Interactions of Sequence Variants in *Interleukin-1 Receptor–Associated Kinase4* and the *Toll-Like Receptor 6-1-10* Gene Cluster Increase Prostate Cancer Risk

Jielin Sun,[1] Fredrik Wiklund,[3,4] Fang-Chi Hsu,[2] Katarina Bälter,[4] S. Lilly Zheng,[1] Jan-Erik Johansson,[5] Baoli Chang,[1] Wennuan Liu,[1] Tao Li,[1] Aubrey R. Turner,[1] Liwu Li,[1] Ge Li,[1] Hans-Olov Adami,[4] William B. Isaacs,[6] Jianfeng Xu,[1,2] and Henrik Grönberg[3,4]

[1]Center for Human Genomics and [2]Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina; [3]Department of Radiation Sciences, Oncology, University of Umeå, Umeå, Sweden; [4]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [5]Department of Urology and Clinical Medicine, Örebro University Hospital and Regional Oncological Center, University Hospital Uppsala, Sweden; and [6]Department of Urology, Johns Hopkins Medical Institutions, Baltimore, Maryland

Abstract

Chronic or recurrent inflammation has been suggested as a causal factor in several human malignancies, including prostate cancer. Genetic predisposition is also a strong risk factor in the development of prostate cancer. In particular, Toll-like receptors (TLR), especially the *TLR6-1-10* gene cluster, are involved in prostate cancer development. Interleukin-1 receptor-associated kinases (IRAK) 1 and 4 are critical components in the TLR signaling pathway. In this large case-control study, we tested two hypotheses: (*a*) sequence variants in *IRAK1* and *IRAK4* are associated with prostate cancer risk and (*b*) sequence variants in *IRAK1/4* and *TLR1-6-10* interacts and confers a stronger risk to prostate cancer. We analyzed 11 single nucleotide polymorphisms (four in *IRAK1* and seven in *IRAK4*) among 1,383 newly diagnosed prostate cancer patients and 780 population controls in Sweden. Although the single-nucleotide polymorphisms in *IRAK1* and *IRAK4* alone were not significantly associated with prostate cancer risk, one single-nucleotide polymorphism in *IRAK4*, when combined with the high-risk genotype at *TLR6-1-10*, conferred a significant excess risk of prostate cancer. In particular, men with the risk genotype at *TLR6-1-10* and *IRAK4*-7987 CG/CC had an odds ratio of 9.68 (P = 0.03) when compared with men who had wild-type genotypes. Our findings suggest synergistic effects between sequence variants in *IRAK4* and the *TLR 6-1-10* gene cluster. Although this study was based on *a priori* hypothesis and was designed to address many common issues facing this type of study, our results need confirmation in even larger studies. (Cancer Epidemiol Biomarkers Prev 2006;15(3):480–5)

Introduction

Prostate cancer is the most common cancer and second leading cause of death in the Unites States (1) and in Western Europe (2). Many factors contribute to prostate cancer, and genetic susceptibility is among the most important of these (3). Recently, chronic inflammation has been suggested as a causal component in the development of a variety of malignancies, including cancer of the stomach, liver, and prostate (4-7). The cause of inflammation in the prostate remains unclear, but in some cases may reflect a specific response to microbial pathogens. Two of the three reported prostate cancer susceptibility genes (*RNASEL* and *MSR1*) are involved in innate immunity, supporting the hypothesis that variations in genes regulating innate immunity may modify both host response to infection and susceptibility to prostate cancer (8, 9).

Recently, we found that sequence variants in four Toll-like receptors (*TLR*) genes, the *TLR6-TLR1-TLR10* cluster, are associated with prostate cancer risk (10). Evidence for association between *TLR6-TLR1-TLR10* sequence variants and prostate cancer risk is particularly well supported, as 11 of the 17 variants in this gene cluster are highly significant (10). These findings suggest that innate immunity, particularly the TLR, influences prostate cancer development. The TLR family, recognizing a variety of ligands including pathogens, is involved in pathogen-mediated inflammation (11). The engagement of ligands on these receptors initiates a downstream signaling cascade, leading to the transcription of target genes, including inflammatory cytokines (tumor necrosis factor-α, interleukin-12) and costimulatory molecules.

