Research Article

Cancer Epidemiology, Biomarkers & Prevention

A Prospective Study of Chronic Inflammation in Benign Prostate Tissue and Risk of Prostate Cancer: Linked PCPT and SELECT Cohorts

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Abstract

Background: We leveraged two trials to test the hypothesis of an inflammation–prostate cancer link prospectively in men without indication for biopsy.

Methods: Prostate Cancer Prevention Trial (PCPT) participants who had an end-of-study biopsy performed per protocol that was negative for cancer and who subsequently enrolled in the Selenium and Vitamin E Cancer Prevention Trial (SELECT) were eligible. We selected all 100 cases and sampled 200 frequency-matched controls and used PCPT end-of-study biopsies as "baseline." Five men with PSA > 4 ng/mL at end-of-study biopsy were excluded. Tissue was located for 92 cases and 193 controls. We visually assessed inflammation in benign tissue. We estimated ORs and 95% confidence intervals (CI) using logistic regression adjusting for age and race.

Results: Mean time between biopsy and diagnosis was 5.9 years. In men previously in the PCPT placebo arm, 78.1% of cases ($N = 41$) and 68.2% of controls ($N = 85$) had at least one baseline biopsy core (~5 evaluated per man) with inflammation. The odds of prostate cancer ($N = 41$ cases) appeared to increase with increasing mean percentage of tissue area with inflammation, a trend that was statistically significant for Gleason sum <4+3 disease ($N = 31$ cases; vs. 0%, >0–<1.8% OR = 1.70, 1.8–<5.0% OR = 2.39, ≥5% OR = 3.31, $P_{trend} = 0.047$). In men previously in the finasteride arm, prevalence of inflammation did not differ between cases (76.5%; $N = 51$) and controls (75.0%; $N = 108$).

Conclusions: Benign tissue inflammation was positively associated with prostate cancer.

Impact: This first prospective study of men without biopsy indication supports the hypothesis that inflammation influences prostate cancer development. *Cancer Epidemiol Biomarkers Prev; 26(10); 1549–57. ©2017 AACR.*

Introduction

Conditions that cause continued bouts of epithelial cell injury and cell death, such as chronic infections and chronic inflammatory conditions, result in a sustained abnormal increase in epithelial cell proliferation that can enhance carcinoma development (1–3). These scenarios are established for gastric carcinomas induced by *Helicobacter pylori*, colitis-associated colorectal carcinomas, and viral and other chronic hepatitis-induced hepatocellular carcinomas (2, 4). Such a link between inflammation and prostate cancer has been proposed, but not yet established (5).

Currently, we do not have evidence-based diet, lifestyle, or FDA-approved chemoprevention approaches to prevent prostate cancer, and do not have tools to readily identify men who would most benefit from preventive interventions. If a link can be established between inflammation and prostate cancer, then the development of novel interventions to abrogate intraprostatic inflammation (or its causes) could be a viable primary prevention strategy.

Previously in the Prostate Cancer Prevention Trial (PCPT) placebo arm, we assessed inflammation in benign areas of prostate biopsy cores from 191 cases and 209 controls (6). Men with inflammation in at least one of three biopsy cores assessed had 1.8 times the odds of prostate cancer and 2.4 times

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PCPT and SELECT were Southwest Oncology Group coordinated studies.

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the odds of Gleason sum ≥7 disease compared with men for whom none of their cores had inflammation. The odds increased with proportion of cores with inflammation. An association with Gleason sum <7 disease was also present, but weaker. Prevalence of inflammation was higher in the finasteride arm (92.4%; ref. 7) than in the placebo arm (78.2%; ref. 6), and in contrast to the placebo arm, inflammation was not associated with prostate cancer in the finasteride arm (7). Because of a design feature of the PCPT—prostate biopsies were performed at the end of the trial including on men without indication—our studies had clear strengths: (i) the likelihood of occult prostate cancer was reduced in controls, minimizing attenuation of associations resulting from admixing cases with controls (8); and (ii) inflammation in controls was less likely to be distorted by a common indication for biopsy—elevated PSA—which is higher in men with intraprostatic inflammation, including in the PCPT placebo arm (6, 9).

For inflammation to be a cause, it must temporally precede disease. While our prior results suggest that inflammation influences prostate cancer (in the absence of finasteride), we could not determine whether inflammation preceded prostate cancer because we assessed inflammation in the biopsies used to rule in or out a prostate cancer diagnosis. Thus, the most critical step in establishing the inflammation–prostate cancer association is a prospective study.

Therefore, in this study we linked the PCPT cohort and the Selenium and Vitamin E Cancer Prevention Trial (SELECT) cohort to form a cohort of men who were negative for prostate cancer on PCPT end-of-study biopsies and were eligible, specifically having PSA ≤4 and a normal DRE, and participated in SELECT. We used PCPT end-of-study biopsies as "baseline" biopsies in the linked cohort. Linking these cohorts is the only epidemiologically sound approach to prospectively test the inflammation–prostate cancer association in men without an indication for biopsy now and in the foreseeable future. We hypothesized that men with more inflammation in their baseline biopsies would have a greater prostate cancer risk. We also sought to determine if we could confirm, in a prospective study, our prior observation (7) that inflammation is not associated with risk in men previously treated with finasteride.

