Null Results in Brief

MIC1 and *IL1RN* Genetic Variation and Advanced Prostate Cancer Risk

Iona Cheng,[1] Lisa M. Krumroy,[2] Sarah J. Plummer,[2] Graham Casey,[2] and John S. Witte[1]

[1]Department of Epidemiology and Biostatistics and Center of Human Genetics, University of California, San Francisco, San Francisco, California and [2]Department of Cancer Biology, Lerner Research Institute, The Cleveland Clinic, Cleveland, Ohio

Introduction

Recently, polymorphisms in *macrophage inhibitory cytokine-1* (*MIC1*) and *interleukin 1 receptor antagonist* (*IL1RN*) were identified to be associated with prostate cancer risk (1-3). MIC-1 is a divergent member of the transforming growth factor-β superfamily of cytokines. In the Cancer Prostate in Sweden study, the nonsynonymous *MIC1* H6D polymorphism was associated with a lowered risk of prostate cancer [CG versus CC; odds ratio (OR), 0.80; 95% confidence interval (95% CI), 0.66-0.97; ref. 2], whereas an Australian study found this polymorphism associated with a nonsignificant lowered risk of prostate cancer (CG/GG versus CC; OR, 0.85; 95% CI, 0.70-1.04) yet an increased risk of prostate cancer death (CG/GG versus CC; OR, 1.72; 95% CI, 1.06-2.78; ref. 1).

IL1RN inhibits the proinflammatory response of interleukin 1-α and interleukin 1-β cytokines. The *IL1RN* haplotype (ATGC) was significantly associated with prostate cancer risk in the Cancer Prostate in Sweden study (homozygous carriers versus noncarriers; OR, 1.6; 95% CI, 1.2-2.2), with larger effects observed among advanced disease (homozygous carriers versus noncarriers; OR, 1.8; 95% CI, 1.3-2.5; ref. 3). To further investigate these previous reports, we comprehensively surveyed the common genetic variation of *MIC1* and *IL1RN* and tested whether inherited differences at these loci predispose men to advanced prostate cancer.

Materials and Methods

Study Subjects. This study includes 506 advanced incident prostate cancer cases and 506 controls from the major medical institutions in Cleveland, Ohio. Advanced prostate cancer cases were defined as having either a Gleason score ≥7, tumor-node-metastasis stage $\geq T_{2c}$, or prostate-specific antigen at diagnosis >10 ng/mL. Controls were frequency matched to cases by age (within 5 years), racial/ethnic group, and medical institution. Detailed information about this study has been reported previously (4). Institutional Review Board approval was obtained from the participating medical institutions, and informed consent was obtained from all study participants.

Genetic Characterization and Tag Single Nucleotide Polymorphism Selection. We determined the genetic structure of *MIC1* and *IL1RN* by using publicly available genotype data from the International HapMap project[3] (5). For *MIC1*, we evaluated 12 single nucleotide polymorphisms (SNP; minor allele frequency, >5% among Caucasian pedigrees; average density, 1 SNP/477 bps) that spanned ~3 kbs upstream of the transcription start site and ~2 kb downstream of the 3′ untranslated region. For *IL1RN*, we examined 41 SNPs (average density, 1 SNP/440 bps) that spanned ~1 kb upstream and ~700 bps downstream. We did not capture the genetic variation of African populations because our sample size did not have sufficient power for African American–specific analyses.

To capture the common genetic variation for *MIC1* and *IL1RN*, we identified tag SNPs using the Tagger software[4] (6). We selected 6 and 7 tag SNPs for *MIC1* and *IL1RN*, respectively, which had a minimum $r^2 > 0.8$ with the unmeasured SNPs for each gene (Supplementary Table A). For *MIC1*, we ''forced in'' the previously associated H6D polymorphism (rs1058587) to be selected as a tag SNP. The 6 tag SNPs for *MIC1* and 7 tag SNPs for *IL1RN* captured all 12 and 41 SNPs, respectively, with an average r^2 of 98.8% and 95.9%, respectively.