Interleukin-1 receptor–associated kinases (IRAK) are essential components in the Toll/interleukin-1 receptor (TIR) signaling pathway. Until now, four IRAKs have been identified in mammals. Whereas IRAK1 and IRAK4 are expressed in all tissues, IRAK2 and IRAK-M have a narrower cellular distribution (12-15). A large body of biological evidence suggests that IRAKs play important roles in the TIR signaling pathway. *IRAK4* knockout mice are completely nonresponsive to all TIR signaling (16). In contrast, when *IRAK1* knockout mice were stimulated with IL-1 and lipopolysaccharide, the response was reduced but was not completely abolished (17, 18), suggesting that *IRAK1* is involved in fine-tuning the TIR signaling pathway. Abnormalities in these two kinase genes are likely to be responsible for impaired TIR signaling, resulting in increased innate immunity responses and increased normal inflammatory responses; this, in turn, may contribute to the onset of various inflammatory diseases such as prostate cancer.

Received 8/18/05; revised 12/5/05; accepted 1/20/06.

Grant support: This study was partially supported by National Institutes of Health grants CA 105055 (to J.X.), and CA 95052 (to J.X.).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Jianfeng Xu, Medical Center Boulevard, Winston-Salem, NC 27157. Phone: 336-713-7500; Fax: 336-713-7566. E-mail: jxu@wfubmc.edu

Copyright © 2006 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-05-0645

Downloaded from http://aacrjournals.org/cebp/article-pdf/15/3/480/2244708/480.pdf by guest on 06 October 2024

Considering the critical role of IRAKs in the TLR signaling pathway, we hypothesized that sequence variants in the *IRAK1* and *IRAK4* genes are associated with prostate cancer risk. Moreover, considering the intertwined roles of the TLR and IRAK genes in this signaling pathway and the significant association between sequence variants in the *TLR6-TLR1-TLR10* gene cluster and prostate cancer risk, we further hypothesized *a priori* that interactions between variants in *TLR6-TLR1-TLR10* gene cluster and these two IRAK genes increase prostate cancer risk.

To test these hypotheses, we systematically evaluated two IRAK genes, *IRAK1* and *IRAK4*, in a large and well-characterized prostate cancer case and control population in Sweden. This is the first published report on the potential interaction between these genes in any disease.

Materials and Methods

Study Population. The study base for the first phase of Cancer Prostate in Sweden-1 has been described in detail elsewhere (19). Briefly, Cancer Prostate in Sweden-1 is a large-scale, population-based case-control study in Sweden. Prostate cancer patients were identified and recruited from four of six regional cancer registries in Sweden. The inclusion criteria for case subjects in Cancer Prostate in Sweden-1 were pathology- or cytology-verified adenocarcinoma of the prostate, diagnosed between July 1, 2001, and September 30, 2002. Control subjects were randomly selected from the continuously updated Swedish Population Register and frequency-matched according to age (within 5 years) and geographic origin of the case subjects. In total, 1,444 case and 866 control subjects were recruited. Among all participants, DNA samples and questionnaires were available for 1,383 case and 780 control subjects, representing an 83% and 52% participation rate among all eligible case and control subjects, respectively.

Clinical information, such as tumor-node-metastasis stage (20), Gleason grade, and prostate-specific antigen levels at diagnosis were available from either cancer registries or the National Prostate Cancer Registry for 94% of the case subjects. The clinical characteristics of the study subjects are presented in Table 1. The case subjects were classified as having advanced disease if they met any of the following criteria: $T_{3/4}$, N_+, M_+, grade 3, Gleason score sum of 8 to 10, or a prostate-specific antigen level of >50 ng/mL. All other case subjects were classified as having localized disease.

The study received institutional approval at Umeå University and Karolinska Institutet in Sweden, and Wake Forest University in United States. All participants provided written informed consent.

Genotyping Methods. Genotyping was done using the MassARRAY system (Sequenom, Inc., San Diego, CA). For the MassARRAY assay, PCR and extension primers for sequence variants were designed using SpectroDesigner software (Sequenom). The primer information is available at the corresponding author's web site (www.wfubmc.edu/genomics). PCR and extension reactions were done according to the instructions of the manufacturer, and extension product sizes were determined by mass spectrometry.

Selection of Polymorphisms. The *IRAK1* and *IRAK4* genes are mapped to Xq28 and 12q12, respectively. The length of transcripts for *IRAK1* and *IRAK4* are ~9 and 29 kb. *IRAK1* is composed of 14 exons, whereas the *IRAK4* gene contains 12 exons. The single nucleotide polymorphism (SNP) information for *IRAK1* was obtained from the web site http://innate-immunity.net/data/homology (October 2003). The SNP information for *IRAK4* was obtained from two web sites, http://pga.gs.washington.edu (October 2003) and http://snpper.chip.org (21). We used two criteria to select SNPs from a large number of SNPs in these Web sites. (*a*) We selected SNPs with a minor allele frequency of at least 5% at a resolution of one SNP per kb across the genomic region of each gene, including 2.5 kb of the promoter, all exons, introns, and 3′ untranslated region. For the *IRAK4* gene, a resolution of 1 SNP per 2 kb was used because of the large size of this gene. (*b*) We also selected all of the nonsynonymous SNPs in these genes, regardless of resolution.

