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Key genes involved in the immune response are generally not associated with intraprostatic inflammation in men without a prostate cancer diagnosis: Results from the Prostate Cancer Prevention Trial

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Abstract

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BACKGROUND—We previously reported that both intraprostatic inflammation and SNPs in genes involved in the immune response are associated with prostate cancer risk and disease grade. In the present study, we evaluated the association between these SNPs and intraprostatic inflammation in men without a prostate cancer diagnosis.

METHODS—Included in this cross-sectional study were 205 white controls from a case-control study nested in the placebo arm of the Prostate Cancer Prevention Trial. We analyzed inflammation data from the review of H&E-stained prostate tissue sections from biopsies performed per protocol at the end of the trial irrespective of clinical indication, and data for 16 SNPs in key genes involved in the immune response (*IL1β, IL2, IL4, IL6, IL8, IL10, IL12(p40), IFNG, MSR1, RNASEL, TLR4, TNFA*; 7 tagSNPs in *IL10*). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between carrying at least one minor allele and having at least one biopsy core (of a mean of 3 reviewed) with inflammation.

RESULTS—None of the SNPs evaluated was statistically significantly associated with having at least one core with inflammation. However, possible inverse associations were present for carrying the minor allele of rs2069762 (G) in *IL2* (OR=0.51, 95% CI 0.25–1.02); carrying two copies of the minor allele of rs1800871 (T) of *IL10* (OR=0.29, 95% CI 0.08–1.00); and carrying the minor allele of rs486907 (A) in *RNASEL* (OR=0.52, 95% CI 0.26–1.06). After creating a genetic risk score from the 3 SNPs possibly associated with inflammation, the odds of inflammation increased with increasing number of risk alleles (P-trend=0.008).

CONCLUSION—While our findings do not generally support a cross-sectional link between individual SNPs in key genes involved in the immune response and intraprostatic inflammation in men without a prostate cancer diagnosis, they do suggest that some of these variants when in combination may be associated with intraprostatic inflammation in benign tissue.

INTRODUCTION

Inflammation in the prostate is hypothesized to be a cause of prostate cancer [1]. Indeed, in the Prostate Cancer Prevention Trial (PCPT), we previously reported that the prevalence and extent of inflammation [2] and variants in genes involved in the immune response [3] were associated with an increased risk of the disease, including higher-grade disease, and with serum prostate specific antigen (PSA) concentration.

In the present study, also based in PCPT, we directly investigated whether select SNPs in key genes involved in the immune response are associated with intraprostatic inflammation in men without a diagnosis of prostate cancer. Men not diagnosed with prostate cancer during the trial were requested to undergo a biopsy at the end of the trial irrespective of their PSA concentration. Given that 1) some SNPs have been found to be associated with PSA concentration in controls [4–6], 2) men with inflammation tend to have higher PSA concentrations [2], and 3) some controls in the PCPT had an elevated PSA at the time of the end of study biopsy, to minimize the likelihood of resulting detection bias, we additionally evaluated the association between SNPs and intraprostatic inflammation in controls with low PSA (<2 ng/mL). We hypothesized that the SNPs involved in the immune response (i.e., rs3212227 in *IL12*(p40), rs4073 in *IL8*, and tagSNPs rs1800890 and rs3021094 in *IL10*) that

we previously observed to be associated with prostate cancer risk would also be associated with intraprostatic inflammation and that these associations would not be due to detection bias.

METHODS

In this cross-sectional study, we analyzed data from 205 white controls from the prostate cancer case-control study nested [7] in the placebo arm of the PCPT, a SWOG-Coordinated Study S9217 [8]. The controls were men who underwent an end-of-study biopsy, irrespective of clinical indication, per trial protocol, and were negative for cancer. The Institutional Review Boards at the participating trial sites approved the PCPT. The Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health and the Colorado Multiple Institutional Review Board approved this study on inflammation.

The data used in this analysis were from our previous studies [2,3]. The inflammation data were obtained from a review of H&E-stained prostate tissue sections from the biopsies as described previously [2]. A mean of 3 (of the 6–10 cores taken) cores were reviewed for the presence of any inflammatory cells whether acute (e.g., polymorphonuclear cells) or chronic (e.g., cells with an appearance consistent with that of lymphocytes and macrophages). We classified the men as having at least one biopsy core with inflammation or no cores with inflammation. The genotype data were obtained as follows: SNPs were selected and genotyped in *IL1b, IL2, IL4, IL6, IL8, IL10, IL12(p40), IFNG, MSR1, RNASEL, TLR4*, and *TNFA* and 7 tagSNPs in *IL10* using DNA extracted from buffy coat or from serum [3]. In our prior study, we chose genes with a role in innate immunity and T cell activation and/or response, and then preferentially chose SNPs in those genes thought to affect the production or activity of the gene product and/or have been found to be associated with prostate cancer, gastric cancer, or colitis (see Supplement Table 1 in Winchester *et al.* [3]).

