Association of Prostate Cancer Risk Variants with Clinicopathologic Characteristics of the Disease

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Abstract

Purpose: Fifteen independent genetic variants have been implicated in prostate cancer risk by recent genome-wide association studies. However, their association with clinicopathologic features of prostate cancer is uncertain.

Experimental Design: We systematically evaluated these 15 variants in 1,563 prostate cancer patients undergoing radical prostatectomy, taking advantage of the uniform tumor stage and grade information available for each of these cases. Associations of these variants with aggressiveness, pathologic Gleason scores, pathologic stage, age at diagnosis, or serum prostate-specific antigen (PSA) levels were tested.

Results: After adjusting for multiple testing, none of the single nucleotide polymorphisms was individually or cumulatively associated with aggressiveness or individual clinicopathologic variables of prostate cancer such as Gleason scores, pathologic stage, or age at diagnosis of prostate cancer. The reported risk allele (G) for single nucleotide polymorphism rs2735839 in the *KLK3* gene at 19q13 was more frequent in less aggressive prostate cancer patients (0.89) than in more aggressive prostate cancer patients (0.86; nominal $P = 0.03$) or in controls (0.86; nominal $P = 0.04$). Considering that this allele was also significantly associated with higher serum PSA levels among controls (nominal $P = 0.003$), the observed trend of higher frequency of this risk allele between less and more aggressive prostate cancer, or between less aggressive and controls may be due to detection bias of PSA screening.

Conclusions: Prostate cancer risk variants recently discovered from genome-wide case-control association studies are not associated with clinicopathologic variables in this population. Case-case studies are urgently needed to discover genetic variants that predict tumor aggressiveness.

Recently, several single nucleotide polymorphisms (SNP) have been implicated in prostate cancer risk by genome-wide association studies (1–9). In contrast to the difficulty in replicating association for previously reported prostate cancer risk variants (10), these novel risk variants can be consistently replicated in multiple study populations (11–20). However, results on associations of these risk variants with clinicopathologic features of prostate cancer were inconsistent. Although there is a moderate trend of risk alleles observed more often in prostate cancer patients with early age at diagnosis, higher Gleason grade and/or stage or more aggressive form of the disease in some reports (1, 3, 5, 11–14, 15), no such associations were found in other studies (2, 4, 16, 17). These inconsistent results may be partially due to the heterogeneous nature in evaluating clinical and pathologic features of prostate cancer.

To better understand associations of these variants with clinicopathologic variables of prostate cancer, we systematical evaluated all prostate cancer risk variants reported to date from recent genome-wide association studies in a population of 1,563 cases who underwent radical prostatectomy for treatment of prostate cancer at Johns Hopkins Hospital. Because each patients' cancer was extensively and accurately graded and staged using uniform criteria in this case series, it offers an excellent ability to assess associations of genetic variants with clinicopathologic characteristics of prostate cancer.

Materials and Methods

Study subjects. The JHH study population was described in detail elsewhere (12). Briefly, the prostate cancer patients were 1,563 men of European descent (by self report) who underwent radical prostatectomy for treatment of prostate cancer at The Johns Hopkins Hospital from January 1, 1999, through December 31, 2006. Because the prostate

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Received 4/9/08; revised 5/30/08; accepted 6/3/08.

Grant support: National Cancer Institute grants CA106523 and CA95052 (J. Xu); CA112517 and CA58236 (W.B. Isaacs); CA86323 (A.W. Partin) and by Department of Defense grant PC051264 (J. Xu).

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doi:10.1158/1078-0432.CCR-08-0934

Translational Relevance

Because most prostate cancers have a favorable outcome, molecular markers capable of providing prognostic information are urgently needed to assist in the identification of the subset of patients who will benefit from aggressive treatment. In this study, we assessed the possible association between recently identified genetic markers of prostate cancer risk and pathologic indicators of cancer aggressiveness. Using pathologic stage and grade to divide men undergoing radical prostatectomy into more or less aggressive cancer groups, we compared the frequencies of alleles at 15 single nucleotide polymorphisms previously confirmed to be associated with risk of prostate cancer. Although two single nucleotide polymorphisms showed different frequencies between the groups with more or less aggressive prostate cancer, no single nucleotide polymorphisms were significantly different when the results were adjusted for multiple tests. Although these markers seem to be informative for the identification of men who may be at elevated risk for a prostate cancer diagnosis, they do not seem to be helpful in identifying men at risk for developing a more aggressive prostate cancer. Additional studies directly comparing cases with more or less aggressive disease in the discovery phase should be pursued to identify genetic variants that predict aggressive characteristics of prostate cancer.

