Research Article

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Variation in *IL10* and Other Genes Involved in the Immune Response and in Oxidation and Prostate Cancer Recurrence

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Abstract

Background: To evaluate the association of variation in genes involved in immune response, including *IL10*, production and detoxification of reactive oxygen species, and repair of oxidative DNA damage with risk of recurrence after surgery for localized prostate cancer.

Methods: We conducted a nested case-control study of men who had a radical prostatectomy in 1993 to 2001. A total of 484 recurrence cases and 484 controls were matched on age, race, and pathologic stage and grade. Germline DNA was extracted from paraffin-embedded unaffected lymph nodes. We genotyped candidate single-nucleotide polymorphisms (SNP) in *IL10*, *CRP*, *GPX1*, *GSR*, *GSTP1*, *hOGG1*, *IL1B*, *IL1RN*, *IL6*, *IL8*, *MPO*, *NOS2*, *NOS3*, *SOD1*, *SOD2*, *SOD3*, *TLR4*, and *TNF* and tagging SNPs in *IL10*, *CRP*, *GSR*, *IL1RN*, *IL6*, *NOS2*, and *NOS3*. We used conditional logistic regression to estimate OR and 95% confidence intervals (CI).

Results: The minor allele (A) in *IL10* rs1800872, known to produce less interleukin-10 (IL-10), was associated with a higher risk of recurrence (OR = 1.76, 95% CI: 1.00–3.10), and the minor allele (G) in rs1800896, known to produce more IL-10, was associated with a lower risk of recurrence (OR = 0.66, 95% CI: 0.48–0.91). We also observed associations for candidate SNPs in *CRP*, *GSTP1*, and *IL1B*. A common *IL10* haplotype and 2 common *NOS2* haplotypes were associated with recurrence.

Conclusion: Variation in *IL10*, *CRP*, *GSTP1*, *IL1B*, and *NOS2* was associated with prostate cancer recurrence independent of pathologic prognostic factors.

Impact: This study supports that genetic variation in immune response and oxidation influence prostate cancer recurrence risk and suggests genetic variation in these pathways may inform prognosis. *Cancer Epidemiol Biomarkers Prev; 21(10); 1774–82. ©2012 AACR.*

Introduction

Inflammation is likely a risk factor for prostate cancer incidence (1), suggesting variation in genes involved in the immune response in conjunction with environmental exposures, including those causing oxidative damage, may influence prostate carcinogenesis. Long-standing chronic inflammation has already been shown to be associated with cancers of other organ systems including the liver, stomach, colon, urinary bladder, and bile ducts (2, 3). Inflammation found within regions of the prostate could initiate carcinogenesis through a process of damaging DNA, or promote carcinogenesis through alterations in the cell cycle (1). Although much less studied, intraprostatic inflammation may also influence outcomes in men diagnosed with prostate cancer (4), including after prostatectomy (5). In the case of recurrence after prostatectomy, this increased risk resulting from inflammation may occur by influencing proliferation and possibly increasing cancer cell invasion and dissemination before surgical intervention.

Variation in genes involved in the immune response and oxidation has been studied in relation to incident prostate cancer. In particular, variation in *IL10*, the gene encoding the antiinflammatory cytokine interleukin-10 (IL-10), has been associated with prostate cancer risk in some studies (6–8), and with risk of recurrence in the only study conducted on this outcome to date (9). Thus, to investigate further the influence of inflammation and

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oxidation on the risk of prostate cancer recurrence, we evaluated the association of variation in *IL10* and other candidate genes involved in the immune response, production, and detoxification of reactive oxygen species (ROS), and the repair of oxidative DNA damage with the risk of recurrence, and examined their association with recurrent disease in a case-control study nested in a cohort of men who were surgically treated for clinically localized prostate cancer. In addition to *IL10*, we examined single nucleotide polymorphisms in *CRP*, *GPX1*, *GSR*, *GSTP1*, *hOGG1*, *IL1B*, *IL1RN*, *IL6*, *IL8*, *MPO*, *NOS2*, *NOS3*, *SOD1*, *SOD2*, *SOD3*, *TLR4*, and *TNF*. We also examined haplotype-based associations for *IL10*, *CRP*, *GSR*, *IL1RN*, *IL6*, *NOS2*, and *NOS3*.

