

## Chronic Inflammation in Benign Prostate Tissue Is Associated with High-Grade Prostate Cancer in the Placebo Arm of the Prostate Cancer Prevention Trial

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### Abstract

**Background:** Chronic inflammation is hypothesized to influence prostate cancer development, although a definitive link has not been established.

**Methods:** Prostate cancer cases ( $N = 191$ ) detected on a for-cause (clinically indicated) or end-of-study (protocol directed) biopsy, and frequency-matched controls ( $N = 209$ ), defined as negative for cancer on an end-of-study biopsy, were sampled from the placebo arm of the Prostate Cancer Prevention Trial. Inflammation prevalence and extent in benign areas of biopsy cores were visually assessed using digital images of hematoxylin and eosin-stained sections. Logistic regression was used to estimate associations.

**Results:** Of note, 86.2% of cases and 78.2% of controls had at least one biopsy core (of three assessed) with inflammation in benign areas, most of which was chronic. Men who had at least one biopsy core with inflammation had 1.78 [95% confidence interval (CI), 1.04–3.06] times the odds of prostate cancer compared with men who had zero cores with inflammation. The association was stronger for high-grade disease (Gleason sum 7–10,  $N = 94$ ; OR, 2.24; 95% CI, 1.06–4.71). These patterns were present when restricting to cases and controls in whom intraprostatic inflammation was the least likely to have influenced biopsy recommendation because their prostate-specific antigen (PSA) was low (<2 ng/mL at biopsy).

**Conclusion:** Inflammation, most of which was chronic, was common in benign prostate tissue, and was positively associated with prostate cancer, especially high grade. The association did not seem to be due to detection bias.

**Impact:** This study supports an etiologic link between inflammation and prostate carcinogenesis, and suggests an avenue for prevention by mitigating intraprostatic inflammation. *Cancer Epidemiol Biomarkers Prev*; 23(5); 847–56. ©2014 AACR.

### Introduction

Chronic infections and chronic inflammatory diseases are known to causally influence the development of epithelial malignancies, including liver, stomach, urinary bladder, and large-intestine cancers (1, 2). Inflammation contributes to carcinogenesis during disease initiation, growth in the localized environment, tumor cell invasion,

angiogenesis, and metastatic dissemination (3). More recently, chronic inflammation has been hypothesized to be a cause of prostate cancer (3). If so, then intraprostatic inflammation should be highly prevalent given that prostate cancer is so common (4). Indeed, inflammatory infiltrates are frequently found in biopsies performed for elevated prostate-specific antigen (PSA) or abnormal

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digital rectal examination (DRE; ref. 5), in radical prostatectomy specimens (6), and in tissue resected for benign prostatic hyperplasia (7, 8). However, little is known about the presence of inflammation in prostate tissue in older men without prostate conditions because this tissue is difficult to obtain. And, it remains to be shown whether the presence or amount of inflammation in benign prostate tissue is, indeed, related to prostate cancer risk.

To address these important questions, we conducted a case-control study nested in the placebo arm of the Prostate Cancer Prevention Trial (PCPT). These questions could be uniquely addressed in the PCPT because, as part of this trial, all men underwent annual PSA screening and DRE, and men not diagnosed with prostate cancer by the end of the 7-year follow-up period were asked to undergo an "end-of-study" prostate biopsy (9). Given these PCPT features, we were also able to address these questions in men with lower serum PSA concentration and in men without indication for biopsy; that is, men in whom detection bias resulting from any link between intraprostatic inflammation and indication for biopsy (e.g., elevated PSA) is the least likely.

## Materials and Methods

### Study design and population

Included in this study were participants in the multisite PCPT (9). The purpose of the trial was to determine whether the 5 $\alpha$ -reductase type II inhibitor, finasteride, prevents prostate cancer. From 1993 to 1997, 18,882 men enrolled in the trial. To be eligible, men had to be at least 55 years old and have a normal DRE, a serum PSA  $\leq$ 3 ng/mL, and an American Urological Association Symptom Index  $<$ 20. Men were randomized to receive finasteride (5 mg/d) or placebo for 7 years. At trial entry, men completed questionnaires on demographic, lifestyle, and medical factors, including cigarette smoking history, first-degree family history of prostate cancer, and history of a diagnosis of diabetes. Also at trial entry, weight and height were measured, and the body mass index (BMI; kg/m<sup>2</sup>) was calculated.

Men were screened for prostate cancer by PSA and DRE at each of 7 annual visits. If serum PSA concentration was  $>$ 4 ng/mL or the DRE was abnormal, a prostate biopsy was recommended. Cancers detected on such biopsies were considered to be "for-cause" biopsy detected. All men not diagnosed with prostate cancer during the trial were requested to undergo prostate biopsy after 7 years on the trial irrespective of their PSA concentration or DRE status. Cancers detected on such biopsies were considered to be "for-cause" biopsy detected if serum PSA concentration was  $>$ 4 ng/mL or the DRE was abnormal, otherwise these cancers were considered to be "end-of-study" biopsy detected. The diagnosis made at the study site was confirmed and determination of Gleason sum was made centrally at the Prostate Diagnostic Laboratory, University of Colorado (Boulder, CO); the pathologists were blinded to trial arm and exposure information. The Data

Safety and Monitoring Board recommended that the trial be stopped early because the primary study objective had been met (9).