Genotyping. Genotyping was done by the Taqman allelic discrimination assay. One SNP (rs1058587) could not be assayed by Taqman and was genotyped using the Amplifluor SNPs HT Genotyping system. All assays were read on a 7900HT Sequence Detection System. All assays were undertaken by individuals blinded to case-control status. For *MIC1* and *IL1RN*, the average genotyping success rate was 100% and 99.9%, respectively, and the concordance rate for 2% replicate samples was 100% for both genes. There were no deviations from Hardy-Weinberg equilibrium ($P > 0.01$).

Statistical Analysis. ORs and 95% CI were estimated by unconditional logistic regression to examine the association between *MIC1* and *IL1RN* SNPs and multimarker haplotypes and prostate cancer risk. We estimated multimarker haplotype frequencies by the expectation-maximization algorithm using the tagSNP software (7). OR estimates were adjusted for the matching variables: age, racial/ethnic group, and medical institution. All reported *P* values are two sided.

Results

MIC1. For the previously associated H6D polymorphism, the allele frequency among Caucasians (controls/cases, 27.2%/

(Cancer Epidemiol Biomarkers Prev 2007;16(6):1309–11)

Received 2/21/07; accepted 4/10/07.

Grant support: NIH grants CA88164, CA94211, and CA98683 and NIH R25T training grant CA112355-01A1 (I. Cheng).

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.

Note: Supplementary data for this article are available at Cancer Epidemiology Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Requests for reprints: John S. Witte, Department of Epidemiology and Biostatistics, University of California, San Francisco, CA 94143-0794. E-mail: wittej@humgen.ucsf.edu

doi:10.1158/1055-9965.EPI-07-0165

[3] http://www.hapmap.org
[4] http://www.broad.mit.edu/tagger

26.5%) was similar to the Swedish (controls/cases, 28.8%/27.7%) and Australian (controls/cases, 26.5%/24.3%) studies (1, 2). Our analysis detected a statistically nonsignificant inverse association between the H6D polymorphism and prostate cancer risk (CG/GG versus CC; OR, 0.85; 95% CI, 0.66-1.09; $P = 0.20$). A weaker nonsignificant association was seen among Caucasians (CG/GG versus CC; OR, 0.93; 95% CI, 0.71-1.22; $P = 0.58$; Supplementary Table B). There were no significant associations between the five remaining *MIC1* SNPs and prostate cancer risk (Table 1).

IL1RN. The allele frequencies for *IL1RN* between prostate cancer cases and controls were similar (*P*s for allele frequency differences > 0.13). There were no significant association between the 7 *IL1RN* tag SNPs and 4 multimarker haplotypes and prostate cancer risk (Table 2; Supplementary Table C).

Discussion

In this comprehensive evaluation of the *MIC1* and *IL1RN*, we found no substantial influence of common genetic variation in these two genes on prostate cancer risk. We did observe a nonsignificant inverse association between the *MIC1* H6D polymorphism and prostate cancer as seen in the Australian study (1). To further clarify this effect, we genotyped the H6D polymorphism in a sibling study of 439 prostate cancer cases and 479 unaffected brothers (~90% White, 9% African American, and 1% Asian or Latino; ref. 8). By using a sibling-based design, we exclude the potential of bias due to population stratification as controls and cases were ascertained from the same genetic source. In this sibling study, there was no association between the H6D polymorphism and prostate cancer risk (CG/CC versus GG; OR, 1.18; 95% CI, 0.82-1.7; $P = 0.37$).

