Research Article

Cancer Epidemiology, Biomarkers & Prevention

Prostate Cancer Susceptibility Variants Confer Increased Risk of Disease Progression

Iona Cheng[1], Sarah J. Plummer[2], Christine Neslund-Dudas[3], Eric A. Klein[4], Graham Casey[2], Benjamin A. Rybicki[3], and John S. Witte[5]

Abstract

Background: Genome-wide association studies have identified numerous single nucleotide polymorphisms (SNP) associated with the risk of prostate cancer. Our objective was to determine whether these SNPs affect the progression of prostate cancer.

Methods: We genotyped 26 SNPs previously associated with prostate cancer risk among 788 aggressive prostate cancer patients who were treated by radical prostatectomy or radiation therapy. Prostate cancer progression was defined as biochemical recurrence based on posttreatment prostate-specific antigen levels of >0.3 ng/mL for radical prostatectomy patients or a 2-ng/mL increase above the nadir for radiation therapy patients, initiation of hormone treatment, or metastases. We assessed the association between independent and combined SNPs and disease progression by Cox proportional hazards regression.

Results: Five SNPs showed independent associations with prostate cancer progression (rs12621278, rs629242, rs9364554, rs4430796, and rs5945572) based on stepwise regression analysis. The strongest SNP was rs12621278 in the *ITGA6* locus, which was associated with a 2.4-fold increased risk of progression (P = 0.0003). When considering the sum of risk alleles across these five SNPs, each additional allele was associated with a 29% increase in risk of progression (95% confidence interval, 1.12-1-47).

Conclusions: We found that five of the recently highlighted prostate cancer susceptibility loci also influence prostate cancer progression beyond the known clinicopathologic predictors. If confirmed, these genetic variants might help clarify which tumors are likely to progress and require more aggressive treatment in contrast to those that might not have substantial effects on morbidity or mortality.

Impact: Genetic susceptibility variants for prostate cancer development may also inform disease progression. *Cancer Epidemiol Biomarkers Prev; 19(9); 2124–32. ©2010 AACR.*

Introduction

Prostate cancer is the most commonly diagnosed cancer in men in the United States and is the second leading cause of cancer death. The widespread use of serum prostate-specific antigen (PSA) for prostate cancer screening and the use of extended pattern biopsy techniques have led to a profound stage migration, with the majority of prostate tumors detected today being of low grade, limited volume, clinically localized, and associated with low serum PSA (1). Nevertheless, it remains unclear exactly which men should receive the most aggressive treatment and which tumors are most likely to progress following treatment, raising the possibility of overtreatment of prostate cancer (2).

At present, there are clinical and pathologic measures such as Gleason grade, tumor stage, and prognostic nonograms that help predict which prostate tumors have poor prognosis and the highest potential for recurrence and/or death (3). Even with such prognostic measures, most men diagnosed with this disease undergo radical prostatectomy or radiation treatment. Side effects due to treatment and their effect on quality of life highlight the importance of sparing men from unnecessary treatment and the need for specific markers affecting disease prognosis. There is clear clinical utility in identifying additional factors that can distinguish between prostate tumors that will likely recur, progress rapidly, and be life-threatening versus those that might not have substantial effect on morbidity or mortality once treated.

Authors' Affiliations: [1]Epidemiology Program, Cancer Research Center of Hawai`i, University of Hawai`i, Honolulu, Hawaii; [2]Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California; [3]Department of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, Michigan; [4]Department of Urology, Cleveland Clinic Taussig Cancer Institute and Glickman Urological and Kidney Institute, Cleveland, Ohio; and [5]Departments of Epidemiology & Biostatistics and Urology, Institute for Human Genetics, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California

Corresponding Author: John S. Witte, Departments of Epidemiology & Biostatistics and Urology, University of California, San Francisco, San Francisco, CA 94158-9001. Phone: 415-505-3066; E-mail: jwitte@ucsf.edu

doi: 10.1158/1055-9965.EPI-10-0268

Table 1. Twenty-six prostate cancer risk SNPs identified by GWAS of prostate cancer

SNP	Chromosome	Associated			Associated Allele frequency cases	
		Gene	Allele	Alleles	African-American	White
rs721048*	2	*EHBP1*	A (10)	A, G	0.048	0.177
rs1465618	2	*THADA*	A (16)	A, G	0.143	0.243
rs12621278	2	*ITGA6*	G (16)	A, G	0.009	0.039
rs2660753	3	Intergenic	T (11)	C, T	0.543	0.157
rs7652331	3	*FYC01*	T (25)	C, T	0.095	0.399
rs1545985	3	*FYC01*	G (25)	A, G	0.205	0.406
rs13149290	4	*LOC152485*	T (25)	C, T	0.261	0.233
rs629242	4	*KIAA1211*	T (25)	T, C	0.305	0.200
rs7679673	4	Intergenic	A (16)	C, A	0.556	0.404
rs17021918*	4	*PDL1M5*	T (16)	C, T	0.183	0.354
rs251177	5	*FCHSD1*	C (25)	T, C	0.371	0.245
rs9364554	6	*SLCC22A3*	T (11)	C, T	0.054	0.277
rs6465657	7	*LMTK2*	C (16)	T, C	0.155	0.459
rs10486567	7	*JAZF1*	G (9)	A, G	0.731	0.803
rs12155172	7	Intergenic	A (16)	G, A	0.127	0.236
rs1512268	8	Intergenic	A (16)	A, G	0.642	0.466
rs1571801	9	*DAB2IP*	A (25)	C, A	0.145	0.240
rs10993994	10	*MSMB*	T (9, 11)	C, T	0.390	0.474
rs4962416	10	*CTBP2*	C (9)	T, C	0.201	0.304
rs10896449	11	Intergenic	G (9)	G, A	0.684	0.570
rs7127900	11	Intergenic	A (16)	G, A	0.394	0.200
rs10492519	13	*FAM124A*	G (25)	A, G	0.090	0.427
rs1859962	17	Intergenic	G (11, 16, 17)	T, G	0.325	0.516
rs4430796	17	*HNF1B, TCF*	A (9, 17, 24)	G, A	0.353	0.570
rs2735839	19	*KLK2/KLK3*	G (11)	G, A	0.328	0.138
rs5945572	X	*NUDT10,NUDT11*	A (10)	G, A	0.263	0.385

