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Association of *IL10* and Other Immune Response- and Obesity-Related Genes with Prostate Cancer in CLUE II

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Abstract

BACKGROUND—Chronic intra-prostatic inflammation and obesity are thought to influence prostate carcinogenesis. Thus, variants in genes in these pathways could be associated with prostate cancer risk.

METHODS—We genotyped 17 common single nucleotide polymorphisms (SNPs) in *RNASEL*, *TLR4*, *IL1B*, *IL6*, *IL8*, *IL10*, *TNF*, *CRP*, *ADIPOQ*, *LEP*, *PPARG*, and *TCF7L2* in 258 white prostate cancer cases and 258 matched controls nested in CLUE II. Single-locus analyses were conducted using conditional logistic regression. TagSNPs were selected in *IL10*, *CRP*, and *TLR4* and haplotype analyses were done.

RESULTS—The A allele of *IL10 -1082G>A* (rs1800896), known to result in lower levels of this anti-inflammatory cytokine, was positively associated with risk (AG vs. GG, OR=1.69, 95% CI: 1.10–2.60; AA vs. GG, OR=1.81, 95% CI: 1.11–2.96; P_{trend}=0.02). Associations of *IL10* haplotypes with prostate cancer were explained by high linkage disequilibrium between two tagSNPs (rs1800890 and rs3024496) and *-1082G>A*. A *TLR4* candidate SNP (rs4986790; AG/GG vs. AA, OR=0.60, 95% CI: 0.33–1.08; P_{trend}=0.09), known to have decreased expression and be associated with lower circulating levels of inflammatory mediators, and tagSNP (rs10116253; CC vs. TT, OR=3.05, 95% CI: 1.11–8.41), but not haplotypes, were associated with risk. None of the other candidate SNPs or haplotypes was statistically significantly associated with risk.

CONCLUSION—Our prospective study suggests that genetic variation in *IL10* and possibly *TLR4* is associated with prostate cancer risk. Although none of the SNPs in the obesity genes tested was associated, this does not rule out a complex role of obesity and its metabolic consequences in prostate cancer etiology.

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Keywords

prostate cancer; single nucleotide polymorphism; inflammation; obesity

INTRODUCTION

Recent studies have focused on the role of inflammation, and more generally the host immune response, as well as, on obesity and energy regulation, including insulin secretion and sensitivity, in the etiology of prostate cancer [1, 2]. Variants in genes encoding components of these pathways have been evaluated for prostate cancer risk. Some interesting candidates include *RNASEL*, which encodes a ribonuclease involved in the immune response to [3], and *TLR4*, which encodes a toll-like receptor involved in the innate immune response to bacteria [4]. Cytokines play a crucial role in the innate and adaptive immune response, including the response to tumors [5]. Promoter polymorphisms in genes related to the pro- and anti-inflammatory response (e.g., *IL1B*, *TNF*, *IL6*, *IL8* and *IL10*), immune cell chemoattraction or angiogenesis (e.g., *IL8*), and signal transduction or tumor cell growth regulation (e.g., *IL6*) could influence production of cytokines and thus, risk of prostate cancer [6, 7]. C-reactive protein, a nonspecific circulating marker of inflammation, has been observed to be positively associated with metastatic prostate cancer and correlated with PSA concentration independent of stage [8], although not associated with risk of developing prostate cancer [9]. Genetic variants in *CRP* and their haplotypes influence usual serum C-reactive protein concentration and its concentration during inflammation [10].

Obesity is associated with an increased risk of prostate cancer death, pathological characteristics indicative of worse prognosis, and risk of biochemical recurrence after radical prostatectomy, although it is possibly inversely associated with risk of prostate cancer overall in the PSA era [11]. Adiponectin and leptin are proteins synthesized by adipocytes that take part in energy regulation and apoptosis [12]. Some evidence suggests an association between adipocyte-derived proteins and risk or severity of prostate cancer [13]. Polymorphisms in *PPARG*, encoding peroxisome proliferators-activated receptor gamma, which influences insulin sensitivity, and *TCF7L2*, encoding transcription factor 7-like2 (*TCF7L2*), which influences insulin secretion, could affect prostate cancer risk because of the well-documented inverse association between diabetes mellitus type 2 and prostate cancer [14] and variants in these genes have been found to be associated with diabetes [15, 16].

To further investigate how variants in genes encoding components of the innate and adaptive immune response and those related to obesity and energy regulation, including insulin sensitivity and secretion, may influence prostate cancer, we tested common single nucleotide polymorphisms (SNPs) in *RNASEL*, *TLR4*, *IL1B*, *IL6*, *IL8*, *IL10*, *TNF*, *CRP*, *ADIPOQ*, *LEP*, *PPARG*, and *TCF7L2* for their association with prostate cancer risk using a case-control study nested within a prospective community-based cohort. In addition, haplotype-based associations were assessed using tagSNPs selected for coverage of the variation in *IL10*, *TLR4*, and *CRP*, genes that were selected based on early promising findings for candidate SNPs in these genes for prostate and colorectal cancers in this cohort.

SUBJECTS AND METHODS

Study Population

Incident prostate cancer cases and controls were identified among members of CLUE II, an ongoing prospective cohort established in May 1989. Participants were 32,898 men and women, of whom 22,887 were adult (≥18 years old) residents of Washington County, MD USA. Forty-one percent of the cohort is male and, similar to the county population, 98% is

white. Participants provided a blood sample and completed a brief medical and exposure history and a food frequency questionnaire [17] at baseline. Blood was collected in tubes containing heparin and was chilled until centrifuged, aliquotted into plasma, red blood cells, and buffy coat and frozen at −70°C. Loss to follow-up is <5% among cohort members who were ≥45 years at baseline, the at-risk age range for prostate cancer. To be eligible to be selected as a case or control, a participant had to be male and free of a cancer diagnosis (except non-melanoma skin cancer) prior to blood sampling. The Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health approved this study.