Materials and Methods

Recurrence cases and controls

The source population for this study was the 4,860 men who underwent radical prostatectomy for treatment of clinically localized prostate cancer at the Johns Hopkins Hospital from 1993 to 2001 and who were followed through 2004 for a median of 4.0 years, and had not had preoperative or immediate adjuvant hormonal or radiation therapy. Surveillance for recurrence was conducted by the men's primary care physicians using digital rectal examination and measurement of serum prostate-specific antigen (PSA) concentration routinely checked at 3 months and at least yearly thereafter.

Recurrence cases were defined as men who experienced biochemical recurrence (i.e., PSA concentration of >0.2 ng/mL on 2 or more occasions after a previously undetectable level after prostatectomy), local recurrence or distant metastasis, or prostate cancer death. All 524 eligible recurrence cases were selected. For each recurrence case, one control was selected using incidence-density sampling matched on age, race, pathologic stage and grade, and by definition, time since prostatectomy (10). Thus from the 4,860 eligible men, 524 matched case-control pairs were selected using this process, consisting of 742 unique individuals as some men were selected as control multiple times or selected as control before they became a case. This study was approved by the Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health.

Single-nucleotide polymorphism selection and genotyping

More than 100 single-nucleotide polymorphisms (SNP) in 18 genes involved in the immune response, production of ROS, detoxification of ROS, and repair of oxidative DNA damage that were known to be polymorphic in whites were initially selected on the basis of their reported functionality or possible involvement in prostate cancer incidence. Because *IL10* was of particular interest (7), as were *CRP* (11), and *IL6* (12), we also selected haplotype-tagging SNPs to capture variation across these genes. For *GPX*, *GSR*, *NOS2*, *NOS3*, and *IL1_RN*, we hypothesized an association for genetic variation with recurrence, but did not have *a priori* expectations for particular SNPs, and thus we selected tagging SNPs. Tagging SNPs were selected using Tagger (13). The targeted genomic regions included the entire candidate gene, 10 kb before transcription start site and 5 kb after the transcription end site, based on annotation in NCBI Build 35 (14). A pairwise r^2 threshold of 0.8 and a minor allele frequency $\geq$5% were used. Twenty-seven of these SNPs could not be multiplexed or in validation steps had poor success, leaving 72 SNPs in 17 candidate genes for genotyping (Supplementary Table S1).

Germline DNA was obtained from formalin-fixed paraffin-embedded lymph nodes removed during prostatectomy. Tissue blocks were retrieved for 730 of the 742 unique men from the pathology archive at the Johns Hopkins Hospital. Johns Hopkins Pathology Tissue Core pathologists reviewed hematoxylin and eosin-stained sections cut from blocks containing the lymph nodes to confirm that the material did not contain cancer. The blocks were then cored to obtain tissue for DNA extraction. After deparaffinizing the samples, genomic DNA was extracted using the GeneQuickTM kit by a commercial laboratory (BioServe Biotechnologies). Insufficient DNA was extracted for 8 of the 730 samples. DNA for the remaining 722 samples was sent for genotyping.

Genotyping was done using high-throughput MassArray (Sequenom, Inc.) in the Center for Human Genomics at Wake Forest University (Winston-Salem, NC). Laboratory personnel were blinded to recurrence status and all samples were labeled with a code number. On average, 96% of the men were successfully genotyped for any given SNP; none of the SNPs could be genotyped for 4 men. Thus, of the 742 unique men sampled for the study, genotype data was not available for 24 men. Excluding the matched pairs for these 24 men left 484 matched case-control pairs for the analysis. Although the expected minor allele frequency in white men unselected for prostate cancer is 4% based on the HapMap release 28 CEU [CEPH (Utah residents with ancestry from northern and western Europe)] population panel (15), *IL6* rs2069860 was monomorphic in this sample set. Thus, the analysis included 71 SNPs with a minimum of 421 and maximum of 472 case-control pairs depending on the SNP. Given the minimum and maximum number of pairs, we had 80% power at a 2-sided alpha level = 0.05 to detect a minimum OR ranging from 1.50 to approximately 2.35 with prevalence of carrying at least 1 allele ranging from 0.50 to 0.05, respectively.

Statistical analysis

Conditional logistic regression was used to estimate associations between SNPs and prostate cancer recurrence. Matched ORs and 95% confidence intervals (CI) were calculated assuming codominant, dominant, and additive models. The additive model was used to test for trend across the number of minor alleles. When either the recurrence case or the control had missing data for a given SNP, both the individual with missing data and the matched pair were excluded. In subanalyses, we (i) restricted to white men, and stratified by (ii) median age

(<59, ≥59 years old), (iii) stage (pT2/pT3a, pT3b/N1), (iv) pathologic Gleason sum (<8, ≥8), (v) the combination of pathologic stage and grade, (vi) PSA level (<10, ≥10 ng/mL), and (vii) restricted to negative surgical margins. We also evaluated associations by type of recurrence: biochemical only ($N = 312$), or the combination of local recurrence ($N = 37$), distant metastases ($N = 76$), and prostate cancer death ($N = 12$). In addition, we checked for confounding by positive surgical margins and PSA using the change-in-estimate approach, but neither covariate was found to bias the estimates of effect substantially. These analyses were conducted using SAS v. 9.2 (SAS Institute, Cary, NC).