We calculated the prevalence of carrying at least one minor allele for men who had at least one biopsy core with inflammation and men with no cores with inflammation and then compared between the two groups using the Chi-square test. We used logistic regression to estimate the odds ratio (OR) and 95% confidence intervals (CI) of at least one biopsy core with inflammation associated with the SNPs adjusting for age at biopsy. SNPs were modeled in three ways: indicator variables for genotype (codominant), a binary variable for carrying least one minor allele (dominant), and an ordinal variable for the number of minor alleles (log-additive model). We stratified the analysis for carrying at least one minor allele by BMI (leaner: <25 kg/m^2; heavier ≥ 25 kg/m^2). We also restricted the analysis for carrying at least one minor allele to men without a history of diabetes, and to men with a low PSA concentration (<2 ng/mL) at the time of biopsy. The presence of a statistical interaction between carrying at least one minor allele for each SNP and BMI was evaluated by entering into the model terms for their main effects and their product, the coefficient for which was evaluated by the Wald test. Post hoc, we calculated a genetic risk score by summing the number of risk alleles for the SNPs that were possibly associated with inflammation. We used logistic regression adjusting for age to estimate the association between number of risk alleles and inflammation using the median number of risk alleles among the men as the reference. Statistical analyses were performed using Stata (version 13.1, College Station,

Texas 77845 USA). P-values are from 2-sided tests and P-values <0.05 were considered to be statistically significant.

RESULTS

The characteristics of the 205 white controls are shown in Table 1. Men with inflammation were significantly older (P=0.02) and had higher PSA levels (P=0.03) than those without inflammation. After adjusting for age, men with and without inflammation did not notably differ on any of the characteristics assessed.

Overall, the prevalence of carrying at least one copy of the minor allele for the studied SNPs did not differ between men with and without inflammation, with two possible exceptions (Table 2). The prevalence of carrying at least one copy of the minor allele (G) of rs2069762 in *IL2* was lower in men with inflammation (47.7%) than men without inflammation (64.4%; P=0.05). Likewise, the prevalence of carrying the minor allele (A) of rs486907 in *RNASEL* was lower in men with inflammation (55.4%) than men without inflammation (70.2%; P=0.07).

We next modeled the association between genotypes and inflammation (Table 3). Men with one (OR=0.51, 95% CI 0.25–1.05) or two (OR=0.51, 95% CI 0.15–1.68) copies of the minor allele (G) of rs2069762 in *IL2* had a lower odds of inflammation than men with two copies of the major allele (P-trend=0.09). Men with two copies of the minor allele (T) of rs1800871 in *IL10* had a lower odds of inflammation (OR=0.29, 95% CI 0.08–1.00); no association was present for carrying only one copy of the minor allele. Men with one copy of the minor allele (A) of rs486907 in *RNASEL* had a lower odds of inflammation (OR=0.48, 95% CI 0.23–1.00) than men who carried two copies of the major allele; few men carried two copies of the minor allele. None of the other candidate SNPs or tagSNPs when modeled based on genotype or based on the log-additive model was statistically significantly associated with inflammation (Table 3).

Table 4 gives the ORs for having at least one biopsy core with inflammation associated with carrying at least one minor allele in the men overall, and in men with low PSA, who are leaner, who are heavier, and who did not have diabetes. Consistent with the analysis by genotype, carrying at least one minor allele (G) of rs2069762 in *IL2* and carrying at least one minor allele (A) rs486907 in *RNASEL* were inversely associated with inflammation. Within participant subgroups, the direction of the associations was generally similar to overall, but the strength of association differed for some SNPs in some subgroups. In particular, among non-diabetics, the inverse association for the minor allele (G) of rs2069762 in *IL2* remained, and was of the same magnitude and statistically significant. Also, carrying the minor allele (T) of rs1800871 in *IL10* was more inversely associated with inflammation (OR=0.50, CI 0.23–1.10) in men with low PSA (<2 ng/mL) than overall (OR=0.71, CI 0.35–1.44). Also, carrying at least one minor allele (A) of rs486907 in *RNASEL* was more strongly inversely associated with inflammation in leaner (OR=0.33, CI 0.11–1.00) than in heavier (OR=0.79, 95% CI 0.28–1.92) men. However, no statistically significant interactions were observed, including by BMI for rs486907 (P-interaction=0.27).

When we created a genetic risk score from those SNPs in Table 3 that were possibly associated with inflammation – rs2069762 in *IL2*, rs1800871 in *IL10*, and rs486907 in *RNASEL* – we observed that the more risk alleles the men had the greater the odds of inflammation (P-trend=0.008; Table 5).