gland was removed entirely for each patient, each tumor was accurately and systematically graded using the Gleason scoring system (21) and staged using the tumor-node-metastasis system (22), as described previously (23). We defined more aggressive and less aggressive disease based on pathologic tumor stage and Gleason score (Table 1). Tumors with pathologic Gleason Scores of 7 or higher, or pathologic stage T_3 or higher, or N+ or M+ (i.e., either high-grade or nonorgan-confined disease) were oversampled from this patient population and defined as more aggressive disease (n = 1,017). Tumors with pathologic Gleason scores of 6 or lower and pathologic stage $T_2/N_0/M_0$ (i.e., cancer confined to the prostate) were defined as less aggressive disease (n = 546). In a recent study with postoperative follow-up for >2,500 patients with pathologically organ-confined, Gleason score 6 or less prostate cancer, biochemical recurrence and local recurrence after radical prostatectomy were extremely rare, and no patients experienced distant metastases or prostate cancer specific mortality (24). Normal seminal vesicle tissue that was obtained and frozen at the time of surgery was used to isolate DNA for genotyping of case patients. As a reference group, men undergoing screening for

Table 1. Clinicopathologic variables of prostate cancer patients

	More aggressive cases	Less aggressive cases
No. of subjects	1,017	528
Mean age (SD), y	60.1 (6.89)	56.8 (6.46)
Age at diagnosis, *n* (%)		
<65 y	708 (72)	463 (88)
≥65 y	274 (28)	64 (12)
Serum PSA levels, *n* (%)		
<4.0 ng/mL	76 (10)	191 (36)
≥4.0 ng/mL	660 (90)	333 (64)
Pathologic stage, *n* (%)		
T_2N_0	186 (27)	528 (100)
Td or N+ or M+	502 (73)	0 (0)
Pathologic Gleason score, *n* (%)		
≥6	72 (8)	528 (100)
7	606 (63)	0 (0)
8	148 (15)	0 (0)
≥9	131 (14)	0 (0)

Table 2. Association of 15 reported prostate cancer risk SNPs with clinicopathologic variables in prostate cancer patients

SNPs	Chr	Position*	Alleles†		Frequency of risk allele in controls *n* = 486	Aggressiveness		
			Reference	Risk		Frequency of risk alleles		*P*
						More *n* = 1,017	Less *n* = 528	
rs2660753	3p12	87,193,364	C	T	0.12	0.14	0.13	0.53
rs9364554	6q25	160,804,075	C	T	0.27	0.31	0.27	0.04
rs10486567	7p15	27,749,803	T	C	0.80	0.78	0.78	0.65
rs6465657	7q21	97,654,263	T	C	0.46	0.48	0.49	0.57
rs16901979	8q24 (2)	128,194,098	C	A	0.04	0.05	0.05	0.63
rs6983267	8q24 (3)	128,482,487	T	G	0.50	0.56	0.58	0.40
rs1447295	8q24 (1)	128,554,220	C	A	0.08	0.14	0.12	0.27
rs1571801	9q33	121,506,927	G	T	0.23	0.26	0.26	0.64
rs10993994	10q11	51,219,502	C	T	0.43	0.47	0.46	0.59
rs4962416	10q26	126,686,862	A	G	0.28	0.31	0.33	0.38
rs10896449	11q13	68,751,243	A	G	0.52	0.57	0.57	0.86
rs4430796	17q12	33,172,153	C	T	0.51	0.58	0.58	0.74
rs1859962	17q24.3	66,620,348	T	G	0.48	0.51	0.51	0.95
rs2735839	19q13	56,056,435	A	G	0.86	0.86	0.89	0.03
rs5945619	Xp11	51,074,708	A	G	0.33	0.42	0.36	0.02

Abbreviation: Chr, chromosome.
*Build35.
† Risk alleles are based on the previously published studies.