Selection of Prostate Cancer Cases and Controls

Prostate cancer cases were identified through linkage with the Washington County Cancer Registry, and since 1992 with the Maryland Cancer Registry. Age, date, stage, and Gleason sum at diagnosis were abstracted. A total of 269 men were histologically confirmed as having adenocarcinoma of the prostate after blood donation through December 2002. Cases were classified as clinically organ-confined (T1 or T2) or advanced (extraprostatic extension or metastatic [T3, T4, N1, M1], or fatal), and as low (Gleason sum <7) or high (≥7) grade. We further classified cases by combinations of stage and grade.

For each case, one control was selected who was still alive at the date of the case's diagnosis and who did not have a subsequent cancer diagnosis. Controls were individually matched to cases on age at blood donation, race, date of blood draw, and number of hours between last meal and blood draw.

Genotyping

Buffy coat DNA was extracted by alkaline lysis. Genotyping was performed using Applied Biosystems Taqman 5′ exonuclease assays, Taqman Universal PCR Master Mix, No AmpErase UNG, and 2.5 ng of genomic DNA. The thermal cycling conditions consisted of an initial hold at 95°C for 10 min followed by 50 cycles of a 15 s 92°C denaturing step and a 1 min 60°C annealing and extension step. The ABI Prism 7900HT Sequence Detection System was used to detect the nucleic acids and the Sequence Detection Software v2.2 was used to discriminate alleles and call genotypes (Applied Biosystems, Foster City, CA).

We performed the study in two stages. First, we selected candidate SNPs in genes involved in the two pre-specified pathways based on whether they were known or thought to be functional or because they previously were associated with risk of prostate or other cancers in some studies (including [3, 4, 6, 7, 18–21]). After conducting preliminary analyses and observing possible associations for SNPs in some of the genes, in stage 2 we selected haplotype tagging SNPs (ta/gSNPs) for those genes.

Stage 1: Candidate SNPs

Seventeen SNPs in 12 genes involved in the two pathways were selected from public databases. Selected SNPs had variant allele frequencies of at least 5% in whites. *RNASEL* 1385G>A (*Arg462Gln*, rs486907), *TLR4* 896A>G (*Asp299Gly*, rs4986790), *TLR4 11381G>C* (rs11536889), *IL1B -551T>C* (rs1143627), *IL6 -174G>C* (rs1800795), *IL6 -573G>C* (rs1800796), *IL6 -596G>A* (rs1800797), *IL8 -251T>A* (rs4073), *IL10 -592C>A* (rs1800872), *IL10 -1082G>A* (rs1800896), *TNF -308G>A* (rs1800629), *CRP 1082C>T* (rs1205), *CRP 1059G>C* (rs1800947), *ADIPOQ 276G>T* (rs1501299), *LEP 19G>A* (rs2167270), *PPARG* C>G intronic (*Pro12Ala*, rs1801282), and *TCF7L2 C>T* intronic (rs7903146). rs486907, rs4986790, and rs1801282 in *RNASEL, TLR4* and *PPARG*, respectively are nonsynonymous. Laboratory personnel were masked to case-control status. Genotyping was successful for 93 to 99% of participants for each candidate SNP.

Stage 2: TagSNPs for *IL10*, *TLR4*, and *CRP*

We selected tagSNPs that would cover most of the genetic variation in *IL10*, *TLR4*, and *CRP* and possibly point to additional regions of these genes that should be investigated in relation to prostate cancer risk. TagSNPs were chosen using Tagger (http://www.broad.mit.edu/mpg/tagger/server.html). Targeted genomic regions included the entire gene 10 kb before the transcription start site and 5 kb after the transcription end site based on the National Center for Biotechnology Information NCBI Build 35 and the phased HapMap release 21 CEU population panel. Criteria for selection were a pairwise r^2 threshold of 0.8 and variant allele frequency ≥ 5%. Seven tagSNPs were chosen for *IL10*, eight for *TLR4*, and four for *CRP* (TABLE I). Genotyping was successful for >95% of *IL10* and *CRP* tagSNPs, but was less so for *TLR4* tagSNPs (Table I).

Assessment of Other Factors

Baseline characteristics of cases and controls were obtained from the questionnaire completed at blood donation, including age; self-reported race; educational attainment; current and past smoking status; use of diabetes medications and non-steroidal anti-inflammatory drugs (NSAIDs) in the past 48 hours; current height and weight, and weight at age 21. Current BMI and BMI at age 21 were calculated as weight in kilograms divided by height in meters squared. Diabetes medications included oral medications, such as sulfonylurea, as well as, insulin. NSAIDs included over-the-counter and prescription aspirin, ibuprofen, and other non-steroidal anti-inflammatory agents. Dietary factors, including intake of energy and calcium, and use of supplements, including multivitamins and minerals, were estimated for the 82% of participants who returned the food frequency questionnaire. First degree family history of prostate cancer was obtained from the 1996 follow-up questionnaire, the first one after the brief baseline survey, which was available for 64.8% of men in the analysis. Plasma concentrations of total cholesterol (mg/dL), C-reactive protein (mg/L) [22], and C-peptide (pmol/L) [23] were measured previously using an enzymatic method [24] in a commercial laboratory; using an immunoturbidimetric assay (Roche Diagnostics, Indianapolis, IN), and ELISA (ALPCO Diagnostics, Windham, NH) in the laboratory of Dr. Nader Rifai at Children's Hospital Boston, respectively.