Materials and Methods

Study population, design, and case ascertainment

Men who participated in both of two randomized, multisite chemoprevention trials conducted by Southwest Oncology Group—PCPT and SELECT—were eligible for this study (Fig. 1).

Starting in 1993, PCPT randomized 18,882 men ≥55 years old with a normal DRE and PSA ≤3.0 ng/mL to 5 mg/day finasteride (5α-reductase type II inhibitor) or placebo for 7 years; 3.8% were African American. Because finasteride reduces serum PSA, values in the finasteride arm were adjusted upward in post-baseline samples to keep the proportion of biopsies triggered by PSA screening comparable between arms. All men not diagnosed with prostate cancer during the trial were recommended to undergo biopsy at the end, even if PSA and DRE results were normal, to determine prostate cancer status. If during the trial a man had an elevated PSA, was biopsied, and cancer was not found, then a subsequent PSA had to have an increase of ≥50% the prior level or be >10 ng/mL to be considered as an indication for biopsy, including at the end of the trial. In total, 7,761 men without clinical indication underwent an end-of-study biopsy within 7 years ± 90 days of randomization, and no cancer was found. PCPT found that finasteride reduced the period prevalence of prostate cancer by 25% (10).

Starting in 2001, SELECT randomized 35,533 men ages ≥50 years old, if African American, or ≥55 years old otherwise, with a PSA ≤4.0 ng/mL, normal DRE, and no other cancer diagnoses within the preceding 5 years to receive supplements containing selenium, vitamin E, selenium and vitamin E, or placebo for 7 years. PCPT participants were eligible to subsequently join SELECT if they met the eligibility criteria. Twelve percent were African American. Participants were followed by the local study site for prostate cancer for a median of 5.5 years using community standards for PSA and DRE to recommend prostate biopsy. Diagnosis and Gleason sum were centrally reviewed for 85% of cases. SELECT was stopped early based on interim results showing that selenium and vitamin E supplementation did not decrease prostate cancer risk (11). With 18 months of extended follow-up, risk was 17% higher in the vitamin E arm compared with the placebo arm (12).

The linked cohort consists of men negative for prostate cancer on PCPT end-of-study biopsies and who subsequently enrolled in SELECT. We used PCPT end-of-study biopsies to estimate the prevalence and extent of inflammation at entry into SELECT. By 2011, when we designed this inflammation study, 100 biopsy-confirmed incident prostate cancer cases were diagnosed in 1,780 men in the linked cohort (51% from the finasteride arm). Given SELECT's eligibility criteria, all men in the linked cohort had no clinical indication for biopsy at the start of follow-up. To form a nested case–control study, we selected all 100 incident cases, and sampled 200 controls frequency matched on age at PCPT end-of-study biopsy (</≥ median of 69.3 years), race (white/nonwhite), PCPT arm (finasteride/placebo), and SELECT arm (vitamin E, selenium, both, placebo). Most cases were early stage. For this inflammation study, we classified cases as Gleason sum <4+3 (grade groups 1–2 in the Epstein and colleagues prostate cancer grading system; ref. 13) or ≥4+3 (grade groups 3–5). Although at entry into SELECT none of the men had an indication for biopsy, five had a PSA >4 ng/mL at the PCPT end-of-study biopsy. For this reason, we excluded one control and two cases among men previously in the PCPT placebo arm and two cases among men previously in the PCPT finasteride arm.

The institutional review boards (IRBs) at the participating sites approved PCPT and SELECT. The Johns Hopkins Bloomberg School of Public Health and Department of Defense IRBs approved this linked cohort and research on inflammation and prostate cancer.

Evaluation of intraprostatic inflammation

PCPT end-of-study biopsies were stored at the University of Colorado Prostate Biorepository. Tissue was located for 92 cases (96%) and 193 controls (97%). Joint distribution of PCPT and SELECT treatment arms among cases and controls is shown in Supplementary Table S1; 81% of men had 6 cores and 13.5% had 7–10 cores taken during the prostate biopsy. As we did previously (6), for feasibility we sampled two slides per man (96%), Slides had multiple cores mounted, yielding a mean of 4.6 cores in controls and 4.9 cores in cases.

