Research Article

Cancer Prevention Research

Association of Prostate Cancer Risk Loci with Disease Aggressiveness and Prostate Cancer–Specific Mortality

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Abstract

Genome-wide association studies have detected more than 30 inherited prostate cancer risk variants. While clearly associated with risk, their relationship with clinical outcome, particularly prostate cancer–specific mortality, is less well known. We investigated whether the risk variants are associated with various measures of disease aggressiveness and prostate cancer–specific mortality. In a cohort of 3,945 men of European ancestry with prostate cancer, we genotyped 36 single nucleotide polymorphisms (SNP): 35 known prostate cancer risk variants and one SNP (rs4054823) that was recently reported to be associated with prostate cancer aggressiveness. The majority of subjects had a diagnosis of prostate cancer between 1995 and 2004, and the cohort included a total of 580 prostate cancer–specific deaths. We evaluated associations between the 36 polymorphisms and prostate cancer survival, as well as other clinical parameters including age at diagnosis, prostate-specific antigen (PSA) at diagnosis, and Gleason score. Two SNPs, rs2735839 at chromosome 19q13 and rs7679673 at 4q24, were associated with prostate cancer–specific survival ($P = 7 \times 10^{-4}$ and 0.014, respectively). A total of 12 SNPs were associated with other variables ($P < 0.05$): age at diagnosis, PSA at diagnosis, Gleason score, and/or disease aggressiveness based on D'Amico criteria. Genotype status at rs4054823 was not associated with aggressiveness or outcome. Our results identify two common polymorphisms associated with prostate cancer–specific mortality. *Cancer Prev Res; 4(5); 719–28. ©2011 AACR.*

Introduction

Prostate cancer is the leading nondermatologic cancer diagnosis among men in the United States and the second leading cause of cancer-related death (1). Although the disease will prove lethal for approximately 27,000 men this year (1), it is estimated that almost half of men with prostate-specific antigen (PSA) screening will develop a form of disease that is not life threatening and will likely remain indolent over the course of a lifetime (2). As a result, thousands of men needlessly undergo radical treatment of prostate cancer each year and are exposed unnecessarily to substantial morbidity. Yet, treatment of the substantial minority with deadly forms of disease is vitally important. Accurate prognostication about prostate cancer aggressiveness is therefore critical for patients and their physicians.

Widely accepted prognostic factors include stage of disease, tumor differentiation (Gleason sum), and PSA. These 3 parameters have also been combined for prediction of recurrence after local treatment (3). However, each of these clinical predictors has significant shortcomings. For example, biopsy Gleason sum, the factor most strongly associated with outcome, is inherently subject to selective sampling of tumor. Even when sampling error is not an issue, heterogeneity in outcome is well described, and high Gleason score ($\geq$7), for example, has a positive predictive value for mortality of only 29% (4). Improved predictors of long-term outcome, available at the time of diagnosis, are needed.

Since 2006, several genome-wide association studies (GWAS) have convincingly identified genetic polymorphisms associated with risk of developing prostate cancer. To date, more than 30 risk loci have been described, primarily in populations of European ancestry (5–16). Although certain single nucleotide polymorphisms (SNP) discovered in GWAS are clearly associated with risk, it is less clear whether the SNPs are also associated with clinical variables that help predict outcome. The discovery of germ line risk polymorphisms provides an opportunity to determine the

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effects of these variants on clinically relevant issues related to prostate cancer. Previous studies have shown a modest association between certain risk alleles and parameters such as Gleason score, age at diagnosis, or disease aggressiveness (17–19), whereas other studies have not detected these associations. Most of these studies focused solely on clinical surrogates for outcome. Few studies have evaluated associations between risk allele status and prostate cancer–specific mortality (20–23), and no study has evaluated all of the known risk loci to date for prostate cancer–related death.

In the present study, 3,945 prostate cancer patients were genotyped for 35 SNPs associated with prostate cancer risk. We also genotyped an SNP which recently was reported to be associated specifically with aggressive disease (24). We assessed associations between genotype and prostate cancer–specific survival to determine whether previously identified risk SNPs can differentiate men who develop low-risk, indolent forms of the disease from men who develop lethal prostate cancer.