Software to conduct appropriate haplotype analysis for matched case-control studies in which controls were sampled using incidence density sampling is not available. Thus, we estimated haplotype probabilities for each set of tagging SNPs for each gene using a progressive insertion expectation maximization algorithm using the Haplo.stats v. 1.4.4 package in R v. 2.11.1. Common haplotypes with frequencies of greater than 5% overall were identified and selected for analysis. A haplotype design matrix was then obtained as output in which each man had a weight ranging from 0 to 2, under an additive model, for each common haplotype based on the posterior probability that the man had that specific haplotype given his genotypes and the haplotypes known in the rest of the sample. For example, if a man's genotype indicated that he could only have 2 copies of a single haplotype he would receive a weight equal to 2 for that haplotype and 0 for all other haplotypes. The weight for each haplotype was then reimported into the analysis and modeled as the single predictor of case status in a conditional logistic regression model with a resulting reference group of all other haplotypes.

Results

Characteristics of the recurrence cases and matched controls are shown in Table 1. Cases and controls differed on mean prediagnostic PSA level ($P = 0.05$) and the proportion with positive surgical margins ($P < 0.0001$). However, SNPs subsequently found to be associated with recurrence were not associated with surgical margins or mean PSA levels.

Table 1. Baseline characteristics of prostate cancer recurrence cases and controls, men who underwent radical prostatectomy for clinically localized disease at Johns Hopkins Hospital, 1993 to 2001

	Recurrence cases	Controls	*P*
N	484	484	
Mean ± SD age (y)	58.9 ± 6.2	59.0 ± 5.9	Matched
Race (%)			
White	85.1	88.0	
African-American	9.5	7.9	
Hispanic	1.5	0.6	
Asian	0.4	0.0	
Other	3.5	3.5	Matched
Mean ± SD preoperative PSA (ng/mL)	12.0 ± 9.5	10.9 ± 8.4	0.05
Pathological stage (%)			
T2	13.8	13.6	
T3a	51.5	51.5	
T3b or N1[a]	34.7	34.9	Matched
Pathologic Gleason sum (%)			
5	0.6	0.6	
6	14.3	14.5	
7	61.2	63.0	
8+	24.0	21.9	Matched
Positive surgical margins	35.3	21.1	<0.0001
Mean year of surgery	1997	1995	<0.0001

[a]18.6% of cases and 13.0% of controls had lymph node metastases.

Candidate SNPs

Men with 2 copies of the minor allele (A) in *IL10* rs1800872 had a higher risk of recurrence (OR = 1.76, 95% CI: 1.00–3.10) than men with 2 copies of the major allele (C); heterozygotes (AC) did not have a higher risk (Table 2). Men with 1 (OR = 0.66, 95% CI: 0.48–0.91) or 2 (OR = 0.74, 95% CI: 0.51–1.06) copies of the minor allele (G) in *IL10* rs1800896 had a lower risk of recurrence (P trend = 0.08) than men with 2 copies of the major allele (A), especially older men (1 copy: OR = 0.49, 95% CI: 0.30–0.79; 2 copies: OR = 0.53, 95% CI: 0.30–0.94; P trend = 0.02; data not shown). The associations for *IL10* rs1800872 and rs1800896 seen in the total sample were similar overall in white men and in men with low grade (Gleason sum < 8) or early stage (T2/T3a) disease (data not shown), although these associations were stronger in men with both low-grade and early-stage disease (rs1800872, 2 copies of the minor allele: OR = 2.94, 95% CI: 1.21–7.17; 1800896, 1 copy of the minor allele: OR = 0.44, 95% CI: 0.27–0.72, 2 copies of the minor allele: OR = 0.61, 95% CI: 0.35–1.07, P trend = 0.06). The associations did not differ by whether the type of recurrence was biochemical or more severe (data not shown).

