Plasma Antibodies against *Chlamydia trachomatis*, Human Papillomavirus, and Human Herpesvirus Type 8 in Relation to Prostate Cancer: A Prospective Study

Siobhan Sutcliffe,[1] Edward Giovannucci,[6] Charlotte A. Gaydos,[2] Raphael P. Viscidi,[3] Frank J. Jenkins,[7] Jonathan M. Zenilman,[2] Lisa P. Jacobson,[1] Angelo M. De Marzo,[4,5] Walter C. Willett,[6] and Elizabeth A. Platz[1,4]

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health; [2]Division of Infectious Diseases, Department of Medicine; [3]Stanley Division of Developmental Neurovirology, Department of Pediatrics; [4]James Buchanan Brady Urological Institute and the Sidney Kimmel Comprehensive Cancer Center; and [5]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, Maryland; [6]Departments of Nutrition and Epidemiology, Harvard School of Public Health and the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; and [7]Department of Pathology, School of Medicine, and Departments of Infectious Diseases and Microbiology, School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract

Traditionally, case-control studies of sexually transmitted infections and prostate cancer have focused on gonorrhea and syphilis, with overall positive associations. More recently, researchers have begun to expand their focus to include additional sexually transmitted infections, such as *Chlamydia trachomatis*, human papillomavirus (HPV), and human herpesvirus type 8 (HHV-8) infections. Continuing this investigation, we examined each of these infections in relation to incident prostate cancer in a nested case-control study within the Health Professionals Follow-up Study. Prostate cancer cases were men diagnosed with prostate cancer between the date of blood draw (1993-1995) and 2000 (n = 691). Controls were men free of cancer and alive at the time of case diagnosis who had had at least one prostate-specific antigen test between the date of blood draw and case diagnosis. One control was individually matched to each case by age; year, time of day, and season of blood draw; and prostate-specific antigen screening history before blood draw (n = 691). *C. trachomatis* and HPV-16, HPV-18, and HPV-33 antibody serostatus were assessed by enzyme-based immunoassays and HHV-8 antibody serostatus was assessed by an immunofluorescence assay. No associations were observed between *C. trachomatis* [odds ratio (OR), 1.13; 95% confidence interval (95% CI), 0.65-1.96], HPV-16 (OR, 0.83; 95% CI, 0.57-1.23), HPV-18 (OR, 1.04; 95% CI, 0.66-1.64), and HPV-33 (OR, 1.14; 95% CI, 0.76-1.72) antibody seropositivity and prostate cancer. A significant inverse association was observed between HHV-8 antibody seropositivity and prostate cancer (OR, 0.70; 95% CI, 0.52-0.95). As this study is the first, to our knowledge, to observe such an inverse association, similar additional studies are warranted. (Cancer Epidemiol Biomarkers Prev 2007;16(8):1573–80)

Introduction

In 1950, Ravich and Ravich (1) proposed that sexually transmitted infections may contribute to prostate carcinogenesis. Since that time, several case-control studies have investigated this hypothesis with overall positive associations (2, 3). Traditionally, these studies focused on gonorrhea or syphilis. However, more recent epidemiologic studies have begun to investigate additional sexually transmitted infections, reflecting the expanding number of recognized sexually transmitted pathogens over time. These include *Chlamydia trachomatis*, human papillomavirus (HPV), and human herpesvirus type 8 (HHV-8) infections. *C. trachomatis* is a putative, candidate infection because it is intracellular and is often asymptomatic in men, which may allow it to persist in the male genitourinary tract and possibly ascend to the prostate, where it has been observed to infect prostate epithelial cells and elicit an intraprostatic inflammatory immune response (4-6). HPV and HHV-8 infections are putative candidates because they have both been associated with other cancers (cervical, vulval, anal, and penile carcinomas and Kaposi's sarcoma; refs. 7, 8) and have both been detected (with some debate) in prostate tissue (9-13). Additionally, a recent study observed a correlation between HHV-8 protein expression and a macrophage/monocyte marker in prostate specimens, suggesting that HHV-8 infection may elicit intraprostatic inflammation (12). HHV-8 is also known to express viral interleukin-6, a homologue of human interleukin-6 (8), which has been proposed to play a role in prostate cancer cell proliferation (14) and may thus contribute to prostate cancer progression. To date, results from prospective and retrospective epidemiologic studies of *C. trachomatis*, HPV, and HHV-8 infections and prostate cancer are largely inconclusive, as many earlier positive findings have not been replicated in subsequent studies, and results are generally variable across studies, even those with similar study designs (15-26).

