Association of the Innate Immunity and Inflammation Pathway with Advanced Prostate Cancer Risk

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Abstract

Prostate cancer is the most frequent and second most lethal cancer in men in the United States. Innate immunity and inflammation may increase the risk of prostate cancer. To determine the role of innate immunity and inflammation in advanced prostate cancer, we investigated the association of 320 single nucleotide polymorphisms, located in 46 genes involved in this pathway, with disease risk using 494 cases with advanced disease and 536 controls from Cleveland, Ohio. Taken together, the whole pathway was associated with advanced prostate cancer risk (P = 0.02). Two sub-pathways (intracellular antiviral molecules and extracellular pattern recognition) and four genes in these sub-pathways (TLR7, TLR6, OAS1, and OAS2) were nominally associated with advanced prostate cancer risk and harbor several SNPs nominally associated with advanced prostate cancer risk. Our results suggest that the innate immunity and inflammation pathway may play a modest role in the etiology of advanced prostate cancer through multiple small effects.

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Introduction

Prostate cancer is the most frequent and second most lethal cancer in men in the United States [1]. There is growing evidence that innate immunity and inflammation may play a role in prostate and other cancers [2,3,4]. Chronic inflammation could contribute to prostate cancer through several biological processes: the mutagenesis caused by oxidative stress; the remodeling of the extracellular matrix; the recruitment of immune cells, fibroblasts, and endothelial cells; or the induction of cytokines and growth factors contributing to a proliferative and angiogenic environment [2,3,5].

Compelling evidence supports a role for genes involved in the innate immunity and inflammation pathway in prostate cancer risk. Several genes harboring single nucleotide polymorphisms (SNPs) associated with prostate cancer risk have been identified, including the pattern recognition receptors MSR1, TLR1, TLR4, TLR5, TLR6, and TLR10 [6,7,8,9,10,11,12,13,14,15,16]; the antiviral gene RANASE [9,17,18,19,20,21]; the cytokines MIP1, IL8, TNF, and IL1RY [13,19,22,23,24,25,26]; and the pro-inflammatory gene COX-2 [27,28,29,30]. However, most of the previous studies have focused on individual SNPs or genes and very little is known about the impact of the overall innate immunity and inflammation pathway on developing more advanced prostate cancer.

Moreover, advanced prostate cancer cases have a higher public health burden than less advanced cases. Thus, identifying the components of the innate immunity and inflammatory process that increase the risk of advanced prostate cancer is of major importance.

To determine the role of innate immunity and inflammation in advanced prostate cancer, we investigated the association of 320 SNPs, located in 46 innate immunity and inflammation genes, with advanced prostate cancer risk. We undertook a comprehensive approach evaluating the association between disease risk and SNPs-sets pooled across the whole pathway, sub-pathways, and each gene, as well as individual SNPs.

Materials and Methods

Study Population

The case sample comprised 494 men with newly diagnosed, histologically confirmed prostate cancer, having either a Gleason score ≥7, a clinical stage ≥T2c, or a serum Prostate Serum Antigen (PSA) at diagnosis >10 recruited from the major medical institutions in Cleveland, Ohio (Cleveland Clinic Foundation, University hospitals of Cleveland, and their affiliates) [31]. The control sample comprised 536 men frequency matched to cases by age (within 5 years), ethnicity, and medical institution, who underwent standard annual exams at the major medical institutions in Cleveland, and who did not have a previous history of non-skin cancer. The PSA was measured and found elevated in two controls. Further investigations lead us to reclassify them as advanced cases of prostate cancer, leaving us with a total of 494
advanced prostate cancer cases and 536 controls used here in our analyses. Approval for this study was obtained from the University Hospitals of Cleveland Institutional Review Board and the Cleveland Clinic Foundation Institutional Review Board, and written informed consent was obtained from all study participants. More details about this study population have been previously described [27,29,32,34,35,36].

### SNP Selection, Genotyping and Quality Control

We selected for study 46 candidate genes coding for proteins involved in innate immunity and inflammation, and further grouped these into 6 relevant biological sub-pathways using a previously proposed and published classification [37]. These sub-pathways were: 1) cytokine signaling (26 genes), 2) eicosanoid signaling (1 gene, i.e. COX-2), 3) extracellular pattern recognition (8 genes), 4) intracellular antiviral molecules (4 genes), 5) nuclear kappa-light chain-enhancer or activated B cell (NFKB) signaling (5 genes), and 6) selenoproteins (2 genes). The genes SELS and SEP15 coding for selenoproteins were included because of their potential role in the control of the inflammatory response through regulation of cytokine production [38].