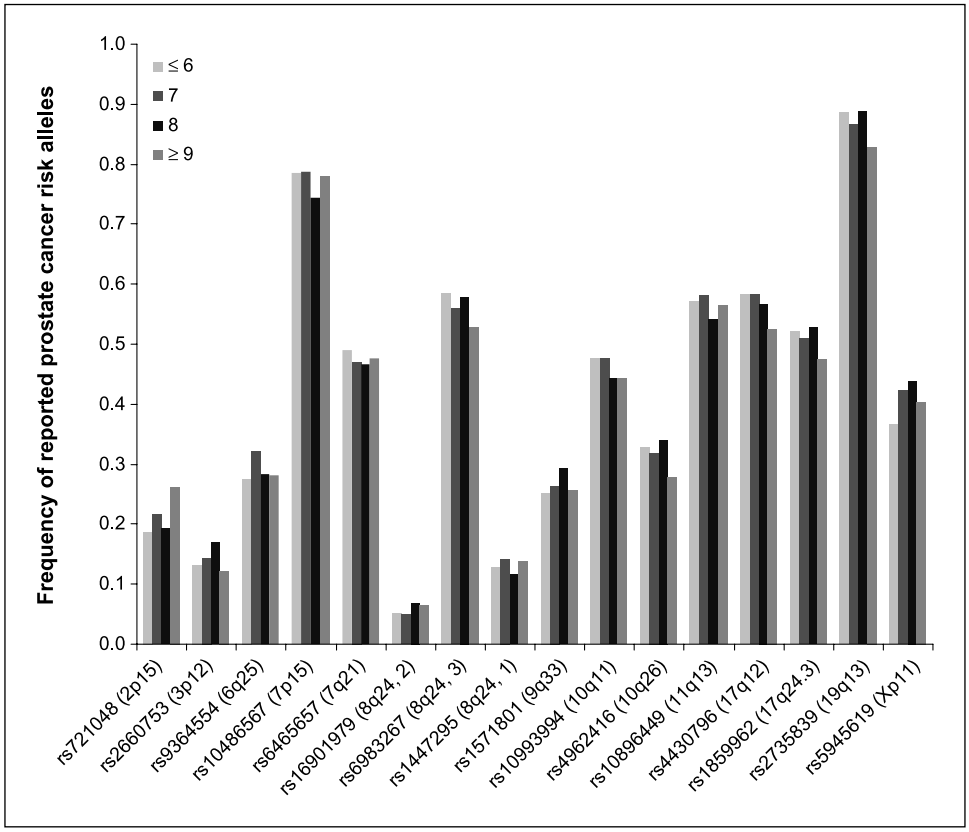

Fig. 1. Frequencies of reported risk alleles of 15 SNPs in patients with Gleason scores of ≤6, 7, 8, or ≥9. No consistent increase or decrease in the patterns of the reported risk alleles with increasing Gleason scores was found.

prostate cancer at The Johns Hopkins Hospital and The Johns Hopkins University Applied Physics Laboratory during the same time period were asked to participate as control subjects. Serum prostate-specific antigen (PSA) levels, digital rectal examination results, and demographic information were available for these subjects. A total of 482 men of European descent (by self report) met our inclusion criteria as control subjects for this study: normal digital rectal examination, PSA levels of <4.0 ng/mL, and age older than 55 y.

Selection of SNPs and SNP genotyping. We selected 15 SNPs that were implicated in four genome-wide association studies and were replicated in at least one independent study population in the original papers (1–9). They included one SNP each from 8q24 (three separate sub regions), 17q12, 17q24.3, 3p12, 6q25, 7p15, 7q21, 9q33, 10q11, 10q26, 11q13, 19q13, and Xp11. The SNP at 2q15 was not included because no evidence for prostate cancer association was found in this study population in the initial report (8).