Methods

Study population

The Gelb Center prostate cancer cohort at Dana-Farber Cancer Institute has been described previously (25) and includes blood samples from more than 6,000 patients followed prospectively. Clinical data are collected from multiple sources, including medical records, institutional laboratory, patient registration, pharmacy systems, and clinician forms. Men included in the study had a known date of diagnosis and information available about PSA at diagnosis, biopsy Gleason score, and clinical stage at diagnosis. Each subject consented for clinical data and provided blood samples for DNA extraction for research use. Only European American subjects were included in the study, and the majority provided reliable ancestry data. Ancestry information was confirmed for a subset of patients and determined for all subjects without self-reported data by genotyping 26 ancestry-informative SNPs (26). A total of 3,945 subjects were included in the study.

Age at diagnosis was considered age at the date of first prostate cancer–positive biopsy. PSA at diagnosis was the last PSA value within 3 months prior to diagnostic biopsy. Gleason score used in the study was based on prostate biopsy. All biopsy samples were reviewed by pathologists at Brigham and Women's Hospital. D'Amico criteria were used to classify aggressiveness of patients' disease at diagnosis as having low-, intermediate-, or high-risk disease (27). The criteria were developed to classify men with localized disease. Because men with metastases at presentation were included in the present study, the criteria were modified to include this subset. Groups were defined as low-risk (PSA <10 ng/mL, Gleason score <6, and clinical stage T1c or T2a), intermediate-risk (PSA 10–20 ng/mL or Gleason 7 disease or clinical stage T2b, and otherwise low-risk features), and high-risk (PSA >20 or Gleason >8 or stage T2c-M1). Aggressiveness based on these criteria could be defined for a total of 3,500 patients. Prostate cancer–specific mortality was considered any death in the setting of castration-resistant prostate cancer and prostate cancer metastases. Records of more than 300 patients who died and did not meet these criteria were reviewed by an oncologist (M.P.) to determine cause of death and establish that prostate cancer was not the immediate cause.

SNP selection

Thirty-five SNPs chosen for analysis met genome-wide statistical significance for association with prostate cancer risk in multistage GWAS analyzing more than 1 independent cohort of cases and controls published between 2006 and 2009 (5, 7–16). If 2 risk SNPs discovered at the same locus were in linkage disequilibrium $r^2 > 0.50$, only 1 was included in the present study. In addition, a SNP, rs4054823, recently reported to be associated with aggressive disease, was included in the analysis (24).

DNA extraction and genotyping

All DNA samples were extracted from peripheral whole blood by using QIAamp DNABlood mini kit (QIAGEN Inc.). Genotyping was carried out using Sequenom iPLEX matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry. All risk SNP assays were combined into 4 multiplex pools. All reactions were carried out in 384-well format. Approximately 5% of samples were randomly selected and genotyping duplicated for quality control. Concordance rate for duplicate genotyping was 100%. Call rate overall was greater than 99%.

Statistical method

Patient clinical and disease characteristics were summarized as median and interquartiles range for continuous variables, as well as number and percentage for categorical variables. Genotype for each SNP was analyzed as 3 categories in all analyses. Both χ^2 and Kruskal–Wallis tests were used to assess associations of SNP genotypes and categorical variables (Gleason score, D'Amico risk classification) and continuous variables (age and PSA at diagnosis), respectively.

The Kaplan–Meier method was used to estimate survival distribution. Prostate cancer–specific survival was defined from prostate cancer diagnosis to time of death, which was related to prostate cancer or censored on the last known alive date, or death due to other causes. HRs and associated 95% CIs for prostate cancer–specific survival among different genotypes were calculated in univariate analysis as well as adjusting for clinical characteristics at diagnosis, using a Cox proportional hazard model for cause-specific hazard. Competing risk for other causes of death was assessed as a sensitivity analysis.

The study had more than 80% power to detect a range of HRs between 1.5 and 1.7 for risk allele frequencies varying from 0.10 to 0.25 with a 0.05 two-sided type I error in 3,945 patients, with 15% events adapting the recessive genetic model (combining men homozygous and heterozygous for the major allele). Data reported here are not corrected for multiplicity of hypothesis testing.