To further investigate associations between sexually transmitted infections and prostate cancer, we conducted a large, nested case-control study of *C. trachomatis*, HPV types 16, 18, and 33, and HHV-8 infections in relation to incident prostate cancer among participants in the Health Professionals Follow-up Study (HPFS). Histories of these infections were assessed by prediagnostic antibody serostatus to capture asymptomatic infections, which comprise a large proportion of *C. trachomatis*, HPV, and HHV-8 infections, and symptomatic infections of

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Requests for reprints: Elizabeth A. Platz, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Room E6132-A, 615 North Wolfe Street, Baltimore, MD 21205. Phone: 410-614-9674; Fax: 410-614-2632. E-mail: eplatz@jhsph.edu

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possibly unrecognized origin. This last attribute is particularly important for *C. trachomatis* infection because it tends to be treated presumptively rather than specifically diagnosed in men and because chlamydia diagnostics have only been commercially available since 1985, after many participants may have already been infected.

Materials and Methods

Study Population and Design. In 1986, American male health professionals of ages 40 to 75 years were invited to participate in the HPFS, an ongoing prospective study of cancer and heart disease in men. A total of 51,529 health professionals agreed to participate by completing a mailed, baseline epidemiologic questionnaire on demographics, lifestyle, and medical history and a semiquantitative food frequency questionnaire. Since 1986, participants have completed questionnaires every 2 years to update exposure and disease information, and every 4 years to update dietary information. Information on death is obtained from the National Death Index and the U.S. Postal Service or next of kin in response to follow-up questionnaires. Between 1993 and 1995, HPFS participants were additionally asked to provide a blood sample for research purposes. Chilled, EDTA-preserved blood specimens were returned to the Harvard School of Public Health via overnight courier by 18,225 participants. On arrival at the school, specimens were centrifuged; separated into plasma, buffy coat, and erythrocyte aliquots; and stored in liquid nitrogen.

All participants who provided a blood sample in 1993-1995, who were free of reported cancer (except nonmelanoma skin cancer) at the time of blood draw and who provided valid baseline food frequency information, were eligible for inclusion in the nested case-control study. Cases were defined as men diagnosed with prostate cancer between the date of blood draw and January 31, 2000 (n = 691). Information on prostate cancer was obtained from biennial follow-up questionnaires, which requested that participants report medical diagnoses, including prostate cancer, in the prior 2 years. More than 90% of prostate cancer diagnoses were subsequently confirmed by medical record and pathology report review with permission from the participant or next of kin. Many of the remaining 10% provided supporting information (e.g., evidence of treatment) for their diagnosis. Information on disease stage (tumor-node-metastasis) and Gleason sum was abstracted from medical records by trained study investigators using a standard form. Participants diagnosed with stage T_{1a} prostate cancers (n = 2) were not included as cases because, by definition, their tumors were detected at transurethral resection of the prostate for benign prostatic hyperplasia and are especially prone to detection bias.

Controls were defined as men alive and free of a diagnosis of cancer (except nonmelanoma skin cancer) at the time of case diagnosis. Controls were also required to have had at least one prostate-specific antigen (PSA) test between the date of blood draw and the 2-year interval of case diagnosis. No restrictions were placed on PSA concentration to avoid excluding men with noncancerous prostate conditions associated with elevated PSA, such as benign prostatic hyperplasia or prostatitis, because these conditions were not excluded from the case definition. Had restrictions been placed on control PSA concentration, a bias may potentially have been introduced if any of the infections considered were associated with either benign prostatic hyperplasia or prostatitis. One control was individually matched to each case by age (±1 year); time (midnight-9 a.m., 9 a.m.-noon, noon-4 p.m., and 4 p.m.-midnight), season (January-March, April-June, July-September, and October-December), and year (exact) of blood draw; and PSA testing history before 1993-1995 (yes/no).

This analysis was approved by the Human Subjects Committee at the Harvard School of Public Health and the Committee on Human Research at the Johns Hopkins Bloomberg School of Public Health.

Plasma Antibody Detection

C. trachomatis *Infection. C. trachomatis* antibody serostatus was assessed with the Ani Labsystems *C. trachomatis* immunoglobulin G enzyme immunoassay (Ani Labsystems, Helsinki, Finland) in the laboratory of Dr. Charlotte A. Gaydos. This assay detects immunoglobulin G antibodies against synthetic peptides derived from variable domain IV of the major outer membrane protein of *C. trachomatis* serovars C, G, E, and L2 (27, 28). In a validation study, this assay had a sensitivity of 56.2% in culture-positive men and a specificity of 99.3% in children [calculated from Narvanen et al. study (27)]. Anti–*C. trachomatis* immunoglobulin G antibodies are believed to be relatively persistent over time, as evidenced by stable antibody titers with increasing age in a cross-sectional survey of elderly Finnish men (29) and by persistent antibody seropositivity in women with past chlamydial salpingitis, a complication of *C. trachomatis* infection (30).

All samples were tested once by enzyme immunoassay. Samples with signal to cutoff ratios between 1.0 and 1.4 were considered equivocal for anti-chlamydial antibodies, and those with signal to cutoff ratios ≥1.4 were considered positive for anti-chlamydial antibodies according to the manufacturer's instructions.