DISCUSSION

Because we previously observed a positive association between intraprostatic inflammation and prostate cancer, especially higher-grade disease in the PCPT [2], and because we previously observed that some the SNPs involved in the immune response that we selected for study were also associated with prostate cancer risk in the PCPT [3], we next hypothesized that intraprostatic inflammation might mediate the association between these SNPs and prostate cancer risk. To begin to address this hypothesis, in the present study we evaluated the association between these same SNPs and intraprostatic inflammation in the controls who overlapped in our team's two prior analyses. We studied only the controls because they represent the source population for the cases, and thus, provide an estimate of the link between these SNPs and intraprostatic inflammation in men at risk for prostate cancer. We found that 16 candidate SNPs in 12 key genes involved in inflammation and the immune response and seven *IL10* tagSNPs were generally not associated with the odds of intraprostatic inflammation. Possible exceptions were inverse associations for the minor alleles of rs2069762 in *IL2*, rs1800871 of *IL10*, and rs486907 in *RNASEL*. These inverse associations were present in men with low PSA (<2 ng/mL), leaner and heavier men, and men without diabetes, especially rs486907 (A) in *RNASEL* in leaner men. When we summed across risk alleles for the SNPs possibly associated with inflammation, we found that the odds of inflammation increased with increasing number of risk alleles. While the findings of this study do not strongly support the hypothesis that the selected SNPs individually influence inflammation in benign prostate tissue, they do suggest that the cumulative number of risk alleles might be influential.

Of the SNPs that were possibly associated with inflammation in the present study the *IL10* and *RNASEL* SNPs, but not *IL2* SNP, appeared to be associated with prostate cancer risk in our prior study [3]. More specifically for *RNASEL*, carrying two copies of the minor allele of rs486907 (A) was possibly inversely associated with prostate cancer, especially higher-grade disease. For *IL10*, the minor allele of rs1800871 (T) was positively associated with lower-grade disease. In our prior study [3], rs3212227 in *IL12*(p40), rs4073 in *IL8*, and tagSNPs rs1800890 and rs3021094 in *IL10* were the most consistently associated with risk of prostate cancer overall or disease grade in the PCPT. However in the present study, none of these SNPs was associated with intraprostatic inflammation in the controls.

We studied IL-2 because it is a major cytokine that is induced following T cell activation, and it is a critical cytokine for T cell survival [9]. In addition to this pro-inflammatory role, IL-2 is important in the induction and maintenance of regulatory T cells (Tregs) [10], a population of T cells that down-regulates both adaptive and innate immune responses, and that have shown to be prevalent in the prostate of men with prostate cancer [11,12]. In the present study, we observed that carriers of the minor allele (G) of rs2069762 in *IL2*, which is associated with higher production of IL-2 [11,12], had a lower odds of intraprostatic

inflammation. This observation is consistent with the notion that IL-2 is supporting intraprostatic CD4+ Tregs to down-modulate inflammation in these men, although specific tissue studies are required to explore that hypothesis. While the higher IL-2 production may explain the inverse association between *IL2* and intraprostatic inflammation, at face value, this observation is not compatible with our prior finding of a positive association between the minor allele of this SNP and serum PSA or with the lack of an association between this SNP and prostate cancer [3].

RNASEL encodes riboendonuclease L (RNASEL), an enzyme activated by the interferon pathway [13]. Upon stimulation by double-stranded viral RNA, the 2'–5' oligoadenylate synthetase (OAS)-RNASEL pathway induces apoptosis in infected cells [13]. Genetic variation in *RNASEL* can affect its enzymatic activity: the minor allele A of rs486907 (amino acid substitution R462Q) results in reduced enzymatic activity. If chronic viral infection [1], indeed, plays a role in chronic intraprostatic inflammation, then deceased ongoing RNASEL-mediated death of infected cells could result in decreased chronic inflammation in the absence of cancer. In that regard, the potential associations among the rs486907 SNP in *RNASEL*, intraprostatic inflammation, serum PSA concentration, and higher-grade prostate cancer are perhaps among the most interesting findings when comparing the current findings with our prior results [3]. In our prior study, we found that men with the minor allele of rs486907 in *RNASEL* appeared to have slightly lower serum PSA concentration than men with the major allele. In Figure 1, we show possible causal and non-causal links between *RNASEL* and higher-grade prostate cancer. It is possible *RNASEL* causally influences risk of higher-grade prostate cancer via an influence on intraprostatic inflammation. However, it is also possible that *RNASEL* non-causally influences risk of higher-grade prostate cancer via its influence on inflammation, which in turn influences serum PSA concentration, changing the likelihood of biopsy and detection of occult prostate cancer. We cannot determine from our studies whether the causal or non-causal explanation is more likely, whether other pathways not depicted in the Figure 1 are explanatory, or whether what we have observed in our studies are due solely to chance. It should also be noted that both rare and common *RNASEL* SNPs have been previously studied in association with prostate cancer, and these studies show disparate results [14,15]; thus a definitive role for the gene and its variants in prostate carcinogenesis is lacking.

IL-10 is a T_H2 cytokine, and is generally associated with the down-modulation of an inflammatory immune response [16]. The minor allele of *IL10* rs1800871 (T) is associated with decreased IL-10 levels [17,18]. Explanations for the links among the minor allele of rs1800871 (T) in *IL10*, inflammation, serum PSA concentration, and the risk of prostate cancer are less clear. Men with two copies of the minor allele (but not one copy) were less likely to have intraprostatic inflammation, but were more likely to be diagnosed with lower-grade prostate cancer, primarily when restricting to men with low PSA. This SNP was not associated with serum PSA concentration in our prior study [3]. Based on the known function of IL-10, we might have expected that the minor allele of the gene encoding this anti-inflammatory cytokine would be associated with both an increased prevalence of intraprostatic inflammation and with increased prostate cancer risk. Although other explanations are possible, it seems likely that our finding that carrying two copies of this

SNP was associated with inflammation may have been observed by chance alone given the low prevalence of this genotype in the population.

