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Advances in Brief

The V89L Polymorphism in the 5α-Reductase Type 2 Gene and Risk of Prostate Cancer[1]

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Abstract

5α-Reductase type 2, the predominant prostatic isozyme of this protein, converts testosterone to dihydrotestosterone. It has been hypothesized that individuals with greater 5α-reductase activity are at increased risk for prostate cancer (CaP). A single nucleotide polymorphism of the 5α-reductase type 2 gene (*SRD5A2*) gives rise to a substitution of leucine (leu) for valine (val) at codon 89 (V89L), the presence of which may affect serum androstanediol glucuronide (AAG) levels. We studied the effect of this polymorphism on the risk of prostate cancer in a prospective, nested, case-control design within the Physicians' Health Study. In all controls ($n = 799$), the *leu* allele frequency was 0.30. Among the 386 controls with plasma AAG levels available, there was no significant association between AAG levels and V89L genotype. We also detected no significant association between risk for CaP and genotype [odds ratio: *val/val* = 1.0 (reference), *leu/val* = 0.96 (95% confidence interval, 0.76–1.20), and *leu/leu* = 0.84 (95% confidence interval, 0.57–1.24)]. These data do not support a moderate to large effect of the *SRD5A2* V89L polymorphism on plasma AAG levels or CaP risk in this predominantly Caucasian cohort, although a small effect cannot be completely excluded.

Introduction

5α-Reductase converts testosterone, the most abundant androgen in the serum, to dihydrotestosterone (1). Two forms of 5α-reductase exist (*SRD5A1* and *SRD5A2*; Ref. 2); *SRD5A2* is the form exclusively expressed in the prostate (3, 4). Because *SRD5A2* is critical to prostate growth and development (5, 6), altered prostatic *SRD5A2* activity attributable to genetic polymorphisms within the *SRD5A2* gene could affect risk for CaP.[3]

A TA-dinucleotide repeat polymorphism is present in the 3′ untranslated region of *SRD5A2* (7, 8). Although this polymorphism has no known effect on protein function, longer alleles are associated with a modest reduction in risk for CaP (9).

More recently, several other polymorphisms in *SRD5A2* have been reported (10). The most common is a G to C transversion, resulting in a valine (val) to leucine (leu) variation at codon 89 (V89L; Ref. 10). In 102 Asian men, the *val/val* genotype was associated with 39% higher AAG levels (a surrogate marker of 5α-reductase activity) compared with *leu/leu* individuals; heterozygote, *leu/val* individuals had intermediate AAG levels (10). This polymorphism may also confer a 33% reduction in the V_{max} of *SRD5A2* as compared with the wild type (11). These preliminary findings prompted our study to determine whether the risk of CaP was associated with the V89L polymorphism using a nested case-control design within the Physicians' Health Study cohort.

Materials and Methods

Study Population. The source population for this analysis was the Physicians' Health Study, a randomized, double-blinded, placebo-controlled trial of aspirin and β-carotene in the prevention of heart disease and cancer (12, 13). The 22,071 men were 40–84 years of age at the start of the trial in 1982, and 97% were Caucasian. Men with a diagnosis of myocardial infarction, stroke, transient ischemic attacks, unstable angina, cancer (except nonmelanoma skin cancer), current renal or liver disease, peptic ulcer or gout, or who had a contraindication for aspirin, other platelet-active agents, or vitamin A supplement use were excluded from the trial. Updated medical history was obtained at 6 months, 1 year, and annually thereafter. Follow-up questionnaires were completed by 99% of the men through 1995, and vital status was known for all randomized men. A blood specimen, which was requested from participants before randomization, was returned by two-thirds of the men ($n = 14,916$; Ref. 9). CaP cases and controls were drawn from among the participants who supplied a blood specimen.

