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Genetic variation in the Toll-Like Receptor 4 and prostate cancer incidence and mortality

Irene M. Shui¹, Jennifer R. Stark^{1,3,4}, Kathryn L. Penney^{1,3}, Fredrick R. Schumacher⁵, Mara M. Epstein^{1,3}, Michael J. Pitt³, Meir J. Stampfer^{1,2,3}, Rulla M. Tamimi^{1,3}, Sara Lindstrom¹, Howard D. Sesso⁶, Katja Fall^{1,7}, Jing Ma³, Peter Kraft¹, Edward Giovannucci^{1,2,3}, and Lorelei A. Mucci^{1,3,8}

¹ Department of Epidemiology, Harvard School of Public Health, Boston MA ² Department of Nutrition, Harvard School of Public Health, Boston MA ³ Channing Laboratory Brigham and Women's Hospital, Harvard Medical School, Boston MA ⁴ Department of Urology, University of Orebro, Orebro, Sweden ⁵ Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA ⁶ Divisions of Preventive Medicine and Aging, Brigham and Women's Hospital, Harvard Medical School, Boston MA ⁷ Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden ⁸ Centre for Public Health Sciences, University of Iceland, Reykjavik, Iceland

Abstract

Background—Common genetic variants in the Toll-like receptor 4 (TLR4), which is involved in inflammation and immune response pathways, may be important for prostate cancer.

Methods—In a large nested case-control study of prostate cancer in the Physicians' Health Study (1982–2004), 10 single nucleotide polymorphisms (SNPs) were selected and genotyped to capture common variation within the TLR4 gene as well as 5 kilobases up and downstream. Unconditional logistic regression was used to assess associations of these SNPs with total prostate cancer incidence, and with prostate cancers defined as advanced stage/lethal (T3/T4, M1/N1(T1-T4), lethal) or high Gleason grade (7 (4+3) or greater). Cox-proportional hazards regression was used to assess progression to metastases and death among prostate cancer cases.

Results—The study included 1267 controls and 1286 incident prostate cancer cases, including 248 advanced stage/lethal and 306 high grade cases. During a median follow-up of 10.6 years, 183 men died of prostate cancer or developed distant metastases. No statistically significant associations between the TLR4 SNPs were found for total prostate cancer incidence, including SNPs for which an association was reported in other published studies. Additionally, there were no significant associations with TLR4 SNPs and the incidence of advanced stage/lethal, or high grade cancers; nor was there evidence among prostate cancer cases for associations of TLR4 SNPs with progression to prostate cancer specific mortality or bony metastases.

Conclusions—Results from this prospective nested case-control study suggest that genetic variation across TLR4 alone is not strongly associated with prostate cancer risk or mortality.

Keywords

TLR4; prostate cancer; inflammation; molecular epidemiology

Corresponding Author: Irene Shui, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Ave, 9th Floor, Boston, MA 02115, ishui@hsph.harvard.edu.

Conflict of Interest

The authors declare no conflicts of interest

Introduction

Several lines of evidence support the roles of inflammation and infection in prostate cancer development and progression[1]. Chronic inflammation, frequently observed in prostate tumor specimens, can lead to epithelial cell damage and may create a tissue microenvironment favorable for tumor growth and progression[2,3]. There are many potential sources of intraprostatic inflammation, including exposure to certain infectious agents[2]. Common genetic variants involved in pathways related to the host innate immune response could potentially influence prostate cancer risk and progression.

The family of toll-like receptors plays an important role in the innate immune response, particularly in inflammatory responses against exogenous pathogens such as bacteria, virus, fungi and parasites. The toll-like receptor 4 (TLR4) is known to be expressed in the prostate and is a cell surface receptor that recognizes LPS (endotoxin) on gram-negative bacteria, as well as other ligands. Stimulation of TLR4 results in NF-kappaB activation, pro-inflammatory cytokine production, and interferon expression creating an anti-apoptotic and pro-growth microenvironment in the prostate epithelium[4–7]. This experimental evidence has prompted investigation into whether genetic variants in the TLR4 could influence prostate cancer risk through the action of the receptor in response to infection or resulting inflammation[4].