Results

The study cohort consisted of 3,945 prostate cancer patients of European ancestry. Characteristics of the study subjects are summarized in Table 1. The majority of subjects had a diagnosis of prostate cancer between 1995 and 2004 (range, 1976–2007), and median follow-up was 5.3 years. Most patients (52%) presented with PSA levels between 4 and 10 ng/mL, reflecting a widely PSA-screened population. More than half of the subjects had a diagnosis of intermediate or highly aggressive disease, based on Gleason score or D'Amico criteria. There were 580 prostate cancer–specific deaths, defined as death in the setting of metastatic, castration-refractory disease, accounting for 66% of all deaths ($N = 883$). A total of 36 SNPs (35 risk SNPs and 1 SNP reported to be associated with aggressiveness) were chosen for analysis as described in Methods and listed in Table 2. Risk allele frequencies are similar to those detected in prostate cancer cases from previous GWAS conducted in men of European ancestry.

Each SNP was initially analyzed using a multiplicative model for association between genotype and the following clinical variables at the time of diagnosis: age, PSA, biopsy Gleason score, and aggressiveness groups based on D'Amico criteria (27). In addition, each SNP was analyzed for association with prostate cancer–specific survival. Twelve SNPs achieved a value of $P < 0.05$ for association with at least 1 clinical variable analyzed (Table 3). The complete list of SNP genotypes and their associations with clinical parameters can be found in Supplementary Tables S1–S5.

The SNP rs2735839 at chromosome 19q13 was significantly associated with prostate cancer–specific mortality (Fig. 1A, Table 4). The protective (non-risk) allele (A) was associated with increased risk of dying of the disease ($P = 7 \times 10^{-4}$). The unadjusted HR for prostate cancer–specific death among patients carrying an A allele (i.e., heterozygous for the SNP) was 1.26 (95% CI, 1.05–1.52) when compared with homozygotes for the risk allele (GG). For AA homozygotes, the HR was 2.14 (95% CI, 1.33–3.44). The non–risk allele at rs2735839 was also associated with earlier onset of disease, higher Gleason score, and higher-risk disease (Table 3). Risk allele status at rs2735839 was not associated with PSA at diagnosis. When adjusting for PSA level, the association with prostate cancer–specific survival was still present ($P = 0.03$), though the association was no longer present when adjusting for Gleason score ($P = 0.1$). After adjusting for aggressiveness according to D'Amico criteria and age at diagnosis, HRs for prostate cancer–specific mortality was 1.23 (95% CI, 1.01–1.47) for heterozyotes (AG) and was 1.92 (95% CI, 1.20–3.09) for AA homozygotes when compared with homozygotes for the risk allele (GG; $P = 0.005$; Table 4).

Recent data implicate the risk variant at the chromosome 19q13 locus in the regulation of PSA level (10, 28–34). In a cohort consisting of men who received diagnosis of prostate cancer largely via PSA screening, an association with PSA level could lead to ascertainment bias when evaluating associations with outcome. To account for this possibility, prostate cancer–specific survival was analyzed in a subgroup of patients ($n = 237$) who had a diagnosis of prostate cancer prior to 1993, the beginning of the PSA era. It is presumed that few in this subgroup underwent PSA screening. With 50% power to detect an HR of 2.0, no association with mortality was observed: HR for the AG genotype was 1.13 when compared with the GG genotype ($P = 0.56$).

The SNP rs7679673 at chromosome 4q24 was also associated with prostate cancer–related death (Fig. 1B, Table 4). The risk allele (C) was associated with an increased hazard of dying of prostate cancer ($P = 0.014$). Compared with men carrying the non–risk AA genotype, the unadjusted HR for men heterozyogous for this SNP was 1.22 (95% CI, 0.95–1.57) and was 1.44 (95% CI, 1.11–1.86) for men homozygous for the risk allele (C). After adjusting for aggressiveness and age at diagnosis, the association remained significant ($P = 0.002$). The adjusted