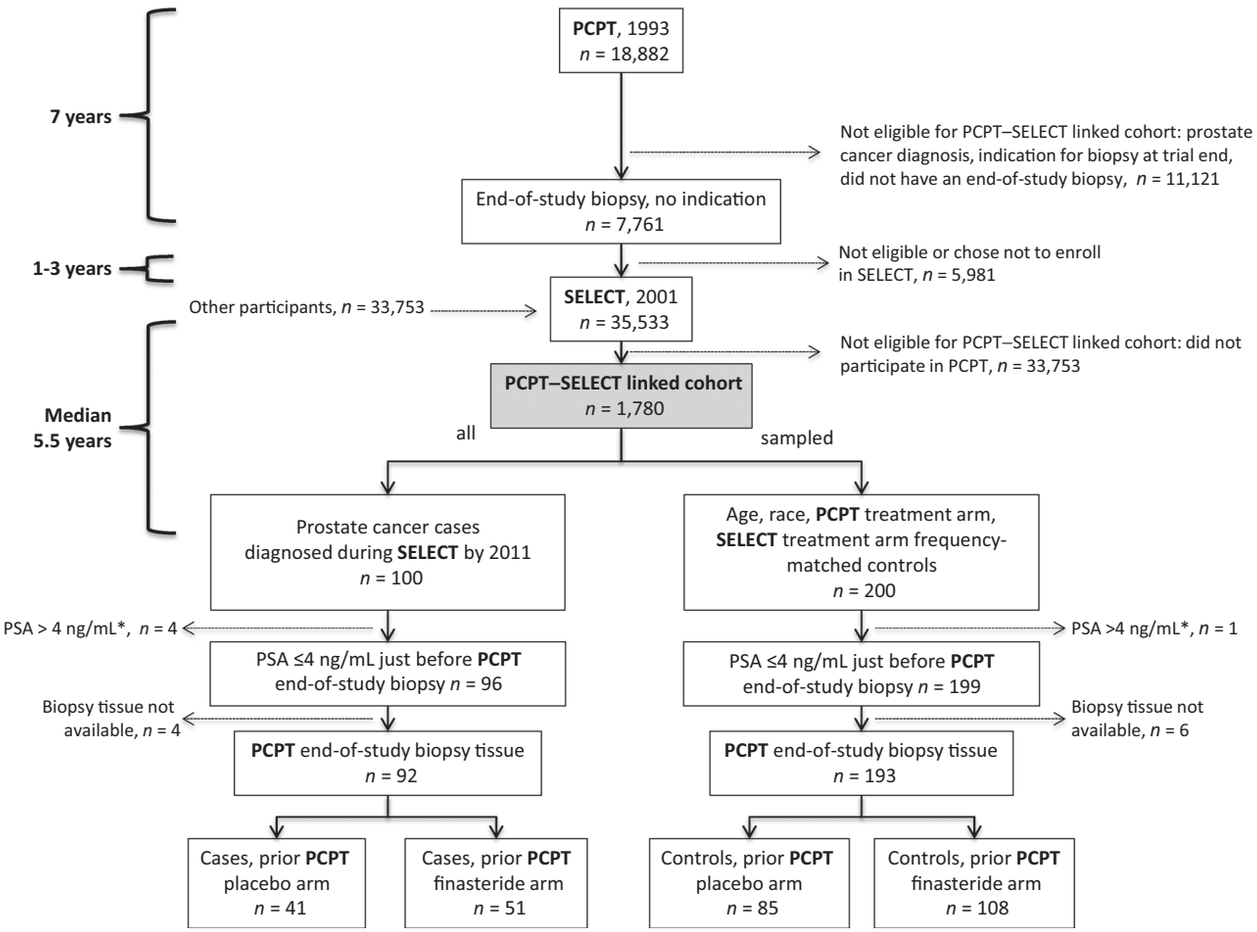

Figure 1.

Inclusion and exclusion of participants from the PCPT and SELECT trials to form the PCPT–SELECT linked cohort. Cases and controls nested in the PCPT–SELECT linked cohort participated for 7 years in PCPT and spent 1 to 3 years before enrollment in SELECT. Men eligible to be selected as controls participated for 5.5 years in SELECT. Cases diagnosed during SELECT participated for up to 5.5 years. The PCPT end-of-study biopsies served as the baseline biopsies for the PCPT–SELECT linked cohort. Cases were diagnosed a mean of 5.9 years after the PCPT end-of-study biopsy. *, A total of 5 men who were eligible for and enrolled in SELECT (had PSA $\leq$4 ng/mL and normal DRE) were considered to not have an indication for biopsy at the time of the PCPT end-of-study biopsy even though they had a PSA >4 ng/mL. This resulted from the following PCPT study policy: if during the trial a man had an elevated PSA, was biopsied, and cancer was not found, then a subsequent PSA had to have an increase of $\geq$50% the prior level or be >10 ng/mL to be considered as an indication for biopsy, including at the end of the trial.

Images were visually assessed using the Aperio ImageScope Viewer Software package to determine presence of total inflammatory cells, acute inflammatory cells (e.g., polymorphonuclear cells), and chronic inflammatory cells (e.g., cells with an appearance consistent with that of lymphocytes and macrophages), and quantify the percentage of the biopsy core tissue area per slide that had involvement of any inflammatory cells (6). The pathologist (I. Kulac) trained to score inflammation was blinded to case–control status.

Collection of other data

We used data collected at SELECT entry to characterize cases and controls by risk and other factors, including family history, body mass index (BMI), smoking status, and energy intake. We used serum PSA concentration measured closest in time before the PCPT end-of-study biopsy. For men previously in the finasteride arm, we used data on continued use of the study drug at the end-of-study biopsy.

Statistical analysis

Based on our prior findings (6, 7), we performed analyses separately by previous PCPT treatment assignment. We compared participant characteristics, including measures of inflammation, by case and control status using linear and logistic regression. In controls, we compared participant characteristics by proportion of biopsy cores with inflammation (none, some, all). We estimated ORs and 95% confidence intervals (CIs) of total and Gleason sum <4+3 (groups 1 and 2) prostate cancer by prevalence (at least one biopsy core with inflammation), proportion of biopsy cores with inflammation (none, some, all), and mean percentage of tissue area with inflammation (0%, tertiles of the distribution >0% with cutoff points based on controls) using

logistic regression. We could not evaluate associations for Gleason sum ≥4+3 (grade groups 3–5) disease due to small number (N = 9). We adjusted for age at PCPT end-of-study biopsy and race in the main analysis, and confirmed that additional adjustment for family history, height, BMI, smoking, and energy intake did not notably change the results. To test for trend, we used an ordinal term for proportion of cores with inflammation [none (0), some (1), all (2) cores] or mean percentage of tissue area with inflammation [none (0), tertile 1 (1), tertile 2 (2), tertile 3 (3)] and evaluated the coefficient by the Wald test. To determine whether associations differed by previous PCPT treatment arm, we used the likelihood ratio test to compare models with and without an interaction term between treatment arm and inflammation. Although vitamin E and selenium did not reduce prostate cancer risk in SELECT (11, 12) and inflammation was assessed in tissue collected before the men took SELECT supplements, we confirmed that trial arm was not an effect modifier using this same method.