The Institutional Review Boards at the participating trial sites approved the PCPT. The Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health and the Colorado Multiple Institutional Review Board approved this inflammation study.

### Prostate cancer cases and controls

We previously developed a case-control study nested in the PCPT that included all 1,809 eligible men diagnosed with prostate cancer (cases), detected either on a for-cause or end-of-study biopsy, and a sample of 1,809 men who were negative for prostate cancer on the end-of-study biopsy (controls), irrespective of whether they had a clinical indication for biopsy (10). To achieve 1,809 controls and to enrich for non-White race for more powerful race-specific analyses, we selected all 372 non-White men, and then sampled 1,437 men from the remaining white controls. Controls were sampled such that they were frequency matched to the cases on age at baseline, first-degree family history of prostate cancer at baseline, and treatment arm. From these 3,600 men, we sampled 600 cases and 600 controls for serum-based studies (11). For this tissue-based study, our goal was to obtain from these 1,200 men about 200 of the cases and 200 controls from the placebo arm for this labor-intensive tissue-based study. Sufficient tissue could not be obtained for some men and thus, from the placebo arm we included 191 cases and 209 controls. To enhance statistical efficiency, for the cases, we sampled approximately equal numbers by grade (Gleason sum, low  $<$ 7 and high 7–10) and by reason for biopsy (for-cause, end-of-study).

### Assessment of inflammation in benign prostate tissue from biopsies

To test the hypothesis that inflammation in benign prostate tissue is associated with prostate cancer risk, we used the hematoxylin and eosin (H&E)-stained slides that were used to make or exclude the diagnosis of prostate cancer. Typically, six to 10 needle biopsy cores were taken per man and multiple cores were mounted on each slide. To achieve about three biopsy cores per man for review, we sampled a mean of two H&E-stained slides per man (16.0% had 1, 68.5% had 2, and 15.5% had 3 slides), which yielded a mean of 3.3 biopsy cores per man (0.3% had 1, 5.8% had 2, 70.0% had 3, 16.0% had 4, 6.0% had 5, and 2.0% had 6–8 cores). Most cores were from the apex or mid-gland.

To ensure blinding of the pathologist who assessed inflammation to case-control status, all areas of adenocarcinoma (cases) and arbitrary benign areas on cores without cancer (cases and controls) were masked with ink on the slide cover slips. The H&E-stained slides were then digitized in their entirety using the Aperio ScanScope slide scanner (Aperio) and uploaded into the Spectrum Digital Pathology Information Management System

(Aperio). The slide images were reviewed for inflammation visually online using the Aperio ImageScope Viewer Software package.

Only benign areas of the biopsy cores were evaluated for inflammation. First, we recorded the presence of any inflammatory cells, any acute inflammatory cells (e.g., polymorphonuclear cells), and any chronic inflammatory cells (e.g., cells with an appearance consistent with that of lymphocytes and macrophages) in the benign tissue for each biopsy core on each slide. Second, we visually estimated the proportion of the total benign (unmasked) biopsy core area per slide that had involvement of any inflammatory cells irrespective of the compartment. Third, we visually scored inflammation in the benign tissue using a modified version of the histopathologic classification system developed by Nickel and colleagues (12). For each slide, the extent (1, focal; 2, multifocal; and 3, diffuse) and grade (1, mild; 2, moderate; and 3, severe) of acute and chronic inflammation present was recorded separately for the luminal, intraepithelial, and stromal compartments of the benign prostate tissue (Fig. 1). To capture the combination of extent and grade, we calculated an intensity score for acute and chronic inflammation in each compartment for each of the slide of the man. To do so, we multiplied each grade that was present by its extent and summed over these products. These steps yielded the compartment-specific acute or chronic inflammation intensity score