Table 1. Clinical characteristics of the study cohort

	n (%)
Total	**3,945**
Median follow-up, mo	63.7
Year of diagnosis	
Before 1990	59 (1.5)
1990–1994	529 (13.4)
1995–1999	1,093 (27.7)
2000–2004	1,570 (39.8)
2005–2009	643 (16.3)
Unknown	51 (1.3)
Age at diagnosis	
Median	61
Range	39–91
PSA at diagnosis	
Median (interquartile range), ng/mL	6.4 (4.6–11)
<4	552 (14)
4–10	2,060 (52)
10–20	544 (14)
>20	468 (12)
Unknown	321 (8)
Biopsy Gleason score	
≤6	1,790 (45)
7	1,288 (33)
8–10	716 (18)
Unknown	151 (4)
D'Amico risk criteria	
Total no. evaluated	3,500
Low risk	1,307 (37)
Intermediate risk	1,370 (39)
High risk	823 (24)
Mortality	
Total no. of deaths	883
Prostate cancer-specific	580
Other causes	303

Table 2. Prostate cancer risk polymorphisms genotyped in the study cohort

SNP	Locus	Alleles	Risk allele	Risk allele frequency
rs12621278	chr2:173019799	A/G	G	0.050
rs1465618	chr2:43407453	A/G	A	0.215
rs721048	chr2:62985235	A/G	A	0.195
rs10934853	chr3:129521063	A/C	A	0.282
rs2660753	chr3:87193364	C/T	T	0.151
rs7679673	chr4:106280983	A/C	C	0.609
rs12500426	chr4:95733632	A/C	A	0.484
rs17021918	chr4:95781900	C/T	C	0.687
rs401681	chr5:1375087	A/G	G	0.558
rs9364554	chr6:160753654	C/T	T	0.285
rs10486567	chr7:27943088	A/G	G	0.782
rs6465657	chr7:97654263	C/T	C	0.474
rs7841060	chr8:128165659	G/T	G	0.233
rs13254738	chr8:128173525	A/C	C	0.340
rs16901979	chr8:128194098	A/C	A	0.046
rs16902094	chr8:128389528	A/G	G	0.151
rs445114	chr8:128392363	C/T	T	0.653
rs620861	chr8:128404855	A/G	G	0.659
rs6983267	chr8:128482487	G/T	G	0.551
rs7000448	chr8:128510352	C/T	T	0.399
rs10090154	chr8:128601319	C/T	T	0.123
rs2928679	chr8:23494920	C/T	T	0.481
rs1512268	chr8:23582408	A/G	A	0.462
rs4962416	chr10:12668686	C/T	C	0.301
rs10993994	chr10:51219502	C/T	T	0.469
rs7127900	chr11:2190150	A/G	A	0.218
rs11228565	chr11:68735156	A/G	A	0.254
rs7931342	chr11:68751073	G/T	G	0.585
rs4054823	chr17:13565749	C/T	T	0.555
rs11649743	chr17:33149092	A/G	G	0.837
rs4430796	chr17:33172153	A/G	A	0.550
rs1859962	chr17:66620348	G/T	G	0.526
rs8102476	chr19:43427453	A/G	G	0.587
rs2735839	chr19:56056435	A/G	G	0.863
rs5759167	chr22:41830156	G/T	G	0.544
rs5945619	chrX:51258412	A/G	G	0.394

HR for CC was 1.56 (95% CI, 1.20–2.02) and 1.27 (95% CI, 1.00–1.63) for AC when compared with homozygote AA (Table 4). The rs7679673 risk SNP was also associated with earlier onset of disease ($P = 0.0013$).

The cumulative incidence method for competing risk of death from other causes was also carried out. The cumulative incidence of an event of interest (prostate cancer–specific mortality) was calculated in the presence of competing risks (death from other causes). For both rs2735839 and rs7679673, the direction, magnitude, and significance of effects on prostate cancer–specific survival were consistent with the results described earlier (data not shown).

The SNP rs4054823, reported to be associated with prostate cancer aggressiveness, was not associated with any prostate cancer–associated clinical parameter, including D'Amico criteria ($P = 0.697$) and prostate cancer–specific mortality ($P = 0.12$; Supplementary Tables S1–S5). Xu and colleagues reported that for the putative aggressiveness marker, aggressive disease was defined in 3 different ways in the populations analyzed (24). As in the present study, criteria for aggressiveness were based on Gleason score, stage, and PSA. When the definition for aggressiveness used by Xu and colleagues in their discovery set was applied to our cohort, no association was observed with genotype at rs4054823 (data not shown).