Table 2. Association of 15 reported prostate cancer risk SNPs with clinicopathologic variables in prostate cancer patients (Cont'd)

Stage			Age at diagnosis (y)		
Frequency of risk alleles		*P*	Frequency of risk alleles		*P*
$T_3/N+/M+$ *n* = 714	$T_2/N_0/M_0$ *n* = 528		≥65	<65	
0.13	0.14	0.75	0.14	0.14	0.72
0.30	0.28	0.35	0.28	0.30	0.47
0.78	0.78	0.79	0.76	0.79	0.11
0.48	0.48	0.79	0.46	0.49	0.22
0.05	0.06	0.34	0.04	0.06	0.26
0.56	0.58	0.22	0.59	0.56	0.20
0.14	0.13	0.15	0.12	0.13	0.50
0.26	0.27	0.55	0.27	0.26	0.42
0.49	0.46	0.18	0.45	0.47	0.29
0.32	0.32	0.81	0.32	0.32	0.89
0.57	0.58	0.66	0.57	0.57	0.79
0.57	0.58	0.70	0.56	0.58	0.33
0.53	0.51	0.28	0.50	0.52	0.45
0.86	0.89	0.02	0.87	0.88	0.56
0.43	0.37	0.05	0.40	0.40	0.98

Table 3. Association of 15 SNPs with serum PSA levels in cases (preoperative) and controls

Variable	Chr	Position*	Alleles†		Cases				Controls			
			W	R	WW	WR	RR	*P*	WW	WR	RR	*P*
rs2660753	3p12	87,193,364	C	T	8.80	8.66	13.09	0.35	1.09	1.17	1.78	0.55
rs9364554	6q25	160,804,075	C	T	8.70	8.62	11.15	0.18	1.14	1.08	1.19	0.64
rs10486567	7p15	27,749,803	T	C	10.04	8.78	8.84	0.77	1.15	1.07	1.14	0.72
rs6465657	7q21	97,654,263	T	C	8.37	8.88	9.30	0.61	1.13	1.08	1.17	0.66
rs16901979	8q24 (2)	128,194,098	C	A	8.86	8.49	16.01	0.40	1.10	1.16	2.77	0.20
rs6983267	8q24 (3)	128,482,487	T	G	8.75	8.78	9.03	0.95	1.04	1.12	1.16	0.57
rs1447295	8q24 (1)	128,554,220	C	A	8.77	9.43	7.29	0.52	1.10	1.14	0.55	0.27
rs1571801	9q33	121,506,927	G	T	8.88	9.09	7.68	0.52	1.06	1.13	1.42	0.10
rs10993994	10q11	51,219,502	C	T	9.80	8.56	8.47	0.23	1.09	1.07	1.29	0.12
rs4962416	10q26	126,686,862	A	G	9.29	8.31	8.95	0.35	1.11	1.10	1.10	0.99
rs10896449	11q13	68,751,243	A	G	10.15	8.49	8.73	0.19	1.05	1.17	1.07	0.34
rs4430796	17q12	33,172,153	C	T	8.75	9.23	8.40	0.50	1.11	1.06	1.20	0.34
rs1859962	17q24.3	66,620,348	T	G	9.23	8.73	8.86	0.83	1.21	1.08	1.03	0.19
rs2735839	19q13	56,056,435	A	G	9.09	8.34	8.99	0.69	0.79	0.86	1.23	2.9E-03
rs5945619	Xp11	51,074,708	A	G	8.76		8.94	0.93	1.10	1.00	1.12	0.82

Abbreviations: W, wild-type; R, risk.
*Build35.
† Wild-type and risk alleles are based on the previously published studies.

These 15 SNPs were genotyped using a MassARRAY QGE iPLEX system (Sequenom, Inc.). PCR and extension primers for these SNPs were designed using MassARRAY Assay Design 3.0 software (Sequenom, Inc.). The primer information is available at the Wake Forest University Baptist Medical Center Web site.[6] PCR and extension reactions were done according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. Duplicate test samples and two water samples (PCR-negative controls) that were blinded to the technician were included in each 96-well plate. The average genotype call rate for these SNPs was 98.1% and the average concordance rate was 99.8% among 100 duplicated quality control samples. Each of the SNPs in the autosomal chromosomes was in Hardy-Weinberg equilibrium ($P \geq 0.05$).

