterion was used to ensure that controls had the opportunity to have an occult prostate cancer diagnosed. The matching criteria were year of birth (± 1 year), PSA test before blood draw (yes/no), and timing of blood draw: time of day (midnight to before 9 am, 9 am to before noon, noon to before 4 pm, 4 pm to before midnight), season (winter, spring, summer, and fall), and year (exact). Ninety-two percent of the controls had a PSA test within 2.5 years of their matched case's date of diagnosis.

Laboratory Assays. Plasma concentrations of sex steroid hormones and SHBG were measured in the laboratory of Dr. Nader Rifai at the Children's Hospital, Boston, MA using the following methods: total testosterone, a chemiluminescent immunoassay (Elecsys autoanalyzer, Roche Diagnostics, Indianapolis, IN); free testosterone, enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX); dihydrotestosterone, RIA (Diagnostic Systems Laboratory); androstanediol glucuronide, solid phase RIA (Diagnostic Systems laboratory); estradiol, third-generation RIA (Diagnostic Systems Laboratory); and SHBG, coated tube noncompetitive immunoradiometric assay (Diagnostic Systems Laboratory). The androgen receptor gene CAG repeat length was determined by running the PCR-amplified fragments on a denaturing polyacrylamide gel with automated fluorescence detection of the fragments and sizing by Genescan in the laboratory of Dr. Philip Kantoff at Dana-Farber Cancer Institute as done previously (11).

Case-control pairs were analyzed together, and laboratory personnel were blinded to case-control status. Cases diagnosed from the date of blood draw through January 1996 and their matched pairs and cases diagnosed from February 1996 through January 1998 and their matched pairs were assayed in separate analytic runs. We included in the statistical analysis 448 case-control pairs with complete data for all of the sex steroid hormones and SHBG and 460 pairs for the androgen receptor gene CAG repeat. Missing data occurred because samples were too lipemic for the free testosterone assay (7 cases and 5 controls), remaining volume was too low (3 cases), or fragment elution time for the CAG repeats could not be detected (1 case and 2 controls). The mean intrapair coefficients of variation calculated from blinded quality control samples were 4.9% for total testosterone, 8.4% for free testosterone, 9.7% for dihydrotestosterone, 6.7% for androstanediol glucuronide, 5.2% for estradiol, 10.7% for SHBG, and 1.8% for the CAG repeat.

We assessed the intraperson consistency of the sex steroid hormones and SHBG over time by measuring for 144 men from the Health Professional Follow-up Study cohort who were free of a cancer diagnosis plasma concentrations in specimens collected in 1993 and again in 1997 (mean of 3.03 ± 0.46 years apart). Adjusting for age and race, the Spearman correlation coefficients between the two time points were 0.68 for total testosterone, 0.39 for free testosterone, 0.66 for dihydrotestosterone, 0.74 for androstanediol glucuronide, 0.55 for estradiol, and 0.74 for SHBG (all $P < 0.0001$).

As part of the National Cancer Institute's Cohort Consortium, DNA was extracted from a second split of the case and control samples and genotyped subsequently (Dr. David Kwiatkowski, Director of SNP Genotyping Facility, Harvard Partners Center for Genetics and Genomics, Boston, MA). The PCR-amplified fragments were separated on an ABI 3100 machine (Applied Biosystems, Foster City, CA) with automated fluorescence detection by ABI Prism Genescan and sizing by ABI Prism Genotyper. The two runs of genotyping produced repeat lengths in good agreement (91.5% were within one repeat), and the estimates from the statistical analysis were similar. We present the estimates from the original genotyping.

Statistical Analysis. We compared mean sex steroid hormone and SHBG concentrations and mean length of the androgen receptor gene CAG repeat between matched cases and controls using the paired t test. Results were similar using

the nonparametric Wilcoxon sign rank test. We used conditional logistic regression to estimate matched odds ratios (OR) and 95% confidence intervals (CI) for total prostate cancer, by stage, by grade, and by clinical presentation. We used indicator variables for quartiles of the plasma hormone concentrations or for categories of length of the CAG repeat, with cut points based on the distributions among the controls. Because the samples were assayed in two analytic runs, we used separate quartile cut points for the plasma markers defined by analytic run. To take into account that testosterone, dihydrotestosterone, and estradiol compete for binding to SHBG, in multivariable models we adjusted these sex steroid hormones for SHBG and also mutually. Adjustment for factors previously identified to be associated with total or advanced prostate cancer in this cohort (father or brother with prostate cancer, height, vigorous physical activity, diabetes mellitus, vasectomy, cigarette smoking in the past 10 years, intake of energy, red meat, and fish, intake of energy-adjusted lycopene, calcium, fructose, and α-linolenic acid, and supplemental vitamin E and selenium) did not notably alter the results. Thus, fully adjusted results are not presented. To test for trend, we entered into the model a single ordinal variable with values of 1 to 4 corresponding to the quartile or corresponding to the median of the category of CAG repeat length into which an individual fell.

To evaluate whether the associations varied by age at diagnosis [<64 years (25th percentile) versus ≥64 years], family history of prostate cancer (yes/no), or body mass index (<25 versus ≥25 kg/m^2), we ran stratified conditional logistic regression models (age at diagnosis) or stratified logistic regression models adjusting for the matching factors. To test for statistical interaction, we entered into the appropriate multivariable model the main effect terms for the hormonal system component (value of 1-4 corresponding to quartiles) and the covariate (binary) along with a term for their product, the coefficient for which was evaluated by the Wald test. All analyses were conducted using SAS release 8.2 (SAS Institute, Cary, NC). We report two-sided P values for all hypothesis tests.

