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PROSPECTIVE STUDY OF HUMAN HERPESVIRUS TYPE 8 SEROSTATUS AND PROSTATE CANCER RISK IN THE PLACEBO ARM OF THE PROSTATE CANCER PREVENTION TRIAL

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Abstract

Background—Human herpesvirus type 8 (HHV-8), a gamma herpesvirus associated with Kaposi's sarcoma, has been proposed as a candidate risk factor for prostate cancer (PCa) because of its detection in benign and malignant prostate specimens, and its relation with histologic prostatic inflammation. We investigated the possible relation between pre-diagnostic HHV-8 infection and PCa risk in a case-control study sampled from the placebo arm of the Prostate Cancer Prevention Trial (PCPT).

Methods—We defined cases as men with a confirmed diagnosis of PCa after visit 2 (n=315) and controls as men not diagnosed with PCa during the trial who also had a negative end-of-study prostate biopsy (n=315). We tested sera from visit 2 for IgG antibodies against HHV-8 using a monoclonal antibody-enhanced immunofluorescence assay against multiple lytic HHV-8 antigens.

Results—The adjusted seroprevalence of HHV-8 infection was 11.6% for cases and 11.0% for controls (p=0.81). No association was observed between HHV-8 seropositivity and PCa risk (OR=1.06, 95% CI: 0.65-1.76).

Conclusion—Our findings of a null association between HHV-8 seropositivity and PCa risk do not support an association between HHV-8 infection and PCa development, consistent with the general tendency of the epidemiologic literature to date.

INTRODUCTION

Human herpesvirus type 8 (HHV-8) is a gamma herpesvirus associated with Kaposi's sarcoma (KS) and primary effusion lymphoma (1). This virus has also been proposed as a candidate risk factor for prostate cancer (PCa) because of its detection in benign and malignant prostate tissue and secretions (2-7), and because of its relation with histologic prostatic inflammation (3), a possible cause of PCa (8). In one of the first studies to investigate HHV-8 infection and PCa, Hoffman and colleagues (9) observed a positive association between HHV-8 seropositivity and PCa in a series of case-control studies, most notably one conducted among Afro-Caribbean men from Tobago (Table 1). Subsequent studies, however, have observed null and sometimes even suggestive inverse findings between HHV-8 seropositivity and PCa (10-15), particularly those published before the execution of the present study (10-12). Therefore, to further inform the possible relation between HHV-8 infection and PCa, and to investigate the reproducibility of previous suggestive inverse findings, we conducted a case-control study sampled from the placebo arm of the Prostate Cancer Prevention Trial (PCPT). This study population is particularly appropriate for investigations of infections and PCa risk because participants were screened annually for PCa and, if not diagnosed during the trial, were recommended for end-of-study prostate biopsy (16). Both of these design features reduce the possibility of detection bias caused by: 1) differential rates of screening by infection status; 2) infection-mediated prostate specific antigen (PSA) elevation, which has been observed for HHV-8 infection (14); and 3) and infection-mediated induction of any other prostate abnormalities that might trigger a prostate biopsy.

MATERIAL AND METHODS

Study population and design

The PCPT was a large, multi-site randomized clinical trial designed to determine whether the drug, finasteride, a 5 α -reductase type II inhibitor, prevents PCa (17). Generally healthy men $\geq$ 55 years of age who had no evidence of PCa (i.e., PSA concentration $\leq$ 3 ng/mL and a normal digital rectal examination (DRE)) or other clinically-significant chronic conditions, including severe benign prostatic hyperplasia (BPH, defined as an International Prostate Symptom Score (IPSS) $\geq$ 20), were eligible for the trial. Between 1994 and 1997, 18,882 of these men were randomized into either the placebo or finasteride arm of the trial, and were

then followed actively for seven years for PCa diagnosis. Participants were screened annually for PCa by DRE and PSA testing, and those found to have abnormal DRE results or elevated PSA (>4 ng/mL) were recommended for prostate biopsy (“for-cause” biopsy). To ensure that approximately equal proportions of men were recommended for biopsy in each arm, PSA concentrations were adjusted among men in the finasteride arm because finasteride is known to lower serum PSA. Serum remaining after PSA testing in both arms was stored for research purposes. After seven years of participation in the trial, men who had not been diagnosed with PCa were offered a prostate biopsy as part of the trial protocol (“end-of-study” biopsy). This biopsy was included to ensure that biopsy referral patterns were not biased by use of finasteride. PCa cases detected on a biopsy recommended because of an abnormal PSA/DRE that coincided with the end-of-study biopsy window were considered to have had a for-cause biopsy.

