5α-Reductase Activity and Prostate Cancer: A Case-Control Study Using Stored Sera

Harry A. Guess,[1] Gary D. Friedman, Marianne C. Sadler, Frank Z. Stanczyk, Joseph H. Vogelman, Julianne Imperato-McGinley, Rogerio A. Lobo, and Norman Orentreich

Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27599-7400 and Merck Research Laboratories, Blue Bell, Pennsylvania 19422 [H. A. G.]; Kaiser Permanente Medical Care Program, Division of Research, Oakland, California [G. D. F., M. C. S.]; Department of Obstetrics and Gynecology, School of Medicine, University of Southern California, Los Angeles, California [F. Z. S.]; Orentreich Foundation for the Advancement of Science, Inc., Cold Spring-on-Hudson, New York [J. H. V., N. O.]; Department of Medicine, Cornell University, New York, New York [J. I-M.]; and Department of Obstetrics and Gynecology, Columbia University, College of Physicians and Surgeons, New York, New York [R. A. L.]

Abstract

We report a nested case-control study of serum biomarkers of 5α-reductase activity and the incidence of prostate cancer. From a cohort of more than 125,000 members of the Kaiser Permanente Medical Care Program who underwent multiphasic health examinations during 1964–1971, we selected 106 incident prostate cancer cases. A control was pair matched to each case on age, date of serum sampling, and clinic location. Serum levels of total testosterone, free testosterone, androsterone glucuronide, and 5α-androstane-3α,17β androstanediol glucuronide (3α-diol G) were measured on the stored samples and scored as quartiles. Potential confounders included alcohol, smoking, and body mass index. The adjusted odds ratios and 95% confidence intervals for a one quartile score increase were 1.00 (0.75–1.34) for total testosterone, 1.14 (0.86–1.50) for free testosterone, 1.13 (0.84–1.53) for androsterone glucuronide, and 1.16 (0.86–1.56) for 3α-diol G. A limitation of this study is that there are two different 5α-reductase isoenzymes, only one of which is expressed in high levels within the prostate, yet both of which may affect serum biomarkers. Since the two isoenzymes are encoded on different chromosomes, variation in one would act as an independent source of measurement error in any analysis of serum biomarker effects of the other. Consequently, the odds ratios may be underestimated and the study, although negative, cannot exclude the previously hypothesized possibility that a positive relationship between intraprostatic 5α-reductase activity and prostate cancer may exist. A clinical trial to test this hypothesis is under way.

Introduction

Normal prostate growth is mediated by androgens and treatment of prostate cancer with androgen deprivation produces tumor regression. However, epidemiological studies relating serum testosterone levels to prostate cancer risk have yielded inconsistent results (1). Within the prostate, testosterone undergoes irreversible 5α reduction to dihydrotestosterone, whose binding affinity to the prostatic androgen receptor is five times higher than that of testosterone (2–4). A recent study showed that young Japanese men have lower 5α-reductase activity than young Caucasian-American and African-American men, based on assays of serum biomarkers of 5α-reductase activity (5). The authors suggested that the lower 5α-reductase activity in Japanese men may at least partially explain the lower incidence of prostate cancer in Japanese men than in Caucasian-American and African-American men. In addition, human clinical trials show that pharmacological inhibition of intraprostatic 5α-reductase activity decreases prostate size and glandular epithelial activity both in benign prostatic hyperplasia and in prostate cancer (6, 7). All of these results make it biologically plausible that lowering intraprostatic 5α reductase activity may reduce the incidence of prostate cancer.

To provide a definitive test of the hypothesis that pharmacological inhibition of intraprostatic 5α-reductase activity will reduce the incidence of prostate cancer, a randomized, double-blind, placebo-controlled clinical trial has recently been undertaken (8). In this trial, 18,000 healthy men 55 years of age and older have been randomized in equal proportions to a specific 5α-reductase inhibitor (finasteride) or placebo and are being followed up for 7 years for the primary end point of biopsy-proven presence or absence of prostate cancer. The trial is designed to have 92% power to detect a 25% reduction in the prevalence of biopsy-proven prostate cancer using a two-sided α of 0.05.

Because results of this trial will not be available until 2002, we undertook a nested case-control study to test the hypothesis that serum biomarkers of 5α-reductase activity are related to the risk of prostate cancer. The study used prospectively collected stored serum samples from a well-characterized cohort for which follow-up for cancer diagnoses was considered to be virtually complete.