Men with 1 copy (OR = 0.65, 95% CI: 0.49–0.86) of the minor allele (A) of *CRP* rs1205, but not 2 copies (OR = 1.21, 95% CI: 0.76–1.94), had a lower risk of recurrence compared with men with 2 copies of the major allele (GG). Men with at least 1 copy of the minor allele (C) in *CRP* rs1800947 had a lower risk of recurrence (OR = 0.53, 95% CI: 0.36–0.79) compared with men with 2 copies of the major allele (G; Table 2). Similar associations were observed in the subgroup analyses (data not shown).

Table 2. Matched ORs and 95% CIs of prostate cancer recurrence for candidate SNPs in genes involved in immune response, production of ROS, detoxification of ROS, and repair of oxidative DNA damage, men who underwent prostatectomy at John Hopkins Hospital, 1993 to 2001

		Number of minor alleles							
		None	1 copy		2 copies			At least 1 minor allele	
Gene	dbSNP	Cases/controls (genotype)	Cases/controls (genotype)	OR (95% CI)	Cases/controls (genotype)	OR (95% CI)	P^b	Cases/controls (genotype)	OR (95% CI)
IL10									
	rs1800872	236/253 (CC)	171/168 (AC)	1.09 (0.83–1.44)	35/21 (AA)	1.76 (1.00–3.10)	0.09	206/189 (A-carrier)	1.17 (0.90–1.52)
	rs1800896	146/112 (AA)	212/242 (AG)	0.66 (0.48–0.91)	100/104 (GG)	0.74 (0.51–1.06)	0.08	312/346 (G-carrier)	0.69 (0.51–0.92)
CRP									
	rs1205	221/186 (GG)	171/219 (AG)	0.65 (0.49–0.86)	51/38 (AA)	1.21 (0.76–1.94)	0.27	222/257 (A-carrier)	0.74 (0.57–0.96)
	rs1800947	418/385 (GG)	40/75 (CG)	0.50 (0.33–0.75)	2/0 (CC)	N/A	0.004	42/75 (C-carrier)	0.53 (0.36–0.79)
GSTP1									
	rs1695	204/240 (AA)	196/165 (AG)	1.40 (1.06–1.86)	46/61 (GG)	1.35 (0.84–2.16)	0.004	242/226 (G-carrier)	1.39 (1.06–1.82)
hOGG1									
	rs293795	312/320 (AA)	134/126 (AG)	1.09 (0.82–1.45)	16/16 (GG)	1.03 (0.50–2.11)	0.63	150/142 (G-carrier)	1.08 (0.82–1.43)
IL1B									
	rs1143627	209/174 (TT)	188/196 (CT)	0.80 (0.60–1.06)	53/80 (CC)	0.54 (0.36–0.81)	0.003	241/276 (C-carrier)	0.73 (0.56–0.95)
IL6									
	rs1800795[a]	211/206 (GG)	200/191 (CG)	1.02 (0.78–1.35)	54/68 (CC)	0.77 (0.51–1.16)	0.36	254/259 (C-carrier)	0.96 (0.74–1.24)
IL8									
	rs4073	121/133 (TT)	218/207 (AT)	1.14 (0.85–1.54)	107/106 (AA)	1.10 (0.76–1.59)	0.55	325/313 (A-carrier)	1.13 (0.85–1.50)
MPO									
	rs12452417	334/323 (CC)	96/99 (CT)	0.95 (0.69–1.31)	15/23 (TT)	0.65 (0.34–1.24)	0.26	211/222 (T-carrier)	0.89 (0.66–1.19)
	rs12944679	259/250 (GG)	135/138 (AG)	0.95 (0.71–1.27)	41/47 (AA)	0.85 (0.54–1.33)	0.46	176/185 (A-carrier)	0.92 (0.71–1.20)
	rs12451466	334/317 (CC)	94/102 (CT)	0.88 (0.64–1.22)	14/23 (TT)	0.60 (0.31–1.17)	0.12	108/125 (T-carrier)	0.82 (0.61–1.11)
NOS3									
	rs1799983[a]	230/235 (GG)	159/173 (GT)	0.95 (0.72–1.26)	44/25 (TT)	1.75 (1.04–2.93)	0.20	203/198 (T-carrier)	1.05 (0.80–1.37)
SOD1									
	rs2070424[a]	369/370 (AA)	69/66 (AG)	1.06 (0.72–1.56)	5/7 (GG)	0.72 (0.23–2.26)	0.93	74/73 (G-carrier)	1.02 (0.70–1.47)
SOD2									
	rs4880	131/117 (TT)	233/236 (CT)	0.89 (0.66–1.20)	108/119 (CC)	0.81 (0.56–1.16)	0.24	341/355 (C-carrier)	0.86 (0.65–1.15)
SOD3									
	rs699473	194/178 (TT)	206/223 (CT)	0.85 (0.64–1.12)	71/70 (CC)	0.92 (0.63–1.36)	0.48	277/293 (C-carrier)	0.86 (0.66–1.13)
	rs1799895	452/456 (CC)	–/–	N/A	15/11 (GG)	1.36 (0.63–2.97)	0.43	15/11 (G-carrier)	1.36 (0.63–2.97)
	rs2855262	182/164 (CC)	193/213 (CT)	0.80 (0.59–1.08)	72/70 (TT)	0.91 (0.60–1.39)	0.41	265/283 (T-carrier)	0.82 (0.62–1.10)
TLR4									
	rs4986790	393/387 (AA)	55/62 (AG)	0.88 (0.60–1.30)	3/2 (GG)	1.39 (0.23–8.46)	0.64	58/64 (G-carrier)	0.89 (0.60–1.31)
TNF									
	rs1800629	341/336 (GG)	113/126 (AG)	0.88 (0.65–1.20)	14/6 (AA)	2.25 (0.86–5.88)	0.84	127/132 (A-carrier)	0.95 (0.71–1.27)