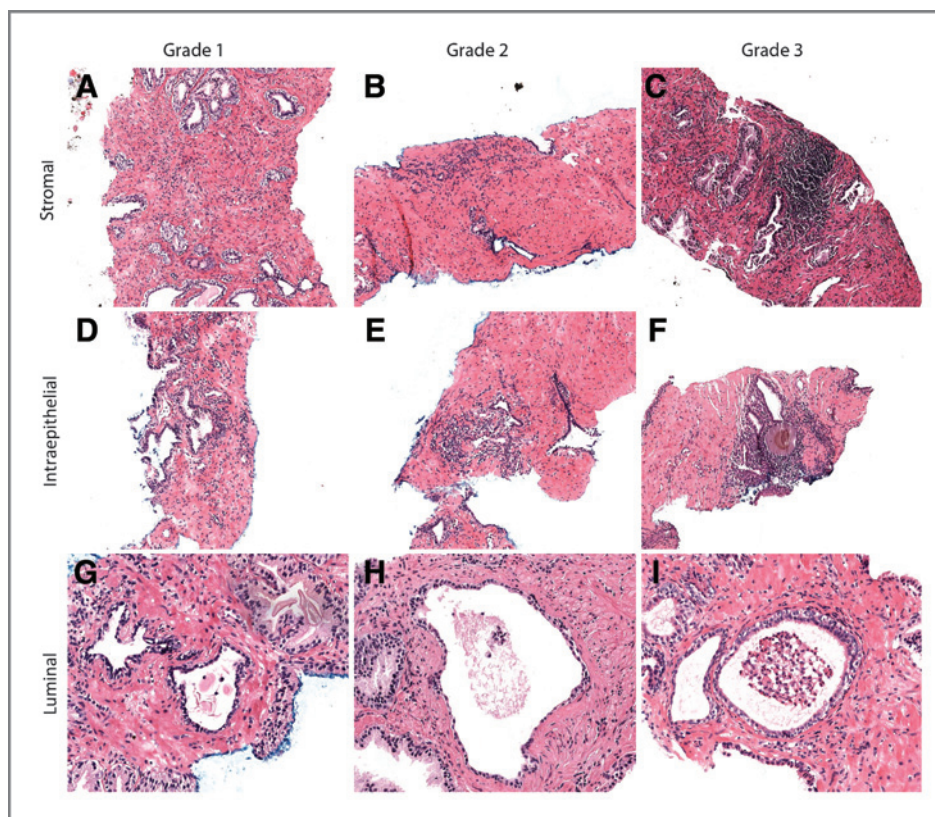
for each slide. Then, to obtain the acute or chronic inflammation intensity score for each of the slides of the man, we summed over the three compartment-specific acute or chronic inflammation intensity scores. For consistency, a single pathologist (B. Gurel), who was trained to score inflammation using each of these methods and who was blinded to cancer status, reviewed all of the images for this study.

### Statistical analysis

We used generalized linear models to calculate health- and tissue-related characteristics for the cases and controls adjusted for baseline age, family history of prostate cancer, and race. Measures of tissue inflammation included: the prevalence (at least one biopsy core with inflammation, at least one biopsy core with grade 3 chronic inflammation, and at least one biopsy core with grade 3 acute inflammation) and extent of inflammation in benign tissue [number of cores with inflammation (zero, some, or all); the mean percentage of tissue area with inflammation per man; the maximum percentage of tissue area with inflammation per man; mean chronic inflammation intensity score overall and by compartment (intraepithelial, luminal, stromal) per man; and maximum chronic inflammation intensity score per man].

We estimated the OR and 95% confidence interval (CI) for the associations of overall, high-grade, and low-grade prostate cancer with measures of the prevalence and

**Figure 1.** Scoring of chronic inflammation in prostate biopsy cores by severity (grade) and tissue compartment. All images are of benign areas obtained as screen shots from whole slide scanned images. A to C, increasing severity of inflammation in the stroma; D to F, increasing severity of intraepithelial inflammation; G to I, increasing severity of intraluminal inflammation. A to F, original magnification  $\times 100$ . G to I, original magnification  $\times 200$ .



extent of inflammation using logistic regression. All models included terms for the oversampling of non-White controls and frequency matching on baseline age and family history. In separate models, we additionally adjusted for BMI, pack-years smoked, and history of diabetes.

We conducted several analyses to address the potential for detection bias resulting from any influence of intra-prostatic inflammation on indication for biopsy (e.g., elevated PSA). Using linear regression models to adjust for baseline age, we calculated mean serum PSA concentration at biopsy by the prevalence and extent of inflammation in cases overall and separately by indication for biopsy, and in controls overall and separately in controls without an indication for biopsy. Next, we repeated these analyses restricting to (i) cases and controls with lower serum PSA concentration (<2 ng/mL) at biopsy; and (ii) cases detected on an end-of-study biopsy and controls who did not have a clinical indication for biopsy at trial end.

Statistical analyses were conducted using SAS release 9.3 (SAS Institute). We report two-sided *P* values.

## Results

### Characteristics of prostate cancer cases and controls

Table 1 gives the characteristics of the 191 prostate cancer cases and 209 controls sampled from the placebo

arm of the PCPT. The cases and controls were similar on baseline age and family history (both of which were frequency matching factors), and by design the controls were more likely to be non-White. After adjusting for age, family history, and race, cases and controls did not differ on baseline BMI or cigarette smoking status, although among current and former smokers, cases had smoked fewer pack-years than controls. Cases had a nonsignificantly lower prevalence of diabetes than controls. Cases had higher serum PSA concentrations at baseline and at biopsy, and a higher PSA velocity over follow-up than controls.

### Prevalence and extent of inflammation in benign prostate tissue in controls

Table 2 gives the age, family history, and race-adjusted prevalence and extent of inflammation in benign prostate tissue from controls. Of note, 78.2% of controls had at least one biopsy core with inflammation in benign tissue. On average, 52.1% of the cores evaluated for each man had inflammation of any grade. Of men who had at least one biopsy core with inflammation, on average, 14.7% of the benign tissue area for each of these men had inflammation. Values for controls who did not have an indication for biopsy at the end of the trial (191 of 209 controls; 91.4%) were similar to all controls (data not shown).