Recognizing the complexity of the immune system, we speculated that products of the genes we selected may work in concert and thus, small differences in the production or function of those gene products could alter the prostate inflammatory milieu. In the post-hoc analysis in which we summed across the risk alleles for the SNPs we observed to be possibly associated with inflammation in this study, we found that the odds of inflammation increased with increasing number of risk alleles. The positive dose-response we observed was expected given that the SNPs we studied are independently inherited and we only included the SNPs we observed to be associated with intraprostatic inflammation in the genetic risk score. Nevertheless, this result may point to the importance of certain gene products working together in the production of intraprostatic inflammation.

To our knowledge, this study is the first to directly evaluate the cross-sectional link between variation in key genes involved in the immune response and intraprostatic inflammation in men without prostate cancer. The men included in the analysis were sampled from the placebo arm of the PCPT. In the PCPT, men not diagnosed with prostate cancer during the trial were requested to undergo an end-of-study biopsy irrespective of clinical indication [8]. Thus, tissue was available for the assessment of inflammation unbiased by the links among genes, intraprostatic inflammation, and PSA [2].

We used data that we previously collected for studies on genes, inflammation, and prostate cancer. In our previous study, SNP selection was hypothesis driven, but was limited to candidate SNPs (aside from *IL10*, for which we also selected tagSNPs). We cannot rule out that other SNPs in the same pathway may influence intraprostatic inflammation. While we selected purported functional SNPs in genes involved in the innate immunity and T cell activation and/or response, our overall null results could be explained by the SNPs/genes that we selected not sufficiently capturing the propensity to mount an inflammatory response in general or a response specific to particular stimuli or insults in the prostate. Additionally, we cannot rule out that we did not detect an association between these select SNPs and intraprostatic inflammation because we did not evaluate this association within the setting of a nascent cancer, which might elicit a stronger inflammatory response on some genetic backgrounds. Also, we did not assess the presence or density of particular immune cell types and thus, our bulk assessment of inflammatory cells may not have allowed us to detect associations between SNPs in genes involved in particular aspects of the immune response and overall inflammatory infiltrates. Because we used biopsy cores, which were sampled primarily the peripheral zone and only a small portion of the total prostate, we cannot be certain that the cores we assessed are representative of the entire inflammatory milieu of the prostate [2]. Given the modest sample size, associations were less precise for carrying two copies of the minor allele for some SNPs. We did not analytically correct for multiple testing because none of the primary associations was statistically significant. We created the genetic risk score from those SNPs that were possibly associated with inflammation. The finding from this post-hoc score warrants further investigation. Finally, the sample size was too small to study SNP-inflammation associations in non-white men.

In conclusion, our findings generally do not support a cross-sectional link between individual SNPs in key genes involved in the immune response and intraprostatic inflammation in men without a prostate cancer diagnosis. However, our findings do suggest that variants in key genes when in combination may be associated with intraprostatic inflammation in benign tissue and may indicate joint pathways for further investigation. Additionally, future studies are needed to address whether modifiable factors and/and autoimmunity including coupled with genetic variation, rather than genetics alone, may explain why some men have intraprostatic inflammation and others do not. The long-term goal of this and the future research is to be able to intervene on the causes of intraprostatic inflammation as a possible way of preventing prostate cancer.