HPV-16, HPV-18, and *HPV-33 Infections.* HPV-16, HPV-18, and HPV-33 immunoglobulin G antibody serostatus were assessed by three in-house ELISAs in the laboratory of Dr. Raphael P. Viscidi (31). These assays detect immunoglobulin G antibodies against HPV-16, HPV-18, and HPV-33 virus-like particles, respectively. In previous studies, these assays had sensitivities of ≥50% in DNA-positive women and specificities of ≥98% in virginal women and children. Whereas some women in these studies lost antibody seropositivity over time, others have been observed to maintain antibody seropositivity for at least a decade, a phenomenon likely dependent, at least in part, on characteristics of their HPV infection(s) (e.g., duration; refs. 32, 33).

All samples were tested in duplicate with repeat duplicate testing for duplicates with absorbance coefficients of variation >25% and at least one value above the absorbance cutoff point for seropositivity (defined below; n = 44 for HPV-16, n = 42 for HPV-18, and n = 25 for HPV-33). Mean absorbance values were calculated for each participant based on duplicate test values or, in the case in which four replicates were tested, based on the average of the three values in closest agreement. Absorbance cutoff points of 0.85, 0.70, and 0.50 were used to assign seropositivity for HPV-16, HPV-18, and HPV-33, respectively, based on previously determined cutoff points in self-reported female virgins (31).

HHV-8 Infection. HHV-8 antibody serostatus was assessed by an in-house monoclonal antibody–enhanced immunofluorescent assay against multiple lytic HHV-8 antigens in the laboratory of Dr. Frank J. Jenkins (34). In a comparative study of HHV-8 serologic tests, this assay had a sensitivity of 100% among patients with Kaposi's sarcoma and an estimated sensitivity of 53.4% and specificity of 96.6% among blood donors (35). Once detected, anti–HHV-8 antibodies have been observed to persist for at least 17 years and to increase in titer and range of epitope recognition over time (36), consistent with episodic viral reactivation of this life-long infection.

All samples were tested in duplicate and assessed microscopically by the same reader. In the case of discrepant duplicate results, a third replicate was tested and the results of the two replicates in agreement were used. Samples

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positive at a dilution of 1:100 were considered positive for anti–HHV-8 antibodies. Antibody titers were determined for positive samples using serially diluted serum samples. End-point titers were calculated as the reciprocal of the last positive dilution.

For each assay, samples were tested in random case-control pair order, with case and matching control samples adjacent to one another but in random within-pair order. Laboratory technicians were blinded to the case-control status of each sample. For HPV-16, HPV-18, and HHV-8 testing, blinded samples of known serostatus were included in the testing sequence (two seropositive and two seronegative samples per assay) to assess the reliability of each serologic test. Known seropositive and seronegative samples were used as opposed to duplicate samples because of the expected low seroprevalence of each infection in this population. Reliability was high for each of these tests (κ = 1.00 for HPV-16, HPV-18, and HHV-8 infections). For *C. trachomatis* and HPV-33 testing, reliability was estimated using duplicate, blinded quality control samples unselected for *C. trachomatis* and HPV-33 seropositivity (four samples per assay) due to difficulties in locating sufficient volumes of known seropositive serum (κ = 0.82 for *C. trachomatis* and κ = 0.91 for HPV-33 infections).

Statistical Analysis. To characterize participants and begin to investigate potential confounding, means and proportions of known or suspected sexually transmitted infection and prostate cancer correlates or risk factors were calculated for prostate cancer cases and controls. Known or suspected sexually transmitted infection correlates and risk factors included histories of gonorrhea, syphilis, trichomonosis, and clinical prostatitis; ejaculation frequency from ages 20 to 29 and 40 to 49 years (times/mo); alcohol consumption from ages 18 to 22 years (drinks/wk); cigarette smoking before age 30 years (pack-years); body mass index at age 21 years (kg/m^2); and vigorous physical activity in high school and college. Covariates previously observed to be associated with prostate cancer risk or progression in the HPFS cohort included race/ethnicity; cumulative family history of prostate cancer through 1996; height (inches); cigarette smoking between 1984 and 1994 (pack-years); total energy (kcal/d), alcohol (g/d), tomato sauce (servings/d), red meat (servings/d), fish (servings/d), calcium (mg/d), and energy-adjusted α-linolenic acid (g/d) in 1994; energy-adjusted fructose intake (g/d) in 1990; vitamin E (<15, ≥15 mg/d) and zinc (<101, ≥101 mg/d) supplementation; vigorous physical activity (metabolic equivalent-hours/wk); and histories of vasectomy and diabetes mellitus type 2 as of 1994.