Subjects and Methods

Study Population. Between 1964 and 1971 serum samples were collected from more than 125,000 adult enrollees in the KPMCP who participated in a multiphasic health screening program (9). These samples were stored at −23°C or colder in Oakland, CA until 1980, when they were packed in dry ice and

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[1] To whom requests for reprints should be addressed, at Department of Epidemiology, CB 7400, McGavran-Greenberg Hall, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400.

[2] The abbreviations used are: KPMCP, Kaiser Permanente Medical Care Program; 3α-diol G, 5α-androstane-3α,17β-androstanediol glucuronide.

shipped to the Orentreich Foundation for the Advancement of Science in New York, where they have been stored at −40°C thereafter. Cancers diagnosed in this population have been ascertained by records from the KPMCP and the California Tumor Registry, as described in previous epidemiological studies of lung cancer (10), gastric cancer (11), and prostate cancer (12–14) that were conducted in this cohort using this source of serum samples. Previous work has documented that nearly all cancers diagnosed among members of KPMCP are likely to be reflected in these records (15).

Cases and Controls. The 106 cases were randomly selected from the Caucasian men in the multiphasic health examination cohort who met all of the following conditions: (*a*) Serum samples were available. (*b*) The initial prostate cancer diagnosis occurred more than 5 years after the serum sample was obtained. (*c*) The men were alive and still enrolled in KPMCP at the time of their initial prostate cancer diagnosis. Approximately 185 men met all three conditions. The restriction to Caucasian men was made because previous studies used most of the serum samples on cases in African-American men. All cases were all diagnosed between September 1970 and November 1987, at a time when prostate-specific antigen testing was not being used clinically for prostate cancer detection within the KPMCP. The median time from serum sampling to prostate cancer diagnosis was 14 years (range, 5–23 years).

Controls were Caucasian men who were pair matched to the case on age (±1 year), date of multiphasic examination at which the serum sample was drawn (±1 month), and location of the clinic where their multiphasic examination was held. Controls were also required to meet the same KPMCP enrollment criteria as was required of cases. In addition, each control was required to be at risk for incident prostate cancer at the time of the incident prostate cancer diagnosis of the case to which the control was matched.

Cases and matched controls were also required to have no history of prostate surgery at the time of their serum sampling and no record of hospitalization for prostatectomy up through the time of diagnosis of initial prostate cancer in the case. The latter requirement was imposed to eliminate all prostate cancer cases diagnosed at prostatectomy. This was done to avoid detection bias due to the possibility that men with higher 5α-reductase activity might be more likely to undergo transurethral prostatectomy for benign prostatic hyperplasia, with an increased likelihood of having stage A prostate cancers detected. Because all cases in this study were diagnosed at a time when prostate-specific antigen testing was not being used clinically for prostate cancer detection within KPMCP, the elimination of prostate cancer cases diagnosed at prostatectomy effectively limited cases to stage B and higher. To avoid selection bias, the same restriction of no prostatectomies was applied to both cases and controls.

Potential Confounders. Information on a number of potential confounders was available for each member of the multiphasic health examination cohort. Age, race, date of serum sampling, and clinic location were controlled by matching. The following other potential confounders were ascertained based on information from the multiphasic health examination when the serum sample used in the analysis was obtained: obesity (measured by tertiles of body mass index), self-reported history of diabetes mellitus at baseline (present, not present), alcohol consumption (nondrinker, two drinks or less per day, three drinks or more per day), and smoking history (nonsmoker, former smoker, current smoker, missing). The alcohol consumption variables were dichotomized as drinking at the level of one or two drinks daily (yes/no) and drinking at the level of three or more drinks daily (yes/no), so that a nondrinker would be represented as having a negative answer to both questions.

The three smoking variables were dichotomized as current smoker (yes/no), former smoker (yes/no), or missing smoking data (yes/no), so that a person who reported never having smoked would be represented as having a negative response on each of the above three variables. Each subject was categorized into one of the above categories based on his responses to questions on the baseline health questionnaire. Current smokers were those who reported current cigarette, cigar, or pipe smoking at the time of their baseline examination.

Laboratory Assays. As a rough check on desiccation, each serum sample was required to have a serum sodium in the normal range. The specific markers of 5α-reductase activity that were assayed included two metabolites of dihydrotestosterone: androsterone glucuronide and 3α-diol G. These markers may reflect 5α-reductase activity more reliably than serum levels of dihydrotestosterone because they are present in higher levels in the circulation (16) and because of substantial binding of dihydrotestosterone to sex hormone-binding globulin. Free testosterone and total testosterone were also measured.