Results

The median age at diagnosis was 69.8 years (range, 47.7-84.3). Of the 67% of cases for whom clinical presentation was known, 69% were detected because of elevated serum PSA concentration only and 28% because of an abnormal digital rectal examination with or without an elevated serum PSA concentration. For 99 of the remaining cases, the clinical presentation was not specified in the medical records that we reviewed. Of the 84% of cases for whom stage was known, 90% were organ confined at diagnosis (T1b-T2b) or had minimal extraprostatic extension (T3a). Among the 84% of cases for whom grade was known, the most common Gleason sums were 5 (19%), 6 (33%), and 7 (29%). At diagnosis, the median PSA concentration for the 64% of cases for whom PSA was available was 7.0 ng/mL. The mean time between blood draw and prostate cancer diagnosis was 2.15 ± 1.22 years. At least one screening PSA test before blood draw was reported by 79% of cases and 80% of controls.

Sex Steroid Hormones. Mean plasma concentrations of sex steroid hormones and SHBG did not differ between the cases and controls, even after adjusting the sex steroids for SHBG (Table 1). No associations between total testosterone, free testosterone, dihydrotestosterone, androstanediol glucuronide, estradiol, or SHBG and total prostate cancer were observed (Table 2). Adjustment of total testosterone, dihydrotestosterone, and estradiol for SHBG did not alter the results (Table 2). In addition, mutually adjusting total testosterone, androstanediol glucuronide, estradiol, and SHBG, again no associations with total prostate cancer were found overall (Table 3) or after excluding cases (remaining pairs = 235) diagnosed within 2 years of blood draw (data not shown). No associations were seen between the molar ratios of total testosterone to SHBG (data not shown), estradiol to SHBG (data not shown), or estradiol to total testosterone and total prostate cancer (Table 3).

Stage at Diagnosis. For regionally invasive or metastatic disease (n = 40 pairs), after mutual adjustment, risk seemed lower with higher total testosterone concentration and higher with higher androstanediol glucuronide, SHBG, and the ratio of estradiol to total testosterone, although none of these was statistically significant (Table 3). No association was present between free testosterone and regionally invasive or metastatic disease (comparing highest versus lowest quartiles: OR, 0.94; 95% CI, 0.33-2.72; P_{trend} = 0.90). There were no associations of the mutually adjusted hormones (Table 3) or free testosterone (comparing highest versus lowest quartiles: OR, 1.13; 95% CI, 0.72-1.76; P_{trend} = 0.44) with cases that were organ confined/minimal extraprostatic extension. No statistically significant patterns were present for the molar ratios of total testosterone to SHBG or estradiol to SHBG by stage (data not shown).

Histologic Grade. For high-grade disease (Gleason sum ≥ 7; n = 148 pairs), risk decreased with increasing total testosterone concentration, increased with increasing molar ratio of estradiol to total testosterone, and suggestively increased with increasing SHBG concentration (Table 3). In addition, risk of high-grade disease was lower in men with a higher free testosterone concentration, although this association was not linearly decreasing (compared with the lowest quartile: quartile 2 OR, 0.68; quartile 3 OR, 0.78; and quartile 4 OR, 0.57; 95% CI, 0.31-1.06; P_{trend} = 0.11). Men whose plasma total

Table 1. Mean plasma sex steroid hormone concentrations and androgen receptor gene CAG repeat length in prostate cancer cases and matched controls nested in the Health Professionals Follow-up Study, 1993-1998

	Prostate cancer cases	Controls	P*	$P^{\dagger}$
n	448	448		
Testosterone (ng/mL)	4.83 ± 1.75	4.83 ± 1.68	0.97	0.95
Free testosterone (pg/mL)	27.6 ± 8.3	27.4 ± 8.0	0.78	—
Dihydrotestosterone (pg/mL)	424 ± 255	424 ± 239	0.99	0.97
Androstanediol glucuronide (ng/mL)	5.05 ± 3.14	4.99 ± 3.39	0.78	—
Estradiol (pg/mL)	28.9 ± 8.8	28.8 ± 8.3	0.70	0.74
SHBG (nmol/L)	98.3 ± 45.9	98.7 ± 46.3	0.90	—
n	460	460		
Androgen receptor gene CAG repeat length	22.0 ± 3.2	21.8 ± 3.0	0.48	—

*For the hypothesis test of no difference in means (paired t test) between prostate cancer cases and controls. All tests are two sided.
†For the hypothesis test of no difference in means between prostate cancer cases and controls after adjusting, the sex steroid hormones for SHBG by linear regression. Mutual adjustment was done because the concentrations of some sex steroid hormones and SHBG are moderately correlated (testosterone: Pearson r = 0.71, estradiol: r = −0.32, and dihydrotestosterone: r = 0.32; all P < 0.0001).