Statistical Analysis

Hardy-Weinberg equilibrium in controls was tested using the chi-square test. Differences in proportions of genotypes and other categorical variables between cases and controls were evaluated by McNemar's test. For continuous variables, the paired t-test or Wilcoxon sign rank test, if the distribution was not normal, was used to compare cases and controls. Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) under a codominant model. To test for a trend, a single ordinal variable for number of variant alleles was entered into the model; its coefficient was evaluated by the Wald test. We also conducted these analyses by stage (organ-confined, advanced), grade (low, high) at diagnosis, and by the combinations of stage and grade. To obtain *P*-values that take into account multiple testing, we performed a random permutation test with 10,000 replications [25]. We stratified by age at diagnosis (<25th percentile, ≥25th percentile) and family history of prostate cancer because we hypothesized that these men are enriched with inherited form of prostate cancer. We also stratified by factors expected to influence or be a component of the inflammation and obesity pathways: BMI (<25, ≥25 kg/m^2), NSAID use (yes, no), and plasma concentrations of cholesterol (<200, ≥200 mg/dL), C-reactive protein (cutpoint at median: <0.129, ≥0.129 mg/L), and C-peptide (cutpoint at median: <2310, ≥2310 pmol/L). To evaluate effect modification by all of these factors except age, we dropped case-control matching and ran logistic regression models adjusting for the matching factors. To evaluate effect modification by age, we ran conditional logistic regression models. Tests for interaction were conducted by comparing nested models with and without

a cross-product term for genotype and the stratification variable using the likelihood ratio test. Two-sided *P*-values are reported. These analyses were performed using SAS version 9.1 (SAS institute, Cary, NC).

D′ [26] and r^2 [27] were used to estimate pairwise linkage disequilibrium for tagSNPs in *IL10, TLR4,* and *CRP* using PROC ALLELE in SAS Genetics (SAS institute, Cary, NC). Haplotype frequencies were estimated using the Expectation-Maximization algorithm. We used a global score test to assess the difference in haplotype frequency distributions between cases and controls [28]; permutated *P*-values were calculated from an empirical distribution created from a minimum of 10,000 permutated data sets. The association between each haplotype versus the most common haplotype and prostate cancer was estimated by regression substitution assuming an additive association in Haplo Stat (http://www.mayo.edu/hsr/Sfunc.html). Unadjusted results are presented; results were similar after adjusting for the matching factors.

RESULTS

Baseline characteristics were similar between cases and controls (TABLE II), except that cases were less likely to use diabetes medications. Consistent with this difference and as we have shown previously [23], cases also had a slightly lower C-peptide concentration, a marker of insulin secretion. Among prostate cancer cases, 68.7% were diagnosed as clinically organ-confined and 60.9% were low grade. Mean age at diagnosis was 69.9 ± 7.9 years. Mean time between blood draw and diagnosis was 5.5 ± 3.1 years.

Distribution of allele frequencies in controls did not appear to differ from that of the HapMap CEPH population. A candidate SNP (*IL1B* - rs1143627) and three tagSNPs (*CRP* - rs2794521, *CRP* - rs2808630, *TLR4* - rs10116253) were not consistent with the Hardy-Weinberg assumption in controls ($P \leq 0.05$) (TABLE I). To limit the potential effect of population stratification, the primary analysis was restricted to whites (n=258 pairs); the results were the same when the six African-American case-control pairs were included.

Stage 1: Candidate SNPs

The association of the candidate SNPs with prostate cancer is shown in TABLE III. Only *IL10 -1082G>A* (rs1800896) was statistically significantly associated with prostate cancer risk: compared with wild type homozygotes, men carrying one (OR=1.69, 95% CI: 1.10–2.60) or two (OR=1.81, 95% CI: 1.11–2.96) copies of the variant A allele had an increased risk of prostate cancer (P_{trend}=0.02; adjusting for number of other tests P_{trend}=0.13). Men carrying at least one variant A allele in *IL10 -1082G>A* had an increased risk of organ-confined (OR=1.94, 95% CI: 1.08–3.49) and low-grade (OR=2.00, 95% CI: 1.08–3.72) disease (TABLE IV); these associations were in the same direction for advanced and high-grade disease, but were not statistically significant. The association was strongest for disease that was both organ-confined and low-grade (OR=2.67, 95% CI: 1.04–6.82) (TABLE IV). The association between *IL10 -1082*G>A and prostate cancer did not vary by age or by cholesterol and C-peptide concentrations. However, the association appeared stronger in NSAID users (≥1 A allele versus GG, OR=3.02, 95% CI: 1.34–6.78) than nonusers (OR=1.40, 95% CI: 0.88–2.23; $P_{interaction}$=0.12) and in men with BMI <25 kg/m^2 (OR=2.35, 95% CI: 1.16–4.79) than men with BMI ≥25 kg/m^2 (OR=1.49, 95% CI: 0.92–2.40; $P_{interaction}$=0.32), although interactions were not statistically significant.