Statistical analyses. Allele frequency differences between two groups of patients (more versus less aggressive, early versus late age at diagnosis, or organ confined versus nonorgan confined) were tested for each SNP using a χ^2 test with 1 degree of freedom. Associations of increasing Gleason scores (≤6, 7, 8, or ≥9) with risk genotypes of each SNP, assuming a dominant or recessive model, were tested using the Cochran-Armitage test for trend with 1 degree of freedom. Associations of serum PSA levels with each SNP were tested separately in patients (preoperative PSA) or in controls (at the time of sampling) using an additive model. PSA levels were logarithm transformed before tests.

We tested the cumulative effects of prostate cancer risk SNPs on aggressiveness of prostate cancer by counting the number of prostate cancer–associated alleles (based on the best-fitting genetic model from single SNP analysis) of these 15 SNPs in each subject. The odds ratio for more aggressive prostate cancer for men carrying more prostate cancer risk alleles (2nd, 3rd, 4th, and 5th quintiles) was estimated by comparing to men carrying the lowest quintile (1st) using logistic regression analysis. The Cochran-Armitage test for trend was also done to test increasing risk for aggressive prostate cancer with increasing quintile of number of risk alleles.

All reported *P* values were based on a two-sided test.

[6] http://www.wfubmc.edu/genomics

Results

As a primary analysis, we tested associations of these 15 SNPs with aggressiveness of prostate cancer defined using tumor stage and grade as determined for the radical prostatectomy specimen (Table 2). Three SNPs reached a nominal *P* value of <0.05, including rs9364554 at 6q25 ($P = 0.04$), rs2735839 at 19q13 ($P = 0.03$), and rs5945619 at Xp11 ($P = 0.02$). The reported risk alleles were more frequent among more aggressive prostate cancer patients for SNPs at 6q25 and Xp11. In contrast, the reported risk allele (G) for the SNP at 19q13 was more frequent in less aggressive prostate cancer patients (0.89) than in more aggressive prostate cancer patients (0.86; $P = 0.03$) and in controls (0.86; $P = 0.04$). However, when a Bonferroni correction was applied to account for 15 independent tests, none of the SNPs reached an adjusted *P* value of 0.003 that is required for a 5% study-wise significance level. Results were similar when we removed cases who have Gleason score of 7 and localized disease from the group of more aggressive prostate cancer.

As a secondary analysis, we tested associations of these 15 SNPs with individual clinicopathologic characteristics of prostate cancer (Table 2). For pathologic stage, only rs2735839 at 19q13 reached a nominal *P* value of <0.05; the reported risk allele (G) was more frequent in organ-confined than nonorgan-confined prostate cancer patients ($P = 0.02$). For age at diagnosis, none of the 15 SNPs reached a nominal *P* value of 0.05. Similarly, we did not find any significant trend of association between reported risk genotypes (using either dominant or recessive model) and each increasing Gleason score (≤ 6, 7, 8, or ≥ 9). As shown in Fig. 1, we observed no consistent increase or decrease in the patterns of the reported risk alleles with increasing Gleason scores.

We also tested association of these 15 SNPs with preoperative serum PSA levels among all patients. Not unexpectedly, no significant association, at a nominal *P* value of <0.05, was found for any of these 15 SNPs (Table 3). To assess the association of

Table 3. Association of 15 SNPs with serum PSA levels in cases (preoperative) and controls (Cont'd)