All SNPs located within and 2 kb upstream and 1 kb downstream of the sequence of the 46 candidate genes were identified through the International HapMap Project (www.hapmap.org) and the Genome Variation Server (SeattleSNPs) (http://gvs.gs.washington.edu/). Then, tagging SNPs were selected using the multimarker test criteria in the Tagger software program [39] to capture all common SNPs (minor allele frequency, MAF $>0.05$) with an $r^2 \geq 0.8$ across each candidate gene among European ancestry populations, forcing SNPs that are missense, non-synonymous and previously associated with prostate cancer to be included. Only one missense SNP was included for the genes TLR3 and IL6R.

Moreover, 39 ancestry informative markers (AIMs) [40] were genotyped and principal component analysis was used to estimate genetic ancestry and account for population stratification [41]. The first principal component of this analysis distinguished African Americans from Caucasians and was used as an estimate of genetic ancestry.

Genotyping of the 330 SNPs was done on DNA extracted from blood samples using either the Illumina 500G BeadStation coupled with the GoldenGate assay, or the Applied Biosystems Taqman assay. Further quality control procedures were done separately for each of the two platforms and for each of the two ethnic groups (African-Americans and Caucasians). Ten SNPs that had a call rate $<0.90$, deviated from the expected Hardy-Weinberg proportions in both ethnic groups (P $<0.01$), or had a MAF below 0.01 in both ethnic groups were excluded. Individuals who had a call rate $<0.90$ were also excluded. After the quality control procedure, the data in the case-control sample used to test for association with risk of advanced prostate cancer included 320 tagging SNPs (Table S1) and 39 AIMs.

### Table 1. Study characteristics of the advanced prostate cancer cases and controls.

|                     | Cases     | Controls  | P-value of heterogeneity$^a$ |
|---------------------|-----------|-----------|-----------------------------|
| Age (year), mean (SD) | 65.90 (8.34) | 65.85 (8.54) | 0.91 |
| Ethnicity, n (%)     |           |           |                             |
| African American    | 90 (18.2)  | 104 (19.4) | 0.68 |
| Caucasian           | 404 (81.8) | 432 (80.6) |                 |
| Prostate cancer in first degree relative, n (%)$^b$ |           |           |                             |
| Negative            | 381 (77.3) | 472 (88.9) | $<2 \times 10^{-16}$ |
| Positive            | 112 (22.7) | 59 (11.1)  |                 |
| PSA at diagnosis (ng/mL), mean (SD) | 14.38 (27.67) | 1.74 (1.71) | $<2 \times 10^{-16}$ |
| Categories of PSA at diagnosis, n (%) |           |           |                             |
| $<4.0$              | 25 (5.1)   | –         | –                           |
| 4.0–9.9             | 249 (50.4) | –         | –                           |
| 10–19.9             | 152 (30.8) | –         | –                           |
| 20–49.9             | 53 (10.7)  | –         | –                           |
| $>50$               | 15 (3.0)   | –         | –                           |
| Gleason score, n (%) |           |           |                             |
| $\leq 6$            | 74 (15.0)  | –         | –                           |
| 3+4                 | 217 (43.9) | –         | –                           |
| 4+3 or $\geq 8$     | 203 (41.1) | –         | –                           |
| Clinical stage, n (%)$^b$ |           |           |                             |
| T1                  | 306 (64.7) | –         | –                           |
| T2a–T2b             | 127 (26.8) | –         | –                           |
| T2c                 | 15 (3.2)   | –         | –                           |
| T3–T4               | 25 (5.3)   | –         | –                           |

$^a$P-values obtained using either a Student t-test (quantitative coding) or a Chi-square test (qualitative coding).

$^b$The sum of all categories does not add to the total due to missing data.