Table 1. Characteristics of study subjects in Cancer Prostate in Sweden-1

Variables	Case subjects, *n* (%)	Control subjects, *n* (%)
Age at study (y)		
<60	268 (19.38)	111 (14.23)
60-69	607 (43.89)	330 (42.31)
70-79	508 (36.73)	339 (43.36)
T stage*		
T_0	6 (0.46)	NA
T_1	459 (35.15)	NA
T_2	442 (33.84)	NA
T_3	351 (26.88)	NA
T_4	48 (3.68)	NA
N stage		
N_0	223 (83.21)	NA
N_1	45 (16.79)	NA
M stage		
M_0	578 (81.52)	NA
M_1	131 (18.48)	NA
Gleason score		
≤4	53 (4.45)	NA
5	142 (11.92)	NA
6	421 (35.34)	NA
7	365 (30.65)	NA
8	123 (10.32)	NA
9	77 (6.46)	NA
10	10 (0.84)	NA
Differential grade		
1	69 (13.37)	NA
2	305 (59.11)	NA
3	142 (27.52)	NA
Prostate-specific antigen levels (ng/mL)		
<4	75 (5.79)	NA
4-9.99	433 (33.41)	NA
10-19.99	291 (22.45)	NA
20-49.99	220 (16.97)	NA
50-99.99	126 (9.7)	NA
≥100	151 (11.65)	NA

*Tumor-node-metastasis staging according to ref. 21.

Haplotype Block Construction. The haplotypes of the seven SNPs in the *IRAK4* gene were estimated using a computer program, PHASE 2.0 (www.stats.ox.ac.uk/mathgen/software.html) based on genotypes of the 780 controls. Haplotype blocks in this region were constructed using a web-based program Haploblockfinder (http://cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi/). We used the threshold of minimal pair-wise $D' \geq 0.8$ to define haplotype blocks.

Statistical Analysis. Tests of Hardy-Weinberg equilibrium and pairwise linkage disequilibrium for each of the SNPs in *IRAK4* were carried out using an exact test (22). Empirical *P* values for the Hardy-Weinberg equilibrium test were based on 10,000 permutation tests.

For each SNP, allele frequency differences between cases and controls were tested using a χ^2 test with 1 degree of freedom. The genotype frequency differences were tested using a χ^2 test with 2 degrees of freedom. Odds ratios (OR) of prostate cancer for the variant-allele carriers versus wild-type allele carriers were estimated by logistic regression after adjusting for age. Association between the haplotypes and prostate cancer risk was done using a likelihood ratio test and a χ^2 test implemented in SAS/Genetics.

Downloaded from http://aacrjournals.org/cebp/article-pdf/15/3/480/2244708/480.pdf by guest on 06 October 2024

The combined (interaction) effect of the three genes in the *TLR6-TLR1-TLR10* cluster and the four *IRAK* genes on prostate cancer risk was assessed using a logistic regression framework, with adjustment for age. Because of strong linkage disequilibrium among SNPs in the *TLR6-1-10* gene cluster, we were able to use only one SNP, TLR1 −6399C/T, which was in the middle of several of the most significant SNPs in this gene cluster, to represent this gene cluster and to ultimately to decrease the number of tests. Based on our previous study of the TLR gene cluster (10), we defined *a priori* CC/CT as the risk genotypes for TLR1 (TLR positive), and TT as the nonrisk genotype (TLR negative). For IRAK1/4, we assumed that rare allele genotypes are the high-risk genotypes (IRAK positive) and the homozygous common allele is the low-risk genotype (IRAK negative). Consequently, we classified each individual based on their combined TLR and IRAK genotypes.

Men who carried presumed low-risk genotypes of both genes (−,−) were used as the reference group. The remaining individuals are grouped into three test groups (dummy variables). Men who were TLR negative and IRAK positive comprised group 1 (−,+), TLR positive and IRAK negative group 2 (+,−), and TLR positive and IRAK positive group 3 (+,+). We tested for both additive and multiplicative interaction effects (23) using three dummy variables (groups) in a logistic regression model. In brief, the expected effect of (+,+) considering an additive model is equal to the sum of the effects of (−,+) and (+,−); and the expected effect of (+,+) considering a multiplicative model is equal to the multiplicative product of the effects of (−,+) and (+,−). Additive or multiplicative interaction effects are suggested to be present if the observed effect of (+,+) is larger than the expected value considering additive or multiplicative models. All statistical tests were done using SAS/computer software (version 9.1, SAS Institute, Cary, NC).