log in cases			log in controls								
2.175	2.159	2.572	0.084	0.156	0.574	8.802185	8.662470	13.09198	1.087629	1.168826	1.775354
2.163	2.154	2.411	0.135	0.076	0.177	8.697190	8.619266	11.14510	1.144537	1.078963	1.193631
2.307	2.173	2.179	0.136	0.072	0.131	10.04424	8.784598	8.837464	1.145682	1.074655	1.139968
2.125	2.184	2.230	0.125	0.080	0.155	8.372897	8.881762	9.299866	1.133148	1.083287	1.167658
2.181	2.139	2.773	0.095	0.150	1.020	8.855156	8.490942	16.00658	1.099659	1.161834	2.773195
2.169	2.173	2.200	0.044	0.109	0.149	8.749530	8.784598	9.025013	1.044982	1.115162	1.160673
2.171	2.244	1.987	0.099	0.127	-0.592	8.767046	9.430979	7.293620	1.104066	1.135417	0.55322
2.184	2.207	2.038	0.061	0.126	0.351	8.881762	9.088410	7.675243	1.062899	1.134282	1.420487
2.282	2.147	2.137	0.089	0.063	0.258	9.796253	8.559142	8.473977	1.093081	1.065027	1.294339
2.229	2.118	2.192	0.103	0.098	0.097	9.290570	8.314491	8.953101	1.108491	1.102963	1.10186
2.317	2.139	2.167	0.050	0.161	0.071	10.14519	8.490942	8.732048	1.051271	1.174685	1.073581
2.169	2.223	2.128	0.107	0.056	0.179	8.749530	9.234994	8.398053	1.112934	1.057598	1.196021
2.223	2.167	2.182	0.193	0.077	0.029	9.234994	8.732048	8.864016	1.212883	1.080042	1.029425
2.207	2.121	2.196	-0.232	-0.155	0.206	9.088410	8.339472	8.988985	0.792946	0.856415	1.228753
2.170		2.190	0.096		0.113	8.758284	1	8.935213	1.100759	1	1.119632

these 15 SNPs with serum PSA levels among men not thought to have prostate cancer, we tested the associations in 482 undiagnosed control subjects. Although this group was preselected to have PSA values below 4 ng/mL, 1 SNP (rs2735839) at 19q13 was significantly associated with PSA levels in these men (P = 0.003); men who are homozygous for the reported risk allele (GG) had higher mean PSA levels (1.23 ng/mL) than carriers of GA (0.86 ng/mL) and AA (0.79 ng/mL). This association is biologically plausible because this SNP is in the 3′ of the *KLK3* gene that encodes for a PSA precursor.

Finally, we tested the cumulative effect of these 15 risk variants on aggressiveness of prostate cancer. The odds ratios for aggressive prostate cancer were estimated among patients who have 2nd, 3rd, 4th, or 5th quintile numbers of prostate cancer risk alleles, respectively, compared with patients who have the lowest quintile of prostate cancer risk alleles (Table 4). There was no significant trend of increasing risk for aggressive prostate cancer with increasing quintile of number of risk alleles (P_{trend} = 0.33).

Discussion

The systematic evaluation of all reported prostate cancer risk variants in this large and uniformly evaluated case series from a single hospital provides compelling evidence that the prostate cancer risk variants discovered to date from genome-wide association studies are not strongly associated with clinicopathologic characteristics of prostate cancer. The null findings from this study were unlikely due to lack of statistical power to detect association. To increase our ability to detect SNP effects on more aggressive disease, we oversampled men with cancers with high-grade or high-stage disease as determined after radical prostatectomy. With 1,017 more aggressive and 546 less aggressive prostate cancer patients in our study, we have >80% power to detect an allele that is 0.2 (0.1) in the population that confers odds ratio of 1.3 (1.4) for more aggressive prostate cancer. Because all the cases examined in this study were candidates for curative surgery, these associations should be also assessed in large populations of men presenting with clinically nonorgan-confined (i.e., metastatic) disease.

Table 4. Association of cumulative effect of risk SNPs with aggressiveness of prostate cancer

Quintile of no.	Aggressiveness of prostate cancer		
Risk alleles	**Odds ratio (95% CI)**	***P***	P_{trend}
1st	1.00		
2nd	1.13 (0.81-1.57)	0.49	
3rd	1.41 (1.01-1.97)	0.04	
4th	1.07 (0.77-1.50)	0.68	
5th	1.15 (0.81-1.64)	0.43	0.33

Abbreviation: 95% CI, 95% confidence interval.