**Table 1.** Characteristics<sup>a</sup> of prostate cancer cases and controls<sup>b</sup>, placebo arm, and the PCPT

	Controls	Prostate cancer cases		
		Total	Low-grade	High-grade
<i>N</i>	209	191	97	94
Mean age at baseline (y)	63.9	63.6	62.7	64.6
Mean age at biopsy (y)	70.8	<b>70.1<sup>c</sup></b>	<b>70.2<sup>c</sup></b>	<b>70.1<sup>c</sup></b>
Non-White (%)	16.3	8.4	<b>6.2<sup>c</sup></b>	10.6
Family history (%)	17.7	16.2	15.5	17.0
Cigarette smoking history (%)				
Current	5.9	7.2	5.5	8.9
Former	60.8	56.5	55.3	57.8
Never	33.3	36.3	39.2	33.3
Mean pack-years smoked, current and former smokers	24.7	<b>21.0<sup>c</sup></b>	<b>19.6<sup>c</sup></b>	22.2
Mean BMI (kg/m <sup>2</sup> )	27.4	27.5	27.2	27.9
History of diabetes (%)	9.1	5.8	4.4	7.2
Mean PSA				
Concentration at baseline (ng/mL)	1.2	<b>1.6<sup>c</sup></b>	<b>1.6<sup>c</sup></b>	<b>1.6<sup>c</sup></b>
Concentration at biopsy (ng/mL)	2.1	<b>3.3<sup>c</sup></b>	3.0	<b>3.7<sup>c</sup></b>
Velocity (ng/mL/y)	0.11	<b>0.27<sup>c</sup></b>	0.20	<b>0.34<sup>c</sup></b>

<sup>a</sup>For all characteristics except baseline age, family history of prostate cancer, and race, from generalized linear models (linear for adjusted proportions and means and logistic for *P* values) adjusting for baseline age, family history, and race.

<sup>b</sup>Cases and controls were frequency matched on baseline age and family history. All non-White controls were sampled. Cases were sampled from the placebo arm of the trial so that half were high grade (Gleason sum  $\geq 7$ ) and half were low grade (Gleason sum  $< 7$ ), and of these half were detected on a biopsy performed for an elevated PSA or an abnormal DRE (for-cause biopsy) and half were detected on a biopsy performed at the end of the trial per trial protocol (end-of-study biopsy). Controls were sampled from men who were negative for prostate cancer on the biopsy performed at the end of the trial per protocol.

<sup>c</sup>*P* < 0.05 compared with controls.

**Table 2.** Prevalence and extent<sup>a</sup> of inflammation assessed in benign prostate tissue from biopsy cores, prostate cancer cases overall and by grade and controls<sup>b</sup>, placebo arm, and the PCPT

	Controls	Prostate cancer cases		
		Total	Low-grade	High-grade
<i>N</i>	209	191	97	94
At least one biopsy core with inflammation (%) <sup>c</sup>	78.2	<b>86.2<sup>e</sup></b>	84.0	<b>88.4<sup>e</sup></b>
Mean of the percentage of biopsy cores with inflammation <sup>c</sup>	52.1	58.7	56.1	<b>61.4<sup>e</sup></b>
Mean of the mean percentage of tissue area with inflammation <sup>d</sup>				
Overall	11.5	10.9	10.8	10.9
In men with at least one biopsy core with inflammation	14.7	12.6	12.8	12.3

<sup>a</sup>From generalized linear models (linear for adjusted proportions and means, logistic for *P* values) adjusting for baseline age, family history of prostate cancer, and race.

<sup>b</sup>Cases and controls were frequency matched on baseline age and family history of prostate cancer. All non-White controls were sampled. Cases were sampled from the placebo arm of the trial so that half were high grade (Gleason sum  $\geq 7$ ) and half were low grade (Gleason sum  $< 7$ ), and of these half were detected on a biopsy performed for an elevated PSA or an abnormal DRE (for-cause biopsy) and half were detected on a biopsy performed at the end of the trial per trial protocol (end-of-study biopsy). Controls were sampled from men who were negative for prostate cancer on the biopsy performed at the end of the trial per protocol.

<sup>c</sup>For each man, the denominator is total number of biopsy cores evaluated.

<sup>d</sup>For each man, the denominator is total benign tissue area across all biopsy cores evaluated on each of the slides of the man.

<sup>e</sup>*P* < 0.05 compared with controls.

Shown in Fig. 1 are images of PCPT biopsy cores with grade 1, 2, and 3 inflammation by stromal, intraepithelial, and luminal compartments. The majority of inflammatory cells present were mononuclear cells, morphologically recognizable as lymphocytes and macrophages (i.e., chronic inflammation). Of note, 24.5% of controls had at least one core with grade 3 chronic inflammation, whereas only 1.0% had at least one core with grade 3 acute inflammation. Among controls, the mean of the mean chronic inflammation intensity score across the slides of each man was 3.8. This score varied by compartment and was highest in the stromal, followed by intraepithelial, and then luminal compartment (data not shown). The intensity of chronic inflammation was correlated among the compartments (stromal and intraepithelial:  $r = 0.80$ ,  $P < 0.0001$ ; stromal and luminal:  $r = 0.25$ ,  $P = 0.0003$ ; and intraepithelial and luminal:  $r = 0.28$ ,  $P < 0.0001$ ).