Results

SNP Selection and Haplotype Block Construction. Based on the two criteria described in Materials and Methods, we selected 16 SNPs in the *IRAK1* gene, including all five of the reported nonsynonymous changes: S532L, C203S, F196S, R194H, and T113I. We also selected 16 SNPs in the *IRAK4* gene, including all three of the reported nonsynonymous changes: S98R, H390R, and A428T. We genotyped these 32 SNPs among 96 randomly selected controls. Nineteen SNPs were monomorphic in our population, with 11 of these in *IRAK1* and eight in *IRAK4*, and thus were excluded from additional study. We also excluded other two SNPs for which PCR results remained inconsistent even after repeated attempts at optimization. Haplotypes of *IRAK1* can be directly evaluated because this gene resides on the X-chromosome. For the seven SNPs we examined in the *IRAK4* gene, we identified two haplotype blocks: the first six SNPs were contained within one block and only the last SNP, 20791A/G, was located in the second block.

Allele, Genotype, and Haplotype Frequencies of IRAK1 and IRAK4 SNPs in Cases and Controls. We genotyped 11 SNPs, including four in *IRAK1* and seven in *IRAK4* in all 1,383 and 780 controls whose DNA samples were available at the time of this study. All seven of the SNPs in *IRAK4* were in Hardy-Weinberg equilibrium among case and control groups, respectively (all $P > 0.05$).

The distribution of genotype frequencies among cases and controls are presented in Table 2. No statistically significant differences in allele or genotype frequencies between cases and controls were observed for any of these SNPs.

For the *IRAK1* gene at Xq28, 10 haplotypes were observed, four of which are common (frequency > 0.01; Table 3). However, no significant differences in haplotype frequencies between cases and controls were observed. For the *IRAK4* gene

Table 2. Distribution of SNPs in *IRAK1* and *IRAK4* for prostate cancer cases and controls

SNPs	Position	Genotype	Cases, *n* (%)	Controls, *n* (%)	*P*	
					Allele	Genotype
IRAK1						
F196S (rs1059702)	Exon	C	1,164 (87.39)	650 (86.90)	NA	0.75
		T	168 (12.61)	98 (13.10)		
4788C/T (rs7061789)	Intron	T	1,106 (82.17)	610 (81.88)	NA	0.87
		C	240 (17.83)	135 (18.12)		
S532L (rs1059703)	Exon	T	1,165 (86.55)	645 (86.11)	NA	0.78
		C	181 (13.45)	104 (13.89)		
9373G/T (rs3027898)	3′	T	1,102 (81.63)	611 (81.47)	NA	0.93
		G	248 (18.37)	139 (18.53)		
IRAK4						
−13656T/C (rs1057190)	Promoter	TT	1,057 (77.78)	612 (80.21)	0.37	0.10
		CT	291 (21.41)	140 (18.35)		
		CC	11 (0.81)	11 (1.44)		
−7225G/T (rs4251431)	Intron	GG	1,058 (82.08)	599 (83.54)	0.48	0.64
		TG	220 (17.07)	111 (15.48)		
		TT	11 (0.85)	7 (0.98)		
−2001A/G (rs4251571)	Intron	AA	1,283 (96.03)	715 (95.33)	0.50	0.53
		AG	52 (3.89)	35 (4.67)		
		GG	1 (0.07)	0 (0.00)		
652T/C (rs4251459)	Intron	TT	1,061 (78.42)	621 (81.28)	0.26	0.07
		TC	281 (20.77)	132 (17.28)		
		CC	11 (0.81)	11 (1.44)		
7987G/C (rs4251487)	Intron	GG	1,319 (96.47)	744 (97.77)	0.08	0.21
		CG	47 (3.46)	17 (2.23)		
		CC	1 (0.07)	0 (0.00)		
A428G (rs4251545)	Exon	GG	1,114 (81.37)	634 (82.77)	0.52	0.60
		GA	245 (17.90)	125 (16.32)		
		AA	10 (0.73)	7 (0.91)		
20791A/G (rs4251559)	3′	AA	429 (32.48)	237 (32.38)	0.80	0.93
		AG	625 (47.31)	342 (46.72)		
		GG	267 (20.21)	153 (20.90)		

NOTE: Positions of *IRAK1* and *IRAK4* are based on the translating start site of ATG.