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REFERENCES

1. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Grönberg H, Drake CG, Nakai Y, Isaacs WB, Nelson WG. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007; 7:256–269. [PubMed: 17384581]
2. Gurel B, Lucia MS, Thompson IM, Goodman PJ, Tangen CM, Kristal AR, Parnes HL, Hoque A, Lippman SM, Sutcliffe S, Peskoe SB, Drake CG, Nelson WG, De Marzo AM, Platz EA. 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; 23:847–856. [PubMed: 24748218]
3. Winchester DA, Till C, Goodman PJ, Tangen CM, Santella RM, Johnson-Pais TL, Leach RJ, Xu J, Zheng SL, Thompson IM, Lucia MS, Lippmann SM, Parnes HL, Dluzniewski PJ, Isaacs WB, De Marzo AM, Drake CG, Platz EA. Variation in genes involved in the immune response and prostate cancer risk in the placebo arm of the Prostate Cancer Prevention Trial. Prostate. Published Online First: 5 June 2015.
4. Xue WM, Coetzee GA, Ross RK, Irvine R, Kolonel L, Henderson BE, Ingles SA. Genetic determinants of serum prostate-specific antigen levels in healthy men from a multiethnic cohort. Cancer Epidemiol Biomarkers Prev. 2001; 10:575–579. [PubMed: 11401905]
5. Wiklund F, Zheng SL, Sun J, Adami H-O, Lilja H, Hsu F-C, Stattin P, Adolfsson J, Cramer SD, Duggan D, Carpten JD, Chang B-L, Isaacs WB, Grönberg H, Xu J. Association of reported prostate cancer risk alleles with PSA levels among men without a diagnosis of prostate cancer. Prostate. 2009; 69:419–427. [PubMed: 19116992]
6. Gudmundsson J, Besenbacher S, Sulem P, Gudbjartsson DF, Olafsson I, Arinbjarnarson S, Agnarsson BA, Benediktsdottir KR, Isaksson HJ, Kostic JP, Gudjonsson SA, Stacey SN, Gylfason A, Sigurdsson A, Holm H, Bjornsdottir US, Eyjolfsson GI, Navarrete S, Fuertes F, Garcia-Prats MD, Polo E, Checherita IA, Jinga M, Badea P, Aben KK, Schalken JA, van Oort IM, Sweep FC, Helfand BT, Davis M, Donovan JL, Hamdy FC, Kristjansson K, Gulcher JR, Masson G, Kong A, Catalona WJ, Mayordomo JI, Geirsson G, Einarsson GV, Barkardottir RB, Jonsson E, Jinga V, Mates D, Kiemeney LA, Neal DE, Thorsteinsdottir U, Rafnar T, Stefansson K. Genetic correction of PSA values using sequence variants associated with PSA levels. Sci Transl Med. 2010; 2:62–92.
7. Goodman PJ, Tangen CM, Kristal AR, Thompson IM, Lucia MS, Platz EA, Figg WD, Hoque A, Hsing A, Neuhouser ML, Parnes HL, Reichardt JKV, Santella RM, Till C, Lippman SM. Transition of a clinical trial into translational research: the prostate cancer prevention trial experience. Cancer Prev Res (Phila). 2010; 3:1523–1533. [PubMed: 21149329]

8. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA. The influence of finasteride on the development of prostate cancer. N Engl J Med. 2003; 349:215–224. [PubMed: 12824459]
9. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-2. Nat Rev Immunol. 2004; 4:665–674. [PubMed: 15343366]
10. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008; 8:523–532. [PubMed: 18566595]
11. Sfanos KS, Bruno TC, Maris CH, Xu L, Thoburn CJ, DeMarzo AM, Meeker AK, Isaacs WB, Drake CG. Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. Clin Cancer Res. 2008; 14:3254–3261. [PubMed: 18519750]
12. Ebelt K, Babaryka G, Frankenberger B, Stief CG, Eisenmenger W, Kirchner T, Schendel DJ, Noessner E. Prostate cancer lesions are surrounded by FOXP3+, PD-1+ and B7-H1+ lymphocyte clusters. Eur J Cancer. 2009; 45:1664–1672. [PubMed: 19318244]
13. Meyer MS, Penney KL, Stark JR, Schumacher FR, Sesso HD, Loda M, Fiorentino M, Finn S, Flavin RJ, Kurth T, Price AL, Giovannucci EL, Fall K, Stampfer MJ, Ma J, Mucci LA. Genetic variation in RNASEL associated with prostate cancer risk and progression. Carcinogenesis. 2010; 31:1597–1603. [PubMed: 20576793]
14. Li H, Tai BC. RNASEL gene polymorphisms and the risk of prostate cancer: a meta-analysis. Clin Cancer Res. 2006; 12:5713–5719. [PubMed: 17020975]
15. Wei B, Xu Z, Ruan J, Zhu M, Jin K, Zhou D, Yan Z, Xuan F, Zhou H, Huang X, Zhang J, Lu P, Shao J. RNASEL Asp541Glu and Arg462Gln polymorphisms in prostate cancer risk: evidences from a meta-analysis. Mol Biol Rep. 2012; 39:2347–2353. [PubMed: 21656378]
16. Zhou L, Chong MMW, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009; 30:646–655. [PubMed: 19464987]
17. Ding Q, Fan B, Fan Z, Ding L, Li F, Tu W, Jin X, Shi Y, Wang J. Interleukin-10-819C>T polymorphism contributed to cancer risk: evidence from 29 studies. Cytokine. 2013; 61:139–145. [PubMed: 23046616]
18. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet. 1997; 24:1–8. [PubMed: 9043871]