*rs2710646 used as proxy for rs721048 (r^2 = 1); rs3762876 used as a proxy for rs17021918 (r^2 = 0.96).

Genetic factors offer a potentially promising avenue for further clarification of prostate cancer aggressiveness (4, 5). Most studies of inherited genetic variants, however, have focused on risk factors for disease development and not progression among men diagnosed with this disease. For example, genome-wide association studies (GWAS) have clearly identified genetic variants that affect prostate cancer susceptibility (5, 6). Some of these promising variants are in genes on chromosome 10q11.23 (*MSMB*), 17q12 (*TCF2, HNFB1*), and Xp11.22 (*NUDT10, NUDT11*) as well as at loci on 8q24 with indeterminate biological effects (7-11). To date, most of these susceptibility loci do not seem to be associated with clinicopathologic features (9-17) or prostate cancer mortality (12, 18). Although these findings suggest that these loci affect the early stages of prostate cancer development, their effects on disease progression have only been recently investigated, as one study reported two variants at 10q11 and one variant at 8q24 influencing biochemical recurrence following radical prostatectomy (19). In the present study, we examined 26 prostate cancer risk loci, previously identified by GWAS, for their association with prostate cancer progression following treatment of aggressive prostate cancer.

Materials and Methods

Study subjects were identified from two previously described hospital-based prostate case-control studies from Cleveland, OH and Detroit, MI (dates of diagnoses from January 2001 to August 2004 and August 1999 to July 2004, respectively; refs. 20, 21). Cases were restricted to men with histologically confirmed tumors meeting the D'Amico (22) intermediate or high-risk criteria to focus on the most clinically relevant forms of prostate cancer with a high potential for disease progression. Specifically, the prostate cancer cases had a Gleason score of >7, tumor-node-metastasis stage >T2c, or a diagnostic PSA level of >10 ng/mL. For Gleason scores and stage information, we used values from prostatectomy whenever available; otherwise, biopsy values were used. A total of 788 "aggressive" prostate cancer cases were identified that had follow-up PSA and disease management

information available from medical records. The overall median follow-up time was 50.2 months (4.2 y).

Biochemical recurrence as manifested by PSA relapse was defined according to treatment regimen. For radical prostatectomy patients, biochemical recurrence was defined as a posttreatment PSA of ≥0.3 ng/mL. For radiotherapy patients, biochemical recurrence was defined by a 2-ng/mL increase in PSA above the posttreatment nadir according to the American Society for Therapeutic Radiology and Oncology Phoenix Consensus definition (23). For both treatments, the initiation of salvage hormones/radiation treatment or the presence of metastases also indicated prostate cancer progression. Herein, we use the term prostate cancer progression to reflect biochemical recurrence, initiation of salvage hormones/radiation treatment, or the presence of metastases following radical prostatectomy or radiation treatment. Time of prostate cancer progression was the earliest time observed for each treatment definition of PSA relapse, initiation of salvage hormones/radiation treatment, or presence of metastases. Patients with no evidence of progression were censored at the date of the last PSA test. Institutional review board approval was obtained for the study, and informed consent was acquired from all participants.

Single nucleotide polymorphism selection and genotyping

We evaluated 19 single nucleotide polymorphisms (SNP) identified by recent GWAS of prostate cancer susceptibility (Table 1; refs. 9-11, 16, 17, 24) as well as 7 putative risk SNPs from a GWAS of aggressive prostate cancer (25) given our focus on progression of more aggressive forms of disease. All SNPs were genotyped using the TaqMan allelic discrimination assay except rs629242, which was genotyped by the Illumina GoldenGate assay. All assays were undertaken by individuals blinded to the status of prostate cancer progression. The average genotyping success rate across the 26 SNPs was 98.7%, and the average genotype concordance rate for 2% duplicates was 99.0%. All SNP\s were in Hardy-Weinberg equilibrium among all patients for each racial/ethnic group ($P > 0.01$).

Statistical analysis

The association between each SNP and prostate cancer progression was assessed with hazard ratios (HR) and 95% confidence intervals (CI) estimated by Cox proportional hazards regression. The SNPs were evaluated with codominant and log-additive genetic models. All analyses were adjusted for age, institution, and estimates of genetic ancestry (discussed below), stage, Gleason score, PSA at diagnosis, and treatment. Progression-free survival rates were estimated using the Kaplan-Meier method, and differences in survival distributions by genotypes were evaluated with log rank tests.