The lack of association between these prostate cancer risk variants and aggressiveness of prostate cancer was also found in the data from National Cancer Institute Cancer Genetic Markers of Susceptibility Initiative (CGEMS; ref. 4). The CGEMS study subjects, including 1,172 prostate cancer patients and 1,157 control subjects of European Americans, were selected from the Prostate, Lung, Colon, and Ovarian Cancer Screening Trial. Among the prostate cancer patients, 737 were classified as having more aggressive prostate cancer, defined as clinical stage T_3/T_4 or Gleason Score of $\geq$7 based on biopsy specimen, and 624 were classified as having less aggressive disease, defined as clinical Gleason Score of <7 and stage of less than III. We downloaded individual genotype data from CGEMS Web site[7] and tested association of these 15 SNPs with aggressiveness of prostate cancer. Fourteen of these 15 SNPs were directly genotyped as part of the ~528,000 SNPs in the CGEMS genome-wide association study. One SNP (rs16901979) was imputed from the adjacent genotyped SNPs at 8q24 using the computer program IMPUTE (25). Among these 15 SNPs, only the SNP rs2735839 at 19q13 was significantly associated with aggressiveness of prostate cancer in the CGEMS study (nominal P = 0.003). The association remained significant after adjusting for 15 independent tests using a Bonferroni correction.

[7] http://cgems.cancer.gov/data/

Interestingly, similar to the findings in our study, the reported risk allele (G) of this SNP was also more frequent in less aggressive prostate cancer (0.89) than in more aggressive prostate cancer (0.85) and unaffected controls (0.84).

The lack of association between prostate cancer risk variants and clinicopathologic characteristics observed in this study has important implications. From a mechanistic perspective, this lack of association with stage or grade may indicate that the initiating events for both more and less aggressive prostate cancers are more similar rather than disparate, and that other factors are more important in determining the aggressive nature of individual prostate cancers. Whether these other factors are genetic, environmental, or stochastic in nature will need to be determined by appropriately designed studies. From a clinical perspective, the unfortunate implication is that these SNPs only provide information about who may be at risk for any prostate cancer, as opposed to a cancer with a predilection for more or less aggressive behavior. Considering that a significant number, yet only ~20% of all prostate cancer patients, die of the disease, it is important to identify markers that predict risk for more aggressive prostate cancer. This subset of patients need to be diagnosed earlier and treated aggressively.

It is interesting to observe that prostate cancer risk allele (G) of rs2735839 in the *KLK3* gene was consistently higher among less aggressive (0.89) prostate cancers than more aggressive prostate cancers (0.85-0.86) and unaffected controls (0.84-0.86) in both ours and the CGEMS study. Although the basis for this difference is unknown, it may be due to the association of this allele with higher PSA levels per se, rather than with prostate cancer risk. Because the vast majority of patients in this study were diagnosed as a result of elevated PSA levels, any alleles that contribute to such elevated levels will tend to be present in higher frequency among cases, regardless if they are directly associated with prostate cancer risk or not. It is possible that patients with less aggressive prostate cancer are more likely to carry such PSA-elevating alleles than are nonorgan-confined patients whose PSA levels are elevated as a direct result of more invasive prostate cancer, rather than the allele associated with higher PSA level. If these speculations are correct, the association of this SNP with aggressiveness of prostate cancer merely represents a detection bias due to PSA screening. Based on the same argument, this PSA-related detection bias may also account for the different allele frequencies of this SNP between prostate cancer patients as a group and controls. Obviously, further studies will be necessary to dissect these relationships.

It is important to note that failure to detect association between these prostate cancer risk SNPs and clinicopathologic variables of prostate cancer does not imply lack of such genetic variants in the genome. These 15 prostate cancer risk variants were all discovered using a case-control design. Different study designs, such as case-case studies that compare genetic variants among more or less aggressive prostate cancer cases, should be more efficient to identify genetic variants that predict aggressive characteristics of prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank all the study subjects who participated in this study and the National Cancer Institute CGEMS for making the data available publicly. The authors take full responsibility for the study design, data collection, analysis and interpretation of the data, the decision to submit the manuscript for publication, and the writing of the manuscript. The support of Kevin Jaffe to W.B. Isaacs is gratefully acknowledged.

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