Serum levels of total testosterone, 3α-diol G, and androsterone glucuronide were quantified by validated specific radioimmunoassays in the laboratory of two coinvestigators (F. Z. S., R. A. L.). Testosterone was first extracted from serum with hexane:ethyl acetate (1:1) and subjected to Celite column partition chromatography prior to RIA (17); 3α-diol G was measured directly in serum (18). Androsterone glucuronide was first hydrolyzed using a specific β-glucuronidase (Sigma Chemical Company, St. Louis, MO), and the product, androsterone, was then extracted with diethyl ether and chromatographed on Celite prior to RIA (19). The appropriate factor was used to correct for procedural loss and molecular weight differences due to the hydrolysis step.

Free testosterone (fraction not bound to sex hormone-binding globulin) was quantified by determining its percentage of total testosterone present in serum using ammonium sulfate precipitation to remove the globulins, as described previously (20). The concentration of free testosterone was then calculated by multiplying the percentage of free testosterone times the total testosterone concentration.

Statistical Analysis. Serum levels of each of the biomarkers were divided into quartiles for purposes of analysis. This was done to avoid methods of statistical analysis that could be overly sensitive to extreme outliers, which could result from storage conditions. The primary method of analysis was conditional logistic regression for matched pairs, which is mathematically equivalent to Cox regression for matched pairs. The odds ratio was calculated for the quartile score for a case to be higher than the quartile score for the pair-matched control. The planned sample size was 100 matched pairs (actual number, 106 matched pairs). This provided 80% power to detect an odds ratio of 1.8 at a two-sided α level of 0.05 (21).

Results

Cases and controls were relatively similar with respect to the potential confounders measured (Table 1). The tendency of the biomarker levels to fall in the lower end of the normal range is consistent with the fact that the laboratory normal range is based on measurements using blood samples from younger men (<40 years old; Table 2). It is well recognized that the levels of 3α-diol G and androsterone glucuronide decline symmetrically with advancing age (22).

Table 1 Characteristics of cases and controls

	Cases (n = 106)	Controls (n = 106)
Dates of case diagnoses	September 1970–November 1987	
Time from serum sampling to diagnosis		
Median (yr)	14	
Range (yr)	5–23	
Age at case diagnosis		
Median (yr)	70	70
Range (yr)	54–86	53–86
Body mass index		
Mean ± SE	25.4 ± 0.27	25.3 ± 0.30
Smoking history		
Nonsmokers	22	23
Former smokers	18	21
Current smokers	52	45
Missing data smoking	14	17
Alcohol consumption		
Nondrinker	17	16
≤2 drinks/day	46	59
≥3 drinks/day	28	13
Missing data	15	18
History of diabetes mellitus		
No. with positive history	5	4

Table 2 Range of biomarkers in cases and controls[a]

	Laboratory normal ranges	Percentiles (5-50-95)	Percentiles (5-50-95)
	(ng/ml)	Cases(ng/ml)	Controls(ng/ml)
Androstanediol glucuronide	3.4–22.0	3.1–6.7–13.2	3.2–6.3–13.5
Androsterone glucuronide	78.0–128.0	26.2–58.0–189.0	29.5–57.3–168.1
Total testosterone	3.0–10.0	2.6–4.5–7.5	2.0–4.3–7.5
Free testosterone	0.66–4.2	0.5–1.1–2.3	0.5–1.1–2.1

[a] Androstanediol glucuronide and total testosterone measurements were available on all 106 cases and 106 controls. The androsterone glucuronide measurement was missing on one case, and the free testosterone measurements were missing on two cases.

Table 3 Odds ratios for androsterone glucuronide, total testosterone, free testosterone[a]

	Crude odds ratio	Adjusted odds ratio
Androstanediol glucuronide	1.11 (0.85–1.45)	1.16 (0.86–1.56)
Androsterone glucuronide	1.04 (0.81–1.35)	1.13 (0.84–1.53)
Total testosterone	0.95 (0.74–1.22)	1.00 (0.75–1.34)
Free testosterone	1.08 (0.84–1.38)	1.14 (0.86–1.50)

[a] Matched on age, date of serum sampling, and clinic location. All cases and controls were Caucasian men. The adjusted odds ratios were adjusted for baseline history of alcohol consumption, body mass index, smoking, and diabetes mellitus. The 95% confidence intervals are shown in parentheses.