The Swedish study of *IL1RN* observed no association between *IL1RN* SNPs and prostate cancer risk, although the common haplotype (ATGC) was significantly associated with prostate cancer and greater effects were observed among advanced disease (3). In our study, there was no association between *IL1RN* SNPs and prostate cancer. Furthermore, we estimated the common haplotypes (>5%) across *IL1RN* and observed no significant effects (*P*s > 0.16; data not shown). In contrast to the Swedish study, which used a haplotype tagging approach to capture the genetic variation of the locus from 16 polymorphisms, our study used a tagging approach (6) that reconstructed 41 common polymorphisms across the *IL1RN* locus.

In summary, our study does not support the role of common genetic variation at *MIC1* and *IL1RN* in prostate cancer susceptibility. We had 80% power to detect a minimum OR of 1.48 for a SNP with a 10% allele frequency ($\alpha = 0.05$; two-sided hypothesis test; log-linear model; ref. 9). Future studies should investigate whether other inflammatory genes or the combined effects of several genes in the inflammatory pathway are more likely to influence prostate cancer risk.

Table 1. Association between *MIC1* SNPs and prostate cancer risk (*N* = 1,012)

	Controls, *n* (%)	Case, *n* (%)	OR (95% CI)*	*P*
H6D† (rs1058587)				
CC	273 (54.1)	292 (58.1)	1	
CG	205 (40.6)	180 (35.8)	0.82 (0.63-1.07)	0.14
GG	27 (5.4)	31 (6.2)	1.07 (0.62-1.85)	0.81
CG/GG	232 (45.9)	211 (42.0)	0.85 (0.66-1.09)	0.20
rs1059519				
CC	245 (48.4)	224 (44.3)	1.00	
CG	206 (40.7)	225 (44.5)	1.05 (0.69-1.60)	0.81
GG	55 (10.9)	57 (11.3)	0.88 (0.58-1.33)	0.54
rs1059369				
TT	317 (62.8)	314 (62.2)	1.00	
AT	161 (31.9)	156 (30.9)	0.98 (0.75-1.28)	0.88
AA	27 (5.4)	35 (6.9)	1.31 (0.77-2.22)	0.32
rs1227731				
GG	382 (75.5)	388 (76.7)	1.00	
AG	118 (23.3)	114 (22.5)	0.95 (0.71-1.28)	0.73
AA	6 (1.2)	4 (0.8)	0.65 (0.18-2.34)	0.51
rs16982345				
GG	277 (54.7)	293 (58.0)	1.00	
AG	204 (40.3)	178 (35.3)	0.82 (0.63-1.07)	0.14
AA	25 (4.9)	34 (6.7)	1.29 (0.75-2.22)	0.37
rs8101249				
AA	309 (61.2)	318 (63.0)	1.00	
AC	160 (31.7)	160 (31.7)	0.95 (0.72-1.26)	0.74
CC	36 (7.1)	27 (5.4)	0.69 (0.39-1.21)	0.19

*Adjusted for age, racial/ethnic group, and institution.
†CG is the reverse strand.

Table 2. Association between *IL1RN* variants and prostate cancer risk (*N* = 1,012)