Table 2. Association of plasma sex steroid hormones and SHBG concentrations with prostate cancer among 448 matched pairs nested in the Health Professionals Follow-up Study, 1993-1998

	Quartile*				P_{trend}
	1	2	3	4	
Testosterone					
No. cases/controls	121/113	96/115	138/112	93/108	
OR† (95% CI)	1.00‡	0.78 (0.54-1.13)	1.18 (0.82-1.69)	0.81 (0.56-1.18)	0.72
OR§ (95% CI)	1.00‡	0.77 (0.52-1.14)	1.17 (0.77-1.79)	0.78 (0.48-1.28)	0.73
Free testosterone					
No. cases/controls	113/112	107/112	107/111	121/113	
OR† (95% CI)	1.00‡	0.95 (0.66-1.36)	0.95 (0.65-1.40)	1.07 (0.74-1.54)	0.74
Dihydrotestosterone					
No. cases/controls	126/114	100/112	115/115	107/107	
OR† (95% CI)	1.00‡	0.79 (0.54-1.17)	0.89 (0.61-1.29)	0.88 (0.59-1.33)	0.70
OR§ (95% CI)	1.00‡	0.78 (0.52-1.16)	0.88 (0.58-1.34)	0.87 (0.55-1.39)	0.73
Androstanediol glucuronide					
No. cases/controls	104/115	117/109	115/113	112/111	
OR† (95% CI)	1.00‡	1.18 (0.82-1.71)	1.12 (0.77-1.64)	1.11 (0.76-1.63)	0.64
Estradiol					
No. cases/controls	116/112	117/113	108/113	107/110	
OR† (95% CI)	1.00‡	0.99 (0.68-1.44)	0.91 (0.61-1.35)	0.92 (0.61-1.39)	0.62
OR§ (95% CI)	1.00‡	0.98 (0.67-1.43)	0.91 (0.61-1.35)	0.92 (0.61-1.39)	0.62
SHBG					
No. cases/controls	113/113	121/115	105/112	109/108	
OR† (95% CI)	1.00‡	1.05 (0.72-1.52)	0.93 (0.63-1.38)	1.01 (0.69-1.47)	0.88

*The case-control pairs were assayed in two analytic batches, cases diagnosed after the date of blood draw through 1/1996, and their matched controls and cases diagnosed from 2/1996 through 1/1998 and their matched controls. Quartile cut points for batch 1 were testosterone 4.18, 5.27, 6.69 ng/mL; free testosterone 21.7, 26.5, 32.2 pg/mL; dihydrotestosterone 215, 357, 510 pg/mL; androstanediol glucuronide 3.57, 4.97, 6.97 ng/mL; estradiol 18.9, 24.0, 27.9 pg/mL; and SHBG 91.8, 121, 154 nmol/L. Quartile cut points for batch 2 were testosterone 3.25, 4.32, 5.43 ng/mL; free testosterone 22.0, 26.7, 32.8 pg/mL; dihydrotestosterone 307, 426, 595 pg/mL; androstanediol glucuronide 2.71, 3.76, 5.01 ng/mL; estradiol 28.6, 32.7, 37.7 pg/mL; and SHBG 53.7, 72.4, 91.5 nmol/L.
†Matched on age, time of day and year of blood draw, and PSA test before blood draw.
‡Reference group.
§Adjusted for SHBG.

testosterone was clinically low (<3 ng/mL; 24 cases and 13 controls) had a higher risk of high-grade prostate cancer (OR, 2.59; 95% CI, 1.12-5.00; adjusted for the other hormones and SHBG). For low-grade disease (Gleason sum < 7; n = 229 pairs), there was a statistically significant increasing association for total testosterone and a statistically significant decreasing association for the molar ratio of estradiol to total testosterone (Table 3). In addition, the risk of low-grade disease increased with increasing quartiles of free testosterone (compared with the lowest quartile: quartile 2 OR, 0.99; quartile 3 OR, 1.45; and quartile 4 OR, 1.76; 95% CI, 1.01-3.05; P_{trend} = 0.03). The OR of low-grade disease for clinically low total testosterone (30 cases and 37 controls) was 0.70 (95% CI 0.38-1.29, adjusted for the other hormones and SHBG). The patterns of association of the molar ratios of total testosterone and estradiol to SHBG with grade were compatible with the associations of total testosterone and estradiol controlling for SHBG with grade but were not statistically significant (data not shown). The associations by quartiles of testosterone (comparing highest with lowest quartiles: Gleason ≥ 7: OR, 0.21; 95% CI, 0.07-0.65; P_{trend} = 0.009; Gleason < 7: OR, 2.62; 95% CI, 1.15-5.96; P_{trend} = 0.007) and the other hormones (data not shown) persisted, or were stronger, when limiting the grade analyses to only men with organ-confined disease or minimal extraprostatic extension.

Among cases only, mean total testosterone concentration was higher in men with low-grade disease (5.04 ± 0.08 ng/mL) than in men with high-grade disease (4.67 ± 0.10 ng/mL) after adjusting for age and SHBG concentration (P < 0.005). Similarly, mean free testosterone concentration was higher for low-grade disease (28.5 ± 0.53 ng/mL) than for high-grade disease (26.5 ± 0.66 ng/mL) after adjusting for age (P = 0.02). Concentrations of the other hormones were not statistically significantly different between men with high- and low-grade disease (data not shown).

Clinical Presentation. Among men whose clinical presentation was an elevated PSA concentration only (n = 209 cases), the risk of prostate cancer nonstatistically significantly increased with increasing total testosterone concentration (comparing highest with lowest quartiles: OR, 1.66; 95% CI, 0.77-3.58; P_{trend} = 0.20). In contrast, for men whose clinical presentation was an abnormal digital rectal examination with or without an elevated serum PSA (n = 84 cases), a direct association was not observed for total testosterone concentration (OR, 0.22; 95% CI, 0.05-1.10; P_{trend} = 0.35). These patterns were similar for free testosterone and for both total testosterone and free testosterone when restricting to cases with organ-confined disease or minimal extraprostatic extension (data not shown).