To determine which combination of the 26 SNPs were independently associated with prostate cancer progression, a forward stepwise Cox regression analysis was conducted ($\alpha = 0.10$ for inclusion and exclusion) with each SNP coded according to a log-additive model. The risk allele was modeled as the allele conferring a significantly increased risk of progression among our study population. To examine the combined effects of these SNPs, a prognosis score was created by adding the alleles of each SNP (log additive) which were independently associated with progression from stepwise regression analysis.

To correct for potential population stratification within our study population, genetic ancestry was estimated by principal component analysis and included in our regression models (26). Specifically, 37 ancestry-informative markers for European and African ancestry were selected from the DNA Test Panel from Illumina and were genotyped on all subjects by the Illumina GoldenGate assay. Genotype data were analyzed by the EIGENSOFT software, and the leading principal components distinguishing European and African ancestry were estimated (26). The first principal component from this analysis delineated individuals of European and African descent and was used as an estimate of genetic ancestry. To investigate the potential heterogeneity of genotype effects across race/ethnicity and treatment regimens, we included interaction terms between genotype and race/ethnicity, and genotype and treatment (radical prostatectomy/radiation

Table 2. Study characteristics of 788 more aggressive prostate cancer patients according to progression status

	Prostate cancer progression	No prostate cancer progression
No. (%)	228 (28.9)	560 (71.1)
Age, mean (SD)	62.4 (6.9)	63.7 (7.7)
Race/ethnicity, *n* (%)		
White	162 (71.1)	393 (70.2)
African-American	66 (29.0)	167 (29.8)
Gleason score, *n* (%)		
≤6	29 (12.7)	149 (26.6)
3 + 4	88 (38.6)	240 (42.9)
4 + 3 or >8	111 (48.7)	171 (30.5)
Tumor stage, *n* (%)*		
T_1	66 (29.0)	221 (39.5)
T_{2a}-T_{2b}	39 (17.1)	87 (15.6)
T_{2c}	66 (29.0)	215 (38.5)
≥T_3	57 (25.0)	36 (6.4)
PSA at diagnosis; ng/mL, mean (±SD)	12.7 (±24.3)	8.6 (±7.0)
Treatment		
Radical prostatectomy	170 (74.6)	343 (61.3)
Radiotherapy	58 (25.4)	217 (38.8)

*Does not add to 788 due to missing information.

therapy) in our regression models. All reported P values are two-sided.

Results

The characteristics of the 788 cases in our study are presented in Table 2. Approximately 70% of this study population is White, and 30% is African-American. Of the 788 cases, 228 patients experienced prostate cancer progression and 560 patients did not progress. Patients experiencing prostate cancer progression were more likely to be younger, have higher Gleason score/tumor stage/PSA levels at diagnosis, and received radical prostatectomy treatment compared with the 560 patients who did not experience prostate cancer progression. The median follow-up times for patients that progressed and those that did not were 26.8 and 56.8 months, respectively.

Nine of the 26 SNPs tested were statistically significantly associated with prostate cancer progression: rs12621278, rs7652331, rs1545985, rs629242, rs9364554, rs12155172, rs1512268, rs10896449, and rs5945572 (Table 3). Of the 26 SNPs, there was no evidence of heterogeneity in effects by race/ethnicity ($P > 0.10$), and one SNP (rs17021918) displayed heterogeneous effects by treatment regimen ($P = 0.02$). For rs17021918, there was no association with progression among radical prostatectomy patients (HR per increase in minor allele, 0.85; 95% CI, 0.66-1.10), although a positive association was observed among radiotherapy patients (HR per increase in minor allele, 1.53; 95% CI, 1.02-2.30).

The *ITGA6* locus, rs12621278, displayed the most statistically significant association, with a 2.4-fold increased risk of prostate cancer progression associated with the AG genotype compared with the AA genotype (95% CI, 1.51-3.92). Applying a conservative Bonferroni correction to adjust for the 26 tested SNPs ($P = 0.002$), rs12621278 reached a study-wide significance with $P = 2.6 \times 10^{-4}$. The median progression-free survival times for patients carrying the rs12621278 AG versus the AA genotypes were 46.4 and 50.3 months, respectively ($P = 0.01$).

Five of the nine associated SNPs (rs12621278, rs629242, rs9364554, rs4430796, and rs5945572) showed independent associations with progression based on stepwise regression analysis. By summing the alleles of these five independent SNPs (allele range, 0-7), we created a risk score and estimated a 29% increase in risk of prostate cancer progression per additional risk allele (HR, 1.29; 95% CI, 1.12-1.47; $P = 3.0 \times 10^{-4}$; Table 4). Patients who carried four to seven risk alleles (22.1% of patients that progressed and 15.2% of patients that did not progress) had a 2.19-fold increased risk of prostate cancer progression compared with patients with less than one risk allele (95% CI, 1.40-3.42; $P = 6.0 \times 10^{-4}$). In a stratified analysis by race/ethnicity, similar associations were seen for the risk score for Whites and African-Americans (per additional risk allele: HR_{Whites}, 1.27; 95% CI, 1.08-1.48; and $HR_{African\text{-}Americans}$, 1.31; 95% CI, 1.01-1.72).

Statistically significant differences in survival distributions ($P = 0.01$) were observed when comparing patients with a risk score of <1, 2, 3, and 4 to 7 prognosis alleles (Fig. 1). We observed a decrease in progression-free survival time associated with an increase in the number of risk alleles. Specifically, the median progression-free survival times were 54.3, 49.6, 47.5, and 43.5 months for patients with prognosis scores of <1, 2, 3, and 4 to 7 alleles, respectively.