Several case-control studies have investigated TLR4 genetic variation and prostate cancer incidence[8–10]. Table I shows single nucleotide polymorphisms (SNPs) that were significantly associated with prostate cancer risk in at least one study. The Cancer Prostate in Sweden study (CaPS) found a positive association between overall prostate cancer incidence and one SNP (rs11536889)[9]. Conversely, the Health Professionals Follow-up Study (HPFS) found several inverse associations; however, many of the SNPs in this study were in high linkage disequilibrium with one another[10]. The Cleveland Clinic study, which focused on more advanced prostate cancer, showed one significant inverse association (rs5030728) and one significant positive association (rs10759932)[8]. None of the above associations were replicated across the three studies. Lindstrom et al[11], published a pooled analysis from 3 case-control studies, including CaPS, HPFS, as well as the Prostate Lung Colon and Ovarian Cancer Screening Trial (PLCO). This pooled analysis did not yield any significant findings for total prostate cancer and the 58 genotyped and imputed SNPs[11]. Only CaPS has considered prostate cancer specific mortality as an endpoint and did not find any significant associations with TLR4 variation[12]. Given the diversity of findings and the heterogeneity of prostate cancer outcomes, the objectives of this study were to extend this work and examine whether genetic polymorphisms in and around the TLR4 gene are associated with 1) prostate cancer risk, including analyses stratified by stage and grade, using a nested case-control study design or 2) prostate cancer mortality using a case-only survival analysis design.

Materials and methods

Study design and population

Cases and controls for this study are nested in the Physicians' Health Study (PHS) blood cohort. The design and data collection methods of the PHS has been published in detail[13]. Briefly, the PHS originated as a randomized, double-blind, placebo-controlled trial for investigating aspirin and beta-carotene in the prevention of cardiovascular disease and cancer. After the PHS trial ended, participants continued to be followed as an observational cohort. At baseline, 22,071 healthy US male physicians aged 40 to 84 free of diagnosed cardiovascular disease and cancer were randomized, and blood samples were obtained from

14,916 (68%) of the participants. All participants are followed with annual questionnaires to collect data on medical history and other health and lifestyle behaviors. An additional postcard is sent out every 6 months to update information on health endpoints, including prostate cancer. Self-reported cases of prostate cancer are confirmed through systematic medical record review, and information on PSA at diagnosis, tumor stage, and Gleason score is abstracted. We followed men with prostate cancer using questionnaires to collect specific information on the clinical course of disease, treatments, and development of metastases. We validated self-reported metastases in a subsample of physicians with medical records as the standard and observed 100% specificity and 92% sensitivity. Mortality follow-up for cause-specific deaths was obtained through death certificates and next of kin and all prostate cancer specific deaths were further confirmed by medical record review. Follow-up rates are high for both cancer incidence (96%) and mortality (99%).

For this nested case-control study, confirmed incident cases of prostate cancer diagnosed from 1982 to 2004 were identified from men in the blood sub-cohort. Risk set sampling was used to match cases of incident prostate cancer with controls on baseline age (± 1 year if aged ≤ 55 years and ± 5 years if aged > 55 years) and smoking status (current, former, or never smoker). Advanced stage/lethal prostate cancer was defined as those having a tumor stage at diagnosis of T3/T4, M1/N1(T1–T4) or progression to bony metastases/prostate cancer specific death in follow-up through March 2009. High Gleason-grade prostate cancer were those with Gleason grade of 7 (4+3) or greater. For the case-only study, follow-up for progression began at the time of prostate cancer diagnosis and continued through March 2009. Men were followed until they developed the main endpoint of lethal prostate cancer (metastases to bone or death from prostate cancer), or were censored either at the end of follow up (March 2009) or if they died from causes other than prostate cancer. To reduce the potential for population stratification we restricted the study population to Caucasians, who make up 94% of the PHS population.