Among men whose clinical presentation was an elevated PSA, the findings for Gleason sum were similar in direction to those irrespective of clinical presentation; risk of high-grade disease (n = 78 cases) was nonstatistically significantly lower (OR, 0.32; 95% CI, 0.06-1.65; P_{trend} = 0.35) and risk of low-grade disease (n = 113 cases) was higher (OR, 4.77; 95% CI, 1.45-15.65; P_{trend} = 0.008) in men with higher total testosterone concentration. No such pattern was observed for high-grade (n = 33 cases) and low-grade (n = 49 cases) disease among men whose clinical presentation was an abnormal digital rectal examination, although these analyses were based on small numbers of cases.

Effect Modification. Because men who are young at diagnosis or who have a family history of prostate cancer may be those who are more or less susceptible to hormonal exposures because of their underlying genetic predisposition, we evaluated whether the associations of sex hormones with prostate cancer varied by age at diagnosis or family history (Table 4). Among men diagnosed with prostate cancer at a younger age (<64 years old, n = 112 cases) but not at an older age (≥64 years old, n = 336) and adjusting for total testosterone, estradiol, and SHBG, risk of total prostate cancer seemed to increase across quartiles of androstanediol glucuronide ($P_{interaction}$ = 0.03). Among men with a family history of prostate cancer (n = 66 cases) but not in men without a family

Table 3. ORs of stage and grade of prostate cancer according to quartiles of mutually adjusted plasma sex steroid hormone concentrations or the molar ratio of estradiol to testosterone, 448 matched pairs nested in the Health Professionals Follow-up Study, 1993-1998

	Quartile				P_{trend}
	1	2	3	4	
Total prostate cancer (448 cases/448 controls)					
Testosterone	1.00*	0.77	1.18	0.79 (0.48-1.31)†	0.79
Androstanediol glucuronide	1.00*	1.19	1.16	1.19 (0.80-1.77)	0.57
Estradiol	1.00*	0.98	0.90	0.90 (0.59-1.39)	0.58
SHBG	1.00*	1.09	0.95	1.09 (0.66-1.82)	0.97
Estradiol to testosterone	1.00*	1.17	0.86	1.25 (0.79-1.99)	0.64
Regionally invasive or metastatic (40 cases/40 controls)					
Testosterone	1.00*	0.44	0.89	0.48 (0.06-3.69)	0.28
Androstanediol glucuronide	1.00*	13.86	4.38	6.38 (0.89-45.9)	0.13
Estradiol	1.00*	3.10	1.74	1.11 (0.17-7.23)	0.31
SHBG	1.00*	3.08	3.13	4.85 (0.33-71.9)	0.17
Estradiol to testosterone	1.00*	3.34	3.33	4.57 (0.86-24.2)	0.11
Organ confined or minimal extraprostatic extension (339 cases/339 controls)					
Testosterone	1.00*	0.87	1.21	1.00 (0.56-1.89)	0.73
Androstanediol glucuronide	1.00*	1.06	1.03	1.10 (0.70-1.72)	0.85
Estradiol	1.00*	0.86	0.75	0.87 (0.52-1.46)	0.51
SHBG	1.00*	1.12	0.93	1.08 (0.61-1.94)	0.94
Estradiol to testosterone	1.00*	1.09	0.64	1.00 (0.58-1.72)	0.54
Gleason sum ≥ 7 (148 cases/148 controls)					
Testosterone	1.00*	0.60	0.50	0.26 (0.10-0.66)	0.01
Androstanediol glucuronide	1.00*	1.93	1.81	0.82 (0.38-1.75)	0.87
Estradiol	1.00*	1.20	0.97	1.15 (0.50-2.65)	0.65
SHBG	1.00*	1.82	2.90	2.72 (1.02-7.24)	0.05
Estradiol to testosterone	1.00*	2.06	0.90	3.02 (1.29-7.04)	0.03
Gleason sum < 7 (229 cases/229 controls)					
Testosterone	1.00*	0.88	2.04	1.91 (0.89-4.07)	0.02
Androstanediol glucuronide	1.00*	1.20	1.20	1.49 (0.84-2.65)	0.32
Estradiol	1.00*	0.58	0.52	0.60 (0.31-1.13)	0.20
SHBG	1.00*	1.09	0.59	0.71 (0.34-1.50)	0.13
Estradiol to testosterone	1.00*	0.66	0.57	0.47 (0.23-0.96)	0.04

NOTE: Matched on age, time of day and year of blood draw, and PSA test before blood draw. Mutually adjusted (except for estradiol to testosterone ratio) and estimated from conditional logistic regression models.
*Reference group.
†95% CI.

history (n = 382), risk of total prostate cancer was elevated in the top three quartiles of total testosterone ($P_{interaction}$ = 0.02). Also in men with a family history, risk of prostate cancer was lower in the top three quartiles of estradiol, although the trend was not decreasing (P_{trend} = 0.38).

Because hormonal systems are perturbed in obese men, we evaluated whether the association of sex hormones with prostate cancer varied by body mass index (Table 4). Among men with a normal body mass index (<25 kg/m^2, n = 189 cases) but not in men who were overweight or obese (body mass index ≥ 25 kg/m^2, n = 259 cases), risk of total prostate cancer was suggestively increased in the top three quartiles of androstanediol glucuronide; however, the interaction was not statistically significant ($P_{interaction}$ = 0.56).