Discussion

Our study examined 26 prostate cancer susceptibility loci for their effect on prostate cancer progression. We found that 9 of 26 prostate cancer susceptibility loci were associated with prostate cancer progression after accounting for known predictors of prostate cancer outcomes—diagnostic PSA, Gleason score, stage, and primary treatment. Moreover, five loci (*ITGA6, NUDT10/NUDT11, KIAA1211, SLCC22A3*, and *HNF1B/TCF*) showed independent associations with progression.

The strongest associations were observed at the *ITGA6* locus (rs12621278) that encodes for α6 integrin, a protein involved in important features of tumor invasion—cell adhesion, migration, and signaling. This locus was recently identified by Eeles et al. (16) in a follow-up study of their stage 1 GWAS, whereby the G allele of rs12621278 was associated with a reduced risk of prostate cancer (odds ratio, 0.75; 95% CI, 0.70-0.80; $P = 8.7 \times 10^{-23}$). Interestingly, the G allele in our study was associated with a poor prognosis of prostate cancer. Although it might be expected that the protective effect of the G allele for prostate cancer development would align with improved prognosis, our association with worse prognosis might relate to pleiotropy in the biological effects of α6 integrin. Alteration in integrin function as a result of genetic variation may affect numerous downstream signaling effects as well as cell adhesion and migration activity. Prostate tumors persistently express α6 integrin (27-29), and *in vitro* and *in vivo* studies have linked its expression to increased tumor cell invasion, migration, and metastasis (30-32). This supports the role of *ITGA6* in prostate cancer progression, and other downstream effects of integrin signaling might have other specific effects on disease development.

We created a prognosis score to understand the collective effects of the five independent loci on prostate cancer progression which were identified by stepwise regression. Patients with four to seven risk alleles had a 2.2-fold increased risk, and an 11-month shorter time to prostate cancer progression, than patients with no risk alleles. We recognize that a prognosis score based on associated SNPs may exaggerate the level of statistical significance, and replication of the proposed associations is key.

Penney et al. (12) and Wiklund et al. (18) created similar cumulative scores based on prostate cancer susceptibility variants to examine the association with prostate cancer mortality. Both studies reported no associations

Table 3. Association between 26 prostate cancer risk SNPs and prostate cancer progression

Locus	SNP		Prostate cancer progression patients, *n* (%)	Non–prostate cancer progression patients, *n* (%)	HR (95% CI)*	*P*
Chr 2: *EHBP1*	rs721048	GG	161 (70.9)	416 (75.8)	1.00	
		GA	59 (26.0)	124 (22.6)	1.12 (0.81-1.54)	0.49
		AA	7 (3.1)	9 (1.6)	0.96 (0.42-2.21)	0.92
		Trend			1.06 (0.82-1.39)	0.65
Chr 2: THADA	rs1465618	GG	139 (61.5)	342 (62.2)	1.00	
		GA	76 (33.6)	183 (33.3)	1.00 (0.75-1.34)	0.99
		AA	11 (4.9)	25 (4.6)	1.18 (0.61-2.29)	0.62
		Trend			1.03 (0.81-1.31)	0.79
Chr 2: ITGA6	rs12621278	AA	207 (91.2)	524 (95.3)	1.00	
		AG	20 (8.8)	26 (4.7)	**2.43 (1.51-3.92)**	**0.00026**
Chr 3: Intergenic	rs2660753	CC	120 (52.9)	319 (57.8)	1.00	
		CT	80 (35.2)	176 (31.9)	1.13 (0.82-1.54)	0.45
		TT	27 (11.9)	57 (10.3)	1.27 (0.78-2.07)	0.34
		Trend			1.13 (0.90-1.41)	0.30
Chr 3: *FYC01*	rs7652331	CC	103 (45.4)	279 (50.5)	1.00	
		CT	100 (44.1)	214 (38.8)	**1.39 (1.02-1.89)**	**0.036**
		TT	24 (10.6)	59 (10.7)	1.09 (0.67-1.77)	0.72
		Trend			1.13 (0.92-1.40)	0.26
Chr 3: *FYC01*	rs1545985	AA	87 (38.3)	241 (43.9)	1.00	
		AG	115 (50.7)	244 (44.4)	**1.38 (1.03-1.86)**	**0.034**
		GG	25 (11.0)	64 (11.7)	1.12 (0.70-1.80)	0.64
		Trend			1.14 (0.93-1.40)	0.21
Chr 4: *LOC152485*	rs13149290	CC	135 (59.5)	326 (58.7)	1.00	
		CT	73 (32.2)	192 (34.6)	0.88 (0.66-1.18)	0.39
		TT	19 (8.4)	37 (6.7)	1.06 (0.65-1.73)	0.83
		Trend			0.96 (0.78-1.19)	0.73
Chr 4: *KIAA1211*	rs629242	CC	126 (57.5)	333 (61.3)	1.00	
		CT	73 (33.3)	180 (33.2)	1.17 (0.87-1.57)	0.30
		TT	20 (9.1)	30 (5.5)	**1.63 (1.01-2.64)**	**0.047**
		Trend			**1.23 (1.00-1.52)**	**0.050**
Chr 4: Intergenic	rs7679673	CC	70 (30.8)	172 (31.2)	1.00	
		AC	113 (49.8)	260 (47.2)	1.00 (0.74-1.36)	0.99
		AA	44 (19.4)	119 (21.6)	0.88 (0.59-1.30)	0.51
		Trend			0.94 (0.78-1.14)	0.55
Chr 4: *PDL1M5*	rs17021918	CC	109 (48.0)	272 (48.9)	1.00	
		CT	96 (42.3)	233 (41.9)	0.91 (0.69-1.21)	0.52
		TT	22 (9.7)	51 (9.2)	1.00 (0.63-1.61)	0.99
		Trend			0.97 (0.78-1.19)	0.75
Chr 5: *FCHSD1*	rs251177	TT	119 (52.4)	283 (50.8)	1.00	
		TC	98 (43.2)	223 (40.0)	1.04 (0.79-1.37)	0.78
		CC	10 (4.4)	51 (9.2)	0.63 (0.32-1.21)	0.16
		Trend			0.92 (0.74-1.15)	0.75
Chr 6: *SLCC22A3*	rs9364554	CC	137 (60.4)	355 (64.6)	1.00	
		CT	73 (32.2)	170 (30.9)	1.25 (0.91-1.72)	0.17
		TT	17 (7.5)	25 (4.6)	1.65 (0.97-2.82)	0.07
		Trend			**1.27 (1.01-1.61)**	**0.041**
Chr 7: *LMTK2*	rs6465657	CC	80 (35.2)	198 (35.7)	1.00	
		CT	102 (44.9)	238 (43.0)	1.18 (0.84-1.65)	0.33
		TT	45 (19.8)	118 (21.3)	1.29 (0.86-1.95)	0.22
		Trend			1.14 (0.93-1.40)	0.21