To test whether we could confirm the link between inflammation and PSA that we previously observed in the PCPT placebo arm (6, 9) and the lack of a link in the finasteride arm (7), we calculated mean serum PSA concentration closest in time before the PCPT end-of-study biopsy by prevalence of inflammation and by proportion of biopsy cores with inflammation separately in controls and cases. We used linear regression and adjusted for age at end-of-study biopsy and race. We conducted analyses using SAS version 9.4 and report P values from two-sided tests.

Results

Placebo arm (previous PCPT assignment)

This analysis included 41 prostate cancer cases (31 cases <4+3 = grade groups 1 and 2; 5 cases ≥4+3 (grade groups 3–5); 5 missing grade) and 85 controls. Cases and frequency-matched controls did not differ on baseline characteristics except possibly for family history (Table 1). Control characteristics did not differ across proportion of biopsy cores with inflammation (Table 2). 78.1% of cases (84.1% of cases with grade <4+3) and 68.2% of controls had at least one baseline biopsy core (of ∼5 reviewed) with inflammation (Table 3). In controls, on average, 32.1% of cores per man had inflammation of any grade. Of controls with at least one biopsy core with inflammation, on average, 4.4% of tissue area had inflammation. Most inflammation was chronic.

>Compared with men whose mean percentage of tissue area with inflammation was 0% (no biopsy core had inflammation), the age and race-adjusted OR of Gleason sum <4+3 prostate cancer, statistically significantly increased with increasing mean percentage of biopsy core tissue area with inflammation (vs. 0%, >0 to <1.8% OR = 1.70, 1.8% to <5.0% OR = 2.39, ≥5% OR = 3.31, P_{trend} = 0.047). The pattern was similar for total (Table 4), and for Gleason sum 3+4 (N = 11), and <7 (N = 20) prostate cancer. Although not statistically significant, ORs of total (OR = 1.66; 95% CI, 0.70–3.96) and Gleason sum <4+3 (OR = 2.44; 95% CI, 0.84–7.07) prostate cancer were in the positive direction for having at least one biopsy core with inflammation (Table 4). Also, ORs of total and Gleason sum <4+3 prostate cancer tended to increase across proportion of biopsy cores with inflammation (Table 4). Results were comparable after additional adjustment for prostate cancer risk factors.

In both controls and cases, PSA concentration at the PCPT end-of-study biopsy did not differ between men with at least one biopsy core with inflammation and men with no cores with inflammation, although men with all cores with inflammation tended to have higher PSA than man with none or some cores with inflammation (Table 5).

Finasteride arm (previous PCPT assignment)

This analysis included 51 cases (34 cases <4+3 = grade groups 1 and 2; 4 cases ≥4+3 (grade groups 3–5); 13 missing grade) and 108 controls. In men previously assigned to finasteride, prostate cancer cases and frequency-matched controls did not significantly differ on baseline characteristics except possibly for family history and energy intake (Table 1). The proportion of cases and controls

Table 1. Characteristics of prostate cancer cases and controls,[a] nested in the linked PCPT–SELECT cohort

	Previously in the PCPT placebo arm			Previously in the PCPT finasteride arm		
	Prostate cancer cases	Controls	*P*	Prostate cancer cases	Controls	*P*
N	41	85		51	108	
Mean age[b] (years)	68.2	67.9	0.8	67.7	68.0	0.7
Non-white (%)	2.4	2.4	0.9	9.8	10.2	0.9
Education						
High school (%)	20.0	17.9	0.8	14.0	17.0	0.5
Some college (%)	20.0	27.4	34.0	36.8		
College (%)	60.0	54.7	52.0	46.2		
Missing (*N*)	1	1		1	2	
Mean height (cm)	177.2	176.1	0.4	176.7	177.4	0.5
Mean BMI (kg/m^2)	29.3	27.9	0.2	28.8	28.4	0.6
Missing (*N*)	0	0		0	1	
Smoking history						
Never (%)	41.5	47.1	0.9	47.1	45.3	0.7
Former (%)	58.5	48.2	47.1	53.8		
Current (%)	0	4.7	5.8	0.9		
Missing (*N*)	0	0		0	2	
Family history of prostate cancer (%)	34.2	20.0	0.1	33.3	20.4	0.1
Mean energy intake (kcal/day)	2,377.2	2,353.4	0.9	2,385.7	1,997.3	0.01
Still taking finasteride at PCPT end-of-study biopsy (%)	—	—		84.3	85.2	0.9
Mean time between PCPT end-of-study biopsy and prostate cancer diagnosis in SELECT (years)	6.2	—		5.8	—	

[a]Controls were frequency matched to cases on age at PCPT end-of-study biopsy, race, and PCPT and SELECT treatment arms.
[b]Age at PCPT end-of-study biopsy.