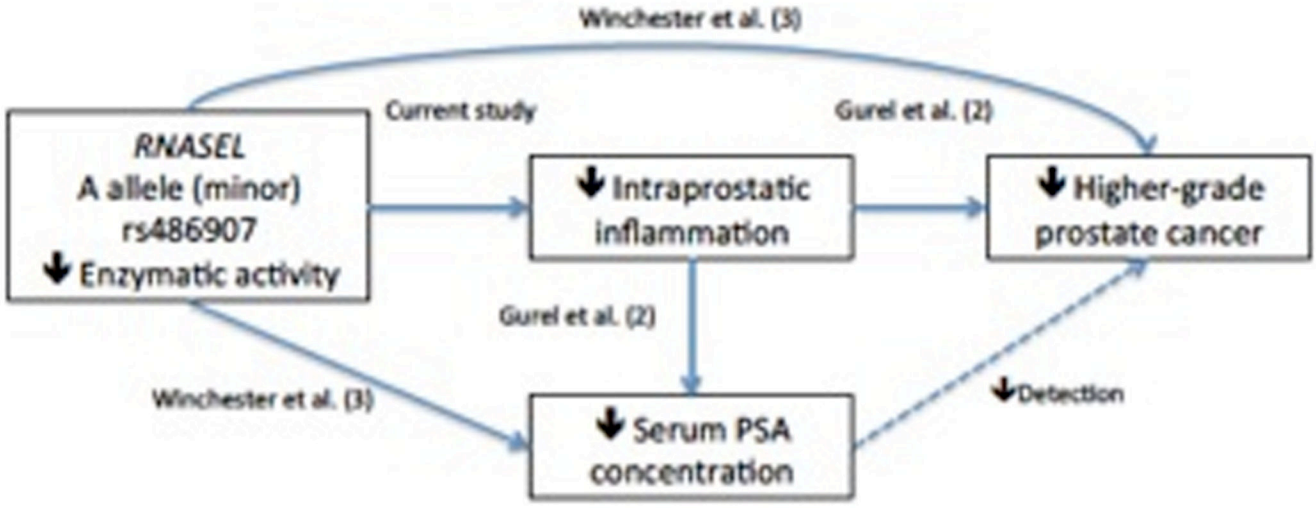

Figure 1.

Possible causal (solid lines) and non-causal (dashed line) associations between the minor allele (A) of rs486907 in *RNASEL* and risk of higher-grade prostate cancer based on studies in the PCPT.

Table 1

Characteristics* of 205 white controls at time of biopsy, placebo arm, PCPT

	At least 1 biopsy core with inflammation**		
	No	**Yes**	**P**
N	47	158	
Mean age at biopsy (years)	69.7	71.8	0.02
Family history (%)	13.6	19.3	0.4
Smoking status (%)			
Current	4.2	5.0	
Former	73.3	59.2	0.2***
Never	22.4	35.7	
Mean pack-years, current and former smokers	22.9	25.6	0.4
Mean BMI (kg/m^2)	27.4	27.4	0.9
History of diabetes (%)	12.8	6.3	0.2
Geometric mean PSA concentration at biopsy (ng/mL)	0.95	1.3	0.03
Mean annual PSA velocity from baseline to biopsy	0.07	0.11	0.6

*All characteristics adjusted for age at biopsy (except age at biopsy) using linear regression for adjusted proportions and means.

**A mean of 3 biopsy cores were reviewed (of 6–10 taken)

***Logistic regression

Table 2

Prevalence of carrying at least 1 minor allele for genes involved in the immune response by whether a man had at least one biopsy core with inflammation, white controls, placebo arm, PCPT

			At least 1 biopsy core with inflammation***				
			No		Yes		
Gene	SNPs	Minor allele	Number of carriers / total	Prevalence of carriers (%)	Number of carriers / total	Prevalence of carriers (%)	P*
IL1β	rs1143634	T	16/47	34.0	60/156	38.5	0.6
IL1β	rs1143627	C	22/43	51.2	82/146	56.2	0.6
IL2	rs2069762	G	29/45	64.4	72/151	47.7	**0.05**
IL4	rs2243250	T	13/42	30.9	40/142	28.2	0.7
IL6	rs1800795	C	30/47	63.8	108/157	68.8	0.5
IL6	rs1800797	A	27/47	57.5	104/156	66.7	0.3
IL8	rs4073	A	32/45	71.1	108/157	68.8	0.8
IL10	rs1800871	T	20/42	47.6	54/144	37.5	0.2
IL10	rs1800872	A	22/46	47.8	61/154	39.6	0.3
IL10	rs1800896	G	31/47	65.9	109/154	70.8	0.5
IL10	rs3024496**	C	26/44	59.1	107/155	69.0	0.2
IL10	rs1800894**	A	2/47	4.3	9/157	5.7	0.7
IL10	rs1800890**	A	25/46	54.4	100/157	63.7	0.3
IL10	rs3024509**	C	6/46	13.0	12/153	7.8	0.3
IL10	rs1554286**	T	15/46	32.6	53/158	33.5	0.9
IL10	rs3021094**	C	8/46	17.4	28/158	17.7	0.9
IL10	rs3024498**	G	18/42	42.9	60/145	41.4	0.9
IL12(p40)	rs3212227	C	19/47	40.4	47/155	30.3	0.2
IFNG	rs2430561	A	31/42	73.8	107/142	75.4	0.8
MSR1	rs3747531	C	8/47	17.0	18/154	11.7	0.3
RNASEL	rs486907	A	33/47	70.2	87/157	55.4	**0.07**
TLR4	rs4986790	G	4/46	8.7	17/155	10.9	0.7

Gene	SNPs	Minor allele	At least 1 biopsy core with inflammation***				
			No		Yes		
			Number of carriers / total	Prevalence of carriers (%)	Number of carriers / total	Prevalence of carriers (%)	P*
TNFA	rs1800629	A	13/46	28.3	50/154	32.3	0.6

* Chi-square test

** TagSNPs

*** A mean of three biopsy cores were reviewed (of 6–10 taken)

Table 3

Association between SNPs in genes involved in the immune response and having at least 1 biopsy core with inflammation, white controls, placebo arm, PCPT