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Table 2. Association of the whole pathway, sub-pathways, and genes of innate immunity and inflammation with advanced prostate cancer risk.

| SNP set                                  | SNP set | SNP count | P-value | African American | Caucasian |
|------------------------------------------|---------|-----------|---------|-----------------|-----------|
| Overall                                  |         |           |         |                 |           |
| Inflammation and innate immunity         |         | 320       | 0.02    | 0.29            | 0.01      |
| • Cytokine signaling (26 genes)          |         | 179       | 0.44    | 0.33            | 0.57      |
| IL10                                     |         | 8         | 0.34    | 0.42            | 0.47      |
| IL12RB2                                  |         | 11        | 0.75    | 0.89            | 0.61      |
| IL5R                                     |         | 1         | -a      | -a              | -a        |
| IL18R1                                   |         | 16        | 0.11    | 0.09            | 0.31      |
| IL1B                                     |         | 4         | 0.53    | 0.58            | 0.59      |
| IL1RN                                    |         | 7         | 0.42    | 0.50            | 0.51      |
| IL12A                                    |         | 4         | 0.12    | 0.66            | 0.13      |
| TGFBR2                                   |         | 33        | 0.75    | 0.22            | 0.78      |
| IL2                                      |         | 5         | 0.81    | 0.41            | 0.63      |
| IL8                                      |         | 4         | 0.18    | 1               | 0.17      |
| IL12B                                    |         | 6         | 0.45    | 0.59            | 0.46      |
| IL13                                     |         | 4         | 0.84    | 0.11            | 0.95      |
| IL4                                      |         | 4         | 0.41    | 0.23            | 0.60      |
| IL5                                      |         | 1         | -a      | -a              | -a        |
| IFNGR1                                   |         | 5         | 0.006   | 0.16            | 0.009     |
| IL17                                     |         | 8         | 0.41    | 0.56            | 0.21      |
| TNF/LTA                                  |         | 11        | 0.72    | 0.44            | 0.92      |
| TGFBR1                                   |         | 6         | 0.49    | 0.40            | 0.52      |
| IL18                                     |         | 8         | 0.048   | 0.07            | 0.08      |
| IFNG                                     |         | 6         | 0.19    | 0.20            | 0.40      |
| IL23A                                    |         | 1         | -a      | -a              | -a        |
| IL12RB1                                  |         | 5         | 0.57    | 0.45            | 0.41      |
| MIC1                                     |         | 6         | 0.94    | 0.10            | 0.51      |
| TGBF1                                    |         | 4         | 0.22    | 0.08            | 0.68      |
| IFNGR2                                   |         | 9         | 0.72    | 0.86            | 0.78      |
| MIF                                      |         | 2         | 0.36    | 1               | 0.23      |
| • Eicosanoid signaling (1 gene: COX2)    |         | 9         | 0.04    | 0.07            | 0.09      |
| • Extracellular pattern recognition (8 genes) |       | 56        | 0.02    | 0.12            | 0.01      |
| TLR5                                     |         | 7         | 0.49    | 0.69            | 0.48      |
| TLR1                                     |         | 7         | 0.002   | 0.09            | 0.004     |
| TLR10                                    |         | 7         | 0.18    | 0.35            | 0.07      |
| TLR2                                     |         | 8         | 0.63    | 0.28            | 0.37      |
| TLR3                                     |         | 1         | -a      | -a              | -a        |
| TLR6                                     |         | 5         | 0.04    | 0.04            | 0.04      |
| MSR1                                     |         | 16        | 0.37    | 0.09            | 0.36      |
| TLR4                                     |         | 5         | 0.11    | 0.05            | 0.19      |
| • Intracellular antiviral molecules (4 genes) |       | 40        | 0.02    | 0.71            | 0.01      |
| RNASEL                                   |         | 7         | 0.31    | 0.24            | 0.43      |
| EIF2AK2                                  |         | 11        | 0.79    | 0.41            | 0.44      |
| OAS1                                     |         | 5         | 0.015   | 0.92            | 0.01      |
| OAS2                                     |         | 17        | 0.019   | 0.79            | 0.01      |
| • NFKB signaling (5 genes)               |         | 27        | 0.32    | 0.04            | 0.48      |
| NFKB1                                    |         | 10        | 0.70    | 0.49            | 0.58      |
| IKKKB                                    |         | 7         | 0.18    | 0.46            | 0.13      |
| CHUK                                     |         | 6         | 0.14    | 0.07            | 0.28      |
Statistical Analysis

To analyze the whole set of 320 SNPs together, or sets of SNPs grouped by sub-pathways or genes, we used the SNP-set kernel-machine association test (SKAT v0.62) [42]. This method uses a logistic kernel-machine model, aggregating individual score test statistics of SNPs, and provides a global P-value for the set of variants tested that takes into account the joint effect of the SNPs in a given SNP-set and allows for incorporating the adjustment covariates: age, institution, and genetic ancestry. One advantage of SKAT over other pathway tests is that it adaptively finds the optimal kernel function. To test for the association between genotyped SNPs, and P-values were estimated using both co-dominant and log-additive models.