As part of a broader investigation of genetic and other serologic exposures in relation to PCa, we conducted a large case-control study within the PCPT. Participants with an adequate baseline serum specimen and a definitive positive or negative diagnosis of PCa (i.e., a confirmed PCa diagnosis or a negative end-of-study biopsy) were eligible for inclusion ($n=8,580$). We defined cases as men diagnosed with PCa on any for-cause or end-of-study biopsy ($n=1,809$). PCa diagnoses were established by agreement between pathologists at the study sites and pathologists at the central laboratory who reviewed all study biopsy material. Clinical stage was provided by the study sites, and Gleason patterns and sum were determined by central pathology review of biopsy tissue. We defined controls as men not diagnosed with PCa at any time during the trial who also had a negative end-of-study biopsy ($n=1,809$). All non-Caucasian men who met this definition were selected as controls to enhance our ability to perform stratified analyses by race/ethnicity. The remaining controls were selected such that the entire distribution of controls was frequency-matched to cases by age (55-59, 60-64, 65-69, and 70 years), treatment arm, and family history of PCa, which we defined as at least one first degree relative with PCa.

For our control sampling strategy, we chose to use cumulative rather than incidence density sampling to provide all participants with the opportunity for occult PCa detection independent of indication for biopsy. This consideration is particularly important for studies of infection and PCa because infection may cause participants’ PSA to rise, as has been observed previously for HHV-8 infection (14), and may thus trigger a prostate biopsy and incidental PCa detection. This same opportunity for incidental PCa detection would not be present for uninfected men. Therefore, incidence density sampling might lead to detection of a false-positive association. Having said that, we recognize that our cumulative density control sampling strategy also had the potential to introduce a survival bias between cases diagnosed by for-cause biopsy and controls found to be negative on end-of-study biopsy. However, as only four cases diagnosed by for-cause biopsy died during follow-up, we do not believe that survival bias was likely. Furthermore, comparison of cases diagnosed by end-of-study biopsy with controls should be free from survival bias, as well as any possible selection biases that may have been introduced by differences in motivation for prostate biopsy between “for-cause” cases and controls (i.e., clinical indication versus study compliance).

For our investigation of HHV-8 infection and PCa risk, we selected a subset of men from the above-described case-control study. We limited this subset to: 1) men in the placebo arm, as these men are more similar to men in the general population and to those in previous studies; and 2) men with an adequate serum specimen from visit 2 to conserve valuable baseline specimens. Therefore, to maintain the prospective nature of the study, we also limited PCa cases to those diagnosed after visit 2 (n=315). Finally, we selected PCa cases such that approximately equal numbers were diagnosed with low-grade (Gleason sum <7) and high-grade (≥7) disease, and by for-cause and end-of-study biopsy to allow for investigation of associations between HHV-8 infection and various PCa outcomes with equal precision. For controls, we selected a subset of the original controls and frequency-matched these men to cases by age and family history of PCa; otherwise, we selected controls at random (n=315). As the original set of controls was enriched for non-Caucasian men, the subset was also enriched for these men.

This study was approved by the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health. The PCPT was approved by the Institutional Review Boards at each of the institutions that randomized a participant.