#### Prevalence and extent of inflammation in benign prostate tissue from biopsies in prostate cancer cases, and comparison with controls

Table 2 gives the age, family history, and race-adjusted prevalence and extent of inflammation in benign tissue for prostate cancer cases. Of note, 86.2% of cases overall and 88.4% of high-grade cases had at least one biopsy core with inflammation, prevalences that were statistically significantly higher than in controls. The prevalence of inflammation in low-grade cases did not differ from that in controls. In high-grade cases, 61.4% of the cores evaluated for each man had inflammation, an extent that was statistically significantly higher than the extent in the controls; the extent in cases overall or in the low-grade cases did not differ from that in controls. Similar to

controls, most of the inflammatory cells present in the benign tissue of cases reflected chronic inflammation. None of the other measures of prevalence or extent of inflammation (including the percentage of tissue area with inflammation; Table 2) differed between cases and controls, although controls were more likely to have values of zero for all of the other histopathologic measures of inflammation (data not shown).

#### Association of the prevalence and extent of inflammation in benign prostate tissue with prostate cancer

Table 3 gives ORs of prostate cancer for inflammation in benign prostate tissue after adjusting for age, family history, and race. Men who had at least one biopsy core with inflammation (a measure of prevalence) had nearly an 80% higher odds of prostate cancer (OR, 1.78; 95% CI, 1.04–3.06) than men who had zero cores with inflammation. This association was more pronounced for high-grade cases (OR, 2.24; 95% CI, 1.06–4.71) than for low-grade cases (OR, 1.57; 95% CI, 0.83–3.00). These statistically significant results were unchanged after adjusting for the number of cores evaluated (data not shown). The results in Table 3 were unchanged after further adjusting for modifiable factors known to be associated with systemic inflammation and thought to be associated with prostate cancer or more aggressive disease; BMI, pack-years smoked, and history of diabetes (total prostate cancer: OR, 1.73; 95% CI, 1.00–2.98; high grade: OR, 2.17; 95% CI, 1.03–4.60).

We divided the number of biopsy cores with inflammation (a measure of extent) into three categories: zero, some, and all cores with inflammation (Table 3).

**Table 3.** Association<sup>a</sup> between inflammation assessed in benign prostate tissue from biopsy cores and prostate cancer risk, overall and by grade, placebo arm, and the PCPT

	Prostate cancer cases		
	Total	Low-grade	High-grade
<i>N</i>	191	97	94
At least one biopsy core with inflammation			
OR	1.78	1.57	2.24
95% CI	1.04–3.06	0.83–3.00	1.06–4.71
Extent of biopsy cores with inflammation <sup>b</sup>			
Zero cores			
OR	1.00	1.00	1.00
95% CI	Reference	Reference	Reference
Some cores			
OR	1.61	1.45	1.97
95% CI	0.92–2.81	0.74–2.84	0.91–4.26
All cores			
OR	2.19	1.87	2.83
95% CI	1.18–4.06	0.88–3.94	1.24–6.44
<i>P</i> <sub>trend</sub>	0.01	0.10	0.01

<sup>a</sup>From the logistic regression model adjusting for the matching factors baseline age and family history of prostate cancer, and for the oversampling of non-White controls. Cases and controls were frequency matched on baseline age and family history of prostate cancer. All non-White controls were sampled. Cases were sampled from the placebo arm of the trial so that half were high grade (Gleason sum  $\geq 7$ ) and half were low grade (Gleason sum  $< 7$ ), and of these half were detected on a biopsy performed for an elevated PSA or an abnormal DRE (for-cause biopsy) and half were detected on a biopsy performed at the end of the trial per trial protocol (end-of-study biopsy). Controls were sampled from men who were negative for prostate cancer on the biopsy performed at the end of the trial per protocol.

<sup>b</sup>A mean of three biopsy cores was assessed per man. Some cores with inflammation usually meant one or two, but not all three cores had inflammation present.

Compared with zero cores, the odds of prostate cancer increased from some (OR, 1.61; 95% CI, 0.92–2.81) to all cores with inflammation (OR, 2.19; 95% CI, 1.18–4.06;  $P_{\text{trend}} = 0.01$ ); these results were the same after adjusting for number of biopsy cores. The OR of low- and high-grade disease also increased with increasing extent of cores with inflammation, but the trend was statistically significant only for high grade ( $P_{\text{trend}} = 0.01$ ).

For the other measures of the prevalence and extent of total and chronic inflammation, when compared with no inflammation (e.g., the percentage of tissue area with inflammation equal to zero), risk was elevated among those with any amount of inflammation. The presence of a dose-response was inconsistent among the measures (data not shown). Because the men with any prevalence or extent of total or chronic inflammation for these other measures tended to be the same men as those with at least one core positive with inflammation, and likewise, because the men with a prevalence or extent of total or chronic inflammation equal to zero for these other measures tended to be the same men as those with zero cores positive for inflammation, in subsequent analyses we considered only two measures: at least one core positive versus zero cores positive; and all or some, versus zero cores positive.