(*Continued on the following page*)

Table 3. Association between 26 prostate cancer risk SNPs and prostate cancer progression (Cont'd)

Locus	SNP		Prostate cancer progression patients, *n* (%)	Non–prostate cancer progression patients, *n* (%)	HR (95% CI)*	*P*
Chr 7: *JAZF1*	rs10486567	GG	340 (61.9)	137 (60.4)	1.00	
		GA	179 (32.6)	80 (35.2)	0.89 (0.67-1.19)	0.43
		AA	30 (5.5)	10 (4.4)	0.70 (0.37-1.33)	0.28
		Trend			0.87 (0.69-1.09)	0.22
Chr 7: Intergenic	rs12155172	GG	147 (64.8)	345 (61.8)	1.00	
		AG	66 (29.1)	200 (35.8)	0.86 (0.64-1.17)	0.35
		AA	14 (6.2)	13 (2.3)	**1.83 (1.05-3.19)**	**0.034**
		Trend			1.07 (0.84-1.35)	0.60
Chr 8: Intergenic	rs1512268	AA	57 (25.1)	142 (25.6)	1.00	
		GA	126 (55.5)	256 (46.1)	**1.46 (1.02-2.08)**	**0.037**
		GG	44 (19.4)	157 (28.3)	1.22 (0.81-1.85)	0.34
		Trend			1.09 (0.90-1.32)	0.39
Chr 9: *DAB2IP*	rs1571801	CC	147 (64.8)	341 (61.7)	1.00	
		CA	70 (30.8)	184 (33.3)	0.91 (0.68-1.22)	0.52
		AA	10 (4.4)	28 (5.1)	0.86 (0.45-1.63)	0.63
		Trend			0.92 (0.72-1.16)	0.46
Chr 10: MSMB	rs10993994	CC	54 (23.9)	133 (24.1)	1.00	
		CT	115 (50.9)	268 (48.5)	1.07 (0.77-1.49)	0.68
		TT	57 (25.2)	152 (27.5)	1.04 (0.71-1.52)	0.85
		Trend			1.02 (0.84-1.23)	0.85
Chr 10: *CTBP2*	rs4962416	TT	118 (52.9)	306 (55.1)	1.00	
		TC	85 (38.0)	199 (35.9)	1.05 (0.79-1.40)	0.74
		CC	21 (9.4)	50 (9.0)	0.99 (0.61-1.60)	0.96
		Trend			1.01 (0.83-1.24)	0.89
Chr 11: Intergenic	rs10896449	AA	26 (11.5)	104 (18.9)	1.00	
		AG	103 (45.4)	251 (45.6)	1.23 (0.79-1.91)	0.36
		GG	98 (43.2)	196 (35.6)	**1.60 (1.03-2.48)**	**0.037**
		Trend	trend 0.0112		**1.28 (1.04-1.56)**	**0.018**
Chr 11: Intergenic	rs7127900	GG	125 (55.1)	296 (53.8)	1.00	
		AG	83 (36.6)	228 (41.5)	0.86 (0.65-1.15)	0.32
		AA	19 (8.4)	26 (4.7)	1.42 (0.86-2.36)	0.18
		Trend			1.03 (0.82-1.29)	0.83
Chr 13: *FAM124A*	rs10492519	AA	106 (46.7)	265 (46.5)	1.00	
		AG	89 (39.2)	212 (38.8)	0.97 (0.70-1.34)	0.86
		GG	32 (14.1)	69 (12.6)	1.16 (0.75-1.79)	0.51
		Trend			1.06 (0.85-1.31)	0.63
Chr 17: Intergenic	rs1859962	TT	67 (29.5)	168 (30.4)	1.00	
		TG	99 (43.6)	274 (49.6)	0.96 (0.69-1.33)	0.80
		GG	61 (26.9)	111 (20.1)	1.24 (0.86-1.80)	0.25
		Trend			1.12 (0.92-1.35)	0.26
Chr 17: *HNF1B, TCF*	rs4430796	GG	59 (26.0)	156 (28.2)	1.00	
		GA	95 (41.9)	247 (44.6)	1.01 (0.72-1.41)	0.98
		AA	73 (32.2)	151 (27.3)	1.29 (0.90-1.87)	0.17
		Trend			1.14 (0.95-1.38)	0.17
Chr 19: *KLK2/KLK3*	rs2735839	GG	150 (65.8)	369 (66.4)	1.00	
		GA	67 (29.4)	158 (28.4)	0.92 (0.68-1.25)	0.58
		AA	11 (4.8)	29 (5.2)	1.17 (0.62-2.24)	0.63
		Trend			0.99 (0.77-1.27)	0.92
Chr X: *NUDT10,NUDT11*	rs5945572	GG	126 (56.0)	380 (68.8)	1.00	
		AA	99 (44.0)	172 (31.2)	**1.45 (1.10-1.91)**	**0.008**