[a]Both a candidate and tagging SNP.

[b]For the additive model.

Men with 1 (OR = 1.40, 95% CI: 1.06–1.86) or 2 (OR = 1.35, 95% CI: 0.84–2.16) copies of the minor allele (G) in *GSTP1* rs1695 had a higher risk of recurrence (*P* trend = 0.004) compared with men with 2 copies of the major allele (A; Table 2). Similar associations to the overall analysis were observed in the subgroup analyses (data not shown).

Men with 1 (OR = 0.80, 95% CI: 0.60–1.06) or 2 (OR = 0.54, 95% CI: 0.36–0.81) copies of the minor allele (C) in *IL1B* rs1143627 had a lower risk of recurrence (*P* trend = 0.003) compared with men with 2 copies of the major allele (T; Table 2). These associations were also present in white men, in men with low-stage and low-grade disease, and for biochemical recurrence (data not shown).

SNPs in *hOGG1*, *IL6*, *IL8*, *MPO*, *NOS3*, *SOD1*, *SOD2*, *SOD3*, *TLR4*, and *TNF* were not associated with the risk of recurrence overall (Table 2) or in subgroups (data not shown).

Tagging SNPs and haplotypes

Of the tagging SNPs, 4 (rs3024498, rs3024496, rs1800894, and rs1800890) of the 7 in *IL10*, 1 (rs3448) of 2 in *GPX1*, 1 (rs878972) of 3 in *IL1RN*, 1 (rs10459953) of 15 in *NOS2*, and 1 (rs5746136) of 2 in *SOD2* were statistically significantly associated with recurrence (Supplementary Table S2). None of the tagging SNPs in *CRP*, *GSR*, *IL6*, *MPO*, *NOS3*, *SOD1*, and *TLR4* was associated with risk of recurrence (Supplementary Table S2).

Five common haplotypes (prevalence > 5% in controls) were observed for *IL10*. Men with at least 1 copy of the G-C-T-C-A-C-A haplotype had a lower risk of recurrence (OR = 0.74, 95% CI: 0.60–0.92) when compared with men with all other haplotypes (Table 3). This association was similar after adjusting for the candidate *IL10* SNPs rs1800872 and rs1800896 (OR = 0.68, 95% CI: 0.51–0.89). For *NOS2*, initially all 15 tagging SNPs were used to construct haplotypes; only 4 common haplotypes were observed. Based on the linkage disequilibrium plot and the associations with tagging SNPs, we reduced the number of SNPs used to construct *NOS2* haplotypes to 4 (rs4795067, rs944725, rs17722851, rs379476). Five common haplotypes were observed. Men with at least 1 copy of the C-A-A-C haplotype had a higher risk of recurrence (OR = 1.30, 95% CI: 1.01–1.67) and men with at least 1 copy of the C-G-A-C haplotype had a lower risk of recurrence (OR = 0.60, 95% CI: 0.40–0.91) when compared with all other haplotypes (Table 3). None of the haplotypes for *CRP*, *GSR*, *IL1RN*, *IL6*, *NOS3* was statistically significantly associated with risk of recurrence (Table 3).