Genotype assessment

Using the HapMap database, 10 linkage disequilibrium tag-SNPs were selected to capture variation with $R^2 > 0.8$ within the TLR4 gene and 5kb up and downstream (See Figure 1 for the linkage disequilibrium (LD) plot). SNP selection was restricted to those with a minor allele frequency (MAF) of $\geq 5\%$ in the International HapMap Phase II CEU (Utah residents of Northern and Western European Ancestry) samples (Version 2, release 21). We imputed 9 additional SNPs using the observed genotypes, the HapMap Phase II CEU data and the MACH imputation program (<http://www.sph.umich.edu/csg/abecasis/MACH/>). The imputation included one SNP not directly tagged (rs10759930), a nonsynonymous SNP that had been genotyped in all prior studies (rs4986790), and an additional 7 SNPs that were significant ($p < 0.05$) in at least one published study. All imputed SNPs had an R^2 quality score of > 0.8 . Table I shows that with the exception of rs11536891, which was not in HapMap Phase II, we were able to capture all SNPs that were shown to have a significant association with prostate cancer in previous studies using the 19 genotyped or imputed SNPs.

All blood samples were labeled with an ID number only and matched case-control pairs were handled identically and assayed in the same batch in a blinded fashion. DNA was extracted from whole blood and genotyping was performed with Sequenom iPLEX matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry technology at the Harvard Medical School-Partners Healthcare Center for Genetics and Genomics. All SNPs had $> 95\%$ genotype completion rates and there was 100% concordance for the blinded quality control samples.

Data analysis

Hardy-Weinberg equilibrium tests for each of the SNPs were performed among controls using the Pearson's goodness-of-fit test using a cutoff of $p < 0.01$. Because we did not have an *a priori* hypothesis regarding the model of inheritance, when the frequency of rare homozygotes was $\geq 5\%$, we present the results for both the co-dominant and additive genotype models. When the frequency of rare homozygotes was $< 5\%$, we present results for only the additive model. Imputation yielded a continuous variable of 0–2 minor alleles for each imputed SNP. Thus, for imputed SNPs, an allele dosage model was used to take into account the uncertainty in the imputation process.

The association between each TLR4 SNP and the incidence of prostate cancer was assessed using unconditional logistic regression models, adjusted for matching factors. Analyses restricted to advanced stage/lethal cases or high-grade cases used all controls. For the survival analysis among the cases, we used Cox-proportional hazards models adjusting for age at diagnosis to estimate the hazard ratios of each SNP for progression to bony metastases or prostate cancer specific death in the case only analysis. All reported p-values are 2-sided and not adjusted for multiple testing. However, given that there are 10 tag SNPs and 9 additional imputed SNPs, we also considered a correction for multiple testing using the method proposed by Gao et al (2008)[14] which takes into account the LD within the set of SNPs being tested to calculate the effective number of independent tests. Analyses were conducted with SAS v9.1 statistical software (SAS Institute, Cary, NC).

Results

Selected characteristics of the study participants are presented in table II. The case-control analysis included 1286 prostate cancer cases and 1267 controls; there were 248 advanced stage/lethal cases and 306 high Gleason grade cases. An additional 45 men who were originally controls subsequently developed prostate cancer and were also included in the case only survival analysis. During a median of 10.6 years of follow-up, 183 men died of prostate cancer or developed metastases to bone and 326 died from other causes.

All SNPs were in Hardy-Weinberg equilibrium ($p > 0.01$). Tables III and IV present the results for the 10 tag SNPs; the 9 imputed SNPs are shown in Supplementary Tables I and II. We did not observe any significant associations between the TLR4 SNPs and total prostate cancer risk, including SNPs for which an association was reported in other published studies (rs11536889, rs6478317, rs10116253, rs1927914, rs1927911, rs2149356, rs7873784, rs1153689). We also did not observe any significant associations between the TLR4 SNPs and advanced stage/lethal (Table III) or high Gleason grade cancers (data not shown). The associations did not differ by age at diagnosis (age < 65 vs. age ≥ 65) (data not shown). The case-only survival analysis (Table IV) also did not yield any significant associations between the TLR4 SNPs and the rate of progression to lethal prostate cancer. Adjusting for clinical characteristics (e.g. stage, Gleason score, and PSA at diagnosis) did not change the interpretation of the results.