Assessment of HHV-8 infection

We assessed HHV-8 infection by titer of anti-HHV-8 serum IgG antibodies. Similar to all herpesvirus infections, HHV-8 infections are lifelong infections that cycle between latent and lytic (or reactivated) states, each with distinct patterns of protein expression. For our study, we used a monoclonal antibody-enhanced immunofluorescence assay (IFA) against multiple lytic antigens to detect HHV-8 infection. We did not measure antibodies against latent antigens because most (>90%) specimens seropositive for latent antigens are also seropositive for lytic antigens using our assay ((18, 19); unpublished data from Frank J. Jenkins). In a study comparing multiple different testing algorithms, our IFA had a sensitivity of 100% in KS patients, and an estimated sensitivity of 53.4% and specificity of 96.6% in blood donors, as compared to estimated sensitivities of 8.7-47.4% and specificities of 97.3-100.0% for other testing algorithms that involved combinations of enzyme immunoassays (EIAs) and IFAs for various different lytic and latent antigens (20). When compared to single assays, our IFA had a sensitivity of 97% among KS patients and 100% among those who developed KS within five years of blood draw, as compared to 87% and 54-85%, respectively, for single peptide lytic EIAs, and 83% and 77%, respectively, for a latent IFA (19). If, similar to KS, PCa is also caused by frequent HHV-8 reactivation, we would expect similarly high sensitivities for these more etiologically relevant HHV-8 infections in older men as in patients who go on to develop KS; less relevant infections that reactivate infrequently and that are associated with initially low antibody titers and waning antibody titers over time would be of less interest to detect (12).

For the present study, we tested visit 2 serum samples for HHV-8 antibodies in duplicate and we assessed each sample microscopically by the same reader. If duplicate results were discrepant, we tested a third replicate and used the results of the two replicates in agreement. Samples positive at a dilution of 1:100 were considered to be HHV-8 seropositive, consistent with all previous studies of HHV-8 infection and PCa that used this same assay

(9, 11, 12, 14). We investigated assay reproducibility by testing 12 sets of six blinded replicate samples from serum remaining after routine syphilis testing at the Baltimore Bureau of Disease Control laboratory. Samples were approved for use by the Baltimore City Health Department public review process and anonymized prior to release. Ten replicate sets had 100% agreement and two had 83.3% agreement.

Assessment of covariates

We obtained information on most covariates from the baseline visit, at which time participants completed a detailed self-administered questionnaire on demographic and lifestyle characteristics. These included race/ethnicity, education, occupation, marital status, cigarette smoking, physical activity, and frequency of sexual activity in the past four weeks, histories of vasectomy and diabetes, and current and past regular aspirin use. Height and weight were also measured at the baseline visit. At the 1-year visit, participants completed a 15-page diet and supplement questionnaire designed specifically for the PCPT. This questionnaire included questions on usual consumption of 99 foods or food groups and nine beverages over the past year; 13 questions on food preparation and purchasing; and three on usual consumption of fruits, vegetables, and fried foods. This information was used to derive usual intakes of energy, protein, carbohydrates, fat, fruit, vegetables, tomato products, red meat, processed meat, fish, calcium and zinc from food and supplements, as well as alcohol (21). We derived a history of symptomatic BPH at baseline from data collected at the baseline clinic visit (IPSS>14 or a history of BPH treatment), and we inferred whether or not participants had military experience from their form of payment (Veterans Affairs-sponsored payment for medical care) and type of study site (Veterans Affairs hospital, Army/Naval hospital, etc). Finally, PSA values and DRE findings from visit 2, the visit at which HHV-8 serostatus was assessed, were also considered in the analysis, as well as serologic evidence of other sexually transmitted or transmissible infections, including *Trichomonas vaginalis* (22), human papillomavirus types 16, 18, and 31 (23), and cytomegalovirus infections (24).

Statistical analysis

As the first step in our analysis, we investigated the potential for confounding by calculating adjusted means and proportions of potential confounding variables by PCa case-control status among all participants and by HHV-8 serostatus among controls. We adjusted means and proportions by age and family history of PCa to account for frequency-matching, and by race to account for over-sampling of non-Caucasian controls in the original parent study design. We considered as potential confounders all variables mentioned in the covariate section (with the exception of PSA results and DRE findings), as well as specimen storage time. To investigate the possible relation between HHV-8 serostatus and PCa risk, we calculated age-, family history-, and race-adjusted seroprevalences of HHV-8 by PCa status, and compared these seroprevalences using linear regression. Next, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) of PCa by HHV-8 serostatus, using unconditional logistic regression and adjusting for age, family history, and race. We investigated confounding further by comparing the results from our base model (age, family history, and race) to results from models including additional covariates (described in the covariate section). As none of these covariates shifted the point estimate for HHV-8 appreciably, we