Discussion

In this nested case-control study in which recurrence cases and controls were matched on the prognostic factors pathologic stage and grade, variation in *IL10* was associated with risk of recurrence after surgical treatment of prostate cancer. Specifically, the minor allele (A) in *IL10* rs1800872, which is located in the promoter region and produces less IL-10 than the major allele (G; 16, 17), was associated with a higher risk of recurrence, and the minor allele (G) in *IL10* rs1800896, also located in the promoter region and produces more IL-10, was associated with a lower risk of recurrence. In addition, a common *IL10* haplotype was associated with recurrence risk independent of these 2 promoter region SNPs. Candidate SNPs in other genes involved in the immune response and oxidation, including *CRP*, *GSTP1*, *IL1B*, and *NOS2* haplotypes were also associated with risk of recurrence. These results support the hypothesis that immune response and oxidative damage influence risk of recurrence after surgical treatment of prostate cancer. Overall, our observed associations were similar in almost all subgroup analyses. However, the measures of association for the candidate SNPs of *IL10* and *IL1B* appeared stronger when restricting to low stage and low grade.

To our knowledge, only one other study has investigated *IL10* SNPs and prostate cancer recurrence: the rs1800871 allele that produces less IL-10 was associated with an increased risk of recurrence (*N* = 28) in 116 Taiwanese men surgically treated for localized disease, even after taking into account stage and grade (9). Most studies investigating this gene have focused on prostate cancer incidence and some have found positive associations with promoter-region SNPs producing less IL-10 (6–8). One of these studies also reported a significant association between *IL10* variation and high-grade disease (7), which has a poorer prognosis. In our current study, we found that the alleles of promoter-region SNPs (rs1800872, rs1800896) that produce less IL-10 were associated with higher recurrence risk taking into account pathologic stage and grade. These 2 SNPs and rs1800871 are in linkage disequilibrium in the CEU HapMap population (15). The C allele of rs1800872 and A allele of rs1800896 are inherited together most often. After adjusting for both rs1800872 and rs1800896, the association between the *IL10* haplotype G-C-T-C-A-C-A and recurrence persisted, supporting a lower risk of recurrence for this haplotype independent of the effects of the promoter SNPs known to influence IL-10 levels. IL-10 may possibly act through a specific signaling pathway that may ultimately prevent tumor metastasis by inhibiting of angiogenesis (18, 19), mechanisms relevant to recurrence as well. Further understanding IL-10's role in recurrence may lead to its use in differentiating men whose disease is more versus less likely to recur, or as a possible therapeutic agent to prevent tumor invasion, angiogenesis, and metastasis (20).

In addition to *IL10*, we found SNPs in *CRP* (rs1205, rs1800947), which result in decreased expression of C-reactive protein (21–23), were associated with a lower risk of recurrence. Circulating C-reactive protein increases substantially during an acute inflammatory response and is also elevated in individuals with chronic inflammatory states. Multiple SNPs and haplotypes in *CRP* are known to be associated with higher C-reactive protein concentration (24). Although C-reactive protein concentration has not been associated with prostate cancer incidence in prospective studies (25–27), higher circulating C-reactive

Table 3. Matched ORs and 95% CIs of prostate cancer recurrence for haplotypes of genes involved in immune response, production of ROS, detoxification of ROS, and repair of oxidative DNA damage, men who underwent prostatectomy at Johns Hopkins Hospital, 1993 to 2001