There was a suggestion of an inverse association for *TLR4 896A>G* (rs4986790) and prostate cancer overall (≥1 G allele versus AA, OR=0.60, 95% CI: 0.33–1.08) (TABLE III) and in younger men (age at diagnosis <65 years, OR=0.26, 95% CI: 0.08–0.87), whereas in older men (≥ 65 years) the inverse association was smaller and not statistically significant

(OR=0.86, 95% CI: 0.46–1.62; $P_{interaction}$=0.09). *TLR4 11381G>C* (rs11536889) was not associated with prostate cancer overall (TABLE III), although it appeared to be positively associated with high grade (≥1 C allele versus GG, OR=1.89, 95% CI: 0.84–4.24) and advanced (OR=1.56, 95% CI: 0.67–3.59) disease. This SNP was inversely associated with total prostate cancer (≥1 C allele versus GG, OR=0.50, 95% CI: 0.28–0.89) in men with normal cholesterol (<200 mg/dL), but positively associated (OR=1.65, 95% CI: 0.98–2.78; $P_{interaction}$=0.003) in men with elevated cholesterol (≥200 mg/dL). No discernable patterns by stage or grade were noted for these two *TLR4* SNPs (data not shown).

Although no statistically significant associations with prostate cancer overall were observed for the other SNPs, possible effect modification was observed for some of these. *IL6 -596G>A* (rs1800797) (and *-174G>C* (rs1800795) in high linkage disequilibrium [r^2=0.94]) variant A allele was inversely associated with prostate cancer (≥1 A allele versus GG, OR=0.39, 95% CI 0.17–0.87) in younger men, whereas no association was found in older men (OR=1.05, 95% CI 0.69–1.59; $P_{interaction}$=0.03). For *PPARG* rs1801282, carrying at least one variant G (Ala) allele was associated with a lower risk (OR=0.48, 95% CI 0.24–0.97) in NSAID users, but not in non-users (OR=1.15, 95% CI 0.69–1.92; $P_{interaction}$=0.04). For *RNASEL 1385G>A* (rs486907), carrying at least one variant A allele was inversely associated with prostate cancer in NSAID users (OR=0.51, 95% CI 0.27–0.96), but not in non-users (OR=0.98, 95% CI 0.63–1.53; $P_{interaction}$=0.09).

Stage 2: Haplotype tagSNPs for *IL10*, *TLR4*, and *CRP*

IL10 rs1800890 (TT vs. AA, OR=0.47, 95% CI: 0.27–0.81) and rs3024496 (CC vs. TT, OR=1.74, 95% CI: 1.06–2.87) were significantly associated with prostate cancer; they were in high linkage disequilibrium with *IL10 -1082G>A* (r^2=0.86 and 1.0, respectively). *TLR4* rs10116253 was significantly positively associated with risk (CC vs. TT, OR=3.05, 95% CI: 1.11–8.41). None of the other *IL10* or *TLR4* or the *CRP* SNPs was associated with risk (data not shown).

Nine common *IL10* haplotypes were reconstructed across seven tagSNPs (TABLE V). Although the distribution of haplotypes between cases and controls was not significant (P_{global}=0.26), haplotypes T-C-A-C-C-T-G (OR=0.72, 95% CI: 0.52–0.99) or T-C-A-C-C-T-A (OR=0.69, 95% CI: 0.46–1.03) were inversely associated with prostate cancer when compared with the most common haplotype (A-C-A-C-C-C-A).

Six common *TLR4* haplotypes were reconstructed across eight tagSNPs. Carrying G-T-G-G-T-G-T-C was non-statistically significantly inversely associated with overall prostate cancer (OR=0.68, 95% CI: 0.40–1.14) when compared to the most common haplotype (A-T-A-G-G-G-T-C), although the global test was not significant (*P*=0.70).

Four common *CRP* haplotypes were reconstructed across four tagSNPs. Neither the distribution of haplotypes (P_{global}=0.71) nor individual haplotypes was associated with risk.

DISCUSSION

We evaluated the association of 17 candidate SNPs in 12 genes involved in immune response and obesity with prostate cancer, and subsequently evaluated 19 tagSNPs in three of these genes. In this prospective study, we observed that carrying the variant A allele at *IL10 -1082G>A* was associated with an increased risk of prostate cancer overall, especially organ-confined/low grade disease. The haplotype analysis did not provide any additional insight beyond the association for the candidate *IL10* SNP. We also noted possible associations for a candidate SNP (rs4986790) and a tagSNP (rs10116253) in *TLR4* with prostate cancer risk. None of the other candidate SNPs was statistically significantly or

consistently associated with prostate cancer, although we cannot rule out associations in subgroups. These findings suggest genetic variation in *IL10* and possibly *TLR4* may influence prostate cancer risk. Although candidate SNPs in the genes related to obesity were not consistently associated with prostate cancer, this does not rule out a role for obesity and its metabolic perturbations in the etiology of prostate cancer.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine regulating both innate and adaptive immunity [29]. IL-10 may influence pathogenesis of prostate cancer by inhibiting production of pro-inflammatory cytokines, including TNF-α, IL-6, and IL-8 [29], and indirectly inhibiting tumor invasion and metastasis [30]. The importance of IL-10 as an anti-inflammatory cytokine is highlighted by the development of enterocolitis in *IL10* knockout mice [31], a model for colorectal carcinogenesis. Prior studies have shown AA homozygotes at −1082 in the promoter region of *IL10* produce less IL-10 [32]. Consistent with its lower production, an association between the *IL10 -1082* AA genotype and prostate cancer risk was previously observed in a hospital-based case-control study (AA vs. GG, OR=1.78, 95% CI: 1.14–2.77) that used bone marrow and organ-donor controls [6], who may or may not reflect allele frequencies in the population that gave rise to the cases. Nevertheless, using controls sampled from the same cohort as the cases, our result in the CLUE II cohort (AA vs GG, OR=1.81, 95% CI: 1.11–2.96) was in agreement with their finding. In contrast, *IL10 -1082G>A* was not associated with prostate cancer in large case-control studies nested in PLCO [33, 34] and ATBC [35], although *IL10 -592C>A* was positively associated with risk in the latter study, but not in CLUE II. The difference in findings among the studies did not appear to be due differences in extent of aggressive and nonaggressive disease; each study evaluated these associations separately by stage and/or grade. Whereas in CLUE II we observed a somewhat stronger association for *IL10 -1082G>A* with organ-confined and/or low-grade disease than for advanced and/or high-grade disease, in PLCO and ATBC no association was present by stage or grade. Other case-control studies have not observed prostate cancer associations for either *IL10 -1082G>A* [36] or *-592C>A* [36, 37] or observed an association in the direction opposite to that expected [38].