SNP	Controls, *n* (%)	Case, *n* (%)	OR (95% CI)*	*P*
rs3087263				
GG	419 (82.8)	433 (85.6)	1.00	
AG	83 (16.4)	69 (13.6)	0.80 (0.57-1.14)	0.22
AA	4 (0.8)	4 (0.8)	0.96 (0.24-3.88)	0.96
rs380092				
AA	201 (39.8)	208 (41.1)	1.00	
AT	214 (42.4)	213 (42.1)	1.07 (0.74-1.54)	0.72
TT	90 (17.8)	85 (16.8)	1.12 (0.76-1.64)	0.56
rs4252019				
CC	341 (67.4)	344 (68.0)	1.00	
CT	141 (27.9)	141 (27.9)	0.99 (0.74-1.31)	0.92
TT	24 (4.7)	21 (4.2)	0.85 (0.45-1.62)	0.63
rs579543				
CC	301 (59.5)	291 (57.5)	1.00	
CT	177 (35.0)	180 (35.6)	0.95 (0.73-1.24)	0.95
TT	28 (5.5)	35 (6.9)	0.77 (0.46-1.30)	0.68
rs315951				
CC	234 (46.3)	231 (45.7)	1.00	
CG	219 (43.3)	218 (43.1)	1.01 (0.78-1.32)	0.95
GG	53 (10.5)	57 (11.3)	1.09 (0.72-1.66)	0.68
rs4252041				
CC	474 (93.7)	470 (93.1)	1.00	
CT	32 (6.3)	33 (6.5)	1.04 (0.63-1.73)	0.88
TT	0 (0)	2 (0.4)	—	—
rs9005				
GG	254 (50.2)	268 (53.0)	1.00	
AG	206 (40.7)	206 (40.7)	0.95 (0.73-1.23)	0.68
AA	46 (9.1)	32 (6.3)	0.66 (0.41-1.07)	0.09
Multimarker haplotype				
rs3087263, rs579543:GG				
0	36 (7.1)	29 (5.7)	1.00	
1 copy	182 (36.0)	178 (35.2)	1.22 (0.72-2.08)	0.46
2 copies	288 (56.9)	299 (59.1)	1.30 (0.77-2.19)	0.32
rs380092, rs579543:AG				
0	201 (39.7)	193 (38.1)	1.00	
1 copy	238 (47.0)	225 (44.5)	0.99 (0.75-1.31)	0.94
2 copies	67 (13.2)	88 (17.4)	1.38 (0.94-2.03)	0.10
rs4252019, rs579543:CG				
0	96 (19.0)	89 (17.6)	1.00	
1 copy	246 (48.6)	237 (46.8)	1.04 (0.74-1.46)	0.84
2 copies	164 (32.4)	180 (36.6)	1.18 (0.83-1.70)	0.36
rs579543, rs315951:GG				
0	47 (9.3)	34 (6.7)	1.00	
1 copy	209 (41.3)	211 (41.7)	1.41 (0.87-2.29)	0.16
2 copies	250 (49.4)	261 (51.6)	1.46 (0.91-2.34)	0.12

*Adjusted for age, racial/ethnic group, and institution.

Acknowledgments

We thank the participants of this study who have contributed to a better understanding of the genetic contributions to prostate cancer susceptibility.

References

1. Hayes VM, Severi G, Southey MC, et al. Macrophage inhibitory cytokine-1 H6D polymorphism, prostate cancer risk, and survival. Cancer Epidemiol Biomarkers Prev 2006;15:1223–5.
2. Lindmark F, Zheng SL, Wiklund F, et al. H6D polymorphism in macrophage-inhibitory cytokine-1 gene associated with prostate cancer. J Natl Cancer Inst 2004;96:1248–54.
3. Lindmark F, Zheng SL, Wiklund F, et al. Interleukin-1 receptor antagonist haplotype associated with prostate cancer risk. Br J Cancer 2005;93:493–7.
4. Liu X, Plummer SJ, Nock NL, et al. Nonsteroidal antiinflammatory drugs and decreased risk of advanced prostate cancer: modification by lymphotoxin α. Am J Epidemiol 2006;164:984–9.
5. Altshuler D, Brooks LD, Chakravarti A, et al. A haplotype map of the human genome. Nature 2005;437:1299–320.
6. de Bakker PI, Yelensky R, Pe'er I, et al. Efficiency and power in genetic association studies. Nat Genet 2005;37:1217–23.
7. Stram DO, Haiman CA, Hirschhorn JN, et al. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. Hum Hered 2003;55:27–36.
8. Plummer SJ, Conti DV, Paris PL, et al. CYP3A4 and CYP3A5 genotypes, haplotypes, and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2003;12:928–32.
9. Gauderman WJ. Sample size requirements for matched case-control studies of gene-environment interaction. Stat Med 2002;21:35–50.