presented the results from our base model only. To investigate the relation between HHV-8 infection and PCa grade and stage, we performed separate analyses for PCa diagnosed by low- and high-grade cancer, and organ-confined disease. In our analyses of high-grade disease, we defined high-grade as either a Gleason sum ≥ 7 , or a Gleason pattern of 4+3 or a Gleason sum ≥ 8 . To investigate the potential for detection bias by infection-mediated PSA elevation or -induction of prostate abnormalities, we performed additional separate analyses for cases diagnosed by for-cause and end-of-study biopsy. As mentioned earlier, these analyses also addressed the potential for survival and selection biases. Finally, we performed further stratified analyses by age at PCa diagnosis, family history of PCa, race, and past regular aspirin use to investigate potential effect modification. We calculated p-values for interaction by the likelihood ratio test.

A priori, we had at least 80% power to detect ORs ≥ 0.52 or ≤ 1.71 for an estimated control seroprevalence of 18% (12). Statistical analysis was performed using SAS version 9.2 (SAS, Cary, NC).

RESULTS

We selected 315 PCa cases and 315 controls for analysis. By design, approximately half of the cases were diagnosed by for-cause biopsy and half by end-of-study biopsy. Approximately half were also diagnosed with low-grade disease and half with high-grade disease, and almost all were diagnosed with organ-confined disease. Comparing cases to controls, cases consumed significantly or non-significantly more energy, protein, carbohydrates, fat, fish, and calcium than controls (Table 2). They also smoked fewer pack-years of cigarettes if they smoked, and were non-significantly less likely to be diabetic than controls. Finally, cases had higher PSA values at visit 2 than controls and were more likely to have abnormal DRE findings. Considering controls only, HHV-8 seropositive men were significantly or non-significantly less likely to have a family history of PCa and to have ever been married than seronegative men. They also consumed more energy, carbohydrates, calcium, and zinc; engaged in more physical activity; and had higher PSA values at visit 2 than seronegative men.

The adjusted seroprevalences of HHV-8 infection were 11.6% for cases and 11.0% for controls ($p=0.81$). No association was observed between HHV-8 serostatus and risk of total PCa, low- or high-grade PCa, and organ-confined disease (Table 3). A slight, non-significant positive association was observed for PCa diagnosed by for-cause biopsy and a slight, non-significant inverse association was observed for PCa diagnosed by end-of-study biopsy, possibly suggestive of detection bias caused by the positive association between HHV-8 serostatus and PSA. No effect modification was observed by age at PCa diagnosis, family history of PCa, past regular aspirin use, and race (all p -interaction ≥ 0.20).

DISCUSSION

In this study of older American men, we observed no association between HHV-8 serostatus and PCa risk. Our null findings differ from both original positive findings in Afro-Caribbean men (9), as well as more recent inverse findings in our previous large study of American

health professionals (12) and unstable, suggestively inverse findings in several smaller studies (10, 11). Our findings are consistent, however, with those from other more recent studies, including a large prospective investigation in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (13) and a recent prospective extension of the original Afro-Caribbean study (14) (see Table 1).

The observed lack of consistency in findings across the HHV-8 and PCa literature may be explained by several possible reasons. First, there may truly be no association between HHV-8 infection and PCa risk, and thus occasional positive and inverse findings may simply be due to chance. Second, differences in the assays used across studies may have contributed to differences in study findings. As detailed in Table 1, some studies used assays that detect single HHV-8 antigens (either lytic or latent) and are considered to be more specific, whereas others used assays that detect multiple antigens and are considered to be more sensitive (19, 20). Some also detected solely lytic or solely latent antigen(s). These differences in assays likely contributed to the wide range of seroprevalence estimates observed across studies because estimates as varied as 6.7% to 27.5% were observed in study populations tested with multiple different assays (11). Differences in geographic location also likely contributed to this range because men from parts of the Mediterranean and Africa tend to have higher seroprevalences of HHV-8 infection than North American men (25). Considering only studies of American men that used the same assay as in our study (i.e., lytic IFA), our seroprevalence estimates were higher than those observed in blood donors (9), comparable to those observed in men with other cancers (9), but lower than those observed in all other study populations (11, 12). Although none of these studies collected information on sexual activity with men, one of the few strong risk factors for HHV-8 infection (26, 27), we have no reason to believe that the prevalence of sexual activity with men varied notably across populations. Therefore, reasons for these differences are unclear. Irrespective of these differences, however, relative comparisons between cases and controls should still be valid. Taking the type of assay used into consideration, we could not discern any consistent pattern in associations between HHV-8 serostatus and PCa across assays. Therefore, we believe that differences in assays are unlikely to explain discrepancies in study findings.