The model for best evaluating the relationship between risk allele status and clinical outcome is not known. Although meaningful associations between genotype at independent risk SNPs and clinical phenotype may exist, the number of risk alleles an individual carries may

Table 3. Prostate cancer risk loci associated with clinical variables at the time of prostate cancer diagnosis ($P < 0.05$)

Age at diagnosis

SNP	Location	Genotype	*n*	Median (q1, q3)		*P*
rs7679673	chr4:106280983					0.0013
		AA	610	62 (55, 68)		
		AC	1,873	61 (56, 68)		
		CC	1,455	60 (55, 66)		
rs9364554	chr6:160753654					0.0004
		CC	2,014	61 (56, 68)		
		CT	1,586	61 (55, 67)		
		TT	324	60 (54, 65)		
rs13254738	chr8:128173525					0.0002
		AA	1,712	60 (55, 67)		
		AC	1,744	62 (56, 68)		
		CC	458	61 (56, 67)		
rs6983267	chr8:128482487					0.006
		GG	1,237	60 (55, 67)		
		GT	1,863	61 (55, 67)		
		TT	831	62 (56, 68)		
rs4430796	chr17:33172153					0.0007
		AA	1,195	60 (55, 66)		
		AG	1,943	61 (56, 67)		
		GG	796	62 (56, 68)		
rs1859962	chr17:66620348					0.017
		GG	1,060	60 (55, 67)		
		GT	2,038	61 (55, 67)		
		TT	837	61 (56, 68)		
rs2735839	chr19:56056435					0.008
		AA	66	64 (58, 68)		
		AG	943	62 (56, 67)		
		GG	2,918	61 (55, 67)		
PSA at diagnosis, ng/mL						
rs16901979	chr8:128194098					0.03
		AA	12	9.2 (6.2, 14.3)		
		AC	304	6.5 (4.9, 12.8)		
		CC	3,264	6.4 (4.6, 10.8)		
rs1859962	chr17:66620348					0.0037
		GG	959	6.2 (4.6, 10.1)		
		GT	1,845	6.3 (4.6, 11.0)		
		TT	776	6.9 (4.9, 12.0)		
Biopsy Gleason score						
rs6983267	chr8:128482487					0.033
		GG	622	389	207	
		GT	873	614	327	
		TT	363	274	174	
rs7000448	chr8:128510352					0.044
		CC	646	465	281	
		CT	888	622	306	
		TT	316	185	119	

Table 3. Prostate cancer risk loci associated with clinical variables at the time of prostate cancer diagnosis ($P < 0.05$) (cont'd)

rs2735839	chr19:56056435					0.00005
		AA	19	22	24	
		AG	396	307	203	
		GG	1,443	945	481	

Risk of aggressive disease

SNP	Location	Genotype	*n*	Median (q1, q3)		*P*
rs445114	chr8:128392363					0.045
		CC	134	159	135	
		CT	565	627	447	
		TT	586	566	399	
rs620861	chr8:128404855					0.016
		AA	130	157	134	
		AG	551	619	445	
		GG	605	580	405	
rs7931342	chr11:68751073					0.03
		GG	469	453	325	
		GT	587	648	445	
		TT	211	220	202	
rs2735839	chr19:56056435					0.0003
		AA	26	26	11	
		AG	267	314	281	
		GG	998	1,009	693	

influence disease aggressiveness. To evaluate this hypothesis, subjects were categorized on the basis of total risk allele burden. An individual can carry a total of 70 risk alleles (35 risk SNPs × 2 alleles per SNP). Subjects in the present cohort carried a median 32 risk alleles (range, 11–47). The cohort was split into quartiles on the basis of risk allele status, and associations with age at diagnosis, PSA at diagnosis, biopsy Gleason score, D'Amico criteria, and prostate cancer–specific mortality were analyzed. An association with age at diagnosis was detected ($P = 4 \times 10^{-5}$); the difference in median age between those carrying the fewest risk alleles (<29) and those carrying the most (≥35) was 2 years (median 62 years vs. 60 years, respectively). No statistically significant trends were observed for prostate cancer–specific mortality or the other clinical parameters evaluated.