Associations between *C. trachomatis*, HPV, and HHV-8 antibody seropositivity and prostate cancer were initially investigated by calculating antibody signal to cutoff ratio means, medians, and proportions for *C. trachomatis* antibody serostatus; absorbance means, medians, and proportions (based on absorbance quartiles among controls and absorbance cutoff points for seropositivity) for HPV antibody serostatus; and proportions for HHV-8 antibody serostatus. Values were compared by paired *t* tests, Wilcoxon signed-rank tests, McNemar's tests, and likelihood ratio tests, as appropriate. Conditional logistic regression was used to calculate matched odds ratios (OR) and 95% confidence intervals (95% CI) for prostate cancer. Confounding was further explored by adding covariates individually and in combination to univariable conditional logistic regression models and comparing with univariable results. Covariates considered were those described above and other antibody serostatus (*C. trachomatis*, other HPV types, and HHV-8, as appropriate). Detection bias was investigated by adding the number of PSA tests before case diagnosis to univariable models and comparing with univariable results. As none of the considered covariates altered any of the point estimates for exposures of interest, only known prostate cancer risk factors [race/ethnicity, family history of prostate cancer, and age (matched)] were retained in the final multivariable model. Total prostate cancer was used as the outcome in the main analyses. Additional analyses using prostate cancer characterized by grade [low grade (Gleason sum <7) and high grade (Gleason sum ≥7)] and stage [organ confined ($\leq T_2$ and N_0M_0) and advanced (T_{3b} or worse)] were also done.

To investigate whether associations between sexually transmitted infections and prostate cancer varied by factors postulated to influence prostatic inflammation, stratified analyses were done by aspirin use since age 20 years, lifetime cigarette smoking, and, in the case of *C. trachomatis* antibody seropositivity, age as a surrogate measure for availability of sulfonamide antibiotics at the time of *C. trachomatis* acquisition. Although *C. trachomatis* infection had not yet been identified as a sexually transmitted infection when sulfonamide antibiotics were first introduced in 1937, it may still have been cured by these antibiotics (which possess anti-chlamydial activity) if acquired concurrently with gonorrhea, a known and prevalent sexually transmitted infection at the time. Variation by underlying genetic susceptibility to prostate cancer was investigated by carrying out analyses stratified by age at prostate cancer diagnosis and family history of prostate cancer. All stratified analyses, with the exception of those for age, were done by unconditional logistic regression including terms for exposures of interest and matching variables (age; time of day, season, and year of blood draw; and PSA screening history before blood draw). The statistical significance of any observed stratum-specific differences was assessed by including an additional cross-product term in the regression model and evaluating this term by the Wald test.

Before conducting the present analysis, we did power calculations to determine the magnitude of minimum detectable associations for each infection using estimates of antibody seroprevalence from the literature. Given a sample size of 691 cases and controls, we estimated sufficient power (80%) to detect observed magnitudes of association between 1.38 and 2.57, assuming a range of antibody seroprevalences from 1.7% to 30% among controls (15, 16). We also did analyses to determine the effect of nondifferential misclassification of exposure on our ability to detect associations. As stated earlier, the sensitivities of the assays used in the present study, which were similar to those used in most previous studies, were low for detecting current uncomplicated or transient infections and may have been even lower for detecting past uncomplicated or transient infections, especially in men. However, we expected that the sensitivities of these assays might be higher for detecting infections of potentially greater relevance for prostate carcinogenesis, such as infections of longer duration that might be more likely to ascend to the prostate, repeated episodes of infection, and those with complications, such as prostatitis. This expectation was based on the observation that women with chlamydial salpingitis, a complication of *C. trachomatis* infection, women with HPV infections of longer duration, men with HHV-8 infections of longer duration, and individuals with Kaposi's sarcoma, a complication of HHV-8 infection, are more likely to have detectable or higher antibody titers than individuals with uncomplicated or transient infections (30, 32, 35, 36). In the case that our expectation of higher sensitivities did not hold, we also did analyses to determine the magnitude of minimum detectable associations using lower-sensitivity assays. Assuming sensitivities of 50% and specificities of 97%, we estimated sufficient power to detect a true magnitude of association of 2.00 (or observed magnitude of association of 1.61), given an observed seroprevalence of 9.6% among controls.

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Table 1. Characteristics of 691 prostate cancer cases and 691 matched controls in the HPFS, 1993-2000