Downloaded from http://aacrjournals.org/cebp/article-pdf/15/3/480/2244708/480.pdf by guest on 06 October 2024

Table 3. Haplotype frequencies in *IRAK* and *IRAK4* among prostate cancer case subjects and control subjects from the Cancer Prostate in Sweden-1 study

	Haplotype	Frequencies		P*
		Case	Control	
IRAK1	CTTT	0.81	0.82	0.60
	TCCG	0.12	0.12	0.83
	CCTG	0.04	0.04	0.62
	CCCG	0.01	0.02	0.37
IRAK4	TGATGGA	0.52	0.52	0.83
	TGATGGG	0.35	0.36	0.58
	CTACGAG	0.07	0.06	0.14
	CTACGAA	0.02	0.03	0.07
	TGGTGGG	0.02	0.02	0.18
	CGACCGA	0.02	0.01	0.24
	Overall			0.44

*Overall P was calculated using a likelihood ratio test; single haplotype P was calculated using the χ^2 test. Both tests were implemented in SAS/Genetics. No overall test was conducted for *IRAK1*.

at 12q12, six common haplotypes were inferred in this population (Table 3). Again, we did not observe significant differences in the haplotype frequencies between cases and controls.

Interaction between the TLR1 (TLR1 −6399C/T) and IRAK1/4 SNPs. When we examined interactions between the TLR gene cluster and IRAK1/4 on prostate cancer risk, we found a multiplicative interaction between a SNP in *IRAK4* (7987G/C) and TLR1. Men who were TLR positive and IRAK positive (+,+) were ~10 times more likely to have prostate cancer when compared with the reference group [−,−; OR, 9.68; P = 0.03; 95% confidence interval (95% CI), 1.27-73.96; Fig. 1B]. The excess risk for men in this group (group 3) was larger than the product of the risk for group 1 (OR, 1.47) and 2 (OR, 1.33), suggesting a multiplicative interaction (Table 4). However, this risk estimate for this joint effect has wide confidence intervals due to small cell count.

We also found an expected additive effect between a *TLR1* SNP and an *IRAK4* SNP (652T/C). Men that were TLR positive and IRAK positive (+,+) had a significant 59% increased prostate cancer risk when compared with the reference group (−,−; OR, 1.59; P = 0.02; 95% CI, 1.06-2.40; Table 4; Fig. 1A). In contrast, we found no significant excess of risk for the joint effect among men in group 1 (−,+; OR, 1.24; P = 0.11; 95% CI, 0.95-1.63). The increased risk of 35% (OR, 1.35; P < 0.01; 95% CI, 1.08-1.69) among men in group 2 (+,−) was statistically significant. The risk in the TLR-positive and IRAK-positive group (+,+) is equal to the sum of the excess risks in groups 1 (−,+) and 2 (+,−), suggesting an expected additive effect considering the additive model.

Discussion

The hypothesis that gene-to-gene interactions play a role in susceptibility to common human diseases is not new (24). Multiple modifier genes, not just an individual gene, are considered responsible for the onset of many common diseases, a theory supported by the ubiquitous biomolecular interactions among gene transcripts and biochemical pathways (25). Functional studies have defined interactions between TLRs, adaptors, and kinases (11). We observed a gene-to-gene interaction between sequence variants in *IRAK4* and *TLR1*; when combined with the risk genotype at *TLR6-1-10*, one SNP in *IRAK4* conferred a multiplicative risk of prostate cancer. Our study is the first evaluation of gene-to-gene interaction effects among these key genes in the TIR signaling pathway in relation to prostate cancer. These results strengthen the hypothesis that inflammation is etiologically relevant in prostate cancer and that several genes in the TIR signaling pathway, instead of a single gene, influence this process.

Four key features of this study that strengthen our findings are the comprehensive evaluation of SNPs in kinase and adaptor genes, the use of a relatively homogeneous study population, a large sample size, as well as a well-defined *a priori* hypothesis. The comprehensive evaluation of 11 SNPs in *IRAK1* and *IRAK4* allows us to capture most of the common genetic variations in these two genes. The relatively homogeneous study population decreases the degree of locus and allelic heterogeneity and, therefore, increases our ability to detect genetic association and minimizes the potential for population stratification that can occur in case control study designs. The large sample size provides greater confidence in the validity of all results except for the multiplicative interaction effect. Finally, the *a priori* hypotheses of this study are well supported from functional studies.