Prostate Cancer Cases and Controls. For men reporting a CaP diagnosis on a follow-up questionnaire, medical records and pathology reports were obtained and reviewed by study physicians to confirm the diagnosis. Through 1995, 592 CaP cancer cases were confirmed. One study physician reviewed the case records to determine stage at diagnosis [modified Whitmore-Jewett classification scheme (14)], tumor grade, and Gleason score (15). If pathological staging was not available, the case was considered to be of indeterminate stage, unless metastasis was clinically evident. We categorized cases as high stage/grade if diagnosed at stages C or D and/or had a Gleason score of ≥7 or poor histological differentiation. We selected 1 or 2 controls/case at random from among those men who returned a blood specimen and met the matching criteria. Matched controls were men who had not undergone a radical prostatectomy, had not been diagnosed with CaP by the date of the case diagnosis, and who were within 1 year of age (±2 years for elderly cases) and of the same smoking status (current, former, or never) at baseline as the case. Smoking history was used as a matching variable because the same set of cases and controls was used for other serum-based analysis that could be affected by smoking status; tobacco use is unlikely to be related to the V89L polymorphism and, thus, is unlikely to affect the results.

Laboratory Analysis. Whole-blood samples from cases and matched controls were received from the Physicians' Health Study, with the laboratory investigators blinded to the name and case/control status. Genomic DNA was obtained from 500 μl of the thawed whole blood using a commercially available kit (QIAamp DNA extraction kit; Qiagen, Chatsworth, CA). DNA concentration and purity were determined by UV absorbance on a Beckman DU640 spectrophotometer. Each sample was diluted to a final concentration of 20 ng/μl and stored at −20°C until analysis.

Adequate DNA quantities were available, and successful amplification was achieved for 584 of the 592 cases (98.6%) and 799 of the 807 controls (99.0%).

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[3] The abbreviations used are: CaP, prostate cancer; AAG, androstanediol glucuronide; OR, odds ratio; CI, confidence interval.

RFLP analysis after PCR amplification in combination with allele-specific PCR were used to identify the alleles for cases and controls. RFLP analysis was used to screen the entire cohort. The G to C transversion, leading to a valine to leucine substitution at codon 89, results in the loss of a *Rsa*I restriction site (GTAC to CTAC). Amplification was performed with primers 5′-GGCCACCTGGGACGTTACTTCTG-3′ (sense) and 5′-AAACGCTAC-CTGTGGAAGTAA-3′ (antisense). The first primer leads to a single nucleotide substitution in the amplified product (G mutated to T) and loss of a second *Rsa*I site. Thus, the polymorphic allele of interest leads to the loss of a restriction site unique within the amplified fragment, and *Rsa*I digestion readily identifies the genotype for each sample. Examples of each allele were directly sequenced to verify the accuracy of this assay (Fig. 1).

The PCR reaction mixture for RFLP analysis included 80 ng of template DNA, 1.0 μM of each primer, 50 mM KCl, 1.5 mM $MgCl_2$, 500 μM each deoxynucleotide triphosphate, and 1.5 units of AmpliTaq (Perkin-Elmer, Norwalk, CT) in a final volume of 22 μl. All amplifications were performed using MicroAmp tubes (Perkin-Elmer).

A Perkin-Elmer GeneAmp PCR System 9600 thermocycler was programmed for three-step PCR. After 5 min at 94°C, samples underwent 35 cycles of melting at 94°C for 30 s, annealing at 55°C for 45 s, and elongation at 65°C for 20 s. There was a final elongation step for 8 min at 65°C, and samples were then cooled to 4°C.

After amplification, 12 μl of amplified product were digested with 5 units of *Rsa*I according to the manufacturer's recommendations (New England Biolabs, Beverly, MA). Digested product was separated using a 2.5% agarose gel containing ethidium bromide. Genotype was based on banding pattern (Fig. 1). A primer dimer, with a length slightly less than that of the undigested *leu* allele, would intermittently obscure results, and for those samples with unclear results, allele-specific PCR was designed.

Allele-specific PCR was performed using two sense primers that were designed and that differed by 2 bp in the final 4 bp from the 3′ end. These primers were: leu (sense), 5′-TACTTCTGGGCCTCTTCTGGC-3′; and val (sense), 5′- TACTTCTGGGCCTCTTCGGCG-3′. The antisense primer was the second primer listed above. The PCR reaction mixture included 40 ng of template DNA, 0.5 μM either val (sense) or leu (sense), and 0.5 μM antisense primer, 50 mM KCl, 1.5 mM $MgCl_2$, 500 μM each deoxynucleotide triphosphate, and 1.8 units of AmpliTaq (Perkin-Elmer) in a final volume of 25 μl. Three-step PCR was used to amplify product. After 5 min at 94°C, samples underwent 35 cycles of melting at 94°C for 30 s, annealing at 55°C for 60 s, and elongation at 65°C for 25 s. There was a final elongation step for 8 min at 72°C, and samples were then cooled to 4°C. Each sample was amplified in reaction mixtures containing val (sense) and leu (sense), and product was separated using a 2.5% agarose gel containing ethidium bromide. Amplification of product identified the presence of either allele (Fig. 1*b*).