NOTE: Boldface indicates significant associations ($P < 0.05$).
*Adjusted for age, genetic ancestry, institution, Gleason grade, stage, treatment, and PSA at diagnosis.

Table 4. Association between prognosis score and prostate cancer progression

No. of risk alleles	Prostate cancer progression patients, *n* (%)	Nonprostate cancer progression patients, *n* (%)	HR (95% CI)*	*P*
≤1	43 (19.8)	156 (29.3)	1.00	
2	66 (30.4)	166 (31.1)	1.55 (1.04-2.31)	0.031
3	60 (27.7)	130 (24.4)	1.90 (1.26-2.88)	0.002
4-7	48 (22.1)	81 (15.2)	2.19 (1.40-3.42)	6.0×10^{-4}
Per unit increase in risk alleles			1.29 (1.12-1.47)	3.0×10^{-4}

NOTE: Prognosis score based on five risk alleles in *ITGA6, NUDT10/NUDT11, KIAA1211, SLCC22A3*, and *HNF1B/TCF*.
*Adjusted for age, genetic ancestry, institution, Gleason score, stage, treatment, and PSA at diagnosis.

with either the cumulative score or individual variants for this outcome (12, 18). Penney et al. (12) examined eight previously associated variants at the 8q24 and 17q loci among 2,746 prostate cancer cases with 243 prostate-specific deaths in the Physicians' Health Study and at the Fred Hutchinson Cancer Research Center. We examined two (rs1859962 and rs4430796) of the eight SNPs and found that these SNPs were also not associated with disease progression. Wiklund et al. (18) tested 16 prostate cancer loci among 2,875 prostate cancer cases with 440 prostate-specific deaths in the Cancer of the Prostate in Sweden study. We tested 12 of these 16 loci, observing significant associations between 2 (rs9364554 and rs10896449) of the 12 loci and prostate cancer progression. Although there seems to be a consistency between prostate cancer mortality and progression for the majority of SNPs tested, the inconsistency for rs9364554 and rs10896449 might relate to differences in biological processes for mortality versus progression, heterogeneity in study populations such as treatment regimens, or chance findings. With biochemical recurrence representing the majority (>95%) of outcome events, it is more reliable in predicting survival following prostatectomy and radiation therapy among high-risk patients such as those with a Gleason score of >7 (similar to our study population; ref. 33), yet it is not equivalent to clinical recurrence nor prostate cancer–specific mortality. Clearly, larger studies are warranted in well-characterized study populations that can evaluate the entire course of the disease from diagnosis to biochemical recurrence, metastasis, and death.

To our knowledge, only one previous study has examined prostate cancer risk loci for their associations with biochemical recurrence (34). In a study of 320 localized prostate cancer patients from Taiwan, three prostate cancer risk variants (rs1447295 at 8q24, and rs7920517 and

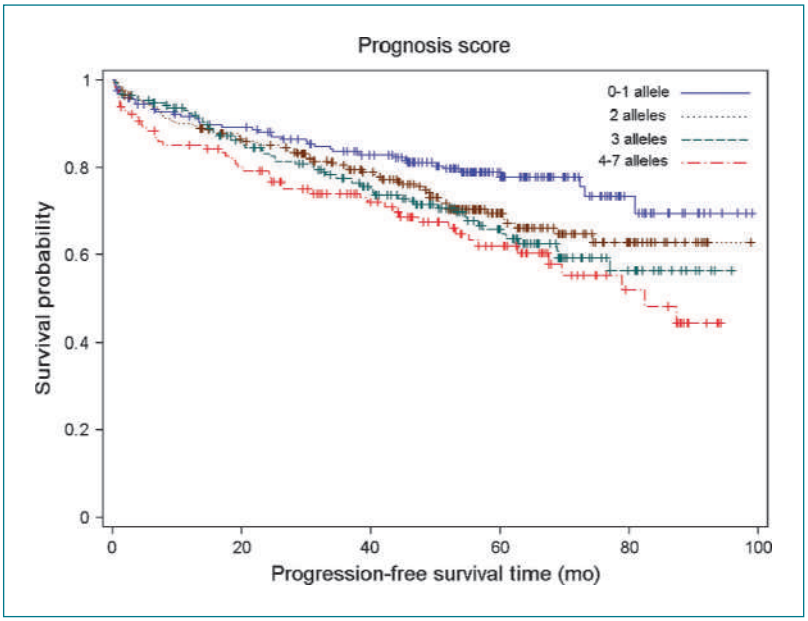

Figure 1. Kaplan-Meier survival curves showing differences in prostate cancer progression up to 100 mo by strata of prognosis score ($P = 0.01$). Score is based on the total number of risk alleles of five independently associated SNPs (rs12621278, rs629242, rs9364554, rs4430796, and rs5945572) from stepwise regression analysis.