The adjusted odds ratios and 95% confidence intervals for a one quartile score increase were 1.00 (0.75–1.34) for total testosterone, 1.14 (0.86–1.50) for free testosterone, 1.13 (0.84–1.53) for androsterone glucuronide, and 1.16 (0.86–1.56) for androstanediol glucuronide (Table 3). The crude odds ratios were nearly identical, although slightly lower in each instance (Table 3). For none of the hormonal measures except androstanediol glucuronide was there evidence of statistical interaction (at the level of $P < 0.10$) with any of the covariates. There was a statistically significant interaction between smoking and androstanediol glucuronide. Upon further examination, this interaction was considered to be artifactual, sensitive to minor differences in how smoking categories were handled in the analysis, and most likely driven by the small size of the "former smokers" group (Table 1). The odds ratio for androstanediol glucuronide was therefore computed without inclusion of the interaction term.

Discussion

None of the hormonal markers evaluated in this study was found to be related in any consistent way to the incidence of prostate cancer. A major limitation of this study has to do with the extent to which serum levels of androsterone glucuronide and 3α-diol G reflect intraprostatic 5α-reductase activity. There are at least two different human 5α-reductases, designated 5α-reductase 1 and 5α-reductase 2 (23). The type 2 isoenzyme is encoded on chromosome 2, is expressed in high levels in the prostate, and is deficient in a syndrome of male pseudohermaphroditism associated with congenital underdevelopment of the prostate. The type 1 isoenzyme is encoded on chromosome 5, is not associated with male pseudohermaphroditism, and does not appear to be expressed in the prostate, although some authors report low levels of type 1 protein in specific cells in the prostate (24).

Because the type 2 isoenzyme is the predominant intraprostatic form, it would be expected that if 5α-reductase activity is related to prostate cancer, then the relationship should be much stronger with the predominately intraprostatic (type 2) isoenzyme than with the predominately extraprostatic (type 1) isoenzyme. However, serum levels of biomarkers of 5α-reductase activity may be affected by both isoenzymes. The variation in serum biomarkers produced by the type 1 isoenzyme may be thought of extraneous measurement variability in the analysis of serum biomarker effects of the type 2 isoenzyme. Consequently, the odds ratios for 3α-diol G and androsterone glucuronide may be biased to the null.

Assay variability introduced by sample degradation would be expected to introduce an additional source of bias, although the actual levels achieved in this study did not suggest any degradation (Table 2). Because cases and controls were pair matched on the date of the examination at which the serum sample for each was obtained, any degradation would be expected to be nondifferential and therefore the expected direction of this bias would be to the null. The classification of hormonal levels by quartile scores should reduce any effect of extreme variation in levels on the analysis because the difference in quartile scores for a case-control pair could range only from −3 to +3.

Finally, a problem common to nearly all epidemiological studies of prostate cancer is the high frequency of undiagnosed prostate cancer that can be expected to be present among as many as 30% of controls (25, 26). Even large prostate cancers are often only detected at autopsy (26). In the present study, the expected effect of undetected prostate cancer among controls would be to produce a further bias to the null.

After this study had been completed and analyzed, we learned of a similar study of serum androgen levels and prostate cancer that had been conducted by Nomura *et al.* (27) as a nested case-control study in a cohort of Japanese-American men. The latter study, which had been conducted entirely independently of the present study, included all of the biomarkers in the present study. Both studies used incident prostate cancer cases, pair-matched to controls, and used similar, although not identical, methods of analyzing elevations in serum

hormone levels based on comparing quartiles between cases and controls. In each study the hormonal measurements for each subject were made on a single prediagnostic stored serum sample.

Although differences in definitions precluded exact comparisons, the two studies had very similar findings and the differences in risk estimates in the two studies appeared to be compatible with sampling variability. In neither study was there evidence to suggest a strong relationship between the serum androgen levels and prostate cancer.

Although the results of the present study and the previous one taken together cannot exclude the possibility that a clinically important relationship between intraprostatic 5α-reductase activity and prostate cancer incidence may exist, the results do suggest that epidemiological studies based on these serum markers of 5α-reductase activity are not likely to be sensitive enough to provide useful information on this relationship. Epidemiological studies based on intraprostatic markers do not appear feasible on ethical grounds. An ongoing randomized, double-blind, placebo-controlled clinical trial of pharmacologically mediated inhibition of intraprostatic 5α-reductase activity in the prevention of prostate cancer may provide a more definitive test of the relationship between intraprostatic 5α-reductase activity and prostate cancer (8).

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