	Cases	Controls	P*
Mean age at blood draw (y)	65.8	65.7	Matching variable
Race/ethnicity (%)			
Southern European	22.0	20.4	
Scandinavian	10.6	9.7	
Other Caucasian	62.1	64.1	0.70
African American	0.9	0.4	
Asian	0.4	0.3	
Other	4.0	5.1	
Family history of prostate cancer (%) †	21.8	16.8	0.02
Mean height in 1986 (in)	70.1	70.1	0.76
Smoked cigarettes in the past 10 y (%)	16.5	17.7	0.61
Mean intakes of:			
Total energy (kcal/d)	2012	2035	0.47
Alcohol (g/d)	11.8	11.5	0.70
Tomato sauce (servings/d) ‡	0.19	0.19	0.89
Red meat (servings/d)‡	1.03	1.01	0.70
Fish (servings/d)‡	0.31	0.33	0.04
Calcium (mg/d)	950	951	0.95
α-Linolenic acid (g/d)§	1.12	1.12	0.93
Fructose in 1990 (g/d)§	49.0	48.9	0.88
Vitamin E supplementation (≥15 mg/d; %)	37.5	34.4	0.28
Zinc supplementation (≥101 mg/d; %)	0.1	0.4	0.62
Regular (2+ times/wk) use of nonsteroidal anti-inflammatory drugs (%)	48.8	46.9	0.52
Any vigorous leisure-time physical activity (%)	58.0	57.4	0.87
Vasectomy (%)	25.9	26.5	0.85
Diabetes mellitus type 2 (%)	6.2	5.8	0.82
Mean no. PSA tests before the date of case diagnosis	2.6	2.5	0.15
History of (%)			
Gonorrhea‖	2.6	2.6	1.00
Syphilis‖	0.1	0.0	1.00
Trichomonosis¶	12.6	9.4	0.07
Clinical prostatitis‖	22.0	18.0	0.07
Mean monthly ejaculation frequency from‖:			
Ages 20-29 y	13.6	14.0	0.17
Ages 40-49 y	10.7	11.1	0.18
Consumed alcohol, ages 18-22 y (%)**	69.6	68.2	0.61
Smoked cigarettes before age 30 y (%) ††	47.9	49.5	0.59
Mean body mass index at age 21 y (kg/m^2) ††	22.8	22.9	0.24
Any vigorous physical activity (%) in‖:			
High school	84.8	85.0	1.00
College	76.6	76.8	0.95

NOTE: Unless otherwise indicated, values are from the 1994 follow-up questionnaire.
*Assessed by paired *t* test for continuous variables, McNemar's test for binary variables, and likelihood ratio test for categorical variables.
†Assessed in 1990 through 1996.
‡Cumulative mean intake between 1986 and 1994.
§Adjusted for total energy intake.
‖Assessed in 1992.
¶Assessed by antibody serostatus from blood samples collected in 1993-1995.
**Assessed in 1988.
††Assessed in 1986.

Results

Of the 691 prostate cancer cases included in this analysis, the majority were organ confined (83.9% of 614 cases with prostate cancer stage information) with Gleason sums between 5 and 7 (14.4% Gleason sum 5, 38.8% Gleason sum 6, and 28.9% Gleason sum 7 of 623 cases with grade information). The mean age at diagnosis was 68.9 years (range, 47.7-84.3 years). The mean time from blood draw to diagnosis was 3.1 ± 1.7 years. When compared with controls, prostate cancer cases were more likely to report a family history of prostate cancer, less fish consumption, a slightly greater number of PSA tests before prostate cancer diagnosis, histories of trichomonosis and clinical prostatitis, and lower frequencies of ejaculation from ages 20 to 29 and 40 to 49 years (Table 1).

***C. trachomatis* Infection.** No differences were observed in the distribution of signal to cutoff ratios for *C. trachomatis* antibody serostatus between prostate cancer cases and controls. Four percent of cases were seropositive for *C. trachomatis* infection as compared with 3.5% of controls (Table 2). Null results were also observed after multivariable adjustment and for prostate cancer characterized by grade and stage (Table 3 and data not shown). Too few seropositive participants were diagnosed with advanced-stage cancer to investigate its association with *C. trachomatis* antibody seropositivity. In stratified analyses, no differences were observed in the association between *C. trachomatis* antibody seropositivity and total prostate cancer across strata of aspirin use, cigarette smoking, age as a surrogate measure for availability of antibiotics, age at prostate cancer diagnosis, and family history of prostate cancer (all $P_{interaction} > 0.20$).

HPV Infection. No differences were observed in the mean or median antibody concentration between prostate cancer cases and controls for HPV-16, HPV-18, or HPV-33. Among cases, 7.5% were seropositive for HPV-16, 6.1% for HPV-18, and 7.2% for HPV-33, whereas among controls, these values were 8.8%, 5.8%, and 6.4%, respectively (Table 2). When type-specific HPV information was combined, null results were also observed for any HPV seropositivity and number of HPV types (Table 2). Adjustment for potential confounding variables did not alter any of the results (Table 3 and data not

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shown). Null results were also observed for low-grade and organ-confined prostate cancer. A significant inverse association was observed between HPV-16 seropositivity and high-grade prostate cancer (Table 3). Too few participants were diagnosed with advanced-stage prostate cancer to investigate potential associations between HPV seropositivity and advanced-stage disease. In stratified analyses, no consistent patterns were observed across strata of cigarette smoking, age at prostate cancer diagnosis, and family history of prostate cancer for HPV-16, HPV-18, and HPV-33 (data not shown). A nonsignificant pattern of decreasing magnitudes of association with increasing aspirin use since age 20 years was observed for each HPV type ($P_{interaction}$ for all HPV types combined = 0.16). No other differences were observed when information on HPV types was combined (all $P_{interaction} > 0.20$).