We observed a positive association for the A allele at *IL10 -1082* among NSAID users, but not non-users, although we had expected the opposite given that the NSAID users would already have an anti-inflammatory exposure that might limit the ability to detect genetic effects. This observation is possibly limited by our assessment of NSAIDs, which was limited to use in the 48 hours prior to blood donation. No evidence of modification of the *IL10 -1082* and prostate cancer association by NSAIDs was observed in PLCO [33]. Nevertheless, our observations may be consistent with work demonstrating that NSAID-treated *IL10* knockout mice developed inflammatory infiltrates in colonic mucosa more rapidly and to a greater extent and had increased COX-2 expression compared with their untreated counterparts [39] and by a study showing that *in vitro* exposure of human peripheral blood mononuclear cells to ibuprofen reduced IL10 production [40]. Together, these studies suggest that in some settings NSAIDs may exacerbate an inflammatory state.

Obesity is well recognized as a low-grade inflammatory systemic disease marked, in part, by elevated serum concentrations of C-reactive protein, IL-6, and TNF-α [41]. We hypothesized that the association between *IL10 -1082G>A* and prostate cancer would be stronger in lean than overweight or obese men because of the numerous perturbations in inflammatory and endocrine pathways as sequelae of excess adipose tissue. Our results were consistent with this expectation.

Human toll-like receptor 4 (TLR4) is a transmembrane protein that binds to bacterial lipopolysaccharide activating expression of inflammatory genes (e.g., *IL1*, *IL6*, and *IL8*) through the nuclear factor-kB signaling pathway [42]. *TLR4*-mutant mice have a reduced

ability to fight microbial infection than wild type mice [43, 44] and studies in humans show associations between variants in *TLR4* and higher risk of viral and bacterial infections [45], lower circulating concentrations of inflammatory mediators and lower risk of atherosclerosis [46]. For *TLR4 896A>G*, our slightly inverse, but not statistically significant association was not inconsistent with prior studies [4, 47, 48]; our association was stronger and statistically significant in younger men. This inverse association is compatible with the hypothesis that a reduced propensity to mount an inflammatory response is protective against cancer but incompatible with the hypothesis that a reduced ability to fight infection increases the risk of cancer. We also observed a positive association between the variant C allele for a *TLR4* tagSNP (rs10116253) and prostate cancer, although it was not in Hardy-Weinberg equilibrium. An inverse association was observed for this SNP in a nested case-control study [47]. Other SNPs and haplotypes in *TLR4* have been observed to be associated with prostate cancer previously [4, 47, 48]. The biologic evidence coupled with these epidemiologic findings suggests the possible importance of *TLR4* in prostate cancer carcinogenesis.

Most of the candidate SNPs in genes involved in the immune response investigated here were not associated with prostate cancer risk. Our mostly null results for candidate SNPs in *CRP* and *RNASEL* are consistent with results of recent prospective studies [21, 34, 49]. However, in stratified analysis, we found that men who carried at least one variant allele of *RNASEL 1385G>A* had a lower risk of prostate cancer (OR=0.51, 95% CI: 0.27–0.96) if they also used NSAIDs, otherwise there was no evidence of association for this SNP. This finding is contrary to that expected and no interaction was observed in the PLCO Trial [49].

The candidate SNPs in *ADIPOQ*, *LEP*, and *TCF7L2* were not statistically significantly associated with prostate cancer overall or in any subgroups. A prior case-control study observed a positive association between another SNP, *LEP −2548G>A* and prostate cancer [19], although no association was observed for variants in the leptin receptor gene and prostate cancer risk in a different case-control study [50]. Another study observed a positive association between the variant TT genotype (versus GG) of *TCF7L2* rs12255372, which is in high linkage disequilibrium with the T allele of the SNP we investigated [51], and high stage and high grade disease, but not prostate cancer overall [52]. The intronic SNP in *PPARG* was not statistically significantly associated with prostate cancer overall, however, we observed a significantly lower risk of prostate cancer when carrying variant alleles of this SNP among NSAID users. *PPARG* encodes PPAR-γ, a nuclear hormone receptor belonging to the family of ligand-activated transcription factors that plays a role in adipocyte differentiation and fatty acids metabolism [53]. This receptor has been implicated in prostate cancer progression [54]. The variant Ala allele of *PPARG Pro12Ala* (G nucleotide; rs1801282) has lower transcriptional activity and is inversely associated with risk of diabetes [15, 16] and possibly colorectal cancer [55]. No association with prostate cancer was reported in ATBC[56], although a small case-control study reported a higher risk of prostate cancer for the Ala allele but only in men with a high BMI [20]. Given the inverse association between diabetes and prostate cancer, we would have expected a positive association between this SNP and prostate cancer. However, in CLUE II, carrying at least one variant G allele was inversely associated with total prostate cancer risk possibly overall and significantly in NSAIDs users, but there was no association in non-users. NSAIDs, including ibuprofen, can activate PPAR-γ [57] and might have different effects on men with or without the variant allele of *PPARG Pro12Ala*.