Another possible explanation for discrepant findings is differences in the distribution of PCa cases by stage across study populations, as HHV-8 was recently shown to confer the capacity for androgen-independent growth in androgen-sensitive PCa cells *in vitro* (28). This finding suggests that HHV-8 may play a role in late-stage PCa progression to androgen-independent disease rather than or in addition to earlier stages of PCa development. To our knowledge, only three studies included a large proportion of late-stage cases in their study population, one of which observed no association between HHV-8 seropositivity and PCa (29); another observed unstable findings because of its very low sample size (11); and a third observed a positive association, although this study excluded controls with PSA values >4 ng/mL (9), which may have selectively reduced the HHV-8 seroprevalence in controls but not in cases (14). These differences across studies make it difficult to draw firm conclusions about the possible relation between HHV-8 infection and late-stage PCa.

If the influence of HHV-8 infection on PCa is earlier rather than later in the natural history of PCa – for instance, in PCa initiation or in the early determination of its clinical potential – it could be argued that our collection of blood samples at one time point later in life and our short follow-up relative to the natural history of PCa could have limited our ability to detect associations. However, as HHV-8 serology is generally viewed as a measure of cumulative exposure to this lifelong virus, and as most seropositive men were likely infected several years/decades before enrollment into the trial (27), we believe this argument unlikely. Moreover, even if antibody levels waned in some individuals with predominantly latent infection, we would still expect levels to have remained detectable in other men with potentially more relevant infections for PCa, such as those that reactivate frequently. Finally, our collection of blood samples from older men and our relatively short follow-up is also similar to most previous studies of HHV-8 infection and PCa nested or sampled from cohorts (12-14).

A third possible explanation for discrepant study findings is differences in the race/ethnicity of participants across studies, particularly with respect to African descent. Among African and African-American men, null to possibly inverse associations have been observed (11, 13, 29), whereas among Afro-Caribbean men, whom Jenkins and colleagues (11) hypothesized may be more susceptible genetically to the effects of HHV-8 infection or may be more likely to have region-specific environmental correlates of HHV-8 infection associated with PCa, conflicting findings have been observed (9, 14). In the first of these studies, the authors observed a positive association comparing predominantly late-stage cases to controls with low PSA (9), whereas in their second study, they observed a null association comparing risk of lower stage PCa between HHV-8 seropositive and seronegative men (14). Whether these differences in findings are explained by chance, selection bias, or differences in association by stage is unclear. However, considering the HHV-8 literature as a whole, it does not appear likely that HHV-8 infection increases the risk of PCa in most men.

In summary, we observed no association between HHV-8 seropositivity and PCa risk in our study of American men, consistent with the general tendency of the HHV-8 and PCa epidemiologic literature to date. Strengths of our investigation were our prospective exposure ascertainment, and our ability to limit detection bias through uniform PCa screening protocols and comparison of cases diagnosed by end-of-study biopsy to controls found to be negative on this biopsy. A limitation that might be addressed in future studies is our inability to examine the relation between HHV-8 infection and late-stage disease.