Table 2. Characteristics by proportion of PCPT end-of-study biopsy cores with inflammation in controls,[a] nested in the linked PCPT–SELECT cohort

	Previously in the PCPT placebo arm				Previously in the PCPT finasteride arm			
	Proportion of biopsy cores with inflammation				Proportion of biopsy cores with inflammation			
	None	Some	All	P_{trend}	None	Some	All	P_{trend}
N	27	52	6		27	73	8	
Education								
Only high school (%)	14.8	18.0	34.0	0.6	26.9	14.2	6.6	0.1
Some college (%)	26.3	30.5	0.6		33.3	39.0	27.4	
College (%)	58.9	51.5	65.4		39.8	46.8	66.0	
Missing (N)	0	0	1		0	2	0	
Mean height (cm)	175.6	176.0	178.7	0.4	178.3	177.2	175.7	0.4
Mean BMI (kg/m^2)	27.3	28.1	28.6	0.5	28.9	28.2	28.1	0.5
Missing (*N*)	0	0	0		0	1	0	
Smoking history								
Never (%)	55.7	41.3	56.9	0.7	37.0	44.0	86.2	0.1
Former (%)	37.3	54.7	42.3		63.0	54.6	13.5	
Current (%)	7.0	4.0	0.8		0	1.4	0.3	
Missing (N)	0	0	0		0	2	0	
Energy (kcal)	2,232.2	2,459.9	1,946.2	0.8	1,837.0	2,019.3	2,381.3	0.1
Still taking finasteride at PCPT end-of-study biopsy (%)	—	—	—		85.6	86.2	72.4	0.6

[a]Adjusted for age at PCPT end-of-study biopsy and race.

still taking finasteride at the PCPT end-of-study biopsy was similar. In controls, education, smoking status, and energy intake possibly differed across proportion of biopsy cores with inflammation (Table 2).

Controls previously in the finasteride arm appeared to have a slightly higher prevalence of inflammation than controls previously in the placebo arm (74.8% vs. 68.9%), but did not have a higher percentage of biopsy cores with inflammation or higher percentage of tissue area with inflammation (Table 3). However, Gleason sum <4+3 cases previously in the finasteride arm appeared to have a slightly lower prevalence (76.4% vs. 84.1%), smaller percentage of biopsy cores with inflammation, and smaller percentage of tissue area with inflammation than Gleason <4+3 cases in the placebo arm. Like in the placebo arm, most inflammation was chronic.

Unlike men previously in the PCPT placebo arm, among men previously in the finasteride arm having at least one biopsy core with inflammation was not associated with risk of total or Gleason sum <4+3 prostate cancer when adjusting for age and race (Table 4). We could not rule out a modest positive, non-statistically significant association among men who had all biopsy cores with inflammation or were in the top tertile of mean percentage of tissue area with inflammation (Table 4). Results were unchanged after further adjusting for potentially confounding factors. Results were unchanged after excluding men who were not still taking finasteride at the end-of-study biopsy. While the association between having at least one biopsy core with inflammation and total ($P_{interaction} = 0.5$) and Gleason sum <4+3 ($P_{interaction} = 0.3$) prostate cancer appeared to be different in the two PCPT treatment arms, we did not detect a statistical interaction.

In controls, PSA concentration at the PCPT end-of-study biopsy was higher in men with at least one biopsy core with inflammation and increased across proportion of biopsy cores with inflammation (Table 5). In cases, PSA concentration did not differ between men with and without at least one biopsy core with inflammation and did not change across proportion of biopsy cores with inflammation (Table 5).

Table 3. Amount[a] of inflammation in prostate tissue from PCPT end-of-study biopsy cores, prostate cancer cases overall, and by grade and controls[b] stratified by previous PCPT treatment arm, case–control study nested in the linked PCPT–SELECT cohort

	Previously in the PCPT placebo arm			Previously in the PCPT finasteride arm		
		Prostate cancer cases			Prostate cancer cases	
	Controls	Total	Gleason sum <4+3 (grade groups 1–2)	Controls	Total	Gleason sum <4+3 (grade groups 1–2)
N	85	41	31	108	51	34
At least one biopsy core with inflammation (%)[c]	68.2	78.1	84.1	75.0	76.5	76.4
Mean of the percentage of biopsy cores with inflammation[c]	32.1	40.4	45.8[e]	35.2	38.9	38.5
Mean of the mean percentage of tissue area with inflammation[d]						
Overall	3.0	6.4	8.1[e]	3.4	4.2	4.3
In men with at least one biopsy core with inflammation	4.4	8.2	9.6	4.5	5.5	5.6

[a]From generalized linear models (linear for adjusted proportions and means, logistic for *P* values) adjusting for age at PCPT end-of-study biopsy and race.
[b]Inflammation was assessed in prostate tissue from the biopsies performed at the end of the PCPT. These biopsies serve as "baseline" biopsies for the linked PCPT–SELECT trials. Cases and controls diagnosed subsequently were frequency matched on age at PCPT end-of-study biopsy, race, and PCPT and SELECT treatment arms. Grade groups are from Epstein et al.'s new grading system (13). Eighteen cases had missing Gleason sum.
[c]For each man, the denominator is total number of biopsy cores evaluated: previous placebo arm controls 4.7, placebo arm cases 5.0, finasteride arm controls 4.6, finasteride arm cases 4.8.
[d]For each man, the denominator is total benign tissue area across all biopsy cores evaluated on each of the man's slides (we evaluated two slides for 96% of the men).
[e]$P < 0.05$ compared with controls.