Gene	Haplotype	Cases frequency	Controls frequency	Matched OR (95% CI)[a]
IL10	(rs3024498, rs3024496, rs3024509, rs1554286, rs3021094, rs1800894, rs1800890)			
	A-C-T-C-A-C-A	0.12	0.12	0.99 (0.75–1.31)
	A-T-T-C-A-C-T	0.28	0.26	1.12 (0.92–1.37)
	A-T-T-T-A-C-T	0.11	0.11	0.95 (0.72–1.26)
	A-T-T-T-C-C-T	0.07	0.05	1.26 (0.87–1.83)
	G-C-T-C-A-C-A	0.18	0.23	0.74 (0.60–0.92)
CRP	(rs3093077, rs2808630, rs1417938)			
	G-A-A	0.9	0.8	1.09 (0.81–1.46)
	T-A-A	0.35	0.35	1.00 (0.83–1.20)
	T-A-T	0.26	0.26	1.01 (0.82–1.24)
	T-G-A	0.25	0.25	1.01 (0.82–1.25)
GSR	(rs3594, rs2551715, rs8190996, rs3779647, rs2978663, rs17557435, rs8190893, rs1002149)			
	G-A-C-G-G-G-G-A	0.08	0.10	0.85 (0.63–1.17)
	G-A-T-A-A-A-G-C	0.13	0.11	1.16 (0.88–1.54)
	G-G-C-G-G-A-G-C	0.13	0.11	1.30 (0.96–1.75)
	T-G-C-A-A-A-G-C	0.09	0.08	1.03 (0.73–1.44)
	T-G-T-A-A-A-G-C	0.17	0.18	0.94 (0.74–1.19)
IL1RN	(rs878972, rs3087263, rs315951)			
	A-G-C	0.46	0.42	1.17 (0.98–1.41)
	A-G-G	0.26	0.24	1.04 (0.84–1.29)
	C-A-C	0.06	0.05	1.18 (0.79–1.75)
	C-G-C	0.14	0.16	0.77 (0.59–1.01)
IL6	(rs1800795, rs1474348, rs2069845, rs2069860)			
	C-C-G-A	0.32	0.31	0.96 (0.79–1.15)
	G-G-A-A	0.59	0.55	1.15 (0.97–1.37)
	G-G-G-A	0.04	0.06	0.66 (0.42–1.03)
NOS2	(rs4795067, rs944725, rs17722851, rs379476)			
	C-A-A-C	0.17	0.14	1.30 (1.01–1.67)
	C-G-A-C	0.04	0.07	0.60 (0.40–0.91)
	C-G-T-C	0.10	0.12	0.83 (0.62–1.11)
	T-A-A-T	0.19	0.17	1.12 (0.90–1.40)
	T-G-A-C	0.41	0.38	1.09 (0.91–1.30)
NOS3	(rs2373961, rs6951150, rs12703107, rs1799983, rs3918227, rs2373929)			
	C-C-T-G-C-C	0.13	0.12	1.12 (0.84–1.50)
	C-T-G-G-C-T	0.09	0.11	0.84 (0.61–1.17)
	C-T-G-T-C-C	0.10	0.12	1.25 (0.93–1.69)
	T-C-G-G-C-C	0.21	0.22	0.95 (0.76–1.20)
	T-C-G-G-C-T	0.12	0.10	0.82 (0.58–1.16)

[a]Reference group was set to all other haplotypes.

protein concentration was statistically significantly associated with shorter overall survival in patients with androgen-independent disease in the ASCENT trial independent of prognostic factors (11). In another study, a doubling of C-reactive protein concentration was associated with poorer overall survival in men with castrate resistant prostate cancer, although these results were not adjusted for prognostic indicators (28). Whether these findings reflect the influence of inflammation on recurrence or whether C-reactive protein levels merely reflect greater tumor burden, a predictor of risk of recurrence, is unclear.

We also found carrying at least one minor allele (G) for *GSTP1* rs1695, a nonsynonymous substitution of valine (V) for isoleucine (I) at codon 105 that results in altered gene function (29), was associated with a higher risk of

recurrence. *GSTP1* encodes glutathione *S*-transferase-pi (GST-pi), an enzyme that detoxifies electrophiles whose expression is greatly diminished or absent in nearly all human prostate cancers (30). A meta-analysis of 24 studies reported no association between this SNP and prostate cancer incidence (summary OR = 1.06, 95% CI: 0.91–1.24; 31), although the specific effect the A to G transition at rs1695 has on enzymatic activity is unknown and may be dependent on population-specific exposures to environmental carcinogens (29). An *in vitro* study indicated GST-pi might contribute to growth of androgen-independent human prostate cancer cells, and thus could influence risk of recurrence (32). Given that almost all of these tumors have silenced GSTP1 expression by promoter region DNA hypermethylation, the significance of germline genetic variability in *GSTP1* in prostate cancer cells would be questionable. Nevertheless, many other cell types, including stromal cells and inflammatory cells, express GSTP1 and genetically determined variations in these levels could influence prostate cancer progression (33). Our result differs from that of Agalliu and colleagues, who reported no association between the I105V *GSTP1* minor allele and risk of recurrence in a cohort of men with prostate cancer from Washington state; they did note a possible positive association between the valine allele and prostate-specific death in these men based on a small number of deaths after adjusting for prognostic and other factors (34).

IL1B rs1143627, for which the minor allele (C) has an unknown effect on IL-1 beta, a proinflammatory cytokine (35, 36), was associated with a lower risk of recurrence. *IL1B* variants were not associated with prostate cancer incidence in 2 prospective studies, the Prostate, Lung, Colon, and Ovarian (PLCO) Cancer Screening Trial (rs1143634 and rs16944; 37) or the CLUE II cohort (rs1143627; 8). To our knowledge, no study has previously evaluated variation in *IL1B* and risk of recurrence.