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Table 1
Seroepidemiologic studies of human herpesvirus type 8 (HHV-8) infection and prostate cancer

First author, year	Study design	Study population	No. cases	No. and type of controls	HHV-8 serum assays	Seroprevalence Cases	Controls	Results
Sitas, 1999 (29)	Cross-sectional survey	South African Black cancer patients *	202	3,091 other cancer controls	ORF73 (latent) IFA	33%	----- [†]	NS
Hoffman, 2004 (9)	Case-control	Men from Tobago and Trinidad *	138 (Tobagonian)	140 Tobagonian controls	Lytic IFA	39.9%	22.9%	OR=2.24 (1.29-3.90) [‡]
		Predominantly Caucasian-American men *	100	174 Trinidadian controls			20.1%	OR=2.63 (1.54-4.50)
				99 other cancer controls		20%	13%	OR=1.65 (0.77-3.54) [§]
Korodi, 2005 (10)	Nested case-control	Finnish men	163	288	ORF65 (lytic) ELISA	1.8%	2.4%	OR=4.67 (1.91-11.65)
Jenkins, 2007 (11)	Case-control	Italian men *	10	34 BPH controls	Lytic IFA	40.0%	38.2%	OR=1.08 (0.27-4.33)
					K8.1 (lytic) ELISA	20.0%	23.5%	OR=0.81 (0.17-4.21)
					ORF73 (latent) ELISA	20.0%	29.4%	OR=0.60 (0.12-3.03)
					Lytic IFA	17.1%	19.0%	OR=0.88 (0.35-2.24)
		African-American men	41	98 BPH controls	K8.1 (lytic) ELISA	7.3%	19.4%	OR=0.33 (0.10-1.11)
					ORF73 (latent) ELISA	12.8%	24.5%	OR=0.43 (0.16-1.18)
					Lytic IFA	17.5%	27.5%	OR=0.56 (0.28-1.14)
					K8.1 (lytic) ELISA	2.1%	4.0%	OR=0.52 (0.10-2.66)
		Caucasian-American men	95	75 population-based controls	ORF73 (latent) ELISA	4.2%	6.7%	OR=0.62 (0.17-2.20)
					Lytic IFA	18.7%	24.4%	OR=0.71 (0.36-1.43)
Sutcliffe, 2007 (12)	Nested case-control	Predominantly Caucasian-American men	691	691	K8.1 (lytic) ELISA	2.9%	3.8%	OR=0.76 (0.17-3.40)
					ORF73 (latent) ELISA	1.9%	2.5%	OR=0.77 (0.13-4.44)
Huang, 2008 (13)	Nested case-control	African-American men	103	368	K8.1 (lytic) ELISA	1.9%	6.0%	OR=0.3 (0.1-1.4)
						13.5%	11.3%	OR=1.3 (0.9-1.7)
		Caucasian-American men	765	915				

First author, year	Study design	Study population	No. cases	No. and type of controls	HHV-8 serum assays	Seroprevalence		Results
						Cases	Controls	
McDonald, 2011 (14)	Nested case-cohort	Predominantly Afro-Caribbean, Tobagonian men	96	415	Lytic IFA	17.7%	11.1%	HR=0.88 (0.46-1.69)

BPH = benign prostatic hyperplasia; ELISA = enzyme-linked immunosorbent assay; HR = hazards ratio; IFA = immunofluorescence assay; OR = odds ratio; NS = not statistically significant; PCa = prostate cancer.

* Assumed to comprise >50% advanced stage PCa based on the stated characteristics of the study population, or on the calendar time or location of diagnosis of PCa cases.

[†] Seroprevalence could not be determined from the data presented in the manuscript.

[‡] OR=1.94 (1.12-3.34) when serostatus was determined by an algorithm defining seropositivity as positivity on any one of three tests: a peptide-based enzyme immunoassay (EIA) that detects antibodies against the ORF65 lytic antigen, a peptide-based EIA that detects antibodies against the ORFK8.1 lytic antigen, and the same monoclonal antibody-enhanced IFA that detects antibodies against multiple lytic antigens.

[§] Similar results were observed when serostatus was determined using the algorithm described in the previous footnote.

^{||} Similar results were observed when a correction was performed to account for the low sensitivity of the assay (48%) estimated using serum from acquired immune deficiency syndrome patients with Kaposi's sarcoma.