The strengths of our study include the matched case-control design nested in a prospective cohort. Information on potential effect modifiers, such as BMI and NSAIDs use, were obtained before cancer diagnosis and thus are unlikely to be subject to recall bias or the effects of the disease. In contrast to the standard case-control design, in the nested case-control study, controls are selected from the same cohort as cases increasing the likelihood

that the allele frequencies in controls reflect those in the population that gave rise to cases. We did not measure circulating levels of IL-10 because this cytokine primarily acts locally; however, by use of genotype we may have captured variation in levels that might be made locally among men if an IL-10 response were elicited. A potential limitation of our study is a modest sample size that was small for fully evaluating interactions. Because we investigated several candidate SNPs we adjusted the *P*-value for *IL10 -1082G>A* using a permutation approach; the association for this SNP, which was selected based on knowledge of its functionality and prior association with prostate cancer, was not statistically significant.

We did not select all possible polymorphic genes in the pathways of interest, but instead took a focused candidate gene approach to evaluate key SNPs and we performed limited haplotype assessment. An alternative approach, assessing SNPs across the genome for their association with prostate cancer, was used by the Cancer Genetics Markers of Susceptibility (CGEMS) investigators with the first stage data from PLCO, a PSA screening trial [34]. In addition to *IL10* rs1800896, *TLR4* rs4986790, *RNASEL* rs486907, and *TCF7L2* rs7903146 were also evaluated in the CGEMS, but no significant associations were found for total prostate cancer or for aggressive disease.

In conclusion, we found that the A allele in *IL10 -1082G>A* was associated with an increased risk of prostate cancer; haplotype analysis did not provide additional insight into these associations. Possible associations were also observed for *TLR4*. None of the investigated candidate SNPs in these genes related to host innate and adaptive immune response or in the genes related to obesity, energy regulation, and insulin was clearly associated with prostate cancer overall. Our findings suggest that mechanistic studies involving experimental manipulation of levels of interleukin-10, which is well recognized to be involved in colorectal carcinogenesis, as well as other immune response components such as toll-like receptor 4, in the etiology of prostate cancer are warranted.

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TABLE I

Characteristics of Selected Single Nucleotide Polymorphisms (SNPs) in Genes Involved in the Immune Response and Obesity, CLUE II[a]

					In controls		
Gene	**Reference no.**	**Nucleotide change**	**Genomic location**	**Genotyped (%)**	**Variant allele (%)**	***P*, HWE**	**Variant allele (%)in HapMap/CGEMS**
Candidate SNPs							
RNASEL	rs486907[b]	G/A	1385 exon 1	96.1	39.5	0.17	40.8/36.9
TLR4	rs4986790[b]	A/G	896 exon 4	96.8	7.0	0.24	3.3/5.8
TLR4	rs11536889	G/C	11381 3′-UTR	97.0	15.9	0.72	12.5/–
IL1B	rs1143627	T/C	−551 promoter	93.8	32.4	0.04	36.7/–
IL6	rs1800795	G/C	−174 promoter	96.2	40.9	0.35	53.3/–
IL6	rs1800796	G/C	−573 promoter	96.4	5.2	0.38	4.3/–
IL6	rs1800797	G/A	−597 promoter	96.8	41.6	0.73	48.1/–
IL8	rs4073	T/A	−251 promoter	96.8	49.1	0.13	43.5[c]
IL10	rs1800872	C/A	−592 promoter	98.8	20.0	0.61	20.2/–
IL10	rs1800896	G/A	−1082 promoter	99.2	44.9	0.25	46.7/51.8
TNF	rs1800629	G/A	−308 promoter	96.1	16.9	0.46	21.7/–
CRP	rs1205	C/T	1082 *e*xon 2	97.2	33.8	0.90	33.3/–
CRP	rs1800947	G/C	1059 exon 2	97.7	6.2	0.28	6.7/–
ADIPOQ	rs1501299	G/T	276 intron 2	96.1	32.5	0.12	26.7/–
LEP	rs2167270	G/A	19 exon 1	97.7	37.9	0.62	29.7/–
PPARG	rs1801282[b]	C/G	Intronic	97.6	13.7	0.28	7.5/–
TCF7L2	rs7903146	C/T	Intronic	96.5	30.5	0.13	25/29.6
TagSNPs							
IL10	rs1800890	A/T	−3585 promoter	96.1	46.8	0.66	60.0/–
IL10	rs1800894	C/T	−886 promoter	98.3	3.5	0.56	5.0/2.7
IL10	rs3021094	A/C	Intronic	98.3	5.9	0.89	6.7/–
IL10	rs1554286	C/T	Intronic	99.4	16.0	0.62	16.9/18.1
IL10	rs3024509	C/T	Intronic	98.8	6.5	0.27	7.5/5.5
IL10	rs3024496	T/C	3′-UTR	96.5	45.0	0.19	54.3/–
IL10	rs3024498	A/G	3′-UTR	98.3	31.4	0.21	28.8/26.9
CRP	rs2794521	T/C	−717 promoter	95.5	27.5	0.05	34.8/–