Discussion

Although an estimated 1 in 6 American men will be diagnosed with prostate cancer in their lifetime, less than 3% men are expected to die because of the disease (35). Treatment of prostate cancer is associated with considerable morbidity, motivating better predictors for those who will and who will not benefit from intervention. The most valuable identifiers would distinguish those with a lethal form of the disease from those whose disease is destined to follow an indolent course. Accurate prediction of prostate cancer aggressiveness for many men, however, remains elusive. The recent discovery of multiple germ line prostate cancer risk alleles potentially opens avenues for refining current prognostic models. Germ line variants are particularly attractive biomarkers, as they are present at the time of diagnosis and remain static; polymorphisms are not influenced by the state of the disease or the timing of diagnosis. The clinical utility of prostate cancer risk markers, however, has not been fully determined. Although inherited variation clearly predisposes men to prostate cancer risk, associations between risk alleles and clinical outcome are uncertain.

We evaluated the associations between genotypes at 35 known prostate cancer risk loci and clinical characteristics of 3,945 prostate cancer patients of European ancestry. A significant strength of the study was the size and extensive clinical annotation of the patient cohort. The distribution of Gleason scores across the cohort is similar to other studies evaluating risk SNPs and clinical outcome (22, 23, 28). The cohort includes 580 prostate cancer–specific deaths, making this the largest study of its kind to evaluate this critical outcome. With 3,945 individuals and a 15% event rate, we have 80% power to detect an HR ranges from 1.5 to 1.7 for the risk allele frequency varying from 0.10 to 0.25. We detected 2 alleles that were significantly associated with prostate cancer–specific mortality: the rs2735839 *non*-risk allele (A; $P = 0.0007$) and the rs7679673 risk allele (C; $P = 0.014$).

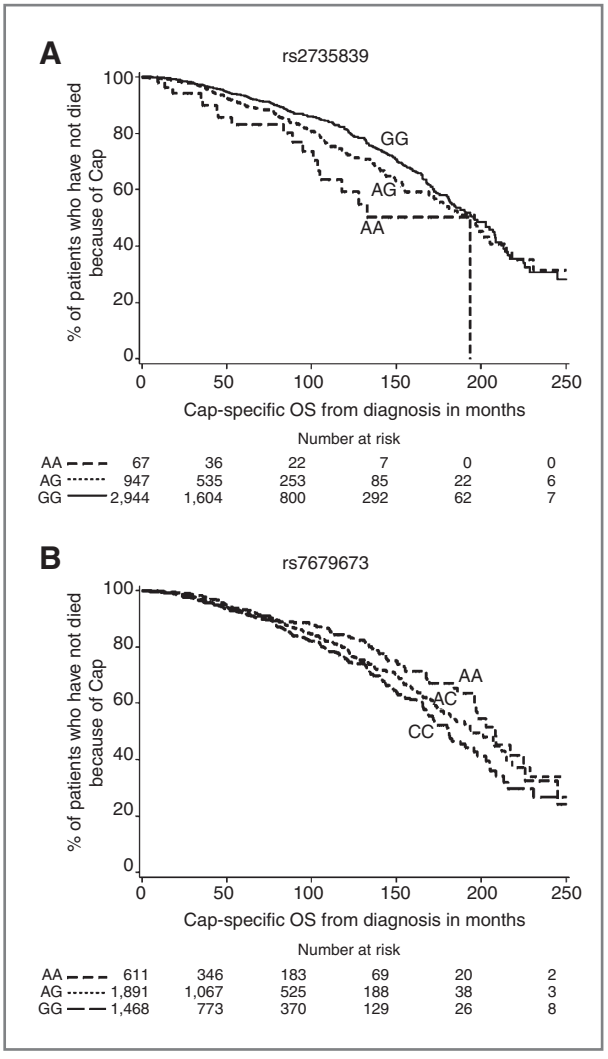

Figure 1. Prostate cancer–specific survival based on genotype at rs2735839 and rs7679673. A, Kaplan–Meier survival curves were plotted for individuals homozygous for the prostate cancer risk allele at rs2735839 (GG), homozygous for the non–risk allele (AA), and heterozygous (AG). The *x*-axis indicates survival from diagnosis of prostate cancer in months, and the *y*-axis represents the percentage of patients who have not died because of prostate cancer. B, similar analysis based on genotype at rs7679673. C is the prostate cancer risk allele and A is the protective (non-risk) allele. CaP, prostate cancer; OS, overall survival.