Allele-specific PCR was performed on samples with unclear genotype after restriction digestion ($n = 341$), and all samples were identified by the restriction digestion assay as *leu/leu* ($n = 82$) to assure results were not attributable to poor restriction digestion. Of the samples determined to be *leu/leu* with the restriction digestion, 96% (79 of 82) were confirmed by the allele-specific assay to be *leu/leu*. The three discordant samples were found to be heterozygous by the allele-specific assay and were analyzed as heterozygotes. Fifty-three samples were analyzed with both methods to test correlation, and only one sample was discordant (98% concordant, $n = 52$ of 53). The discordant sample was identified as *leu/leu* by the restriction assay but was subsequently

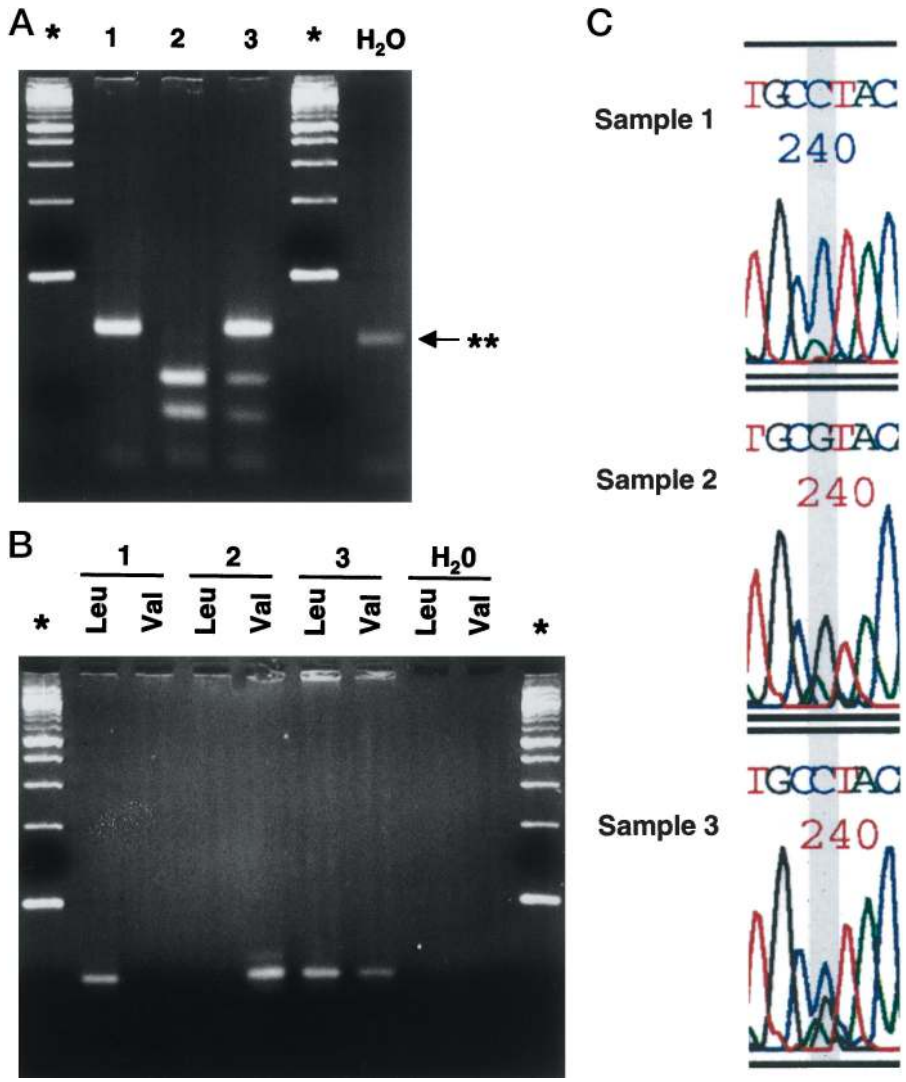

Fig. 1. Restriction-site (*A*) and allele-specific (*B*) PCR-based assays and confirmation by direct sequencing (*C*). Three samples (*Sample 1,* homozygote *leu/leu*; *Sample 2,* homozygote *val/val*; and *Sample 3,* heterozygote *val/leu*) were subjected to both PCR-based assays and sequenced. The more common *val* allele contains a *Rsa*I site (GTAC) that is lost with the *leu* polymorphism (CTAC). Allele-specific primers and parameters are described in the text. A primer-dimer band (**), with a size detectably smaller but very close to the uncut (*leu*) allele, is present in the water lane under optimal conditions. This primer dimer is occasionally present in samples lanes, confounding accurate genotyping. For samples with unclear restriction analysis, allele-specific PCR was used for genotyping. *, 100-bp ladder (NE BioLabs).

found to be heterozygous (*leu/val*) using the allele-specific assay, underscoring the importance of confirming all samples that were determined to be *leu/leu* by the restriction digestion. Plasma AAG levels were determined previously by RIA (16).

Statistical Analysis. We used the χ^2 test to evaluate whether the distribution of genotype varied significantly between cases and controls (17). Using unconditional maximum regression models controlling for the matching variables baseline age (5-year intervals) and smoking status (never, former, or current), we estimated ORs and 95% CIs for the relation of 5α-reductase genotype and total and high stage/grade CaP (18). The OR is an estimation of the relative risk. We included all controls irrespective of whether the matched case was omitted to increase the power to estimate the relation between 5α-reductase genotype and high stage/grade CaP. Similar results were obtained from conditional logistic regression models to account for the matching. We used the Kruskal-Wallis test (17) to assess whether mean concentration of plasma AAG varied by genotype among the controls and, to test for trend, we used the Wald test where genotype was entered as a categorical variable in a linear regression model. We used SAS to conduct all analyses (SAS Institute Inc., Cary, NC).