		Number of minor alleles										
		None			1 copy			2 copies			Log-additive	
Gene	SNPs	Genotype	No. with / without inflammation	OR*	Genotype	No. with / without inflammation	OR* (95% CI)	Genotype	No. with / without inflammation	OR* (95% CI)	OR* (95% CI)	P-trend
IL1β	rs1143634	C/C	96/31	1.00 (ref)	C/T	50/13	1.25 (0.59– 2.62)	T/T	10/3	1.22 (0.31–4.79)	1.17 (0.67–2.03)	0.6
IL1β	rs1143627	T/T	64/21	1.00 (ref)	C/T	67/18	1.14 (0.55–2.37)	C/C	15/4	1.19 (0.35–4.03)	1.12 (0.65–1.89)	0.7
IL2	rs2069762	T/T	79/16	1.00 (ref)	T/G	60/24	**0.51 (0.25–1.05)**	G/G	12/5	0.51 (0.15–1.68)	0.64 (0.38–1.06)	**0.09**
IL4	rs2243250	C/C	102/29	1.00 (ref)	C/T	40/12	0.96 (0.44–2.09)	T/T	0/1	1 (NA-NA)	0.83 (0.40–1.72)	0.6
IL6	rs1800795	G/G	49/17	1.00 (ref)	C/G	76/22	1.19 (0.57–2.49)	C/C	32/8	1.49 (0.57–3.90)	1.22 (0.76–1.94)	0.4
IL6	rs1800797	G/G	52/20	1.00 (ref)	A/G	73/22	1.22 (0.59–2.49)	A/A	31/5	2.34 (0.79–6.94)	1.44 (0.88– 2.32)	0.1
IL8	rs4073	T/T	49/13	1.00 (ref)	A/T	76/24	0.85 (0.39–1.83)	A/A	32/8	0.96 (0.35–2.61)	0.96 (0.59–1.56)	0.9
IL10	rs1800871	C/C	90/22	1.00 (ref)	C/T	47/14	0.89 (0.41–1.92)	T/T	7/6	**0.29 (0.08–1.00)**	0.65 (0.38–1.11)	0.1
IL10	rs1800872	C/C	93/24	1.00 (ref)	A/C	53/16	0.92 (0.45–1.91)	A/A	8/6	0.36 (0.11–1.15)	0.70 (0.42–1.18)	0.2
IL10	rs1800896	A/A	45/16	1.00 (ref)	A/G	75/22	1.20 (0.57–2.55)	G/G	34/9	1.21 (0.47–3.12)	1.11 (0.69 –1.78)	0.7
IL10	rs3024496**	T/T	48/18	1.00 (ref)	C/T	74/17	1.64 (0.76–3.51)	C/C	33/9	1.24 (0.48–3.13)	1.17 (0.73–1.89)	0.5
IL10	rs1800894**	G/G	148/45	1.00 (ref)	A/G	9/2	1.16 (0.23–5.74)	A/A	0/0	-	1.16 (0.23–5.74)	0.9
IL10	rs1800890**	T/T	57/21	1.00 (ref)	A/T	71/19	1.42 (0.69–2.92)	A/A	29/6	1.73 (0.62–4.81)	1.34 (0.83– 2.17)	0.2
IL10	rs3024509**	T/T	141/40	1.00 (ref)	C/T	11/5	0.54 (0.17–1.69)	C/C	1/1	0.21 (0.01–3.58)	0.50 (0.20– 1.26)	0.1
IL10	rs1554286**	C/C	105/31	1.00 (ref)	C/T	45/10	1.51 (0.67– 3.39)	T/T	8/5	0.49 (0.14–1.56)	0.93 (0.54–1.61)	0.8

		Number of minor alleles										
		None			1 copy			2 copies			Log-additive	
Gene	**SNPs**	**Genotype**	**No. with / without inflammation**	**OR***	**Genotype**	**No. with / without inflammation**	**OR* (95% CI)**	**Genotype**	**No. with / without inflammation**	**OR* (95% CI)**	**OR* (95% CI)**	**P-trend**
IL10	rs3021094**	A/A	130/38	1.00 (ref)	A/C	27/8	1.16 (0.67–3.39)	C/C	1/0	-	1.23 (0.52–2.91)	0.6
IL10	rs3024498**	A/A	85/24	1.00 (ref)	A/G	51/17	0.87 (0.42–1.79)	G/G	9/1	2.19 (0.26–18.44)	1.05 (0.58– 1.91)	0.9
IL12(p40)	rs3212227	G/G	108/28	1.00 (ref)	A/C	41/15	0.72 (0.34–1.49)	C/C	6/4	0.36 (0.09–1.43)	0.65 (0.37–1.13)	0.1
IFNG	rs2430561	T/T	35/11	1.00 (ref)	A/T	73/22	1.09 (0.47–2.53)	A/A	34/9	1.19 (0.43–3.28)	1.09 (0.65–1.81)	0.7
MSR1	rs3747531	G/G	136/39	1.00 (ref)	C/G	17/8	0.61 (0.24–1.56)	C/C	1/0	-	0.73 (0.31–1.75)	0.5
RNASEL	rs486907	G/G	70/14	1.00 (ref)	A/G	61/27	**0.48 (0.23–1.00)**	A/A	1/1	0.72 (0.24–2.12)	0.74 (0.46–1.19)	0.2
TLR4	rs4986790	A/A	138/42	1.00 (ref)	A/G	16/3	1.69 (0.47 –6.16)	G/G	1/1	0.22 (0.01–4.47)	1.07 (0.39–2.86)	0.9
TNFA	rs1800629	G/G	104/33	1.00 (ref)	A/G	41/13	1.00 (0.48–2.11)	A/A	9/0	-	1.40 (0.74– 2.66)	0.3