Only a handful of previous studies have evaluated associations between risk allele status and prostate cancer–specific mortality. Penney and colleagues (20) and Salinas and colleagues (21) analyzed the first set of risk markers reported by GWAS, at chromosome 8q24 and 17q, and found no correlation between genotype and survival. Wiklund and colleagues analyzed a cohort that included 440 prostate cancer–related deaths, genotyping a set of 16 risk SNPs, including rs2735839, and did not detect an association with mortality (22). Most recently, Gallagher and colleagues interrogated 29 risk SNPs in a cohort of 798 cases that included 91 prostate cancer–specific deaths (23). As in the present study, the group detected a significant association between the rs2735839 non–risk allele (A) and prostate cancer–specific mortality ($P = 0.0007$). Consistent with this finding, other series have shown that the protective rs2735839 allele (A) is associated with higher Gleason score and increased disease aggressiveness (19, 28).

The risk SNP rs2735839 resides in the intergenic region between *KLK3* and *KLK2* on chromosome 19q13, approximately 600 base pairs (bp) downstream of the 3′ untranslated region of *KLK3* and approximately 12.2 kilobases (kb) upstream of the transcription start site of *KLK2*. *KLK3* (also known as kallikrein 3) encodes PSA, and *KLK2* encodes kallikrein-related peptidase 2 (hK2), which, like PSA, is a known biomarker for prostate cancer. Variation at this locus has been associated with PSA levels in men in several, though not all, studies (10, 28–33, 36), and these changes have been observed primarily in subjects without a diagnosis of prostate cancer. Recently, Gudmundsson and colleagues scanned the entire genome for genetic variants associated with the PSA level. Notably, the risk allele at rs2735839 was highly associated with this phenotype in men without a diagnosis of prostate cancer (34).

Given that carriers of the risk allele have a naturally higher PSA, there has been debate about whether its association with disease risk is authentic or the product of ascertainment bias is due to its association with PSA. It has been reasoned that when men are screened for prostate cancer, carriers of the "risk" allele are more likely to meet a clinician's threshold for prostate biopsy than a noncarrier with similar underlying tumor biology. This results in increased incidence of prostate cancer among carriers. Counterparts carrying the non–risk allele have a lower PSA level and presumably a decreased likelihood of receiving a prostate cancer diagnosis. This was reflected in the study of Eeles and colleagues, the first GWAS describing rs2735839 as a risk locus (10). Stage 1 of that analysis used a control group selected on the basis of low PSA levels. The minor allele frequency of rs2735839 in the control group in stage 1 was appreciably higher than the minor allele frequency in stage 2 for which stringent PSA criteria were not used. In their GWAS analyzing PSA levels, Gudmundsson and colleagues observed an association between variation at 19q13 and prostate cancer risk but only for those with a diagnosis of prostate cancer in the PSA screening era (34).

On the other hand, in the GWAS by Eeles and colleagues, the association between rs2735839 persisted, albeit at a markedly lower significance level, in non–PSA-screened cohorts (10). In addition, functional data have implicated genetic variation at *KLK3* in increased risk of cancer development, consistent with the possibility that the locus is associated with both PSA and disease risk (31).

Bias is a strong consideration when evaluating the results of the present study. If there were an association between the rs2735839 risk allele and the PSA level in men with limited disease burden, risk allele carriers with indolent disease will have a relatively higher screening PSA level and are liable to be referred for prostate biopsy sooner than noncarriers with similar tumor biology. Carriers with

Table 4. Prostate cancer risk loci associated with prostate cancer-specific mortality

Locus	SNP	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)	*P*
Chr19	rs2735839		0.0007		0.005
	AA	2.14 (1.33–3.44)		1.92 (1.20–3.01)	
	AG	1.26 (1.05–1.52)		1.23 (1.01–1.47)	
	GG	1 (reference)		1 (reference)	
Chr4	rs7679673		0.014		0.002
	AA	1 (reference)		1 (reference)	
	AC	1.22 (0.95–1.57)		1.27 (1.00–1.63)	
	CC	1.44 (1.11–1.86)		1.56 (1.30–2.02)	

indolent disease and a borderline abnormal PSA level would therefore receive a prostate cancer diagnosis more often than noncarriers. As a result, our prostate cancer cohort would be enriched with men who carry the rs2735839 risk allele and have low grade, nonaggressive disease. In addition, the diagnosis of non–risk allele carriers whose disease requires prompt attention may be delayed because of a suppressed PSA level. Consistent with a hypothesis of ascertainment bias, subgroup analysis of those with a diagnosis of disease prior to the PSA era showed no association between rs2735839 and mortality.