Table 4. Association[a] between inflammation assessed in prostate tissue from PCPT end-of-study biopsy cores and subsequent total and Gleason sum <4+3 prostate cancer, case–control study nested in linked PCPT–SELECT cohort

	Previously in the PCPT placebo arm		Previously in the PCPT finasteride arm	
	Total	Gleason sum <4+3 (grade groups 1–2)	Total	Gleason sum <4+3 (grade groups 1–2)
No. cases	41	31	51	34
At least one biopsy core with inflammation				
OR	1.66	2.44	1.09	1.09
95% CI	0.70–3.96	0.84–7.07	0.50–2.38	0.44–2.69
Proportion of biopsy cores with inflammation				
None				
OR	1.00	1.00	1.00	1.00
95% CI	Reference	Reference	Reference	Reference
Some				
OR	1.62	2.33	1.05	1.02
95% CI	0.67–3.93	0.79–6.86	0.47–2.32	0.41–2.57
All				
OR	1.95	3.35	1.48	1.75
95% CI	0.43–8.77	0.67–16.89	0.39–5.59	0.41–7.57
P_{trend}	0.3	0.098	0.6	0.6
Mean percentage of tissue area with inflammation[b]				
None				
OR	1.00	1.00	1.00	1.00
95% CI	Reference	Reference	Reference	Reference
>0–<1.8%				
OR	1.42	1.70	0.90	1.00
95% CI	0.47–4.31	0.44–6.50	0.34–2.40	0.33–3.05
1.8%–<5.0%				
OR	1.73	2.39	0.92	0.82
95% CI	0.61–4.88	0.69–8.25	0.34–2.51	0.25–2.70
≥5.0%				
OR	1.83	3.31	1.44	1.42
95% CI	0.63–5.33	0.97–11.25	0.58–3.57	0.50–4.05
P_{trend}	0.2	0.047	0.4	0.6

[a]Adjusted for age at PCPT end-of-study biopsy and race. Cases and controls (85 in placebo arm, 108 in finasteride arm) frequency matched on age at PCPT end-of-study biopsy, race, and treatment arms for PCPT and SELECT trials. Grade groups from Epstein et al.'s grading system (13).
[b]Categories are 0% and tertiles of the distribution of mean percentage of tissue area above 0% among controls.

Discussion

We examined negative prostate biopsies for the presence of inflammation in men in the PCPT who were not biopsied for indication (e.g., had low PSA and negative DRE) and who later enrolled and were followed in SELECT to determine whether inflammation in the biopsy was associated with subsequent prostate cancer. We observed that the odds of detecting Gleason sum <4+3 [grade groups 1 and 2 (13) prostate cancer, the

Table 5. Mean serum PSA concentration at time of end-of-study biopsy[a] by amount of inflammation in prostate tissue from the PCPT end-of-study biopsy cores, in controls and prostate cancer cases, overall and by previous PCPT treatment arm, case–control study nested in the linked PCPT–SELECT cohort

	At least one biopsy core with inflammation			Extent of biopsy cores with inflammation		
	No	Yes	*P*	Some	All	P_{trend}[b]
Previously in the PCPT placebo arm						
Controls						
Total (*N*)	27	58		52	6	
Mean PSA (ng/mL)	1.32	1.37	0.8	1.32	1.81	0.4
Cases						
Total (*N*)	9	32		28	4	
Mean PSA (ng/mL)	2.04	2.02	0.9	1.94	2.62	0.6
Previously in the PCPT finasteride arm						
Controls						
Total (*N*)	27	81		73	8	
Mean PSA (ng/mL)	1.09	1.41	0.06	1.37	1.75	0.02
Cases						
Total (*N*)	12	39		34	5	
Mean PSA (ng/mL)	1.93	1.73	0.5	1.70	1.95	0.8

[a]From linear regression models adjusting for age at PCPT end-of-study biopsy and race. Analysis used the serum PSA concentration measured closest in time to but before the PCPT end-of-study biopsy. All men in this study were without a clinical indication for biopsy at the PCPT end-of-study biopsy.
[b]Across no (zero), some, all biopsy cores with inflammation. Reference is men with "No" (zero) biopsy cores with inflammation.

majority of cases] while on SELECT statistically significantly increased with percent tissue area with inflammation. We previously observed a positive association between inflammation and prostate cancer, including Gleason sum <7 disease (at least one core with inflammation: OR = 1.57, 95% CI, 0.83–3.00; ref. 6) in a different set of men from the PCPT in whom we assessed inflammation in the biopsies that were used to rule in or out cancer. In that prior study, we noted a stronger association for Gleason sum ≥7 prostate cancer (OR = 2.24, 95% CI, 1.06–4.71; across none, some, all cores with inflammation: P_{trend} = 0.01). In this study (prospective PCPT–SELECT), we could not evaluate the association for Gleason ≥4+3 disease because the number of cases was too small. Taken together, our current PCPT–SELECT findings and our prior PCPT findings (6) support the role of intraprostatic inflammation in prostate cancer development.