#### Association between inflammation and prostate cancer after addressing potential PSA-associated detection bias

Given that inflammatory infiltrates are frequently found in biopsies performed for elevated PSA (5), we were concerned that any association between inflammation and prostate cancer could be due to detection bias. To assess this possibility, we first determined mean serum PSA concentration at biopsy by the prevalence and extent of inflammation in controls and cases (Table 4). In controls, mean PSA concentration was statistically significantly higher in men who had at least one core with inflammation than in men who had zero cores with inflammation, and mean concentration statistically significantly increased across zero, some, and all cores with inflammation. These differences were similar when restricting the analysis to controls without an indication for biopsy at the end-of-study biopsy. In contrast, among cases, PSA at the time of biopsy did not differ statistically significantly between men who had at least one core with inflammation and who had zero cores with inflammation, and PSA concentration did not change statistically significantly across zero, some, and all cores with inflammation.

After observing a link between greater intraprostatic inflammation and higher serum PSA concentration in

**Table 4.** Mean serum PSA concentration at biopsy<sup>a</sup> by prevalence and extent of inflammation assessed in benign prostate tissue from biopsy cores in the controls and prostate cancer cases, placebo arm, and the PCPT

	At least one biopsy core with inflammation		<i>P</i>	Extent of biopsy cores with inflammation		<i>P</i> <sub>trend</sub> <sup>b</sup>
	No	Yes		Some	All	
<b>Controls</b>						
Total ( <i>N</i> )	46	163		109	54	
Mean PSA at biopsy (ng/mL)	1.3	2.4	0.003	1.6	3.8	<0.0001
Without indication for biopsy ( <i>N</i> )						
Mean PSA at biopsy (ng/mL)	1.1	1.7	0.001	1.6	1.9	0.0002
<b>Cases</b>						
Total ( <i>N</i> )	26	165		100	65	
Mean PSA at biopsy (ng/mL)	3.3	3.4	0.77	3.4	3.3	0.70
Detected on a for-cause biopsy ( <i>N</i> )						
Mean PSA at biopsy (ng/mL)	4.5	4.7	0.75	4.9	4.2	0.61
Detected on an end-of-study biopsy <sup>c</sup> ( <i>N</i> )						
Mean PSA at biopsy (ng/mL)	2.4	2.0	0.45	1.9	2.3	0.32

<sup>a</sup>From linear regression models adjusting for age at baseline.

<sup>b</sup>Across no (zero), some, all biopsy cores with inflammation. Reference is men with "No" (zero) biopsy cores with inflammation.

<sup>c</sup>Without an indication for biopsy.

controls, we evaluated the association between inflammation and prostate cancer in the men in whom the link between intraprostatic inflammation and serum PSA was the least likely to create a noncausal inflammation–prostate cancer association, namely men with lower PSA at biopsy and, separately, men without indication for biopsy at the end-of-study biopsy. First, when restricting the analysis to cases and controls with low serum PSA level at the time of biopsy (<2 ng/mL), the association of having at least one biopsy core with inflammation with total prostate cancer ( $P = 0.013$ ) and high-grade disease ( $P = 0.066$ ) remained (Fig. 2). Second, when restricting to cases detected on an end-of-study biopsy and to controls without a clinical indication for biopsy at the end of the trial ( $N = 191$ ), the patterns of association for total prostate cancer ( $N = 97$  cases; at least one core with inflammation: OR, 1.55; 95% CI, 0.80–3.01; across extent of cores with inflammation:  $P_{\text{trend}} = 0.07$ ) and for high-grade disease ( $N = 48$  cases; at least one core with inflammation: OR, 1.91; 95% CI, 0.75–4.89; across extent of cores with inflammation:  $P_{\text{trend}} = 0.05$ ) were similar to those in the primary analysis.

## Discussion

In this case–control study nested in the placebo arm of PCPT, the odds of prostate cancer, especially high-grade disease, were higher in men who had inflammation in their benign prostate tissue in needle biopsy cores. The odds of total and high-grade prostate cancer increased with the extent of biopsy cores with inflammation. Inflammation in benign prostate tissue was very common in both

prostate cancer cases and controls, especially in the stroma, and most of the inflammatory cells present were morphologically indicative of chronic inflammation. Although any extent of total or chronic inflammation assessed using the more refined histopathologic measures was associated with prostate cancer, the dose–responses were inconsistent across these measures. When taken together, our results support the hypothesis that chronic, intraprostatic inflammation influences the development of prostate cancer, particularly high-grade disease.

Ours is the first study to directly test the hypothesis that chronic inflammation is associated with prostate cancer in men in whom prostate cancer screening, diagnosis, and Gleason sum determination were standardized and in a setting in which men not diagnosed with prostate cancer during the trial had the opportunity to have occult disease detected on a protocol-driven biopsy at the end of the trial. However, in this study, we cannot determine the timing of the presence of inflammation relative to the onset of cancer because we evaluated inflammation in the biopsy cores obtained to make or exclude the diagnosis of prostate cancer.