Results

Genotyping. Genotypes were determined for 1383 individuals (Table 1). There was no difference in the distribution of genotype between controls and all cases ($P = 0.7$) or high stage/grade cases ($P = 0.9$).

Relationship between Genotype and CaP Risk. The ORs for total CaP and aggressive CaP, in relationship to genotype, are shown in Table 2. Although the likelihood of developing CaP and aggressive CaP was slightly lower in individuals with *leu* alleles compared with those homozygote for the *val* allele, the magnitudes were small and not statistically significant ($P_{trend} = 0.4$ and 0.6, respectively).

Hormone Analysis. Plasma steroid hormone levels were available for 386 (48%) of the controls. Mean AAG levels by genotype are shown in Table 3. Although controls with the *val/val* genotype had a higher mean AAG level than individuals with the *val/leu* genotype or the *leu/leu* genotype, the differences among the means were not statistically significant ($P = 0.21$). The trend for decreasing levels across genotypes was also not statistically significant ($P_{trend} = 0.18$)

Discussion

Androgens are necessary for prostate development and critical in the pathogenesis of CaP (19–21). Variability in the plasma level of androgens may account for some of the observed differences in risk of CaP between racial/ethnic groups (1, 16, 22). In addition, genetic polymorphisms affecting an individual's androgen metabolism and signal transduction may also influence the incidence or behavior of CaP. In this study, we found no significant association between the V89L polymorphism within the *SRD5A2* gene and a measure of 5α-reductase activity or risk of total CaP and aggressive CaP.

There are several possible explanations for the lack of association observed between the V89L polymorphism and plasma AAG levels: (*a*) plasma AAG levels may be poor surrogates for intraprostatic 5α-reductase, type 2 activity. 5α-Reductase type 1 (expressed in the liver) likely contributes to plasma AAG levels and may decrease or eliminate the association between the V89L polymorphism and serum AAG levels. Our study may not have had sufficient statistical power to detect a small to modest effect of the V89L polymorphism on AAG levels, if present.

In the initial report of the V89L polymorphism, Makridakis *et al.* (10) found a statistically significant 39% higher mean AAG level in *val/val* individuals than in *leu/leu* in an Asian cohort. In our cohort of predominately Caucasian men, we found only a 10% higher mean AAG level in men with *val/val* compared with men having the *leu/leu* genotype.