With respect to our second hypothesis, having at least one biopsy core with inflammation was not associated with prostate cancer in men previously assigned to finasteride in PCPT, consistent with what we previously observed (7). However in PCPT–SELECT unlike in PCPT, we could not rule out a modest, non-statistically significant increased odds of Gleason sum <4+3 disease in men who had all cores with inflammation compared with none. In contrast to our study in the PCPT when inflammation and diagnosis of prostate cancer were concurrent with finasteride treatment assignment, in PCPT–SELECT, the inflammation we assessed was concurrent with finasteride treatment assignment (85% were still taking it), but during their follow-up, the men were no longer receiving finasteride. We speculated previously that finasteride-associated intraprostatic inflammation may not be pro-carcinogenic (7).

In PCPT–SELECT, we found that intraprostatic inflammation was common. We hypothesized that if inflammation is a cause of prostate cancer, and given prostate cancer's high incidence, then intraprostatic inflammation also should be common. Prior studies reported that inflammatory infiltrates are very frequently observed in biopsies performed for elevated PSA or abnormal DRE (14), radical prostatectomy specimens (15), and tissue resected for benign prostatic hyperplasia (16, 17). But these studies were conducted using tissue removed for clinical indications, which themselves are associated with or, a consequence of inflammation. By design, our evaluation of the inflammation–prostate cancer association was not biased by any link between clinical indication and inflammation. While the prevalence of inflammation (based on ∼5 cores reviewed per man) that we observed in controls in PCPT–SELECT was high, it was lower than what we previously reported in a different group of controls sampled from PCPT placebo (based on ∼3 cores reviewed per man—78.2%; ref. 6) and finasteride (92.4%; ref. 7) arms. We speculate that if inflammation is associated with prostate cancer risk, by sampling as controls men who reached the end of SELECT follow-up without having been diagnosed with prostate cancer, the sampled controls would have a reduced prevalence of inflammation compared with the source population for the cases and compared with controls sampled using risk set sampling (18). Despite the lower prevalence of inflammation in the current study, as we found before it was higher in those previously in the finasteride arm than placebo arm, which as we indicated (7) is consistent with the observation that androgen deprivation therapy elicits inflammation (19, 20).

Our PCPT–SELECT finding that men in the previous PCPT placebo arm with all cores with inflammation appeared to have higher PSA at the end-of-study biopsy compared with men with no cores with inflammation is compatible with our prior study (6, 9). These findings are supportive of the hypothesis that inflammation leading to higher PSA concentration could distort the association between inflammation and prostate cancer. This pattern was not observed in PCPT–SELECT cases previously in the PCPT placebo arm, or in our prior study (6, 9). Elevations in serum PSA in cases may be more likely to result from cancer than from inflammation, and indeed cases had, on average, a higher PSA concentration measured at the time of the PCPT end-of-study biopsy. Like in our prior study (7), in PCPT–SELECT cases previously in the finasteride arm PSA concentration did not differ by inflammation status. Unlike in our prior PCPT study (7), in PCPT–SELECT controls previously in the PCPT finasteride arm PSA concentration was higher in men with at least one biopsy core with inflammation and increased across proportion of biopsy cores with inflammation. The reason for this latter difference is unknown.

Studies in the Finnish prostate cancer screening trial (21) and REDUCE (22) reported that men who were biopsy negative for prostate cancer had a lower risk of prostate cancer subsequently if they had inflammation (or greater proportion of biopsy cores with inflammation) in their prior negative biopsy (21–23). Results from our current and prior (6) studies and results from the Finnish and REDUCE studies likely differ for many reasons, including that those other analyses required men to have had a negative biopsy following an elevated PSA on the first screen in the Finnish study or a negative biopsy prior to trial entry in REDUCE. In contrast, in PCPT–SELECT, the biopsies we used were from men who did not have an indication for biopsy and also had PSA ≤4 ng/mL at SELECT entry. Men with elevated PSA leading to a biopsy that was negative (criteria for the Finnish analysis (21) 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 is possibly true in REDUCE for men whose negative biopsies before trial entry were performed for 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 (22).

Strengths and limitations

Several study features warrant discussion. (i) In this innovative study, we leveraged cohorts from two completed trials to prospectively test the inflammation–prostate cancer association in men without an indication for biopsy. (ii) We used Epstein and colleagues' new grading system (13) to classify cases for this analysis. (iii) We assessed intraprostatic inflammation 1.2 to 10.5 years (mean 5.9) before prostate cancer diagnosis. However, we do not know whether the inflammation we assessed was in the etiologically relevant window or correlated with it, or was of sufficient duration to influence prostate cancer development. Although the exact duration of chronic inflammation needed for a chronic inflammatory condition (e.g., gastritis-associated stomach cancer) to increase risk is unknown, at least at times, it may take decades. (iv) Because of the sample size, we could not evaluate this association in African American men, an important question given their substantially higher prostate cancer rates. (v) The number of

biopsy cores we reviewed per man (~5) was higher than in our prior study (~3) because more cores were mounted per slide for the end-of-study biopsies than biopsies performed during the PCPT. This likely resulted from the increase over time nationally in number of cores sampled coupled with the PCPT protocol of embedding cores by region into a single block irrespective of number of cores taken from that region. (vi) we confirmed our previous observation for lower-grade prostate cancer, a disease with substantially lower morbidity and mortality. A limitation of our approach was that the sample size was fixed and small, and we could not evaluate important outcomes—lethal and Gleason $\geq$4+3 disease (grade groups 3–5). Finally, (vi) the approach we took allowed us to investigate the association between intraprostatic inflammation and prostate cancer risk in a setting that did not suffer from PSA-associated detection bias. The tradeoff for internal validity may be reduced generalizability in that the men in this analysis had to have participated in two long-term chemoprevention trials and to have been eligible for those trials including by having low PSA at entry into both.