Gene	Reference no.	Nucleotide change	Genomic location	Genotyped (%)	In controls		
					Variant allele (%)	*P*, HWE	Variant allele (%)in HapMap/CGEMS
CRP	rs1417938	T/A	Intronic	95.6	29.4	0.19	33.1/–
CRP	rs2808630	T/C	3′ intergenic	96.9	27.9	0.04	28.3/28.8
CRP	rs3093077	A/C	3′ intergenic	98.8	10.9	0.88	5.1/6.4
TLR4	rs2737190	A/G	5′-UTR	92.0	33.3	0.43	30.8/–
TLR4	rs10116253	T/C	5′-UTR	90.0	22.8	0.04	25.0/–
TLR4	rs1927914	A/G	−6143 promoter	95.5	33.1	0.49	30.8/32.7
TLR4	rs1927911	G/A	Intronic	97.0	25.2	0.07	25.0/–
TLR4	rs2149356	G/T	Intronic	73.6	32.7	0.30	28.3/–
TLR4	rs7873784	G/C	3′-UTR	84.9	12.0	0.89	14.2/–
TLR4	rs11536891	T/C	3′-UTR	93.7	13.0	0.47	–/–
TLR4	rs11536898	C/A	3′-near gene	81.8	10.2	0.80	11.4/–

[a] Analysis restricted to white participants.

[b] Nonsynonymous SNPs: rs486907 (Arg462Gln), rs4986790 (Asp299Gly), rs1801282 (Pro12Ala).

[c] From SNP500 http://snp500cancer.nci.nih.gov/

TABLE II

Baseline Characteristics of Prostate Cancer Cases and Controls, CLUE II

Characteristics	Cases	Controls	P[a]
No.	264	264	
Mean age at blood draw (years)	64.6 ± 9.0	64.6 ± 9.0	Matched
African-American (%)	2.3	2.3	Matched
Family history of prostate cancer (%)[b]	13.5	9.8	0.28
Mean attained education (years)	12.4 ± 3.4	12.1 ± 3.4	0.32
Mean current BMI (kg/m^2)	26.4 ± 3.5	26.7 ± 3.2	0.26
Mean BMI at age 21 (kg/m^2)	22.4 ± 3.2	22.6 ± 3.2	0.36
Cigarette smoking status (%)			
Never	39.8	38.3	Ref
Former	51.9	53.4	0.72
Current	8.3	8.3	0.89
Mean total energy intake (kcal/day)[c]	1583 ± 626	1563 ± 522	0.72
Use of aspirin or other NSAIDs in past 48 hours (%)	32.2	34.9	0.52
Use of diabetes medications in past 48 hours (%)	2.65	6.06	0.06
Mean plasma total cholesterol concentration (mg/dL)	203.4 ± 31.4	205.5 ± 30.3	0.41
Mean plasma C-reactive protein concentration (mg/L)[d]	0.12 ± 0.29	0.14 ± 0.29	0.28
Mean plasma C-peptide concentration (pmol/L)[d]	1378.7 ± 893.7	1495.6 ± 906.2	0.24

[a] Paired t-test (continuous) or McNemar's test (categorical).

[b] Prostate cancer diagnosed in father or brother. Family history missing for 191 out of total 528 (36.2%) because of non-response to the 1996 questionnaire on which family history was assessed.

[c] missing in 45 out of total 528 (8.5%).

[d] missing in 9 out of total 528 (1.7%).

TABLE III

Association of Candidate SNPs in Genes Involved in the Immune Response and Obesity with Prostate Cancer, CLUE II[a]

Gene	Reference no.	Number of copies of variant allele						P_{trend}	≥ 1 copy of variant allele vs 0 OR (95% CI)
		0 copy		1 copy		2 copies			
		Case/control	OR	Case/control	OR (95% CI)	Case/control	OR (95% CI)		
Candidate SNPs									
RNASEL	rs486907	100/88	1.00	121/132	0.78 (0.52–1.16)	27/33	0.81 (0.45–1.45)	0.30	0.78 (0.54–1.15)
TLR4	rs4986790	230/216	1.00	24/35	0.60 (0.33–1.08)	0/0	–	0.09	0.60 (0.33–1.08)
TLR4	rs11536889	178/175	1.00	69/71	0.95 (0.66–1.38)	7/6	1.15 (0.38–3.44)	0.93	0.58 (0.23–1.48)
IL1B	rs1143627	33/32	1.00	96/93	1.07 (0.71–1.59)	114/123	1.03 (0.59–1.78)	0.84	1.06 (0.73–1.53)
IL6	rs1800795	91/84	1.00	116/128	0.82 (0.56–1.21)	43/40	0.99 (0.57–1.73)	0.73	0.85 (0.59–1.24)
IL6	rs1800796	233/225	1.00	19/25	0.75 (0.41–1.38)	1/0	–	0.56	0.79 (0.43–1.45)
IL6	rs1800797	93/85	1.00	116/125	0.82 (0.56–1.20)	41/44	0.82 (0.48–1.41)	0.38	0.82 (0.56–1.19)
IL8	rs4073	58/52	1.00	127/138	0.79 (0.49–1.26)	69/62	1.00 (0.60–1.68)	0.95	0.85 (0.56–1.30)
IL10	rs1800872	150/162	1.00	95/84	1.25 (0.86–1.82)	10/9	1.13 (0.39–3.31)	0.31	1.24 (0.85–1.80)
IL10	rs1800896	56/83	1.00	130/117	1.69 (1.10–2.60)	69/57	1.81 (1.11–2.96)	0.02[b]	1.73[c] (1.15–2.59)
TNF	rs1800629	160/172	1.00	79/69	1.26 (0.85–1.87)	12/9	1.35 (0.53–3.46)	0.22	1.27 (0.87–1.86)
CRP	rs1205	103/111	1.00	127/115	1.18 (0.82–1.72)	22/29	0.75 (0.40–1.39)	0.84	1.08 (0.76–1.54)
CRP	rs1800947	213/227	1.00	37/28	1.39 (0.82–2.33)	3/2	1.71 (0.28–10.4)	0.19	1.40 (0.84–2.34)
ADIPOQ	rs1501299	131/121	1.00	94/104	0.86 (0.60–1.22)	28/28	0.91 (0.50–1.65)	0.49	0.87 (0.63–1.21)
LEP	rs2167270	92/100	1.00	122/119	1.12 (0.77–1.64)	39/38	1.13 (0.67–1.90)	0.57	1.12 (0.79–1.60)
PPARG	rs1801282	198/189	1.00	57/58	0.96 (0.63–1.45)	0/7	–	0.18	0.86 (0.57–1.29)
TCF7L2	rs7903146	131/125	1.00	99/96	0.94 (0.62–1.42)	19/28	0.62 (0.32–1.20)	0.22	0.86 (0.58–1.26)