However, the positive interaction finding of our study should be interpreted with caution. Gene-to-gene interaction studies generally suffer from several limitations, including inflated type I error due to multiple tests and small cell counts as outcomes of many potential genotype combinations. For example, even in our study where we considerably decreased

Downloaded from http://aacrjournals.org/cebp/article-pdf/15/3/480/2244708/480.pdf by guest on 06 October 2024

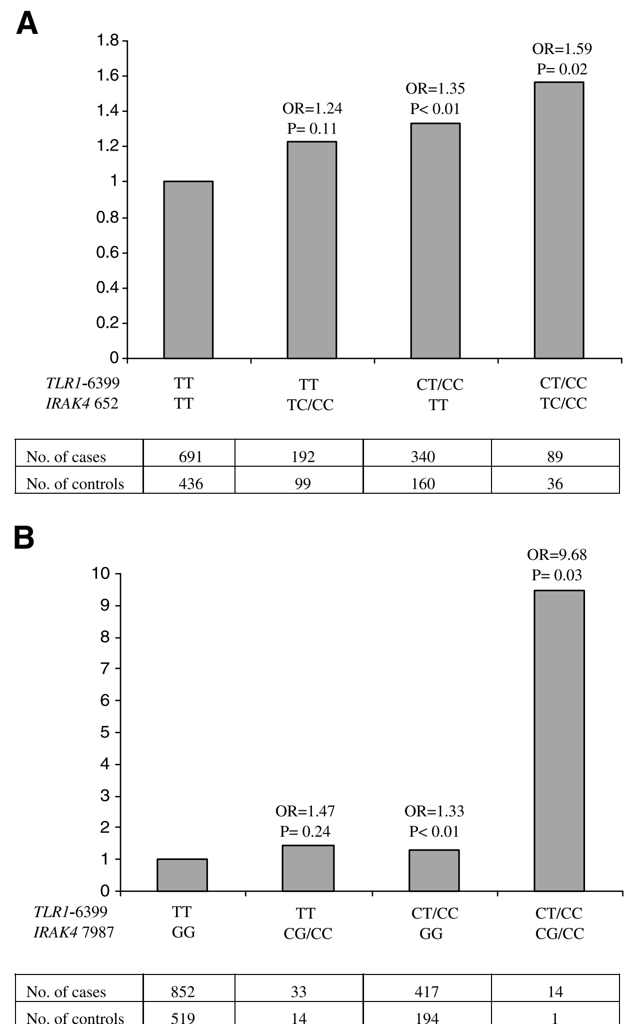

Figure 1. Interaction and additive effect of *TLR1* and *IRAK4* genotypes in prostate cancer cases and controls. *Columns,* ORs between different combinations of genotypes for *TLR1* −6399C/T and *IRAK4* 652T/C (**A**) or *IRAK4* 7978G/C (**B**). The nonrisk genotype for each gene was used as a reference OR.

Table 4. Interaction of TLR1 and IRAK4 genotypes in prostate cancer cases and controls