Even if rs2735839 proves to be associated solely with the PSA level, the findings reported here are noteworthy. In Gudmundsson et al, an argument is made that genotype at 19q13 could inform decisions about PSA cutoff values and referral for biopsy (34). Those carrying the risk allele would have a higher PSA threshold for biopsy and/or carriers of the non–risk allele would have a lower threshold. The results presented here support this notion. The consequences of these effects on PSA levels in a screened population were substantial: carriers of the non–risk allele died more often because of the disease.

On the other hand, other aspects of the present study are consistent with a true association between the protective allele and lethal disease. First, no association between rs2735839 genotype and PSA was observed, albeit in a cohort of prostate cancer cases. Second, the association with prostate cancer–specific mortality was maintained in multivariable analysis that included age at diagnosis and D'Amico risk group (a combination of PSA, Gleason score, and stage). Deciphering the mechanism underlying risk could prove particularly challenging, as the associated variant seems to increase risk of developing prostate cancer but decrease risk of lethal disease. It is possible that 2 causal variants residing at this locus, each in linkage disequilibrium with rs2735839, have differential effects on target genes.

The rs7679673 risk allele at chromosome 4q24 was also associated with prostate cancer–specific mortality on univariate and multivariable analyses. This SNP resides approximately 6.5 kb from the transcription start site of *TET2*. This gene recently has been characterized as a tumor suppressor gene involved in pathogenesis of acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms (37). The next closest gene, located approximately 229 kb from the risk variant, *PP2A* has been implicated in androgen receptor regulation in prostate cancer cell lines (38). No other study, to our knowledge, has evaluated rs7679673 locus for its association with prostate cancer–specific survival.

For the variables age at diagnosis, PSA at diagnosis, Gleason score, and aggressiveness, there was no marked overlap between the findings presented here and data previously published. Two chromosome 17 risk SNPs (rs4430796 and rs1859962) were associated with early onset of disease on univariate analysis here ($P < 0.05$), an association that did not reach statistical significance was also reported by Gudmundsson and colleagues (7). Two groups reported that the rs10993994 risk allele at chromosome 10q was associated with less aggressive disease (19, 28), an association not observed here. Finally, Xu and colleagues analyzed data generated from the Genetic Markers of Susceptibility Study (CGEMS) to identify an SNP, rs4054823, associated with aggressive prostate cancer (24). In our cohort, this SNP was not associated with aggressive disease ($P = 0.697$) or prostate cancer–specific mortality ($P = 0.12$).

We also attempted to evaluate total number of risk alleles carried by an individual and its associations with clinical presentation and outcome. Although it is possible that several inherited risk variants from various loci contribute collectively to the development of a particular phenotype, it is unclear how best to model this. We chose to add the number of risk alleles to quantify an individual's "risk allele burden." Those carrying a greater number of risk variants had a diagnosis of prostate cancer at an earlier age than those carrying fewer risk variants ($P = 4 \times 10^{-5}$). No other clinical parameter was associated with an overall risk allele status. However, it is difficult to determine how best to integrate all of the genetic data. In some cases, as with rs2735839, a non–risk allele may be associated with a more aggressive phenotype, and, in other cases, the risk allele may be associated with more aggressive disease.

Genome-wide scans have successfully identified inherited variants associated with prostate cancer risk. To fully utilize these markers in clinic, it is critical to fully understand the clinical implications of carrying the risk alleles, particularly their impact on mortality. Our study identifies two SNPs associated with prostate cancer–specific disease. Further work is necessary to characterize these risk loci and

determine how to optimally translate these findings into clinical practice.

Disclosure of Potential Conflicts of Interest

No conflict of interest was disclosed.

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