HHV-8 Infection. Prostate cancer cases were significantly less likely to be HHV-8 antibody seropositive than controls (13.5% versus 18.0%, respectively) although no differences were observed in the distribution of antibody titers between seropositive cases and controls (Table 2). Inverse results were also observed after multivariable adjustment and for low-grade and organ-confined prostate cancer (Table 3 and data not shown). Suggestive inverse associations were also observed for high-grade (Table 3) and advanced-stage prostate cancer (OR, 0.48; 95% CI, 0.16-1.42) although the number of seropositive men diagnosed with advanced-stage disease was small ($n = 7$). In stratified analyses, null to suggestive positive, as opposed to inverse, associations were observed for men who used aspirin infrequently since age 20 years (OR, 1.10; 95% CI, 0.58-2.08; $P_{interaction} = 0.17$), men ≥74 years of age (fourth quartile of age at prostate cancer diagnosis, OR, 1.31; 95% CI, 0.72-2.39; $P_{interaction} = 0.02$), and men with a family history of prostate cancer (OR, 1.28; 95% CI, 0.64-2.55; $P_{interaction} = 0.11$); inverse associations were observed for all other strata of men (data not shown).

Table 2. *C. trachomatis*, HPV, and HHV-8 antibody concentrations in 691 prostate cancer cases and 691 matched controls in the HPFS, 1993-2000

	Cases	Controls	P*
C. trachomatis infection			
Mean S/CO	0.228	0.228	0.99
Median S/CO	0.076	0.083	0.49
Score (%)			
S/CO < 1.0	94.8	94.8	
1.0 ≤ S/CO < 1.4	1.2	1.7	0.66
1.4 ≤ S/CO < 2.5	2.3	2.3	
S/CO ≥ 2.5	1.7	1.2	
S/CO ≥ 1.4 (%)	4.0	3.5	0.90
HPV infection			
HPV-16			
Mean absorbance	0.051	0.052	0.77
Median absorbance	0.038	0.037	0.59
Absorbance >0.085 (%) †	7.5	8.8	0.44
HPV-18			
Mean absorbance	0.037	0.040	0.44
Median absorbance	0.030	0.029	0.14
Absorbance >0.070 (%) †	6.1	5.8	0.91
HPV-33			
Mean absorbance	0.030	0.026	0.13
Median absorbance	0.019	0.018	0.19
Absorbance >0.050 (%) †	7.2	6.4	0.60
Any HPV infection ‡ (%)	15.5	16.5	0.66
No. HPV infections ‡ (%)			
0	84.5	83.5	
1	11.6	13.0	0.77
2	2.5	2.5	
3	1.4	1.0	
HHV-8 infection			
Positive§ (%)	13.5	18.0	0.02
Titer (among positive participants; %)			
≤200	49.5	43.6	
400	22.6	29.0	0.49,‖ 0.73¶
800	18.3	11.3	
≥1,600	9.7	16.1	

Abbreviation: S/CO, signal to cutoff ratio.
*Assessed by paired *t* test for mean values, Wilcoxon signed-rank test for median values, McNemar's test for binary variables, and the likelihood ratio test for categorical variables.
†Based on absorbance results from virginal women.
‡HPV-16, HPV-18, or HPV-33.
§Titer ≥100.
‖Based on a comparison of the entire distribution of titers.
¶Based on a comparison of high titers (≥1,600).

Discussion

In this large, nested case-control study of male health professionals, no associations were observed between *C. trachomatis* and HPV-16, HPV-18, and HPV-33 antibody seropositivity and prostate cancer, with the possible exception of a significant inverse association between HPV-16 seropositivity and high-grade prostate cancer. For HHV-8, significant inverse associations were observed for total, organ-confined, and low-grade prostate cancer. Suggestive inverse associations were also observed for HHV-8 antibody seropositivity and high-grade and advanced-stage disease. None of the observed associations were altered by adjustment for histories of other sexually transmitted infections and correlates/risk factors of sexually transmitted infections or prostate cancer.

***C. trachomatis* Infection.** Our null finding for *C. trachomatis* antibody seropositivity is consistent with findings from one previous nested case-control study (15) but differs from those from two additional studies, one of which observed a significant inverse association between *C. trachomatis* antibody seropositivity and prostate cancer across three study sites (22), whereas the other observed a suggestion of an inverse association between self-reported history of chlamydia and prostate cancer (23). Findings from the second study, however, are difficult to interpret because of the low reported cumulative incidence of chlamydia. This low incidence likely underestimates the true incidence because (*a*) chlamydia is frequently asymptomatic in men; (*b*) chlamydia diagnostics were only commercially available after 1985; and (*c*) self-reported incidence of chlamydia was much lower in this study population than reported incidences of gonorrhea, urethritis, and epididymitis, the latter two of which are caused, to a fairly large extent, by *C. trachomatis*.