The AAG levels reported for Asian men by Makridakis *et al.* (10) are 35–48% lower than those observed in our study. The observed difference may be attributable to differences in specimen handling, age, and race/ethnicity of study subjects or interassay variation. Like Makridakis *et al.*, we used a RIA-based test on serum that was frozen prior to use, but the Asian men in their study had, on average, lower levels of AAG than our population of mostly Caucasian men. This racial/ethnic difference agrees with a prior report demonstrating statistically significantly lower AAG levels in young Asian men compared with young Caucasian men, although the differences were less pronounced (23% between Japanese men and White men; Ref. 3). Two other studies of serum AAG levels in Caucasians reported values similar to ours (3, 23), and in a fourth study, AAG levels were at an intermediate level in Japanese-American men (24).

The association found by Makridakis *et al.* (10) between the V89L polymorphism and plasma AAG levels, which is not supported by our study, suggests that the V89L polymorphism may be important in some racial/ethnic populations but not others. Alternatively, the V89L could be in linkage disequilibrium with a locus that alters AAG levels in some racial/ethnic groups but not others (such as another polymorphic locus within the same gene). It will be important to further investigate this polymorphism in Asian (higher prevalence of *leu* allele) and African American (lower prevalence of *leu* allele) men to understand how racial/ethnic differences affect the interaction of this polymorphism with plasma AAG levels and CaP risk or behavior.

We found no statistically significant association between genotype for the V89L polymorphism in *SRD5A2* and CaP risk. We used two PCR-based assays, validated by direct sequencing, to determine genotype for the participants in this study. The overall frequency of the *leu* allele of 0.30 is similar to a prior published report (0.23 among Caucasians; Ref. 10). There was a weak suggestion of a trend toward

Table 1 Distribution of the V89L polymorphism in the 5α-reductase type 2 gene by CaP status in the Physicians' Health Study, 1982–1995

	Genotype			
	leu/leu	*leu/val*	*val/val*	P^a
Controls (*n*)	78	330	391	
Total cases (*n*)	50	239	295	0.7
High stage/grade cases (*n*)	24	110	135	0.9

[a] *P* for χ^2 test for independence.

Table 2 Relationship between V89L polymorphism in the 5α-reductase type 2 gene and CaP in the Physicians' Health Study, 1982–1995

	Genotype		
CaP	*leu/leu*	*leu/val*	*val/val*
Total			
OR[a]	0.84	0.96	1.00
CI[b]	0.57–1.24	0.76–1.20	(Reference)
High stage/grade			
OR	0.88	0.97	1.00
CI	0.54–1.46	0.72–1.30	(Reference)

[a] OR estimated from logistic regression model, controlling for age and smoking status.
[b] 95% CI.

Table 3 Mean ± SD plasma AAG concentration (ng/ml) by the V89L polymorphism in the 5α-reductase type 2 gene in controls in the Physicians' Health Study, 1982–1995

	Genotype			
	leu/leu	*leu/val*	*val/val*	P^a
Controls (*n*)	6.52 ± 2.76 (39)	6.91 ± 3.54 (164)	7.22 ± 3.12 (183)	0.21

[a] *P* for Kruskal-Wallis test for difference of means by genotype.

the *leu* allele being protective (Table 2), agreeing with the *a priori* hypothesis, but it was not statistically significant in this cohort. Thus, we cannot exclude a small association, but a moderate to large effect of this polymorphism on CaP is unlikely.