To adjust for genetic ancestry in all analyses, we included the first principal component of the principal component analysis of the 39 AIMs as covariate. Moreover, to identify SNPs with potential opposite effects in African Americans and Caucasians, we also stratified all analyses by reported ethnicity. Our strategy evaluated disease risk association at multiple levels of SNP groupings (whole set, sub-pathways, genes, and individual SNPs). To account for the multiple tests done while incorporating the adjustment covariates: age, institution, and genetic ancestry. One advantage of SKAT over other pathway tests is that it adaptively finds the genetic similarity between all individuals for the set of SNPs or SNP. Then for each level of SNP groupings, we calculated a family-wise error rate by comparing the P-value of each test to the distribution of the minimum P-values obtained from 1000 permuted data sets. Reported P-values are two-sided and analyses were done using R v2.13.1 [43].

### Results

#### Study Subject Characteristics

The case-control sample included 1,030 subjects whose average age at diagnosis or recruitment was 65.87 (SD: 8.46) years, and was comprised of 194 African Americans (18.9%) and 836 Caucasians (81.2%). Age and ethnicity were similarly distributed in advanced prostate cancer cases and controls (Table 1).

#### Association with Advanced Prostate Cancer Risk

Taken together, the whole set of 320 SNPs in the innate immunity and inflammation pathway was significantly associated with advanced prostate cancer risk (P = 0.02). Of the 6 sub-pathways analyzed, the intracellular antiviral molecules and the extracellular pattern recognition sub-pathways were nominally associated with advanced prostate cancer risk (P = 0.02 for both) but not after correction for multiple testing (P = 0.12 and P = 0.11, respectively).

Interestingly, 4 genes in these 2 sub-pathways were also nominally associated with prostate cancer risk: TLR1 and TLR6 in the extracellular pattern recognition sub-pathway (P = 0.002 and P = 0.04, respectively), and OAS1 and OAS2 in the intracellular antiviral molecules sub-pathway (P = 0.015 and P = 0.019, respectively). In addition, IFNGR1 in the cytokine signaling sub-pathway and COX-2, which is the sole member of the eicosanoid signaling sub-pathway represented in our data set, had nominal P-values of 0.006 and 0.044, respectively (Table 2). However, none of these associations are robust to correction for multiple testing (P = 0.10 for the association with TLR1).

The results of the individual SNP analyses supported the findings obtained with the sub-pathway and gene sets. Indeed, most of the SNPs having a nominal association P-value below 0.01, belong to TLR1, TLR6, OAS1, OAS2, or COX-2 (Table 5). Moreover, many of the other SNPs in these genes have a P-value between 0.01 and 0.05 (Table S2). Interestingly, for all these SNPs, results indicate a protective effect of the minor allele with additive ORs between 0.73 and 0.77. But again, when correcting for multiple testing, these were no longer significant (P = 0.42 for the most significant association).

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### Table 1.

| SNP set   | SNP count | P-value Overall | P-value African American | P-value Caucasian |
|-----------|-----------|-----------------|--------------------------|-------------------|
| RELA      | 2         | 0.16            | 0.04                     | 0.51              |
| NFKBIA    | 2         | 0.67            | 0.24                     | 0.72              |
| Selenoproteins (2 genes) | 9 | 0.67 | 0.93 | 0.44 |
| SEP15     | 5         | 0.37            | 0.74                     | 0.21              |
| SELs      | 4         | 0.95            | 0.86                     | 0.94              |

*Genes with one SNP;
*NFKB: nuclear kappa-light chain-enhancer or activated B cell.