Discussion

Previous studies evaluating TLR4 variation and prostate cancer risk found significant but inconsistent findings that were not replicated (Table I). Additionally, of the 10 significant SNPs found in the HPFS study, several were in strong LD with one another (9 of the SNPs were captured by just 3 SNPs in our study). In this study, by using a linkage disequilibrium tagging approach along with imputation we were able to capture the common genetic variation in TLR4 and 5kb up and downstream, including the majority of SNPs analyzed in previous studies. We did not replicate any previous findings and found no new significant

associations between the TLR4 SNPs assessed and prostate cancer incidence, including for advanced stage, lethal, or high Gleason grade cases. Unlike the CaPS and HPFS studies, we did not find any associations among prostate cancer cases diagnosed among younger men aged <65 years. The inconsistencies of the prior studies and the null findings of the current study likely represent false positive findings in the earlier studies. A recent meta-analysis combining data from the HPFS, CaPS study and PLCO studies also reported a lack of association between genetic variation in TLR4 and prostate cancer incidence[11]. There may be other explanations for the lack of replication, including differences in study population characteristics such as the case mix (e.g. the proportion of clinically significant cancers) or exposure to different environmental factors (e.g. infections) that could modify the relationship between various SNPs and prostate cancer outcomes. Both the HPFS study and our study were limited to Caucasian male health professionals from the United States and were made up mostly of lower grade, localized stage cases, while the CaPS study contained more advanced stage cancers overall. In our study we were able to distinguish high grade and/or advanced stage and lethal incident cancers which represent endpoints that may be more relevant in the era of PSA screening. However, we found a similar lack of association between genetic variation in TLR4 and these more advanced or aggressive incident prostate cancers. Likewise, our results for prostate cancer survival also did not show any significant associations and are consistent with the null results found in the CaPS study.

Strengths of our study include its high follow-up rates, and the careful assessment of prostate cancer clinical characteristics through chart and pathology report review. This study is one of the largest and most comprehensive nested case-control studies on genetic variation of TLR4 and, importantly, had a substantial number of high-grade and advanced stage/lethal cancers, and was able to assess the relationship of TLR4 and prostate cancer survival. However, despite the relatively large sample size, our study is still underpowered to detect smaller magnitude associations of SNPs, especially with lethal prostate cancer. For example, we had 80% power to detect an OR=1.45 for lethal prostate cancer and SNPs with a MAF=0.2 at an alpha=0.05; for SNPs with a MAF=0.05 and lethal prostate cancer, we had 80% power to detect an OR=1.85. Previous studies observed ORs in the range of 1.4 to 4.6 for total prostate cancer, which our study was powered to detect. Finally, our study did not assess rare variants (MAF<0.05) in TLR4 and the results may not be generalizable to non-Caucasian populations who may have different genetic variation across TLR4.

Conclusions

In summary, this study suggests that TLR4 genetic variation alone does not contribute strongly to the risk of prostate cancer incidence or mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

TLR4 SNPs significantly associated ($p < 0.05$) with prostate cancer incidence in at least one published study and in the current study (Physicians' Health Study)

Table 1

SNP	Location	CaPS ^a	HPFS ^b	Cleveland ^c	PHS ^d
rs11536889	3' UTR	Positive	Not significant	Not significant	Not significant
rs6478317 ^e	5' UTR	Not significant	Inverse	Not significant	Not significant
rs10116253 ^e	5' UTR	Not significant	Inverse	Not significant	Not significant
rs1927914 ^e	5' UTR	Not significant	Inverse	Not significant	Not significant
rs1927911 ^e	Intron	Not significant	Inverse	Not significant	Not significant
rs2149356 ^e	Intron	Not in HWE	Inverse	Not significant	Not significant
rs7873784 ^f	3' UTR	Not significant	Inverse	Not significant	Not significant
rs11536891 ^g	3' UTR	Not significant	Inverse	---	---
rs11536898 ^f	3' UTR		Inverse	---	Not significant
rs10759932 ^h	5' UTR	Not significant	Inverse	Positive	Not significant
rs5030728	Intron		Not significant	Inverse	Not significant
rs5030717 ^h	Intron	Not significant	Inverse	Positive	Not significant