References

1. Ross, R., Coetzee, G., Reichardt, J., Skinner, E., and Henderson, B. Does the racial-ethnic variation in prostate cancer risk have a hormonal basis? Cancer (Phila.), *75:* 1778–1782, 1995.
2. Jenkins, E. P., Andersson, S., Imperato-McGinley, J., Wilson, J. D., and Russel, D. W., Genetic and pharmacologic evidence for more than one human steroid 5α-reductase. J. Clin. Investig., *89:* 293–300, 1992.
3. Ross, R., Bernstein, L., Lobo, R., Shimizu, H., Stanczyk, F., Pike, M., and Henderson, B. 5α-Reductase activity and risk of prostate cancer among Japanese and US white and black males. Lancet, *339:* 887–889, 1992.
4. Thigpen, A., Davis, D., Milatovich, A., Mendonca, B., Imperato-McGinley, J., Griffin, J., Francke, U., Wilson, J., and Russell, D. Molecular genetics of steroid 5α-reductase 2 deficiency. J. Clin. Investig., *90:* 799–809, 1992.
5. Andersson, S., Berman, D. M., Jenkins, E. P., and Russell, D. W. Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. Nature (Lond.), *354:* 159–161, 1991.
6. Wilson, J. D., Griffin, J. E., and Russell, D. S. Steroid 5α-reductase 2 deficiency. Endocr. Rev., *14:* 577–593, 1993.
7. Davis, D., and Russell, D., Unusual length polymorphism in human steroid 5α-reductase type 2 gene (*SRD5A2*). Hum. Mol. Genet., *2:* 820, 1993.
8. Reichardt, J., Makridakis, N., Henderson, B., Yu, M., Pike, M., and Ross, R. Genetic variability of the human *SRD5A2* gene: implications for prostate cancer risk. Cancer Res., *55:* 3973–3975, 1995.
9. Kantoff, P., Febbo, P., Giovannucci, E., Krithivas, K., Dahl, D., Chang, G., Hennekens, C., Brown, M., and Stampfer, M. A polymorphism of the 5α-reductase gene and its association with prostate cancer: A case-control analysis. Cancer Epidemiol. Biomark. Prev., *6:* 189–192, 1997.
10. Makridakis, N., Ross, R., Pike, M., Chang, L., Stanczyk, F., Kolonel, L., Shi, C., Yu, M., Henderson, B., and Reichardt, J. A prevalent missense substitution that modulates activity of prostatic steroid 5α-reductase. Cancer Res., *57:* 1020–1022, 1997.
11. Ross, R., Pike, M., Coetzee, G., Reichardt, J., Yu, M., Feigelson, H., Stanczyk, F., Kolonel, L., and Henderson, B. Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. Cancer Res., *58:* 4497–4504, 1998.
12. Steering Committee of the Physicians' Health Study Preliminary Report: findings from the aspirin component of the ongoing Physician's Health Study. N. Engl. J. Med., *318:* 262–264, 1988.
13. Steering Committee of the Physicians' Health Study Report: final report on the aspirin component of the ongoing Physicians' Health Study. N. Engl. J. Med., *321:* 129–135, 1989.
14. Catalona, W., and Avioli, L., Diagnosis, staging, and surgical treatment of prostatic carcinoma. Arch. Intern. Med., *147:* 361–363, 1987.
15. Gittes, R., Carcinoma of the prostate. N. Engl. J. Med., *324:* 236–245, 1991.
16. Gann, P. H., Hennekens, C. H., Ma, J., Longcope, C., and Stampfer, M. J., Prospective study of sex hormone levels and risk of prostate cancer. J. Natl. Cancer Inst., *88:* 1118–1126, 1995.
17. Rosner, B. Fundamentals of Biostatistics, Ed. 2, pp. 467–472. Boston: Duxbury Press, 1986.
18. Kleinbaum, D., Kupper, L., and Morganstern, H., Epidemiologic Research. Principles and Quantitative Methods, pp. 419–446. New York: Van Nostrand Reinhold, 1982.
19. Coffey, D., Physiological control of prostatic growth: an overview. Prostate cancer. Union Int. Contre Cancer Tech. Rep. Ser., *48:* 4–23, 1979.
20. Pollard, M., Luckert, P. H., and Schmidt, M. A. Induction of prostate adenocarcinomas in Lobund Wistar rats by testosterone. Prostate, *3:* 563–568, 1982.
21. Noble, R. L. The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration. Cancer Res., *37:* 1929–1933, 1977.
22. Ross, R., Bernstein, L., Judd, H., Hanisch, R., Pike, M., and Henderson, B. Serum testosterone levels in healthy young black and white men. J. Natl. Cancer Inst., *76:* 45–48, 1986.
23. Guess, H. A., Friedman, G. D., Sadler, M. C., Stanczyk, F. Z., Vogelman, J. H., Imperato-McGinley, J., Lobo, R. A., and Orentreich, N. 5α-Reductase activity and prostate cancer: a case-control study using stored sera. Cancer Epidemiol. Biomark. Prev., *6:* 21–24, 1997.
24. Nomura, A. M., Stemmermann, G. N., Chyou, P-H., Henderson, B., and Stanczyk, F. Z., Serum androgens and prostate cancer. Cancer Epidemiol. Biomark. Prev., *5:* 621–625, 1996.