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### Table 3. Association of SNPs with advanced prostate cancer risk (P-value < 0.01).

| Gene (chromosome) | SNP | Overall | African Americans | Caucasians |
|------------------|-----|---------|-------------------|------------|
|                  |     | OR (95% CI) | P-value | OR (95% CI) | P-value | OR (95% CI) | P-value |
| TLR1 (4)         | rs5743551 | AA 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | AG 0.75 (0.57, 0.99) | 0.044 | 0.86 (0.11, 5.69) | 0.876 | 0.73 (0.55, 0.97) | 0.032 |
|                  |     | GG 0.51 (0.34, 0.78) | 0.002 | 0.52 (0.07, 3.35) | 0.492 | 0.53 (0.32, 0.88) | 0.014 |
|                  |     | trend (G) 0.73 (0.60, 0.88) | 0.001 | 0.64 (0.37, 1.1) | 0.103 | 0.73 (0.59, 0.9) | 0.003 |
| OAS2 (12)        | rs1058480 | CC 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | AG 0.73 (0.56, 0.96) | 0.026 | 1.44 (0.64, 3.29) | 0.379 | 0.67 (0.5, 0.89) | 0.007 |
|                  |     | GG 0.54 (0.35, 0.82) | 0.005 | 1.02 (0.12, 8.88) | 0.988 | 0.53 (0.32, 0.78) | 0.002 |
|                  |     | trend (G) 0.73 (0.60, 0.89) | 0.001 | 1.26 (0.65, 2.48) | 0.495 | 0.7 (0.57, 0.85) | 3.8 \(\times\) 10^{-4} |
| OAS2 (12)        | rs15895 | GG 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | GA 0.76 (0.57, 1.00) | 0.035 | 2.05 (1.35, 3.16) | 0.001 | 5.37 (1.28, 36.55) | 0.038 |
|                  |     | AA 0.53 (0.35, 0.81) | 0.003 | 1.02 (0.78, 3.35) | 0.096 | 0.87 (0.64, 1.18) | 0.006 |
|                  |     | trend (A) 0.74 (0.60, 0.90) | 0.002 | 1.26 (0.65, 2.48) | 0.495 | 0.7 (0.57, 0.85) | 4.6 \(\times\) 10^{-4} |
| TLR1 (4)         | rs4833095 | AA 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | AG 0.75 (0.57, 1.00) | 0.033 | 2.05 (1.35, 3.16) | 0.001 | 5.37 (1.28, 36.55) | 0.038 |
|                  |     | GA 0.74 (0.61, 0.89) | 0.002 | 1.26 (0.65, 2.48) | 0.495 | 0.7 (0.57, 0.85) | 4.6 \(\times\) 10^{-4} |
| TLR6 (4)         | rs5743795 | GG 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | GA 0.73 (0.55, 0.97) | 0.027 | 1.3 (0.44, 3.89) | 0.634 | 0.7 (0.52, 0.93) | 0.016 |
|                  |     | AA 0.53 (0.25, 1.05) | 0.074 | – | – | 0.52 (0.25, 1.03) | 0.068 |
|                  |     | trend (A) 0.73 (0.58, 0.92) | 0.007 | 1.3 (0.44, 3.89) | 0.634 | 0.7 (0.56, 0.9) | 0.004 |
| TLR6 (4)         | rs5743794 | GG 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | GA 0.73 (0.55, 0.97) | 0.029 | 1.3 (0.44, 3.91) | 0.627 | 0.7 (0.52, 0.94) | 0.017 |
|                  |     | AA 0.53 (0.26, 1.05) | 0.075 | – | – | 0.52 (0.25, 1.04) | 0.070 |
|                  |     | trend (A) 0.73 (0.58, 0.92) | 0.008 | 1.3 (0.44, 3.91) | 0.627 | 0.7 (0.56, 0.9) | 0.005 |
| COX-2 (1)        | rs2745557 | GG 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | GA 0.68 (0.51, 0.90) | 0.007 | 0.67 (0.33, 1.32) | 0.249 | 0.67 (0.49, 0.91) | 0.011 |
|                  |     | AA 0.77 (0.36, 1.62) | 0.496 | – | – | 0.91 (0.42, 2.04) | 0.862 |
|                  |     | trend (A) 0.74 (0.58, 0.94) | 0.013 | 0.57 (0.3, 1.05) | 0.074 | 0.77 (0.59, 0.99) | 0.042 |
| OAS1 (12)        | rs2285934 | CC 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | CA 0.64 (0.49, 0.85) | 0.002 | 0.39 (0.17, 0.86) | 0.021 | 0.7 (0.52, 0.95) | 0.022 |
|                  |     | AA 0.71 (0.48, 1.03) | 0.074 | 0.83 (0.35, 1.91) | 0.653 | 0.62 (0.39, 0.96) | 0.033 |
|                  |     | trend (A) 0.79 (0.66, 0.95) | 0.014 | 0.97 (0.64, 1.47) | 0.880 | 0.76 (0.62, 0.93) | 0.009 |
| OAS2 (12)        | rs13311 | CC 1 (Ref) | – | 1 (Ref) | – | 1 (Ref) | – |
|                  |     | CA 1.03 (0.80, 1.33) | 0.080 | 0.78 (0.44, 1.38) | 0.403 | 1.11 (0.84, 1.47) | 0.473 |
|                  |     | AA 3.27 (1.55, 7.55) | 0.003 | – | – | 3.42 (1.61, 7.93) | 0.002 |
|                  |     | trend (A) 1.21 (0.97, 1.51) | 0.091 | 0.78 (0.44, 1.38) | 0.402 | 1.32 (1.03, 1.68) | 0.026 |
with prostate cancer. In our dataset, we observed the same members of the toll-like receptor family [6,12,16]. In particular, TLR1 is specialized in the recognition of gram-positive bacteria. TLR1 and TLR6 also share the ability to form a heterodimer with humans and share structural and functional similarities. Moreover, they are also detected in Caucasians, which represents more than 80% of the sample, but not in African Americans (Tables 2, 3, and Table S2).