^a CaPS: Cancer of the Prostate in Sweden, 1383 cases (Gleason score 8–10: 18%; Stage $\geq$ T3: 30%) Positive association for rs11536889: OR=1.26

^b HPFS: Health Professionals Follow-up Study, 700 cases (Gleason score 8–10: 10%; Stage $\geq$ T3b or N1 or M1 or lethal: 9%) Inverse associations: rs6478317, rs10116253: OR=0.66; rs1927914, rs1927911, rs2149356: OR=0.64; rs7873784: OR=0.51; rs11536891: OR=0.50; rs11536898: OR=0.38; rs10759932: OR=0.73; rs5030717: OR=0.66

^c Cleveland: Cleveland Clinic Case-control Study, 506 advanced cases (Gleason score 8–10 or Stage $\geq$ T2c or PSA at dx of $>10\text{ng/mL}$) Positive association for rs10759932: OR=4.62 (1.55–13.78); rs5030717 tagged by rs10759932 Inverse association for rs5030728: OR=0.60 (0.37–0.97)

^d PHS: Physicians' Health Study, 1286 cases (Gleason score 8–10: 14.7%; Stage $\geq$ T3 or N1 or M1: 11.6%)

--- = not captured

^e In LD with rs2149356

^f In LD with rs7873784

^g Not in HapMap Phase II

^h In LD with rs10759932

Study population characteristics of case-control participants nested in the Physicians' Health Study, 1982–2009

Table II

	Cases (n=1286)	Controls (n=1267)
Age at randomization (years), mean (s.d.)	57.9 (8.4)	57.5 (8.4)
Stage and Grade		
Tumor stage, n (%)		
T1,T2	1079 (88.4)	
T3,T4 or M1/N1(T1–T4)	142 (11.6)	
*missing 65		
Gleason grade, n (%)		
2–6	608 (52)	
7:3+4	239 (21)	
7:4+3	134 (11)	
7: primary score undefined	15 (1)	
8–10	172 (15)	
*missing 118		
Case Only Analysis	Cases (n=1331) ^a	
Age at diagnosis, years mean (s.d.)	70.1 (7.4)	
Deaths/metastases due to PCa, N (%)	183 (14)	
Deaths	168 (13)	
Metastases	15 (1)	
Follow-up time (years)		
To event (PCa deaths/mets), median (interquartile range)	5.7 (3.2, 9.5)	
To censored, median (interquartile range)	10.6 (7.7, 14.5)	

^aIncludes an additional 45 men who were originally selected as controls but later developed prostate cancer during follow-up.

Table III
Genotype frequencies, odds ratios (OR) and 95% confidence intervals (CI) for TLR 4 tagSNPs and prostate cancer incidence Physicians' Health Study