Androgen Receptor Gene CAG Repeat Length. Length of the androgen receptor gene CAG repeat ranged from 13 to 45 in the cases and 7 to 34 in the controls. Mean length did not differ between the cases and controls (Table 1). Risk of total prostate cancer and disease that was regionally invasive/metastatic, organ-confined/minimal extraprostatic extension, Gleason sum ≥ 7, Gleason sum < 7, or that had an elevated serum PSA concentration only as the clinical presentation did not increase with decreasing number of CAG repeats (Table 5). Risk of regionally invasive or metastatic prostate cancer decreased with decreasing number of CAG repeats. No association was seen between short CAG repeat length and total prostate cancer within strata of age at diagnosis ($P_{interaction}$ = 0.31), family history of prostate cancer ($P_{interaction}$ = 0.22), or body mass index ($P_{interaction}$ = 0.72; Table 4), or after excluding cases diagnosed within 2 years of blood collection (data not shown).

Discussion

In this prospective study conducted in the PSA era, we observed no associations of plasma concentrations of sex steroid hormones or length of the androgen receptor gene CAG repeat with total prostate cancer or with stage of disease. However, we found positive associations for plasma total testosterone and free testosterone concentrations with low-grade prostate cancer and inverse associations with high-grade prostate cancer overall, and in men whose clinical presentation was an elevated serum PSA concentration only.

Our findings for total prostate cancer are largely consistent with a meta-analysis of prospective studies on hormones and prostate cancer conducted up to 1999 that reported no case-control differences in concentration of total testosterone, dihydrotestosterone, estradiol, and SHBG (27). In that meta-analysis, the only exception was possibly a slightly higher concentration of androstanediol glucuronide in the cases compared with controls. Plasma concentration of androstanediol glucuronide has been used as an indirect indicator of the activity of 5α-reductase type 2, the enzyme that catalyzes the conversion of testosterone to dihydrotestosterone in the prostate, and thus also may be an indicator of intraprostatic dihydrotestosterone (28). We did not observe an association for androstanediol glucuronide and total prostate cancer overall.

We also did not observe an association between the androgen receptor gene CAG repeat and total prostate cancer. Studies of the androgen receptor gene CAG repeat in relation to total prostate cancer are not fully in agreement, although several studies do support a modest inverse association (8-13). For advanced disease, we observed a suggestive lower risk with

fewer CAG repeats, unlike in the Physicians' Health Study, which showed a monotonically increasing risk with decreasing number of CAG repeats for advanced cases. However, our data for total prostate cancer, most of which was early-stage disease, are more compatible with the null finding for early-stage and low-grade disease in the Physicians' Health Study (comparing ≤18 with ≥26 repeats: OR, 1.03; P_{trend} = 0.86; ref. 11).

The literature on sex steroid hormones, the androgen receptor gene CAG repeat length, and prostate cancer has not been consistent. A possible explanation for the apparent inconsistency is differences among studies in the extent of early versus late stage disease. For example, in the Physicians' Health Study, the findings tended to support associations of sex steroid hormones and the androgen receptor gene CAG repeat with total prostate cancer, whereas findings in the current report are essentially null for these factors overall. The published reports from the Physicians' Health Study included cases diagnosed from 1982 to 1992 (2) or 1982 to 1995 (11). These cases were mainly diagnosed in the pre-PSA era, when the majority of cases were detected because they were palpable or were symptomatic. In contrast, in the Health Professionals Follow-up Study, the prostate cancer cases included in this nested case-control study were diagnosed from 1993 to 1998, all within the PSA era. With the onset of PSA screening, the spectrum of prostate cancer at diagnosis has shifted from clinically detectable (e.g., palpable, symptomatic, metastatic) cases of higher stage to clinically occult, usually small volume early-stage disease (clinical stage T1c; ref. 29). Furthermore, cases diagnosed at an advanced stage in the PSA era may have more favorable characteristics than cases diagnosed in the past. For example, stage C cases that are detected by PSA screening are likely those that have more slowly growing primary tumors and regional metastases; otherwise, these would already have been detected clinically (i.e., length bias).

In our study, most of the cases for whom information was available in the medical records that we obtained were PSA detected and given the high prevalence of PSA screening reported by participants in this cohort and the fact that 90% of the cases were stage T3aN0M0 or below, suggests that many with unspecified clinical presentation may also have been PSA detected. Prostate tumors detected by PSA during their preclinical phase may be of two varieties: those that would have progressed to clinical disease if their natural history had not been interrupted by screening and subsequent treatment and those that never would have progressed to clinical disease during the man's lifetime. At present, it is not possible to distinguish between the two. Nevertheless, we can speculate that our overall null results for cases detected in their

Table 4. Association of plasma sex steroid hormone concentrations and length of the androgen receptor gene CAG repeat with total prostate cancer by age at diagnosis, family history of prostate cancer, and body mass index among matched pairs nested in the Health Professionals Follow-up Study, 1993-1998