We observed that intraprostatic inflammation was more strongly associated with high- than low-grade prostate cancer. We had hypothesized that, as for other cancers, chronic inflammation may cause mutations and damage to prostate cells and promote the proliferation of prostate cells, including those damaged, initiated cells, thus increasing the risk of prostate cancer, especially aggressive disease. Our contention that inflammation would be associated with a more aggressive phenotype

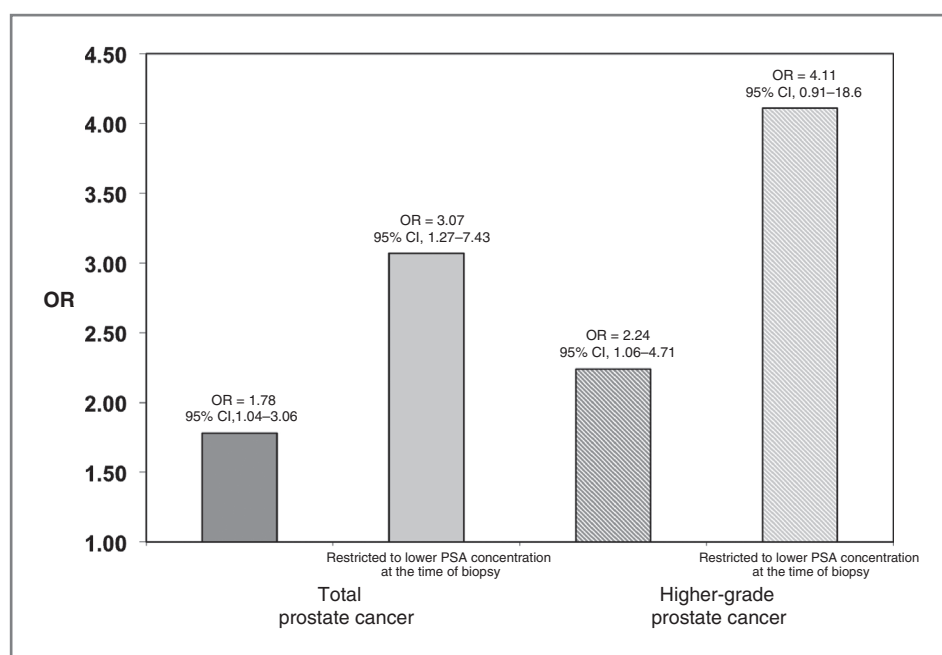


Figure 2. Association between at least one biopsy core with inflammation and total and high-grade prostate cancer overall and when restricting the analysis to cases and controls with lower PSA concentration (<2 ng/mL) at biopsy.

is supported by two prospective studies conducted in patients with prostate cancer that found that men who had a greater extent of intraprostatic inflammation had a higher risk of poor outcome (13, 14).

Two recent studies, one in the Finnish prostate cancer screening trial (15) and another in REDUCE (16), reported that men who were biopsy negative for prostate cancer had a lower risk of prostate cancer subsequently if they had inflammation in their prior negative biopsy (15, 16). In the former study, the initial biopsy was prompted by an elevated PSA concentration (>4 ng/mL) on the first screen of the trial (15). In the latter study, the men had a negative prostate biopsy before trial entry, a PSA concentration 2.5 to 10 ng/mL at trial entry, and underwent 2-year prostate biopsy during the trial (16). In REDUCE, both acute and chronic inflammation in the negative biopsy before trial entry were associated with a lower risk of prostate cancer on the 2-year biopsy, but only acute and not chronic inflammation in the negative biopsy before trial entry was associated with a lower risk in the 4-year biopsy. The results from our study and the results from the Finnish and REDUCE studies are not comparable because of differing distributions of PSA concentration: In the Finnish study, all men had an elevated PSA on the first screen, and in REDUCE the men had PSA concentrations ranging from 2.5 to 10 ng/mL, whereas in the PCPT, all men had a PSA of  $\leq 3$  ng/mL at enrollment. The PCPT study population and those in the Finnish and REDUCE studies also differed on the prior probability of prostate cancer: Men had to have had a negative biopsy following the elevated PSA on the first screen in the Finnish study or a negative biopsy before trial entry in REDUCE, whereas there was no such restriction in the PCPT.

In any study evaluating the association between inflammation and prostate cancer in the PSA era, bias resulting from differential opportunity for prostate cancer detection in men with and without intraprostatic inflammation is possible. In men with an indication for prostate tissue removal (e.g., biopsy, prostatectomy, and transurethral resection of the prostate), PSA is known to be higher in men with greater intraprostatic inflammation (13, 17, 18). In the present study, we showed that a greater prevalence and extent of inflammation in benign tissue was associated with higher serum PSA concentration in controls, and in controls without an indication for biopsy. This observation supports the contention that inflammation leading to higher PSA concentration could distort the association between inflammation and prostate cancer. These patterns were not observed in cases, in whom elevations in serum PSA concentration may result more from the presence of cancer than from inflammation. It is possible, therefore, that men with an elevated PSA and a negative biopsy (criteria for entry into the Finnish study; ref. 15) may indeed be more likely to be negative for cancer on a follow-up biopsy if their initial biopsies showed inflammation because the main determinant of the PSA rise in these men was the inflammation and not cancer. The same was possibly true in REDUCE for those men whose negative biopsies before trial entry were performed for an elevated PSA, although a sensitivity analysis among men with a PSA concentration of 2.5 to 4.0 ng/mL apparently supported the inverse association (16).