Rs number	Genotype	Total Prostate Cancer			Advanced Prostate Cancer ^d			Lethal Prostate Cancer ^d		
		Genotype Frequency ^a cases, n (%)	controls ^b , n (%)	OR (95% CI) ^c	P-value	Genotype Frequency ^a cases, n (%)	OR (95% CI)	P-value	Genotype Frequency ^a cases, n (%)	P-value
rs11536869	AA	1156 (94)	1125 (94)	1.00 (reference)		209 (92)	1.00 (reference)		150 (91)	1.00 (reference)
	AG	72 (6)	77 (6)			16 (7)			14 (8)	
	GG	4 (0)	1 (0)			1 (0)			1 (1)	
	additive			1.00 (0.73, 1.36)	0.97		1.16 (0.68, 2.00)	0.58		1.41 (0.79, 2.51)
rs2148356	CC	579 (49)	576 (50)	1.00 (reference)		107 (50)	1.00 (reference)		76 (48)	1.00 (reference)
	AC	489 (42)	460 (40)	1.06 (0.89, 1.26)		86 (40)	1.02 (0.74, 1.40)		65 (41)	1.10 (0.76, 1.59)
	AA	106 (9)	119 (10)	0.88 (0.66, 1.17)	0.43	23 (11)	1.06 (0.64, 1.76)	0.98	18 (11)	1.15 (0.65, 2.05)
	additive			0.98 (0.87, 1.11)	0.75		1.03 (0.82, 1.28)	0.83		1.08 (0.84, 1.40)
rs7846989	TT	1017 (84)	995 (83)	1.00 (reference)		192 (86)	1.00 (reference)		137 (84)	1.00 (reference)
	TC	193 (16)	189 (16)			29 (13)			24 (15)	
	CC	8 (1)	14 (1)			3 (1)			2 (1)	
	additive			0.94 (0.77, 1.14)	0.52		0.84 (0.58, 1.22)	0.36		0.91 (0.61, 1.38)
rs787384	GG	887 (74)	861 (73)	1.00 (reference)		161 (73)	1.00 (reference)		118 (74)	1.00 (reference)
	GC	295 (24)	302 (26)			57 (26)			39 (25)	
	CC	24 (2)	19 (2)			2 (1)			2 (1)	
	additive			0.98 (0.83, 1.16)	0.84		0.94 (0.69, 1.29)	0.71		0.90 (0.63, 1.29)
rs11536897	GG	1140 (91)	1124 (91)	1.00 (reference)		218 (90)	1.00 (reference)		163 (92)	1.00 (reference)
	GA	115 (9)	102 (8)			25 (10)			15 (8)	
	AA	2 (0)	3 (0)			0 (0)			0 (0)	
	additive			1.07 (0.82, 1.40)	0.60		1.26 (0.80, 2.00)	0.32		0.99 (0.56, 1.75)
rs12351464 (rs11536857)	CC	1122 (89)	1082 (88)	1.00 (reference)		220 (90)	1.00 (reference)		161 (90)	1.00 (reference)
	CT	131 (10)	144 (12)			22 (9)			16 (9)	
	TT	8 (1)	5 (0)			2 (1)			2 (1)	
	additive			0.93 (0.74, 1.17)	0.51		0.89 (0.58, 1.38)	0.61		0.97 (0.59, 1.58)
rs1554973	TT	710 (57)	697 (57)	1.00 (reference)		144 (60)	1.00 (reference)		106 (60)	1.00 (reference)
	TC	467 (37)	438 (36)	1.04 (0.88, 1.23)		82 (34)	0.88 (0.65, 1.20)		62 (35)	0.91 (0.64, 1.28)

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Rs number	Genotype	Total Prostate Cancer				Advanced Prostate Cancer ^d				Lethal Prostate Cancer ^d			
		Genotype Frequency ^a		OR (95% CI) ^c	P-value	Genotype Frequency ^a		OR (95% CI)	P-value	Genotype Frequency ^a		OR (95% CI)	P-value
cases, n (%)	controls ^b , n (%)	cases, n (%)	cases, n (%)			cases, n (%)	cases, n (%)						
rs2737191	CC	71 (6)	84 (7)	0.82 (0.59, 1.15)	0.39	16 (7)	0.97 (0.54, 1.75)	0.72	10 (6)	0.79 (0.39, 1.63)	0.73		
	additive			0.97 (0.86, 1.11)	0.67		0.93 (0.74, 1.18)	0.57		0.90 (0.68, 1.18)	0.44		
	AA	665 (53)	674 (56)	1.00 (reference)		134 (56)	1.00 (reference)		96 (55)	1.00 (reference)			
	AG	490 (39)	445 (37)	1.12 (0.94, 1.32)		91 (38)	1.04 (0.77, 1.41)		68 (39)	1.10 (0.78, 1.56)			
	GG	96 (8)	95 (8)	1.02 (0.76, 1.39)	0.43	16 (7)	0.84 (0.47, 1.50)	0.78	12 (7)	0.89 (0.46, 1.73)	0.77		
rs10789932	additive			1.06 (0.93, 1.20)	0.39		0.97 (0.78, 1.22)	0.82		1.01 (0.78, 1.31)	0.92		
	TT	897 (76)	908 (77)	1.00 (reference)		177 (74)	1.00 (reference)		127 (72)	1.00 (reference)			
	TC	260 (22)	244 (21)			58 (24)			46 (26)				
	CC	27 (2)	27 (2)			5 (2)			4 (2)				
	additive			1.06 (0.90, 1.25)	0.52		1.12 (0.85, 1.50)	0.42		1.22 (0.89, 1.69)	0.22		
rs11536889	GG	909 (74)	897 (74)	1.00 (reference)		176 (73)	1.00 (reference)		134 (76)	1.00 (reference)			
	GC	292 (24)	291 (24)			58 (24)			39 (22)				
	CC	32 (3)	27 (2)			6 (3)			4 (2)				
	additive			1.02 (0.87, 1.19)	0.83		1.06 (0.79, 1.41)	0.71		0.92 (0.65, 1.30)	0.63		