SNPs	Genotype	Genotype of *TLR1* −6399T/C	Group	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	*P*
IRAK1							
F196S (rs1059702)	C	TT	Reference	772 (59.71)	458 (63.88)	1.00	
	T	TT	1	100 (7.73)	69 (9.62)	0.88 (0.64-1.23)	0.46
	C	CT/CC	2	359 (27.76)	163 (22.73)	1.33 (1.07-1.65)	<0.01
	T	CT/CC	3	62 (4.80)	27 (3.77)	1.36 (0.86-2.17)	0.19
4788C/T (rs7061789)	T	TT	Reference	739 (56.58)	431 (60.36)	1.00	
	C	TT	1	144 (11.03)	94 (13.17)	0.94 (0.71-1.25)	0.66
	T	CT/CC	2	336 (25.73)	152 (21.29)	1.35 (1.08-1.69)	<0.01
	C	CT/CC	3	87 (6.66)	37 (5.18)	1.42 (0.95-2.12)	0.09
S532L (rs1059703)	T	TT	Reference	772 (59.07)	453 (63.09)	1.00	
	C	TT	1	106 (8.11)	72 (10.03)	0.90 (0.66-1.24)	0.53
	T	CT/CC	2	361 (27.62)	163 (22.70)	1.35 (1.09-1.67)	<0.01
	C	CT/CC	3	68 (5.20)	30 (4.18)	1.37 (0.88-2.13)	0.17
9373G/T (rs3027898)	T	TT	Reference	738 (56.34)	431 (59.94)	1.00	
	G	TT	1	149 (11.37)	97 (13.49)	0.93 (0.71-1.24)	0.63
	T	CT/CC	2	334 (25.50)	153 (21.28)	1.33 (1.06-1.66)	0.01
	G	CT/CC	3	89 (6.79)	38 (5.29)	1.39 (0.94-2.08)	0.10
IRAK4							
−13656T/C (rs1057190)	TT	TT	Reference	689 (52.36)	433 (59.23)	1.00	
	CT/CC	TT	1	198 (15.05)	101 (13.82)	1.26 (0.97-1.65)	0.09
	TT	CT/CC	2	336 (25.53)	156 (21.34)	1.37 (1.10-1.71)	<0.01
	CT/CC	CT/CC	3	93 (7.07)	41 (5.61)	1.46 (0.99-2.15)	0.05
−7225G/T (rs4251431)	GG	TT	Reference	693 (55.48)	423 (61.39)	1.00	
	TG/TT	TT	1	154 (12.33)	81 (11.76)	1.19 (0.89-1.59)	0.25
	GG	CT/CC	2	334 (26.74)	153 (22.21)	1.35 (1.08-1.69)	<0.01
	TG/TT	CT/CC	3	68 (5.44)	32 (4.64)	1.35 (0.87-2.08)	0.18
−2001A/G (rs4251571)	AA	TT	Reference	840 (64.86)	504 (70.19)	1.00	
	AG/GG	TT	1	36 (2.78)	23 (3.20)	0.95 (0.56-1.63)	0.86
	AA	CT/CC	2	404 (31.20)	180 (25.07)	1.37 (1.11-1.68)	<0.01
	AG/GG	CT/CC	3	15 (1.16)	11 (1.53)	0.80 (0.36-1.76)	0.58
652T/C (rs4251459)	TT	TT	Reference	691 (52.67)	436 (59.64)	1.00	
	TC/CC	TT	1	192 (14.63)	99 (13.54)	1.24 (0.95-1.63)	0.11
	TT	CT/CC	2	340 (25.91)	160 (21.89)	1.35 (1.08-1.68)	<0.01
	TC/CC	CT/CC	3	89 (6.78)	36 (4.92)	1.59 (1.06-2.39)	0.02
7987G/C (rs4251487)	GG	TT	Reference	852 (64.74)	519 (71.29)	1.00	
	CG/CC	TT	1	33 (2.51)	14 (1.92)	1.47 (0.78-2.77)	0.24
	GG	CT/CC	2	417 (31.69)	194 (26.65)	1.33 (1.09-1.62)	<0.01
	CG/CC	CT/CC	3	14 (1.06)	1 (0.14)	9.68 (1.27-73.96)	0.03
A428G (rs4251545)	GG	TT	Reference	727 (54.91)	446 (60.85)	1.00	
	GA/AA	TT	1	165 (12.46)	89 (12.14)	1.17 (0.88-1.55)	0.28
	GG	CT/CC	2	352 (26.59)	162 (22.10)	1.36 (1.09-1.69)	<0.01
	GA/AA	CT/CC	3	80 (6.04)	36 (4.91)	1.40 (0.93-2.10)	0.11
20791A/G (rs4251559)	AA	TT	Reference	275 (21.47)	177 (25.21)	1.00	
	AG/GG	TT	1	591 (46.14)	337 (48.01)	1.20 (0.98-1.48)	0.08
	AA	CT/CC	2	142 (11.09)	56 (7.98)	1.72 (1.21-2.43)	<0.01
	AG/GG	CT/CC	3	273 (21.31)	132 (18.80)	1.41 (1.09-1.83)	0.01

*ORs adjusted by age.

the number of potential tests by fixing on one variant that was implicated from our previous single-gene study (*TLR1*) and only varied the genotyped variants in the *IRAK* genes, there were still 33 tests for interaction effects. A further complication of multiple tests in genetic studies is the difficulty of accurately correcting for multiple tests. For this reason, we were not able to adjust for multiple tests in this study, and our results should be interpreted within this context. Our study also suffered from small cell counts in several tests, even with the large overall sample size. The small cell counts could lead to chance significance or an unstable risk estimate. For example, our major finding of a 9-fold increased risk for the interaction was supported by only 14 cases and 1 control in the cells that were both TLR positive and IRAK positive (+,+). Although caution should be exercised when interpreting this statistically significant result, we cannot exclude the possibility that this statistical interaction reflects an underlying biological interaction between these two SNPs. Our results warrant confirmation in even larger studies.

The interaction and additive effects between the *IRAK4* SNPs (7987G/C and 652T/C) and the *TLR1* SNP −6399C/T may be a direct result of biological interactions between these specific SNPs, or, alternatively, may indirectly reflect other SNPs that are in linkage disequilibrium with these genotyped SNPs. *TLR1* −6399C/T is located in the promoter region and may potentially influence transcription of the *TLR1* gene. Those two *IRAK4* SNPs reside in introns and the nucleotide substitutions in those sites may alter gene splicing. Abnormal expression of TLR1 and IRAK4 directly or indirectly related to these SNPs, may lead to altered responses in the TIR signaling pathway. This, in turn, may affect inflammatory responses that have been implicated in the development of prostate cancer (7). Considering the intriguing genetic findings and the biologically plausible relationship between these genes, replication in other large studies are warranted.