### Discussion

In this integrative analysis of the association of advanced prostate cancer risk with candidate genes involved in innate immunity and inflammation, we studied 320 SNPs and their joint effects across genes and sub-pathways. Taken as a whole, the overall innate immunity and inflammation pathway seems to be involved in advanced prostate cancer, but the individual elements of this association are not clear. Indeed, the whole set of 320 SNPs is significantly associated with advanced prostate cancer risk. However, none of the other evaluated associations with sub-pathways, genes, or individual SNPs were significant, when correcting for multiple testing by making permutation based estimates of the family-wise error rate.

Nonetheless, our results suggest that the extracellular pattern recognition, the intracellular antiviral molecules, and the eicosanoid signaling (ie, COX-2) could be components that play a potential role in advanced prostate cancer risk. Within those sub-pathways, 5 genes (TLR1, TLR6, OAS1, OAS2, and COX-2) were nominally associated with advanced prostate cancer risk. Moreover, these genes harbor several SNPs nominally associated with advanced prostate cancer risk.

TLR1 and TLR6 encode members of the toll-like receptor family. Their role is to recognize molecular patterns associated to infectious pathogens. Both are highly conserved from Drosophila to humans and share structural and functional similarities. Moreover, TLR1 and TLR6 also share the ability to form a heterodimer with TLR2 to recognize peptidoglycan and lipoproteins on pathogens. TLR1 is specialized in the recognition of gram-positive bacteria. Several studies have reported prostate cancer associations with members of the toll-like receptor family [6,12,16]. In particular, Sun et al. [12] observed multiple SNPs in strong linkage disequilibrium located on TLR1, TLR6, and TLR10 associated with prostate cancer. In our dataset, we observed the same association with rs5743551 on TLR1 and rs5743795 on TLR6.

OAS1 and OAS2 encode for two enzymes of the 2-5A synthetase family, involved in the innate immune response to viral infections. These molecules are induced by interferons and activate RNase L (product of RNASEL) which degrades viral RNA and inhibits replication. Recently, Molinaro et al. [44] showed that RNA fractions of prostate cancer cell lines are able to bind and activate OAS molecules, whereas RNA fractions of normal prostate epithelial cells cannot. Also, viral infections, sexually transmitted diseases [45,46,47,48,49,50], and infections with Propionibacterium acnes, a gram positive bacterium, [51,52] have been suggested as triggers in prostate cancer. These infectious agents may be cleared after the acute infection. Nonetheless, these agents could possibly induce carcinogenesis through the activation of a chronic inflammatory response [53]. Only one study of the association between prostate cancer and OAS1 was done on a smaller sample size and 3 SNPs different from our selection where an association with rs2660 was found [54].

COX-2 encodes for the enzyme cyclooxygenase-2 (COX-2). COX-2 converts arachidonic acid to prostaglandin H2, which is a precursor for other tissue-specific inflammatory molecules (prostanoids). COX-2 was found to be overexpressed in prostate cancer tissue compared to the surrounding normal prostate tissue [55,56,57]. The association of genetic variants with prostate cancer risk has also been outlined in previous studies, including in the same dataset [27,28,29,30,58]. However, reports on the association between elevated expression of COX-2 in prostate cancer tissues and high Gleason score and recurrence of the disease have mixed results [59,60,61].