rs10993994 at 10q11) were associated with recurrence (34). In our study, we did not examine rs1447295 and rs7920517, and observed no association with rs10993994. Of the nine variants that were associated with progression in our study, only two (rs9364554 and rs5945572) were tested in the Taiwanese study, and both showed no associations among this study population. The discrepancies in findings may largely reflect heterogeneity in study populations, and/or chance findings. In particular, our study population consisted of more aggressive prostate cancer, receiving radical prostatectomy and radiotherapy treatment, in contrast with the Taiwanese study of localized prostate cancer, which received radical prostatectomy treatment. In addition, heterogeneity in allele frequency is also possible such as rs5945572, which has differing allele frequencies between Asians [minor allele frequency (MAF) = 0.09; ref. 34], African-Americans (MAF = 0.26), and Whites (MAF = 0.38). Furthermore, the number of SNPs (n = 26) tested in our study under both a codominant and log-additive model raises the concern of false-positive associations due to multiple hypothesis testing. False-positive associations are a major concern for genetic association studies, and it is essential that additional studies replicate these findings to establish a clear genetic association.

We investigated whether these 26 prostate cancer risk variants also influenced clinicopathologic features of the disease (data not shown). We observed a few nominal associations with age at diagnosis (rs5945572, P = 0.047; rs10896449, P = 0.031), Gleason score (rs9364554, P = 0.039), and stage (rs2735839, P = 0.006). Three of these SNPs associated with age at diagnosis (rs5945572 and rs10896449) and Gleason score (rs9364554; an important indicator of prostate cancer outcomes), were also associated with progression. In addition, a similar association between rs2735839 (*KLK2/KLK3*) with stage of disease (13) has been previously reported. Notably though, these findings should be interpreted with caution given the large number of tests that were conducted and the chance of false-positive associations.

Seventeen of the prostate cancer risk loci reported by GWAS that were tested in our study were not associated with prostate cancer progression. An implicit premise is that risk factors for disease development would be the same as those involved in progression and mortality; however, there may be different biological pathways for prostate cancer initiation and progression with distinct underlying risk factors. Thus, a locus associated with prostate cancer risk may not necessarily be associated with progression of disease. Another possibility is that the current study was unable to detect weak associations.

Our focus on patients with more aggressive disease, who are eligible to undergo radical prostatectomy or radiation therapy, allows us to examine pathologic types that are most clinically relevant with the greatest likelihood of poor prognosis; however, this range in pathologic types is not representative of the full array of clinical prostate cancers because most cancers detected in the PSA era are of low volume, low grade, and of uncertain biological significance. Ultimately, our results need to be replicated in independent case populations to fully establish their potential clinical utility. In addition, given the limited power and sample size of African-Americans in our study, we were unable to conduct analyses focused on this ethnic group.

There is a great need to further our understanding of the risk factors for prostate cancer progression. This is especially important in light of possible morbidity and detrimental effects on quality of life arising from the standard treatment options for prostate cancer (that is, surgery, androgen deprivation, and/or radiotherapy). Although there are several established clinical measures that help determine which men might have poor prognosis (e.g., tumor stage and grade), there remains room for improvement. Moreover, in light of the large number of men diagnosed with prostate cancer, and the relatively high proportion of men experiencing biochemical recurrence (~33% of radical prostatectomy and radiation therapy patients; refs. 35, 36), additional markers for progression that have reasonable predictive power might be clinically useful. Furthermore, by identifying new progression markers, key insights may be gained in understanding the biological mechanism of disease process.

In summary, our study suggests that five of the recently detected prostate cancer susceptibility loci identified through GWAS also influence a patient's risk of progression beyond the known clinicopathologic predictors. This work builds on the importance of these genetic variants in prostate cancer biology, and it is essential that our findings are replicated in larger studies. By examining how inherited variation affects disease progression, we will further advance our understanding of prostate cancer prognosis, with the ultimate goal of better targeting aggressive treatment to patients most likely to suffer from disease recurrence, metastasis, and death.

Disclosure of Potential Conflicts of Interest

E. Klein: honoraria from the speakers bureau for Abbott and Genomic Health. The other authors disclosed no potential conflicts of interest.

Acknowledgments

We thank the participants of this study, who have contributed to a better understanding of the genetic contributions to prostate cancer, and Chandana Reddy, Alwyn Reuth, Ross Boylan, and Joel Medford for their contributions to this project.

Grant Support

NIH grants (CA88164, CA94211, CA98683, and ES011126).

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.

Received 03/12/2010; revised 05/19/2010; accepted 06/24/2010; published OnlineFirst 07/22/2010.