Our observed *C. trachomatis* seroprevalence of 3.5% to 4.0% is lower than previously published estimates, many of which were estimated in general male Scandinavian populations ranging in age from 18 to 97 years (15, 22, 29) and were based on use of the microimmunofluorescence assay, which is considered to be a more specific but less sensitive test than the Ani Labsystems enzyme immunoassay used in our study (28, 37-39). To our knowledge, no estimates of *C. trachomatis* seroprevalence exist in the general male U.S. population, either based on the microimmunofluorescence assay or enzyme immunoassay, to allow for comparisons between men from the same geographic location. Therefore, it was difficult to compare our estimate to others in the literature and speculate about reasons for their differences. We did, however, consider how our observed seroprevalence might have influenced our ability to detect statistically significant associations between *C. trachomatis* infection and prostate cancer. Given our findings, we are unlikely to have missed strong positive or inverse associations, but we cannot rule out weak to moderate positive (i.e., <1.96) or inverse (i.e., >0.65) associations.

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Table 3. ORs and 95% CIs of prostate cancer by *C. trachomatis*, HPV, and HHV-8 serostatus in 691 matched pairs nested in the HPFS, 1993-2000

	Seronegative		Seropositive		
	Cases/controls	OR (95% CI)*	Cases/controls	OR (95% CI)*	OR (95% CI)†
Total prostate cancer					
C. trachomatis‡	655/655	1.00	28/24	1.15 (0.67-1.99)	1.13 (0.65-1.96)
HPV-16§	639/630	1.00	52/61	0.84 (0.57-1.24)	0.83 (0.57-1.23)
HPV-18‖	649/651	1.00	42/40	1.06 (0.67-1.66)	1.04 (0.66-1.64)
HPV-33¶	641/647	1.00	50/44	1.14 (0.76-1.72)	1.14 (0.76-1.72)
HPV-16, HPV-18, or HPV-33	584/577	1.00	107/114	0.93 (0.70-1.24)	0.92 (0.69-1.22)
HHV-8**	598/567	1.00	93/124	0.70 (0.52-0.95)	0.70 (0.52-0.95)
Low-grade (Gleason sum <7) prostate cancer					
C. trachomatis‡	363/361	1.00	15/14	1.04 (0.50-2.16)	1.01 (0.48-2.11)
HPV-16§	349/350	1.00	31/30	1.04 (0.62-1.74)	1.05 (0.62-1.77)
HPV-18‖	359/363	1.00	21/17	1.29 (0.64-2.58)	1.29 (0.64-2.61)
HPV-33¶	353/356	1.00	27/24	1.13 (0.64-1.98)	1.13 (0.65-1.99)
HPV-16, HPV-18, or HPV-33	321/320	1.00	59/60	0.98 (0.66-1.45)	0.98 (0.66-1.46)
HHV-8**	328/308	1.00	52/72	0.65 (0.43-0.98)	0.64 (0.42-0.96)
High-grade (Gleason sum ≥7) prostate cancer					
C. trachomatis‡	228/228	1.00	11/8	1.34 (0.54-3.34)	1.34 (0.52-3.41)
HPV-16§	230/218	1.00	13/25	0.50 (0.25-1.00)	0.46 (0.23-0.94)
HPV-18‖	224/225	1.00	19/18	1.06 (0.55-2.05)	1.01 (0.52-1.97)
HPV-33¶	226/230	1.00	17/13	1.31 (0.64-2.69)	1.29 (0.62-2.68)
HPV-16, HPV-18, or HPV-33	207/202	1.00	36/41	0.86 (0.53-1.39)	0.81 (0.50-1.33)
HHV-8**	210/199	1.00	33/44	0.72 (0.45-1.17)	0.76 (0.47-1.23)
Organ-confined ($\leq T_2$ and N_0M_0) prostate cancer					
C. trachomatis‡	487/482	1.00	18/16	1.08 (0.55-2.14)	1.05 (0.53-2.08)
HPV-16§	468/461	1.00	39/46	0.84 (0.54-1.30)	0.82 (0.53-1.29)
HPV-18‖	476/474	1.00	31/33	0.93 (0.55-1.57)	0.90 (0.54-1.53)
HPV-33¶	469/475	1.00	38/32	1.19 (0.74-1.92)	1.18 (0.73-1.91)
HPV-16, HPV-18, or HPV-33	425/421	1.00	82/86	0.94 (0.68-1.32)	0.92 (0.66-1.29)
HHV-8**	440/413	1.00	67/94	0.66 (0.47-0.94)	0.66 (0.46-0.93)

*Estimated by conditional logistic regression. Cases and controls were matched on age; time, season, and year of blood draw; and PSA testing history before 1993-1995.
† Estimated by conditional logistic regression including terms for race/ethnicity (Caucasian, non-Caucasian) and cumulative family history of prostate cancer.
‡ Signal-to-cutoff ratio, ≥1.4.
§ Absorbance >0.085.
‖ Absorbance >0.070.
¶ Absorbance >0.050.
** Titer ≥100.