We also found 2 haplotypes of *NOS2*, which encodes the inducible enzyme nitric oxide synthase (NOS), were associated with risk of recurrence. With respect to prostate cancer incidence, in the PLCO the distribution of *NOS2* haplotypes statistically significantly differed between prostate cancer cases defined as aggressive based on stage and grade, and controls controlling for age, time since initial screening and year of blood draw (38). They also observed significant associations between individual SNPs, although different than those studied here, and aggressive disease. It has been shown that inducible NOS activity promotes prostate tumor growth (39), and it is possible variability in the production of NOS leads to more aggressive disease, which may also lead to recurrence independent of stage and grade.

Several aspects of this study warrant discussion. The study had a large sample size. The median follow-up time of the men in the cohort from which the recurrence cases and controls were sampled was 4.0 years in comparison to a median time to biochemical recurrence of 5.0 years in surgically treated men (40). We confirmed that our findings did not differ for earlier versus later recurrence using the median cutoff time of 2 years until recurrence for cases, evidence that genetic variation may affect both immediate and later recurrence. DNA was extracted from formalin-fixed paraffin-embedded unaffected lymph nodes; lymph nodes are a source of abundant DNA given their lymphocyte content. We do not expect these results would have been different using DNA extracted from white blood cells in circulation. The tissues from which we extracted germline DNA for this study were retrieved from the pathology archive retrospectively, including after the case-control pairs had been identified. For 12 of the men originally selected, tissue blocks could not be located; however, it is unlikely that missing tissue blocks was both associated with the man's genotype and risk of recurrence.

Cases and controls were matched on pathologic prognostic indicators, thus we were able to study the genetic influence on recurrence beyond any genetic influence on pathologic characteristics. Controls were selected using incidence density sampling, which has been shown to be an efficient approach that produces unbiased estimates of the relative risk of prostate cancer recurrence in genetic studies (10). However, because controls could be sampled more than once and men who later became cases could have been sampled earlier as controls, missing genetic information was amplified in this matched analysis, resulting in reduction of power. Notably, men excluded from this analysis did not significantly differ on any of the prognostic factors from those included in the analysis. We conducted a large number of statistical tests and when using the conservative Bonferroni correction, none of our reported associations are statistically significant. However, this may not be an appropriate metric given that the results of our SNPs in these candidate genes are likely not independent because markers in the same genes tend to be correlated and our candidate genes have similar roles in the immune response and oxidation and so are unlikely to be independent of each other (e.g., the 2 promoter region SNPs in *IL10*). Other methods that account for the correlation between SNPs have been used to take into account multiple testing in genetic studies, but these methods cannot be directly applied to our data structure. We do not have clear evidence favoring the SNPs that we found to be associated with recurrence over those SNPs that we found not to be associated. Thus, we cannot rule out chance as an explanation for these findings. *A priori* we were most interested in *IL10*'s association with recurrence risk; as such, we have prioritized variants in this gene for future studies.

χ^2 tests showed departure from Hardy–Weinberg equilibrium for 8 of the 71 SNPs among controls at the $\alpha = 0.0001$ level (Supplementary Table S1). Although departure from Hardy–Weinberg equilibrium in the controls might be expected because the controls were men with prostate cancer and some these SNPs have been reported to be associated with prostate cancer incidence, our minor

allele frequencies were similar to those observed in the CEU HapMap population (Supplementary Table S1).

Of the SNPs identified as being associated with recurrence, not all have been found to be associated with incidence. However, this inconsistency in association between genetic variants and prostate cancer incidence and recurrence is not unprecedented: prostate cancer risk alleles identified from genome-wide association studies were not associated with recurrence in this cohort and in other studies (41), but other risk alleles have been associated with biochemical recurrence in different populations (42, 43).

In conclusion, we found SNPs that tend to produce less or produce more *IL10* were associated with a higher and a lower risk of recurrence, respectively, independent of pathologic prognostic factors. We also found associations for SNPs in *CRP*, *GSTP1*, and *IL1B*, and other genes involved in the immune response, production, and detoxification of ROS, and repair of oxidative DNA damage with recurrence. Our findings support a role for the immune response and oxidation in influencing recurrence and, if validated in future studies, suggest variation in these genes may be used to inform prognosis. In addition, if altered immune response and/or the inability to detoxify oxidative species or repair oxidative damage are pathways that lead to prostate cancer recurrence, they may be points for prevention or treatment.

Disclosure of Potential Conflict of Interest

No potential conflicts of interests were disclosed.

Authors' Contributions

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