References

1. Cooperberg MR, Lubeck DP, Mehta SS, Carroll PR. Time trends in clinical risk stratification for prostate cancer: implications for outcomes (data from CaPSURE). J Urol 2003;170:S21–5, discussion S6–7.
2. Welch HG, Albertsen PC. Prostate cancer diagnosis and treatment after the introduction of prostate-specific antigen screening: 1986-2005. J Natl Cancer Inst 2009;101:1325–9.
3. Shariat SF, Karakiewicz PI, Roehrborn CG, Kattan MW. An updated catalog of prostate cancer predictive tools. Cancer 2008; 113:3075–99.
4. Schaid DJ. The complex genetic epidemiology of prostate cancer. Hum Mol Genet 2004;13:103–21.
5. Witte JS. Prostate cancer genomics: towards a new understanding. Nat Rev Genet 2009, published online 23 December 2008; doi:10.1038/nrg2507.
6. Witte JS. Multiple prostate cancer risk variants on 8q24. Nat Genet 2007;39:579–80.
7. Freedman ML, Haiman CA, Patterson N, et al. Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. Proc Natl Acad Sci U S A 2006;103:14068–73.
8. Haiman CA, Patterson N, Freedman ML, et al. Multiple regions within 8q24 independently affect risk for prostate cancer. Nat Genet 2007; 39:638–44.
9. Thomas G, Jacobs KB, Yeager M, et al. Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 2008; 40:310–5.
10. Gudmundsson J, Sulem P, Rafnar T, et al. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. Nat Genet 2008;40:281–3.
11. Eeles RA, Kote-Jarai Z, Giles GG, et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 2008;40: 316–21.
12. Penney KL, Salinas CA, Pomerantz M, et al. Evaluation of 8q24 and 17q risk loci and prostate cancer mortality. Clin Cancer Res 2009;15: 3223–30.
13. Kader AK, Sun J, Isaacs SD, et al. Individual and cumulative effect of prostate cancer risk-associated variants on clinicopathologic variables in 5,895 prostate cancer patients. Prostate 2009;69:1195–205.
14. FitzGerald LM, Patterson B, Thomson R, et al. Identification of a prostate cancer susceptibility gene on chromosome 5p13q12 associated with risk of both familial and sporadic disease. Eur J Hum Genet 2009;17:368–77.
15. Xu J, Isaacs SD, Sun J, et al. Association of prostate cancer risk variants with clinicopathologic characteristics of the disease. Clin Cancer Res 2008;14:5819–24.
16. Eeles RA, Kote-Jarai Z, Al Olama AA, et al. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. Nat Genet 2009;41:1116–21.
17. Gudmundsson J, Sulem P, Steinthorsdottir V, et al. Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat Genet 2007;39:977–83.
18. Wiklund FE, Adami HO, Zheng SL, et al. Established prostate cancer susceptibility variants are not associated with disease outcome. Cancer Epidemiol Biomarkers Prev 2009;18:1659–62.
19. Huang SP, Huang LC, Ting WC, et al. Prognostic significance of prostate cancer susceptibility variants on prostate-specific antigen recurrence after radical prostatectomy. Cancer Epidemiol Biomarkers Prev 2009;18:3068–74.
20. Liu X, Plummer SJ, Nock NL, Casey G, Witte JS. Nonsteroidal anti-inflammatory drugs and decreased risk of advanced prostate cancer: modification by lymphotoxin α. Am J Epidemiol 2006;164:984–9.
21. Rybicki BA, Neslund-Dudas C, Nock NL, et al. Prostate cancer risk from occupational exposure to polycyclic aromatic hydrocarbons interacting with the GSTP1 Ile105Val polymorphism. Cancer Detect Prev 2006;30:412–22.
22. D'Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA 1998;280:969–74.
23. Roach M III, Hanks G, Thames H, Jr., et al. Defining biochemical failure following radiotherapy with or without hormonal therapy in men with clinically localized prostate cancer: recommendations of the RTOG-ASTRO Phoenix Consensus Conference. Int J Radiat Oncol Biol Phys 2006;65:965–74.
24. Gudmundsson J, Sulem P, Gudbjartsson DF, et al. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. Nat Genet 2009;41:1122–6.
25. Duggan D, Zheng SL, Knowlton M, et al. Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. J Natl Cancer Inst 2007;99: 1836–44.
26. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38:904–9.
27. Fornaro M, Manes T, Languino LR. Integrins and prostate cancer metastases. Cancer Metastasis Rev 2001;20:321–31.
28. Cress AE, Rabinovitz I, Zhu W, Nagle RB. The α6β1 and α6β4 integrins in human prostate cancer progression. Cancer Metastasis Rev 1995;14:219–28.
29. Nagle RB, Hao J, Knox JD, Dalkin BL, Clark V, Cress AE. Expression of hemidesmosomal and extracellular matrix proteins by normal and malignant human prostate tissue. Am J Pathol 1995;146:1498–507.
30. Ports MO, Nagle RB, Pond GD, Cress AE. Extracellular engagement of α6 integrin inhibited urokinase-type plasminogen activator-mediated cleavage and delayed human prostate bone metastasis. Cancer Res 2009;69:5007–14.
31. King TE, Pawar SC, Majuta L, et al. The role of α6 integrin in prostate cancer migration and bone pain in a novel xenograft model. PLoS One 2008;3:e3535.
32. Rabinovitz I, Nagle RB, Cress AE. Integrin α6 expression in human prostate carcinoma cells is associated with a migratory and invasive phenotype *in vitro* and *in vivo*. Clin Exp Metastasis 1995;13:481–91.
33. Partin AW, Hanks GE, Klein EA, Moul JW, Nelson WG, Scher HI. Prostate-specific antigen as a marker of disease activity in prostate cancer. Oncology (Williston Park) 2002;16:1024–38, 42; discussion 42, 47–8, 51.
34. Lin HC, Liu CC, Kang WY, et al. Influence of cytokine gene polymorphisms on prostate-specific antigen recurrence in prostate cancer after radical prostatectomy. Urol Int 2009;83:463–70.
35. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. J Urol 2004;172:910–4.
36. Amling CL, Bergstralh EJ, Blute ML, Slezak JM, Zincke H. Defining prostate specific antigen progression after radical prostatectomy: What is the most appropriate cut point? J Urol 2001;165:1146–51.