Given the association between inflammation and PSA observed in controls, we addressed the potential for detection bias in the inflammation–prostate cancer association by restricting analyses to cases and controls with lower PSA at biopsy, and restricting analyses to cases and



controls without an indication for biopsy. These analyses were possible because of the protocol-driven end-of-study biopsies for men not diagnosed with prostate cancer during the trial. About 15% of the men without an elevated PSA or abnormal DRE were diagnosed with prostate cancer at the end of the PCPT (9). In these analyses, the positive association of inflammation with total prostate cancer, and high-grade disease in particular, remained, indicating that the association was not likely fully explained by detection bias. Nevertheless, future studies addressing the association between inflammation and prostate cancer must parse causation from bias. A prospective study (i.e., measure inflammation in prostates of men without cancer and follow them for years for prostate cancer development) and animal models may help disentangle the links among inflammation, PSA, and prostate cancer.

Our study has a number of strengths. First, the PCPT had central pathology confirmation of prostate cancer diagnosis and determination of Gleason sum. Second, we used several methods to quantify prevalence and extent of inflammation in benign prostate tissue from needle biopsies, including a modification of a consensus-developed system (12). Simple assessment of intraprostatic inflammation—having at least one core with inflammation and having increasing number of cores with inflammation—was associated with prostate cancer; in general, the more refined histopathologic measurements, such as the percentage of tissue area with inflammation, did not seem to provide additional information about risk. Future studies addressing the role of inflammation in the etiology of prostate cancer and other prostate diseases may consider using the simple assessment. Third, the pathologist who assessed inflammation was fully blinded to case-control status, which reduces the potential for observation bias. Fourth, prostate cancer cases detected by biopsy but without clinical indication for biopsy have not been studied previously because prostate tissue is usually only available from cases for whom a biopsy is clinically indicated (e.g., an elevated PSA). Also, before this study, no epidemiologic study addressing the inflammation-prostate cancer hypothesis, to our knowledge, has included controls who did not have an indication for biopsy. These unique cases and controls allowed us to address the possibility of detection bias.

Despite its strengths, this study has several aspects that warrant discussion. First, although the PCPT, as a cohort study, is prospective, the study on inflammation was not. We measured inflammation on biopsies that were used to make or exclude the diagnosis of prostate cancer. Thus, we cannot rule out that the inflammation observed in the benign tissue of cases was a response to their cancer. However, the vast majority of men in the PCPT had only one biopsy core positive for cancer (9), and we did not preferentially select the core with cancer to assess inflammation, so most of the cores that we evaluated for the cases were unlikely to contain cancer.

Second, in this study, we used H&E-stained prostate biopsy core sections to visually morphologically identify and quantify inflammatory cells, either chronic or acute. Any reduced accuracy in the quantification of inflammation due to visual assessment relative to image analysis is unlikely to differ between the cases and controls because the pathologist was blinded. The net impact of any such inaccuracy is an underestimate of the association between inflammation and prostate cancer. Although more than 1,000 men in the placebo arm were diagnosed with prostate cancer in the PCPT (9), for feasibility we sampled <20% of cases. However, we did sample such that we had roughly similar numbers of high- and low-grade cases providing approximately equal power to evaluate the association by grade. Also, for feasibility, we sampled only about three of the six to 10 prostate biopsy cores that were obtained for each man. Nevertheless, men who tend to have more intraprostatic inflammation would, on average, be more likely to have a greater extent of inflammation in a given biopsy core selected than men with less intraprostatic inflammation. The net effect of measurement error as a result of sampling would be to attenuate any association toward no association. Prostate biopsies are taken largely from the peripheral zone of the prostate; we could not determine whether inflammation in other areas of the prostate is associated with prostate cancer. Third, because of the intensive prostate cancer screening in the PCPT, we could not address the association between inflammation and prostate cancer that was late stage or fatal. Fourth, we cannot rule out that the PCPT entry criteria (e.g., low PSA) restricted the prevalence or extent of inflammation present in the biopsies, including those performed 7 years after baseline. Fifth, we were not able to evaluate whether the association between intraprostatic inflammation and prostate cancer is the same among racial and ethnic groups; at this time, no large multiracial study has obtained prostate tissue from men without a clinical indication for biopsy. Finally, given the high prevalence of intraprostatic inflammation that we observed, the positive predictive value of the presence versus absence of inflammation in the prostate would be far too low for clinical use. Nevertheless, our findings support the conducting of studies to investigate the specific immune cell milieu of the prostate that may be associated with the development of an aggressive prostate cancer phenotype.

In conclusion, our finding that inflammation, primarily chronic, in benign prostate tissue is associated with an increased odds of prostate cancer, and high-grade prostate cancer in particular, will inform the etiology of this disease. Identifying those men at highest risk of developing aggressive disease is the first step in being able to prevent lethal prostate cancer. Rather than targeting interventions to all healthy men, which may result in unintended harms, if causal, the findings from our work may allow preventive interventions to be targeted to those men who would benefit the most.

### Disclosure of Potential Conflicts of Interest

C.G. Drake is a consultant/advisory board member of BMS and Compugen. W.G. Nelson is a consultant/advisory board member of Glaxo-SmithKline. No potential conflicts of interest were disclosed by the other authors.

### Disclaimer

The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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