Table 2

Baseline characteristics¹ of participants by prostate cancer status and human herpesvirus type 8 serostatus in the Prostate Cancer Prevention Trial

	Case-control status			Human herpesvirus type 8 serostatus (controls only)		
	Case (n=315)	Control (n=315)	P-value ²	Positive (n=34)	Negative (n=281)	P-value ²
Mean age (years)	63.8	64.0	Matched	65.2	63.9	0.17
First-degree relative with prostate cancer (%)	19.4	19.4	Matched	8.8	20.6	0.10
Race (%): ³						
Caucasian	89.8	83.2	Over-sampled	94.1	81.9	0.14
African-American	7.6	7.6		0.0	8.5	
Other race/ethnicity	2.5	9.2		5.9	9.6	
Education (%):						
12 years	18.6	17.6	0.91	11.3	18.6	0.43
13-15 years	28.9	30.4		34.1	30.4	
16 years	52.4	52.0		54.6	51.0	
Occupation (%):						
Professional	66.7	66.7	0.94	69.9	65.9	0.96
Craftsman	13.8	12.3		10.6	13.0	
Sales	7.5	8.4		5.8	8.6	
Farming	1.6	2.2		2.8	2.1	
Other	10.5	10.4		10.9	10.4	
Military experience (%) ⁴	26.6	25.8	0.82	33.7	26.2	0.33
Marital status (%):						
Currently married/marriage-like relationship	87.1	87.5	0.28	76.7	88.6	0.03
Divorced/widowed	10.4	8.4		9.1	8.5	
Never married	2.2	4.2		14.2	2.9	
Mean height (inches)	69.7	69.7	0.78	70.2	69.6	0.27
Mean body mass index (kg/m ²)	27.7	27.4	0.30	27.0	27.4	0.47
Mean intakes/day of:						
Energy (kcal)	2267.9	2131.0	0.05	2358.8	2092.5	0.07
Protein (g)	98.4	92.3	0.07	101.3	90.9	0.15
Carbohydrates (g)	274.4	258.7	0.07	298.7	252.5	0.01
Fat (g)	83.2	77.8	0.10	83.4	76.7	0.34
Fruit (1 serving) ⁵	1.6	1.5	0.35	1.6	1.5	0.63
Vegetables (1 serving)	2.3	2.2	0.65	2.5	2.2	0.18
Tomato products (1 cup serving)	183	171.8	0.39	205.0	166.8	0.16
Red meat (6 oz serving)	240.6	231.6	0.52	236.1	230.2	0.84
Processed meat (6 oz serving)	131.3	134.7	0.75	121.3	136.3	0.50

	Case-control status			Human herpesvirus type 8 serostatus (controls only)		
	Case (n=315)	Control (n=315)	P-value ²	Positive (n=34)	Negative (n=281)	P-value ²
Fish (6 oz serving)	88.6	76.3	0.09	96.1	74.3	0.22
Calcium from food and supplements (mg)	1139	1052.7	0.07	1213.6	1024.5	0.05
Zinc from food and supplements (mg)	26.7	25.4	0.39	30.8	24.5	0.09
Total alcohol (g)	9.5	8.2	0.22	6.2	8.2	0.38
Cigarette smoking:						
Smoked regularly before 25 years of age (%)	61.6	65.7	0.29	69.9	65.2	0.60
Currently smoke (%)	6.6	7.0	0.85	4.0	7.7	0.44
Mean pack-years smoked	14.4	17.2	0.03	18.5	17.1	0.66
Physical activity (%):						
Sedentary	14.7	15.8		9.0	16.7	
Light activity	44.3	40.2	0.33	24.7	41.9	<0.01
Moderate activity	33.0	32.0		40.6	31.0	
Very active	7.4	12.0		25.7	10.4	
Frequency of sexual activity in the past 4 weeks (%):						
Not at all	10.9	13.6		18.4	13.1	
Once	12.8	10.7		5.7	11.1	
2-3 times	27.6	26.0	0.89	23.7	26.3	0.91
Once/week	29.4	29.6		32.3	29.2	
2-3 times/week	16.0	16.7		16.9	16.8	
4 times/week	2.6	2.8		3.0	2.8	
Vasectomy (%)	27.9	28.9	0.78	30.9	27.9	0.72
Mean age at vasectomy	39.2	37.5	0.11	39.5	37.6	0.35
History of diabetes mellitus type 2 (%)	5.3	8.7	0.10	3.7	9.5	0.27
History of benign prostatic hyperplasia (%) ⁶	12.2	12.5	0.91	4.8	13.7	0.15
Aspirin use on a regular basis (%):						
Current use	42.0	41.8	0.95	37.7	41.7	0.65
Past use	6.9	6.1	0.65	1.9	6.9	0.26
Geometric mean prostate-specific antigen concentration at visit 2 (ng/mL)	1.6	1.1	<0.01	1.4	1.1	0.05
Abnormal digital rectal examination findings at visit 2 (%)	7.2	2.3	<0.01	2.4	2.2	0.95
Seropositivity for:						
<i>Trichomonas vaginalis</i> (strong seropositive)	14.8	14.1	0.66 ⁷	22.7	13.3	0.57 ⁷
Human papillomavirus types 16, 18, or 31 (at least one strong seropositive)	4.9	5.0	0.98 ⁷	9.0	4.6	0.11 ⁷