*Odds ratios adjusted for age at biopsy

**TagSNP

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Table 4

Association between carrying at least 1 minor allele in genes involved in the immune response and having at least 1 biopsy core with inflammation overall and by participant characteristics, controls, placebo arm, Prostate Cancer Prevention Trial

		Allmen (158/47)	**PSA <2ng/mL (112/41)**	**Leaner BMI <25 kg/m^2 (81/21)**	**Heavier BMI ≥25 kg/m^2 (77/26)**	**No diabetes (148/41)**
Gene	**SNPs**	**OR* (95% CI)**	**OR* (95% CI)**	**OR* (95% CI)**	**OR* (95% CI)**	**OR* (95% CI)**
IL1β	rs1143634	1.25 (0.62–2.49)	1.13 (0.53– 2.41)	2.11 (0.68– 6.41)	0.85 (0.33–2.14)	1.09 (0.53–2.25)
IL1β	rs1143627	1.15 (0.57–2.29)	1.21 (0.57– 2.55)	1.23 (0.45–3.37)	1.04 (0.39–2.74)	1.14 (0.55–2.35)
IL2	rs2069762	**0.51 (0.25–1.02)**	0.53 (0.25–1.15)	0.46 (0.16–1.33)	0.53 (0.21–1.35)	**0.49 (0.23–1.01)**
IL4	rs2243250	0.90 (0.42–1.93)	0.99 (0.42–2.35)	2.04 (0.53–7.89)	0.56 (0.21–1.52)	1.18 (0.50–2.77)
IL6	rs1800795	1.27 (0.64–2.54)	1.03 (0.48–2.18)	0.77 (0.25–2.37)	1.72 (0.68–4.34)	1.30 0.62–2.74
IL6	rs1800797	1.43 (0.73–2.82)	1.16 (0.56–2.41)	0.99 (0.35–2.79)	1.89 (0.75–4.78)	1.37 (0.66–2.84)
IL8	rs4073	0.87 (0.42–1.83)	0.82 (0.36–1.84)	0.72 (0.25–2.09)	1.1 (0.39–3.10)	0.95 (0.44–2.05)
IL10	rs1800871	0.71 (0.35–1.44)	**0.50 (0.23–1.10)**	0.66 (0.23–1.88)	0.75 (0.29–1.98)	0.77 (0.37–1.64)
IL10	rs1800872	0.76 (0.39–1.50)	0.63 (0.30–1.33)	1.01 (0.38–2.71)	0.59 (0.24–1.51)	0.85 (0.42–1.73)
IL10	rs1800896	1.21 0.59–2.44	1.25 (0.57–2.76)	1.22 (0.45–3.35)	1.25 (0.46–3.40)	1.19 (0.57–2.52)
IL10	rs3024496**	1.49 (0.74–3.01)	1.32 (0.61–2.88)	1.46 (0.54–3.93)	1.57 (0.58–4.22)	1.48 (0.71–3.07)
IL10	rs1800894**	1.16 (0.23–5.74)	1.09 (0.21–5.70)	1.58 (0.18–14.00)	0.71 (0.06–9.12)	1.01 (0.20–5.06)
IL10	rs1800890**	1.49 (0.76–2.93)	1.44 (0.68–3.04)	1.75 (0.65–4.74)	1.42 (0.55–3.67)	1.42 (0.69–2.91)
IL10	rs3024509**	0.48 (0.16–1.41)	1.00 (NA-NA)	0.42 (0.09–1.93)	0.53 (0.11–2.42)	0.42 (0.14–1.25)
IL10	rs1554286**	1.16 (0.57–2.37)	0.96 (0.44–2.08)	1.28 (0.45–3.58)	1.02 (0.38–2.76)	1.35 (0.63– 2.93)

Table 5

Age-adjusted association between number of the risk alleles for rs2069762 in *IL2*, rs1800871 in *IL10*, and rs486907 (A) in *RNASEL* and inflammation in the controls (N=202*) in the placebo arm of the PCPT

Number of risk alleles	At least 1 biopsy core with inflammation	
	Yes/No	OR (95% CI)
1	3/4	0.25 (0.05–1.28)
2	11/5	0.76 (0.23–2.57)
3	31/10	1.04 (0.42–2.62)
4	47/17	1.00 (reference)
5	44/9	1.78 (0.71–4.46)
6	19/2	3.35 (0.69–16.1)
P_{trend}		0.008

*Men with missing information for these three SNPs were excluded (N=3).