One weakness of our study is the unbalanced number of cases and controls due to the significantly low ratio of controls to cases (0.56). As described in Materials and Methods, this is a population-based and frequency-matched (age and geographic) case-control study. The smaller number of controls (n = 780), compared with cases (n = 1,383), reflects a lower response rate among controls (52%). One of the potential problems associated with a low response rate among controls could be a selection bias where control subjects who have a family history of prostate cancer are more likely to participate in the study. This potential bias, together with the decreased statistical power associated with a smaller number of control

Downloaded from http://aacrjournals.org/cebp/article-pdf/15/3/480/2244708/480.pdf by guest on 06 October 2024

subjects, may bias the results toward the null. Although this poses some concern, we feel it does not alter the interpretation of the current study because associations for interaction effect have been observed.

In summary, our results support the hypothesis that interaction effects between sequence variants of *IRAK4* and *TLR1* contribute to the development of prostate cancer. This study, together with our previous observations of association with *TLR1-6-10* and *TLR4* sequence variants, provides further evidence that inflammation plays an important role in the initiation and progression of prostate cancer.

References

1. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. CA Cancer J Clin 2004;54:8 – 29.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74 – 108.
3. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78 – 85.
4. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539 – 45.
5. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860 – 7.
6. Nelson WG, DeWeese TL, DeMarzo AM. The diet, prostate inflammation, and the development of prostate cancer. Cancer Metastasis Rev 2002;21:3 – 16.
7. Nelson WG, De Marzo AM, DeWeese TL, Isaacs WB. The role of inflammation in the pathogenesis of prostate cancer. J Urol 2004;172: S6 – 11.
8. Carpten J, Nupponen N, Isaacs S, et al. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet 2002;302:181 – 4.
9. Xu J, Zheng SL, Komiya A, et al. Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat Genet 2002;32:321 – 5.
10. Sun J, Wiklund F, Zheng SL, et al. Sequence variants in Toll-like receptor gene cluster TLR6-1-TLR10 and prostate cancer risk. J Natl Cancer Inst 2005; 97:525 – 32.
11. Singh BP, Chauhan RS, Singhal LK. Toll-like receptors and their role in innate immunity. Curr Sci 2003;85:1156 – 64.
12. Trofimova M, Sprenkle AB, Green M, Sturgill TW, Goebl MG, Harrington MA. Developmental and tissue-specific expression of mouse pelle-like protein kinase. J Biol Chem 1996;271:17609 – 12.
13. Muzio M, Ni J, Feng P, Dixit VM. IRAK Pelle. family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. Science 1997;278:1612 – 5.
14. Rosati O, Martin MU. Identification and characterization of murine IRAK-2. Biochem Biophys Res Commun 2002;297:52 – 8.
15. Li S, Strelow A, Fontana EJ, Wesche H. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. Proc Natl Acad Sci USA 2002; 99:5567 – 72.
16. Suzuki N, Suzuki S, Duncan GS, et al. Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. Nature 2002;416: 750 – 6.
17. Thomas JA, Allen JL, Tsen M, et al. Impaired cytokine signaling in mice lacking the IL-1 receptor-associated kinase. J Immunol 1999;163:978 – 84.
18. Kanakaraj P, Schafer PH, Cavender DE, et al. Interleukin IL-1 receptor-associated kinase IRAK requirement for optimal induction of multiple IL-1 signaling pathways and IL-6 production. J Exp Med 1998;187:2073 – 9.
19. Zheng SL, Augustsson-Balter K, Chang B, et al. Sequence variants of toll-like receptor 4 are associated with prostate cancer risk: results from the Cancer Prostate in Sweden Study. Cancer Res 2004;64:2918 – 22.
20. Schroder FH, Hermanek P, Denis L, Fair WR, Gospodarowicz MK, Pavone-Macaluso M. The TNM classification of prostate cancer. Prostate Suppl 1992; 4:129 – 38.
21. Riva A, Kohane IS. A SNP-centric database for the investigation of the human genome. BMC Bioinformatics 2004;5:33.
22. Weir BS. Genetic analysis II: methods for discrete population genetic data. Sunderland (MA): Sinauer Association; 1996.
23. Gordis L. Epidemiology. 2nd ed. Philadelphia (PA): W.B. Saunders Co.; 2000.
24. Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Hum Hered 2003;56:73 – 82.
25. Moore JH, Williams SM. Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis. Bioessays 2005;27:637 – 46.

Downloaded from http://aacrjournals.org/cebp/article-pdf/15/3/480/2244708/480.pdf by guest on 06 October 2024