	Category				P_{trend}
	1	2	3	4	
Younger at diagnosis (<64 y, n = 112)					
Testosterone	1.00*	1.07	1.52	1.01 (0.35-2.90)	0.64
Androstanediol glucuronide	1.00*	1.36	1.71	2.29 (0.95-5.52)	0.07
Estradiol	1.00*	0.95	1.40	0.96 (0.34-2.72)	0.93
SHBG	1.00*	0.94	1.00	0.86 (0.31-2.34)	0.77
AR gene CAG repeat	1.10 (0.50-2.42)	1.09	0.79	1.00*	0.78
Older at diagnosis (≥64 y, n = 336)					
Testosterone	1.00*	0.67	1.07	0.66 (0.37-1.20)	0.47
Androstanediol glucuronide	1.00*	1.18	1.06	0.94 (0.59-1.49)	0.67
Estradiol	1.00*	0.98	0.78	0.93 (0.57-1.51)	0.50
SHBG	1.00*	1.19	0.97	1.25 (0.68-2.32)	0.75
AR gene CAG repeat	0.76 (0.49-1.19)	0.76	0.75	1.00*	0.12
Family history of prostate cancer (n = 66)					
Testosterone	1.00*	2.09	3.82	2.58 (0.57-11.72)	0.23
Androstanediol glucuronide	1.00*	3.06	1.44	1.28 (0.36-4.56)	0.86
Estradiol	1.00*	0.64	0.60	0.53 (0.15-1.91)	0.38
SHBG	1.00*	0.64	0.74	1.09 (0.21-5.57)	0.76
AR gene CAG repeat	0.40 (0.09-1.68)	1.18	0.52	1.00*	0.49
No family history of prostate cancer (n = 382)					
Testosterone	1.00*	0.69	1.03	0.67 (0.39-1.14)	0.44
Androstanediol glucuronide	1.00*	1.07	1.10	1.17 (0.77-1.78)	0.50
Estradiol	1.00*	1.09	0.99	0.97 (0.64-1.47)	0.75
SHBG	1.00*	1.09	0.92	1.00 (0.59-1.72)	0.79
AR gene CAG repeat	0.88 (0.59-1.33)	0.80	0.81	1.00*	0.38
Lower body mass index (<25 kg/m^2, n = 189)					
Testosterone	1.00*	1.00	1.11	0.90 (0.39-2.11)	0.81
Androstanediol glucuronide	1.00*	1.89	1.96	1.40 (0.75-2.63)	0.30
Estradiol	1.00*	1.14	0.78	0.79 (0.41-1.51)	0.36
SHBG	1.00*	1.89	1.10	1.29 (0.56-2.95)	0.80
AR gene CAG repeat	0.94 (0.52-1.69)	0.92	0.67	1.00*	0.75
Higher body mass index (≥25 kg/m^2, n = 259)					
Testosterone	1.00*	0.64	1.26	0.72 (0.37-1.41)	0.75
Androstanediol glucuronide	1.00*	0.84	0.81	1.02 (0.61-1.73)	0.95
Estradiol	1.00*	0.93	1.01	0.97 (0.57-1.64)	0.91
SHBG	1.00*	0.79	0.93	1.06 (0.54-2.07)	0.81
AR gene CAG repeat	0.74 (0.44-1.24)	0.75	0.78	1.00*	0.17

NOTE: ORs and 95% CIs were estimated from conditional logistic regression models (when stratified by the matching variable age) or logistic regression models (when stratified by family history and body mass index) separately for the sex steroid hormones and the androgen receptor gene CAG repeat. The sex steroid hormones and SHBG were mutually adjusted. Prostate cancer cases and controls were matched on age, time of day, year, and season of blood draw, and PSA test before blood draw.

Abbreviation: AR, androgen receptor.

*Reference group.

Table 5. Association of androgen receptor gene CAG repeat length with prostate cancer among matched pairs nested in the Health Professionals Follow-up Study, 1993-1998

	Length of androgen receptor gene CAG repeat*				P_{trend}
	≤19	20-21	22-23	≥24	
Total prostate cancer					
No. cases/controls	80/83	145/151	87/100	148/126	
OR (95% CI)	0.84 (0.57-1.23)	0.83 (0.60-1.15)	0.76 (0.53-1.09)	1.00†	0.22
Regionally invasive or metastatic					
No. cases/controls	4/8	13/17	7/6	18/11	
OR (95% CI)	0.38 (0.09-1.50)	0.54 (0.18-1.59)	0.86 (0.23-3.26)	1.00†	0.11
Organ confined or minimal extraprostatic extension					
No. cases/controls	67/65	104/110	70/79	108/95	
OR (95% CI)	0.92 (0.59-1.42)	0.84 (0.57-1.23)	0.80 (0.53-1.20)	1.00†	0.50
Gleason sum ≥ 7					
No. cases/controls	26/30	45/44	32/26	48/51	
OR (95% CI)	0.88 (0.45-1.74)	1.08 (0.62-1.87)	1.31 (0.69-2.48)	1.00†	0.96
Gleason sum < 7					
No. cases	44/37	75/83	43/61	75/56	
OR (95% CI)	0.92 (0.53-1.61)	0.67 (0.41-1.10)	0.55 (0.33-0.91)	1.00†	0.34

NOTE: Matched on age, time of day and year of blood draw, and PSA test before blood draw.
*Medians were 19, 21, 22, and 25 CAG repeats.
†Reference group.

preclinical stage would have resulted (a) if for the former case type, the influence of hormonal systems is important only later in their natural history and (b) if for the latter case type, hormonal systems are not important in their etiology.