Our results are concordant with those reported by Zheng et al. [62] who studied 9,275 SNPs in 1,086 inflammation genes using 200 familial cases and 200 controls of Swedish origin. They observed a significant enrichment in the number of nominal associations observed, suggesting the role of multiple genes with modest effects. However, by using the SKAT, our study is the first analysis of SNP sets pooled across genes and sub-pathways within the innate immunity and inflammation pathway.

None of the SNPs or genes included in our study was reported in any of the genome-wide association studies of prostate cancer listed in the Catalog of Genome-Wide Association studies [63]. Nonetheless, our study has several limitations. First, the limited sample size, and thus limited power, could explain why the association with the whole set of genes is significant while none of the associations with the sub-pathways, genes, or SNPs are significant after correcting for multiple testing. With this sample, the minimum (or maximum for protective) odds ratio detectable with a power of 80% varies between 1.5 (or 0.67) and 2.19 (or 0.46) when the MAF varies between 0.5 and 0.05. Moreover, the limited sample size does not allow evaluating potential heterogeneous effects of variants by ethnicity or other covariates. Second, although a more stringent selection of cases would better describe the role of the innate immunity and inflammation pathway in advanced prostate cancer, it would decrease the sample size – and consequently the power – drastically. Third, our selection of SNPs cannot exclude the possibility for rare functional variants in these candidate genes to play a role in advanced prostate cancer risk.

### Table 3. Cont.

| Gene (chromosome) | SNP | Overall | African Americans | Caucasians |
|-------------------|-----|---------|-------------------|------------|
|                   |     | OR (95% CI) | P-value | OR (95% CI) | P-value | OR (95% CI) | P-value |
| TLR4 (9) rs10759932 | TT 1 (Ref) | 1 (Ref) | 1 (Ref) | 1 (Ref) | 1 (Ref) | 1 (Ref) | 1 (Ref) |
|                   | TC 0.79 (0.60, 1.05) | 0.111 | 0.75 (0.41, 1.38) | 0.360 | 0.81 (0.59, 1.11) | 0.190 |
|                   | CC 4.13 (1.63, 12.6) | 0.005 | 5.67 (1.36, 38.68) | 0.033 | 3.48 (1.05, 13.66) | 0.061 |
|                   | trend (C) | 1.03 (0.81, 1.31) | 0.827 | 1.23 (0.77, 1.99) | 0.388 | 0.97 (0.73, 1.28) | 0.822 |

Odds ratios (OR), 95% confidence intervals (95% CI) and P-values obtained using the unconditional multivariate logistic model adjusted on age, institution, and genetic ancestry (first Principal Component) for SNPs that had at least one of the three tests (heterozygous, rare homozygous or trend) with a P-value below 0.01.

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Third, although the SKAT method provides an ideal framework to test for association with sets of potentially correlated SNPs, it does not measure the increase in risk associated with variants in the set of SNPs.

In conclusion, this study furthers research into prostate cancer genetics by studying SNPs in a candidate pathway at multiple levels of information: whole pathway, sub-pathways, genes, and SNPs. Our results suggest that although it may not be central in the etiology of advanced prostate cancer, the innate immunity and inflammation pathway could play a role in prostate cancer through different genetic variants.

Supporting Information

Table S1 Description of the 320 single nucleotide polymorphisms analyzed. A1: Minor (rare) allele; A2: Other (frequent) allele; A1A1: Rarer homozygous genotype; A1A2: Heterozygous genotype; A2A2: Frequent homozygous genotype; MAF: Minor allele frequency; PHardy-Weinberg: Hardy-Weinberg proportion adequacy test (chi-square test).

Table S2 Association of all SNPs analyzed with advanced prostate cancer risk. The next 3 Excel sheets contain the results of the analyses for the whole sample (Overall) and stratified by ethnicities: African Americans and Caucasians. OR: Odds Ratio; 95% CI: 95% confidence interval; P-value: P-value of the Wald test of association of the heterozygote or rare homozygote genotypes compared to the common homozygote genotype or P-value of the allelic trend test.

Author Contributions

Conceived and designed the experiments: RK JAM IC SJP AML BAR GC JSW. Analyzed the data: RK JAM. Contributed reagents/materials/analysis tools: SJP AML BAR GC. Wrote the paper: RK JAM IC SJP AML BAR GC JSW.

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