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^a Frequencies may differ due to missing genotype information; percentages may not sum to 100 due to rounding

^b All analyses use the same controls

^c Adjusted for matching factors: age at randomization, smoking status, and duration of follow-up

^d Advanced prostate cancer defined as stage T3, T4 or M1/N1 (T1–T4) or lethal; lethal prostate cancer defined as progression to bony metastases or prostate cancer specific death

Genotype frequencies, hazard ratios (HR) and 95% confidence intervals (CI) for TLR4 tagSNPs and prostate cancer deaths and metastases

Table IV

rs number	Genotype	Genotype Frequency ^a			HR (95% CI) ^b	p-value
		Death/mets, n (%)	all other cases, n (%)			
rs11536869	AA	151 (90)	1043 (94)	1.00 (reference)		
	AG	15 (9)	61 (6)			
	GG	1 (1)	3 (0)			
	additive				1.45 (0.90, 2.35)	0.13
rs2149356	CC	77 (48)	525 (50)	1.00 (reference)		
	AC	66 (41)	442 (42)	1.01 (0.73, 1.40)		
	AA	18 (11)	88 (8)	1.34 (0.80, 2.24)		0.52
	additive			1.10 (0.87, 1.40)		0.41
rs7846989	TT	139 (84)	913 (83)	1.00 (reference)		
	TC	24 (15)	176 (16)			
	CC	2 (1)	6 (1)			
	additive			1.03 (0.69, 1.52)		0.90
rs7873784	GG	120 (75)	803 (74)	1.00 (reference)		
	GC	39 (24)	262 (24)			
	CC	2 (1)	22 (2)			
	additive			0.95 (0.69, 1.30)		0.74
rs11536897	GG	165 (92)	1017 (91)	1.00 (reference)		
	GA	15 (8)	102 (9)			
	AA	0 (0)	2 (0)			
	additive			0.91 (0.54, 1.53)		0.72
rs12351464 (rs11536857)	CC	163 (90)	995 (89)	1.00 (reference)		
	CT	16 (9)	123 (11)			
	TT	2 (1)	6 (1)			
	additive			0.92 (0.60, 1.43)		0.72
rs1554973	TT	108 (60)	630 (57)	1.00 (reference)		
	TC	62 (34)	419 (38)	0.87 (0.64, 1.19)		
	CC	10 (6)	63 (6)	1.01 (0.53, 1.94)		0.66

rs number	Genotype Frequency ^a			
	Genotype	Death/mets, n (%)	all other cases, n (%)	HR (95% CI) ^b p-value
rs2737191	additive			0.93 (0.72, 1.19) 0.56
	AA	97 (55)	594 (53)	1.00 (reference)
	AG	69 (39)	435 (39)	0.99 (0.73, 1.34)
	GG	12 (7)	87 (8)	0.91 (0.50, 1.65) 0.95
rs10759932	additive			0.97 (0.77, 1.23) 0.79
	TT	128 (72)	806 (77)	1.00 (reference)
	TC	47 (26)	220 (21)	
	CC	4 (2)	23 (2)	
rs11536889	additive			1.14 (0.86, 1.52) 0.35
	GG	135 (75)	805 (73)	1.00 (reference)
	GC	40 (22)	264 (24)	
	CC	4 (2)	29 (3)	
	additive			0.94 (0.70, 1.27) 0.70

^aFrequency of death/mets prostate cancer may not sum to 183 due to missing genotype information, percentages may not sum to 100 due to rounding

^bAdjusted for age at diagnosis