Although the results of this study were null for total prostate cancer and for stage at diagnosis, we did observe positive associations for plasma total testosterone and free testosterone concentrations with low-grade disease and inverse associations for plasma total testosterone and free testosterone concentrations and high-grade disease overall, and among men whose clinical presentation was an elevated serum PSA concentration only. We also observed that risk of low-grade disease possibly was greater with lower estradiol, SHBG, and the molar ratio of estradiol to total testosterone, whereas for high-grade disease risk was greater for higher SHBG and the molar ratio of estradiol to total testosterone. Our findings for testosterone and grade of disease are consistent with some (30-33) but not all (34) clinical studies of nonmetastatic prostate cancer that have observed that men with low serum testosterone (30, 31) or free testosterone (32, 33) had a higher mean Gleason sum than did men with normal levels. We cannot rule out in our study or in the clinical studies the differential detection of small, well-differentiated tumors because of greater PSA elevations in men with higher circulating total or free testosterone levels compared with men with lower levels.

The findings for total and free testosterone and tumor grade that we observed are also possibly supported by findings from the Prostate Cancer Prevention Trial, in which men were randomized to receive finasteride, a drug that inhibits 5α-reductase type 2, or placebo for 7 years. In that trial, the proportion of all prostate cancers that were of high grade (Gleason sum ≥ 7) was higher in the finasteride arm (35.9%) than in the plcebo arm (20.7%), despite a 24% reduction in the total prevalence of prostate cancer in the finasteride compared to placebo arm (35). However, whether finasteride actually enhances the development of high-grade disease or instead differentially influences the detectability of high-grade disease is currently being evaluated.[7]

Androgens may influence the stage and grade of prostate cancer independently. Grade reflects the differentiation state of that tissue (i.e., maintenance of the normal functional architecture of the tissue). Thus, increased androgenic stimulation may prevent the dedifferentiation of the prostate epithelium in the nascent tumor. In contrast, a reduction in intraprostatic dihydrotestosterone levels may allow for a less differentiated histologic phenotype to emerge. To isolate the influence of androgens on differentiation status, the cases should be restricted to uniform stage. Although we did not intentionally restrict the stage of the cases for inclusion in the present study, variation in stage at diagnosis was limited because the cases included in our analysis were detected in the PSA era, and this narrow stage range may have allowed us to observe the independent association for grade. Furthermore, when we did exclude the small number of men with other than organ-confined disease/minimal extraprostatic extension, the associations for low- and high-grade disease persisted or were strengthened, further supporting the hypothesis of independent action of androgens on grade. More work is needed regarding the action of androgens on the differentiation state of prostate adenocarcinoma.

We also observed possible associations for higher androstanediol glucuronide concentration and total prostate cancer among men who were younger at diagnosis or whose body mass index was in the reference range. In men with a family history of prostate cancer, we observed that prostate cancer risk was positively associated with testosterone and inversely associated with estradiol concentrations, although the associations were not monotonic. Because prostate cancers that occur in younger men or in men with a family history may be enriched with an underlying genetic etiology, one possible explanation for our results is that such genetically determined tumors may be more susceptible to the growth promoting or inhibiting effects of sex steroid hormones. The association of sex steroid hormones with prostate cancer may be more evident in leaner men than in overweight and obese men (36), possibly because insulin metabolism and the balance of sex steroids are perturbed in these latter men, obfuscating the associations for androgens and estrogens.

The null findings for the androgen receptor gene CAG repeat with prostate cancer did not vary by age at diagnosis, family history of prostate cancer, or body mass index. Because the androgen receptor gene is located on the X chromosome, it would have been of interest to consider whether this association varied by father versus brother with prostate cancer; however, few of the men had a brother (2.9% of cases and 1.6% of controls) with this diagnosis.

[7] M. Scott Lucia, Director, PCPT Anatomic Pathology Core Laboratory, Denver, CO, personal communication.

This study has several strengths, including its prospective design and large size. Because of widespread PSA screening, there were few advanced cases at diagnosis. We increased the opportunity for equal detection of prostate cancer and reduced the possibility of undiagnosed prostate cancer in the controls by restricting controls to men who had had a PSA after blood collection. In doing so, we likely limited observation bias that might have resulted if opportunity for disease detection was differential by exposure to the hormonal system components. Although we did not independently confirm staging or Gleason grading, we do not believe that a major error occurred when subclassifying cases into extremes of disease characteristics. In addition, misclassification of grade could not explain our results because errors in grading would have tended to attenuate associations rather than to accentuate associations. We assessed sex steroid hormone concentrations at one time point in midlife, although the etiologically relevant time period is unknown. Nevertheless, in a subset of men in this cohort, we showed good correlation between two measures of each hormone in blood taken an average of 3 years apart, suggesting that a single measurement is reasonably representative of circulating hormone levels in middle age. Finally, the similarity of the estimates when using different technologies for the separation of PCR-amplified fragments of different lengths in two different laboratories indicates that profound measurement error is unlikely an explanation for our null findings for the association between the androgen receptor gene CAG repeat and prostate cancer.

In conclusion, the lack of association of prostate cancer diagnosed in the PSA era with sex steroid hormones and the androgen receptor gene CAG repeat length is compatible with the hypothesis that these factors do not notably influence the development of early-stage disease. Our findings do not preclude that hormonal systems influence the transition from early to late stage, but cases that are regionally invasive or metastatic at diagnosis are rarer in the PSA era. The inverse associations for plasma total testosterone and free testosterone concentrations with high Gleason sum warrants further evaluation.