[1] Analysis restricted to white participants (n=258 pairs); inclusion of nonwhites (n=6 pairs) did not materially alter the results.

[2] After taking into account multiple testing (permutation resampling n=10,000), $P_{adjusted}$=0.13.

[3] p=0.008.

TABLE IV

Association of Carriage of the Variant A Allele in *IL10 -1082 G>A* (rs1800896) with Prostate Cancer Overall and by Stage and Grade at Diagnosis, CLUE II[a]

	0 copy of variant (Referent)	Carriers of variant allele(s)		
	Case/control	Case/control	OR	95% CI
Total prostate cancer	56/83	199/174	1.73	1.15–2.59
Organ-confined	25/83	98/174	1.94	1.08–3.49
Advanced	10/83	45/174	2.17	0.82–5.70
Low grade	24/83	101/174	2.00	1.08–3.72
High grade	20/83	58/174	1.43	0.72–2.83
Organ-confined and low grade	12/83	57/174	2.67	1.04–6.82
Organ-confined and high grade	10/83	27/174	1.25	0.49–3.17
Advanced or high grade	25/83	84/174	1.50	0.83–2.72

[a]Analysis restricted to white participants (n=258 pairs); inclusion of nonwhites (n=6 pairs) did not materially alter the results. Data were too sparse to evaluate associations for advanced and low grade (n=23) and advanced and high grade (n=24) prostate cancer.

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TABLE V

Association of Haplotypes In *IL10*, *TLR4*, and *CRP* with Prostate Cancer, CLUE II[a]

Gene	Haplotype	Cases	Controls	OR (95% CI)	*P, global*	*P*[b]
IL10 (rs1800890, rs1800894, rs3021094, rs1554286, rs3024509, rs3024496, rs3024498)					0.26	
	A-C-A-C-C-C-A	0.338	0.276	1.00 (Ref)		-
	T-C-A-C-C-T-G	0.250	0.292	0.72 (0.52–0.99)		0.05
	T-C-A-C-C-T-A	0.147	0.172	0.69 (0.46–1.03)		0.07
	A-C-A-T-C-C-A	0.113	0.116	0.78 (0.49–1.23)		0.28
	A-C-C-T-C-C-A	0.057	0.049	0.94 (0.52–1.69)		0.84
	A-T-A-C-T-T-A	0.021	0.035	0.49 (0.22–1.10)		0.09
	A-C-A-C-C-T-G	0.030	0.021	1.10 (0.48–2.52)		0.83
	A-C-A-C-T-T-A	0.022	0.028	0.52 (0.22–1.22)		0.13
	A-C-C-C-C-C-A	0.019	0.008	1.33 (0.42–4.23)		0.63
TLR4 (rs2737190, rs10116253, rs1927914, rs1927911, rs2149356, rs7873784, rs11636891, rs11536898)					0.70	
	A-T-A-G-G-G-T-C	0.680	0.671	1.00 (Ref)		-
	G-C-G-A-T-C-C-A	0.115	0.111	1.01 (0.68–1.52)		0.95
	G-C-G-A-T-G-T-C	0.104	0.106	0.97 (0.63–1.51)		0.90
	G-T-G-G-T-G-T-C	0.054	0.078	0.68 (0.40–1.14)		0.15
	G-C-G-A-T-C-C-C	0.028	0.021	1.30 (0.53–3.22)		0.56
	G-T-G-G-G-G-T-C	0.007	0.008	0.94 (0.19–4.67)		0.94
CRP (rs2794521, rs1417938, rs2808630, rs3093077)					0.71	
	T-T-T-A	0.370	0.371	1.00 (Ref)		-
	T-A-T-A	0.285	0.293	0.97 (0.71–1.34)		0.86
	C-T-C-A	0.287	0.277	1.03 (0.76–1.41)		0.84
	T-T-T-C	0.050	0.054	0.92 (0.51–1.64)		0.77

[a] Analysis restricted to white participants (n=258 pairs); inclusion of nonwhites (n=6 pairs) did not materially alter the results.

[b] Versus most common haplotype.