HPV Infection. Our null findings for HPV-16, HPV-18, and HPV-33 infections are consistent with findings from several previous studies but differ from those from several additional studies that observed positive associations, potentially inverse associations, or associations of debatable significance (15-21, 40). In our interpretation of study findings, we considered several noncausal explanations for our results, including limited statistical power and assay validity. With respect to the former, we believe that limited statistical power is unlikely to explain our null findings because our study was as large or larger than previous studies and had similar seroprevalences of infection. With respect to test validity, although the assays we used, which were similar to assays used in all previous studies, had relatively low sensitivities for detecting current or past uncomplicated infections, we expected that they might have higher sensitivities for detecting HPV infections of potentially greater relevance for prostate carcinogenesis, such as infections of longer duration that might be more likely to involve the prostate or repeated episodes of infection. In this case, we expected that the potentially higher sensitivities of the assays would lead to a lesser degree of nondifferential misclassification of exposure and a lesser degree of attenuation. Even in the case in which our assays did not have higher sensitivities for detecting potentially more etiologically relevant infections, we estimated that nondifferential misclassification of exposure could only have resulted in a minor degree of attenuation (e.g., from an OR of 0.78 to 0.84 for HPV-16 infection and an OR of 1.21 to 1.14 for HPV-33 infection, assuming sensitivities of 50% and specificities of 98%).

HHV-8 Infection. Our significant inverse findings for HHV-8 antibody seropositivity and prostate cancer differ from hypothesized findings, particularly for advanced-stage disease, and from positive or null findings from three previous studies (24-26). We considered several possible noncausal explanations for these differences, including selection bias, differential assay validity, and confounding. First, we believe that selection bias due to differential loss of HHV-8^+/HIV^+ co-infected AIDS patients before case-control selection is unlikely to explain our findings because only 36 AIDS deaths (0.5% of all deaths) were observed in the HPFS cohort as of 2000. Second, differences in assay sensitivity and specificity are also unlikely to explain our inverse findings because any of these differences should be nondifferential by case-control status, and thus should only result in an attenuation of study findings toward the null. Third, confounding by Mediterranean heritage, a correlate of classic Kaposi's sarcoma (41), is unlikely to explain our inverse findings because adjustment for Southern European ethnicity did not alter any of the results. Finally, although some studies have observed inverse associations between AIDS and prostate cancer possibly due to decreased frequency of prostate cancer screening in HIV^+ men (42, 43), confounding by HIV/AIDS is also unlikely to explain our findings because of the low expected prevalence of HIV infection in this cohort and because adjustment for prostate cancer screening did not alter any of the results. One possible, albeit speculative, causal explanation for our inverse findings is long-term HHV-8–mediated skewing of the immune response from Th1 toward Th2, possibly by HHV-8 chemokines, vMIP-I, vMIP-II, and vMIP-III (8), which has been hypothesized to protect against prostate cancer (44). Other possible noncausal explanations include confounding by another factor associated with both

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HHV-8 antibody seropositivity and prostate cancer, or chance.

Our predominantly Caucasian study population was composed of cases with typically earlier-stage disease and differed in racial and prostate cancer stage composition from some of the other previously investigated study populations. For instance, one of the two main study populations investigated by Hoffman et al. (25) was predominantly African-Caribbean, and both were composed of a high proportion of advanced-stage cases (high PSA concentration at diagnosis). Therefore, if positive associations are limited to men of African descent, perhaps due to differences in immune response or other genes, or advanced-stage disease, this may explain our inverse, as opposed to positive, findings. We attempted to reduce the likelihood of detecting falsely positive associations by (*a*) designing our study specifically to compare cases and controls from the same source population (i.e., the HPFS cohort) to avoid selection biases; (*b*) matching closely on age (and using a matched analysis) to reduce the likelihood of confounding by age because age may be associated with both increased cumulative incidence of infection and prostate cancer; (*c*) adjusting for race/ethnicity to avoid confounding by Mediterranean heritage (as mentioned previously) and African heritage, both of which are typically associated with higher HHV-8 antibody seroprevalence and varying prostate cancer risk (45); and (*d*) not placing any restrictions on control PSA concentration to avoid excluding controls with benign prostatic hyperplasia or prostatitis because these may potentially be associated with HHV-8 or other associated infections.

In conclusion, no associations were observed between *C. trachomatis* and HPV-16, HPV-18, and HPV-33 antibody seropositivity and prostate cancer, whereas a significant inverse association was observed between HHV-8 antibody seropositivity and prostate cancer in this large population of American male health professionals. As this study is the first, to our knowledge, to observe such an inverse association, similar additional studies are warranted before conclusions can be made about this association.

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