In conclusion, this study—in which biopsy tissue was collected, on average, 6 years before prostate cancer diagnosis, and thus is temporally correct for drawing causal inferences, and that followed men who did not have a clinical indication for the biopsy used as the baseline for assessing inflammation – provides evidence supporting the hypothesis that inflammation influences prostate cancer development. Taken together with our prior PCPT study, our findings inform prostate cancer etiology with an eye toward identifying men at higher risk so that they can be targeted for preventive intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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References

1. Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proc Natl Acad Sci 1995;92:5258–65.
2. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
3. Campisi J. Cancer and ageing: rival demons? Nat Rev Cancer 2003;3:339–49.
4. Vendramini-Costa DB, Carvalho JE. Molecular link mechanisms between inflammation and cancer. Curr Pharm Des 2012;18:3831–52.
5. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Gronberg H, Drake CG, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer 2007;7:256–69.
6. Gurel B, Lucia MS, Thompson IM Jr, Goodman PJ, Tangen CM, Kristal AR, et al. Chronic inflammation in benign prostate tissue is associated with high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev 2014;23:847–56.
7. Murtola TJ, Gurel B, Umbehr M, Lucia MS, Thompson IM Jr, Goodman PJ, et al. Inflammation in benign prostate tissue and prostate cancer in the finasteride arm of the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev 2016;25:463–9.
8. Platz EA, De Marzo AM, Giovannucci E. Prostate cancer association studies: pitfalls and solutions to cancer misclassification in the PSA era. J Cell Biochem 2004;91:553–71.
9. Umbehr MH, Gurel B, Murtola TJ, Sutcliffe S, Peskoe SB, Tangen CM, et al. Intraprostatic inflammation is positively associated with serum PSA in men with PSA <4 ng ml(-1), normal DRE and negative for prostate cancer. Prostate Cancer Prostatic Dis 2015;18:264–9.
10. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med 2003;349:215–24.
11. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009;301:39–51.
12. Klein EA, Thompson IM Jr, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the selenium and vitamin E cancer prevention trial (SELECT). JAMA 2011;306:1549–56.
13. Epstein JI, Zelefsky MJ, Sjoberg DD, Nelson JB, Egevad L, Magi-Galluzzi C, et al. A contemporary prostate cancer grading system: a validated alternative to the Gleason score. Eur Urol 2016;69:428–35.
14. Schatteman PH, Hoekx L, Wyndaele JJ, Jeuris W, Van Marck E. Inflammation in prostate biopsies of men without prostatic malignancy or clinical prostatitis: correlation with total serum PSA and PSA density. Eur Urol 2000;37:404–12.
15. Gerstenbluth RE, Seftel AD, MacLennan GT, Rao RN, Corty EW, Ferguson K, et al. Distribution of chronic prostatitis in radical prostatectomy specimens with up-regulation of bcl-2 in areas of inflammation. J Urol 2002;167:2267–70.
16. Di Silverio F, Gentile V, De Matteis A, Mariotti G, Giuseppe V, Antonio Luigi P, et al. Distribution of inflammation, pre-malignant lesions, incidental carcinoma in histologically confirmed benign prostatic hyperplasia: a retrospective analysis. Eur Urol 2003;43:164–75.
17. Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. BJU Int 1999;84:976–81.
18. Wang MH, Shugart YY, Cole SR, Platz EA. A simulation study of control sampling methods for nested case-control studies of genetic and molecular biomarkers and prostate cancer progression. Cancer Epidemiol Biomarkers Prev 2009;18:706–11.
19. Aragon-Ching JB, Williams KM, Gulley JL. Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer. Front Biosci 2007;12:4957–71.

20. Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proc Natl Acad Sci USA 2001;98:14565–70.
21. Yli-Hemminki TH, Laurila M, Auvinen A, Maattanen L, Huhtala H, Tammela TL, et al. Histological inflammation and risk of subsequent prostate cancer among men with initially elevated serum prostate-specific antigen (PSA) concentration in the Finnish prostate cancer screening trial. BJU Int 2013;112:735–41.
22. Moreira DM, Nickel JC, Gerber L, Muller RL, Andriole GL, Castro-Santamaria R, et al. Baseline prostate inflammation is associated with a reduced risk of prostate cancer in men undergoing repeat prostate biopsy: results from the REDUCE study. Cancer 2014;120:190–6.
23. Moreira DM, Nickel JC, Andriole GL, Castro-Santamaria R, Freedland SJ. Greater extent of prostate inflammation in negative biopsies is associated with lower risk of prostate cancer on repeat biopsy: results from the REDUCE study. Prostate Cancer Prostatic Dis 2016;19:180–4.