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References

1. Hsing AW. Hormones and prostate cancer: what's next? Epidemiol Rev 2001;23:42–58.
2. 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.
3. Jänne OA, Palvimo JJ, Kallio P, Mehto M. Androgen receptor and mechanisms of androgen action. Ann Med 1993;25:83–9.
4. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 1994;22:3181–6.
5. Kazemi-Esfarjani P, Trifiro MA, Pinsky L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenic relevance for the $(CAG)_n$-expanded neuropathies. Hum Mol Genet 1995;4:523–7.
6. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12:241–53.
7. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 2000;92:2009–17.
8. Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 1995;55:1937–40.
9. Ingles SA, Ross RK, Yu MC, et al. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. J Natl Cancer Inst 1997;89:166–70.
10. Stanford JL, Just JJ, Gibbs M, et al. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res 1997; 57:1194–8.
11. Giovannucci E, Stampfer MJ, Krithivas K, et al. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci 1997;94:3320–3.
12. Hsing AW, Gao YT, Wu G, et al. Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. Cancer Res 2000;60:5111–6.
13. Xue W, Irvine RA, Yu MC, Ross RK, Coetzee GA, Ingles SA. Susceptibility to prostate cancer: interaction between genotypes at the androgen receptor and prostate-specific antigen loci. Cancer Res 2000;60:839–41.
14. Mononen N, Ikonen T, Autio V, et al. Androgen receptor CAG polymorphism and prostate cancer risk. Hum Genet 2002;111:166–71.
15. Edwards SM, Badzioch MD, Minter R, Hamoud R, Collins N, Ardern-Jones A. Androgen receptor polymorphisms: association with prostate cancer risk, relapse and overall survival. Int J Cancer 1999;84:458–65.
16. Bratt O, Borg A, Kristofferson U, Lundgren R, Zhang QX, Olsson H. CAG repeat length in the androgen receptor gene is related to age at diagnosis of prostate cancer and response to endocrine therapy, but not to prostate cancer risk. Br J Cancer 1999;81:672–6.
17. Correa-Cerro L, Wohr G, Haussler J, et al. $(CAG)_n$CAA and GGN repeats in the human androgen receptor gene are not associated with prostate cancer in a French-German population. Eur J Hum Genet 1999;7:357–62.
18. Beilin J, Harewood L, Frydenberg M, et al. A case-control study of the androgen receptor gene CAG repeat polymorphism in Australian prostate carcinoma subjects. Cancer 2001;92:941–9.
19. Latil AG, Azzouzi R, Cancel GS, et al. Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. Cancer 2001;92:1130–7.
20. Miller EA, Stanford JL, Hsu L, Noonana E, Ostrander EA. Polymorphic repeats in the androgen receptor in high-risk sibships. Prostate 2001;48:200–5.
21. Modugno F, Weissfeld JL, Trump DL, et al. Allelic variants of aromatase and the androgen and estrogen receptors: toward a multigenic model of prostate cancer risk. Clin Cancer Res 2001;7:3092–6.
22. Chen C, Lamharzi N, Weiss NS, et al. Androgen receptor polymorphisms and the incidence of prostate cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1033–40.
23. Li C, Gronberg H, Matsuyama H, Weber G, et al. Difference between Swedish and Japanese men in the association between AR CAG repeats and prostate cancer suggesting a susceptibility-modifying locus overlapping the androgen receptor gene. Int J Mol Med 2003;11:529–33.
24. dos Santos ML, Sarkis AS, Nishimoto IN, Nagai MA. Androgen receptor CAG repeat polymorphism in prostate cancer from a Brazilian population. Cancer Detect Prev 2003;27:321–6.
25. Giovannucci E. Is the androgen receptor CAG repeat length significant for prostate cancer? Cancer Epidemiol Biomarkers Prev 2002;11:985–6.
26. Stampfer MJ, Willett WC, Speizer FE, et al. Test of the National Death Index. Am J Epidemiol 1984;119:837–9.
27. Eaton NE, Reeves GK, Appleby PN, Key TJ. Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies. Br J Cancer 1999;80:930–4.
28. Ross RK, Bernstein L, Lobo RA, et al. 5-α-reductase activity and risk of prostate cancer among Japanese and US white and black males. Lancet 1992;339:887–9.
29. Hankey BF, Feuer EJ, Clegg LX, et al. Cancer surveillance series: interpreting trends in prostate cancer. Part I. Evidence of the effects of screening in recent prostate cancer incidence, mortality, and survival rates. J Natl Cancer Inst 1999;91:1017–24.
30. Schatzl G, Madersbacher S, Thurridl T, et al. High-grade prostate cancer is associated with low serum testosterone levels. Prostate 2001;47:52–8.
31. Schatzl G, Madersbacher S, Haitel A, et al. Associations of serum testosterone with microvessel density, androgen receptor density and androgen receptor gene polymorphism in prostate cancer. J Urol 2003;169:1312–5.
32. Hoffman MA, DeWolf WC, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? J Urol 2000;163:824–7.
33. Zhang PL, Rosen S, Veeramachaneni R, Kao J, DeWolf WC, Bubley G. Association between prostate cancer and serum testosterone levels. Prostate 2002;53:179–82.
34. Fostad P, Bjoro T, Torlakovic G, Fossa SD. No association of serum gonadal or pituitary hormones with prognostic parameters in stages T1 to T3 pN0Mo prostate cancer. J Urol 2002;168:1188–92.
35. Thompson I, Goodman P, Tangen C, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med 2003;349:215–24.
36. Giovannucci E, Rimm EB, Liu Y, et al. Body mass index and risk of prostate cancer in U.S. Health professionals. J Natl Cancer Inst 2003;95: 1240–4.

Sex Steroid Hormones and the Androgen Receptor Gene CAG Repeat and Subsequent Risk of Prostate Cancer in the Prostate-Specific Antigen Era

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