	Case-control status			Human herpesvirus type 8 serostatus (controls only)		
	Case (n=315)	Control (n=315)	P-value ²	Positive (n=34)	Negative (n=281)	P-value ²
Cytomegalovirus	22.9	20.6	0.48 ⁷	28.3	20.1	0.27 ⁷

¹ Case and control values collected at baseline in 1994-7 or at the first annual visit were adjusted for age, family history of prostate cancer and race (non-Caucasian versus Caucasian) using linear regression.

² P-values were calculated by linear regression for continuous and binary variables, and by generalized logit models for categorical variables.

³ African-American controls and controls of other race/ethnicity were over-sampled in the design of the study. All analyses are adjusted for race to take this feature of the design into account. African-American Hispanics are included in the African-American category. Previous analyses in this case-control study population included African-American Hispanics in the "other race/ethnicity" category.

⁴ Defined as use of Veterans Affairs-sponsored payment for medical care or medical care at a military site. Previous analyses in this case-control study population defined military experience solely by use of Veterans Affairs-sponsored payment for medical care.

⁵ Based on the "5-a-day" definition. Previous analyses in this case-control study population defined fruit consumption by the "summation method".

⁶ Defined as surgical or medical treatment for benign prostatic hyperplasia or an International Prostate Symptom Score >14.

⁷ P-value for the comparison of strong seropositivity to seronegativity.

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) of prostate cancer for human herpesvirus type 8 serostatus in 315 prostate cancer cases and 315 frequency-matched controls in the placebo arm of the Prostate Cancer Prevention Trial

	Cases (n) ¹	Controls (n)	OR (95% CI) ²
<i>Total prostate cancer</i>			
Seronegative	278	281	1.00
Seropositive	37	34	1.06 (0.65-1.76)
<i>Low-grade (Gleason sum <7) prostate cancer</i>			
Seronegative	151	281	1.00
Seropositive	21	34	1.08 (0.60-1.96)
<i>High-grade (Gleason sum ≥ 7) prostate cancer</i>			
Seronegative	127	281	1.00
Seropositive	16	34	1.02 (0.54-1.93)
<i>High-grade (Gleason pattern 4+3 or Gleason sum ≥ 8) prostate cancer</i>			
Seronegative	106	281	1.00
Seropositive	14	34	1.07 (0.55-2.09)
<i>Organ-confined (T2 and N0M0) prostate cancer</i>			
Seronegative	260	281	1.00
Seropositive	35	34	1.08 (0.65-1.80)
<i>Prostate cancer diagnosed by for-cause biopsy³</i>			
Seronegative	141	281	1.00
Seropositive	23	34	1.33 (0.75-2.35)
<i>Prostate cancer diagnosed by end-of-study biopsy⁴</i>			
Seronegative	137	281	1.00
Seropositive	14	34	0.79 (0.41-1.54)

¹ Cases were a sample of men diagnosed with prostate cancer on any biopsy after their second visit or on their end-of-study biopsy (1996-2003).

² Calculated by logistic regression, including terms for age (continuous), family history of prostate cancer, and non-Caucasian race.

³ For-cause biopsy refers to a biopsy performed because of an elevated prostate specific antigen concentration or an abnormal digital rectal examination.

⁴ End-of-study biopsy refers to a biopsy performed without indication after seven years of participation in the study as per trial protocol.