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Individual and cumulative effect of prostate cancer risk-associated variants on clinicopathologic variables in 5,895 prostate cancer patients

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Abstract

Background—More than a dozen single nucleotide polymorphisms (SNPs) have been associated with prostate cancer (PCa) risk from genome-wide association studies (GWAS). Their association with PCa aggressiveness and clinicopathologic variables is inconclusive.

Methods—Twenty PCa risk SNPs implicated in GWAS and fine mapping studies were evaluated in 5,895 PCa cases treated by radical prostatectomy at Johns Hopkins Hospital, where each tumor was uniformly graded and staged using the same protocol.

Results—For 18 of the 20 SNPs examined, no statistically significant differences ($P > 0.05$) were observed in risk allele frequencies between patients with more aggressive (Gleason Scores $\geq 4+3$, or stage $\geq T3b$, or N+) or less aggressive disease (Gleason Scores $\leq 3+4$, and stage $\leq T2$, and N0). For the two SNPs that had significant differences between more and less aggressive disease (rs2735839 in *KLK3* ($P = 8.4 \times 10^{-7}$) and rs10993994 in *MSMB* ($P = 0.046$), the alleles that are associated with increased risk for PCa were more frequent in patients with *less* aggressive disease. Since these SNPs are known to be associated with PSA levels in men without PCa diagnoses, these latter associations may reflect the enrichment of low grade, low stage cases diagnosed by contemporary disease screening with PSA.

Conclusions—The vast majority of PCa risk-associated SNPs are not associated with aggressiveness and clinicopathologic variables of PCa. Correspondingly, they have minimal utility in predicting the risk for developing more or less aggressive forms of PCa.

Keywords

association; prostate cancer; genetics; aggressiveness; Gleason score; stage

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INTRODUCTION

Four GWAS of PCa reported to date have identified more than a dozen genetic variants in the genome that are significantly associated with PCa risk [1-9]. In addition, fine mapping studies in some of the regions containing these variants have revealed several novel PCa risk-associated SNPs [10-16]. Altogether, 20 independent PCa risk-associated SNPs have been consistently implicated in multiple study populations, with overall statistical significance approaching or exceeding the genome-wide significance level. The discovery of these risk-associated SNPs may provide new insights into PCa disease etiology, as the majority of these are not located in or near genes known to be involved in the development of this disease. In addition, the consistent finding of these common risk-associated SNPs among different populations suggests they may be used, in combination, to predict an individual's risk to PCa [17-18].

Given the PCa, a more important clinical question may be whether these inherited SNPs could be used to differentiate men at increased risk to more aggressive PCa which requires earlier and more intensive therapy as compared to less aggressive PCa which may be more conservatively treated and in appropriate cases expectantly managed [19-20]. Several studies have tested the association of these PCa risk SNPs with aggressiveness and clinicopathologic variables [1-18,21-29]. However, results available to date are inconclusive; with some studies reporting stronger associations for some of these SNPs among more aggressive PCa patients, for example those at 8q24 [1,3,10-11,22-28], 2p15 [7], 6q25 [29], 9q33 [9], 17q12 [5], and 22q13 [13], while others did not. The discrepancies may be due in part to small sample size, heterogeneous criteria in classifying aggressive PCa, and, in some studies, reliance on clinical grading and staging techniques that are subject to biopsy-related sampling errors and limited sensitivity of physical examination, respectively.

Herein we report a study designed to address this question by overcoming these known limitations. We systematically evaluated all PCa risk-associated variants reported to date from recent GWAS and fine mapping studies in a large patient cohort (N = 5,895) who underwent radical prostatectomy for treatment of PCa at Johns Hopkins Hospital (JHH). Because the entire prostate of each patient was assessed using uniform grading and staging criteria applied by pathologists at a single center, this cohort offers a unique opportunity to examine associations of genetic variants with aggressiveness and clinicopathologic variables of PCa.

MATERIALS AND METHODS

Study subjects

The initial subset of the JHH study population has been described in detail elsewhere [21]. Additional PCa patients that have been recruited using the same study protocol were included in this study. The PCa patients were 5,895 men of European descent (by self report) who underwent radical prostatectomy for treatment of PCa at The Johns Hopkins Hospital from January 1, 1999, through December 31, 2008. As the entire prostate gland was available for analysis, each tumor was accurately and systematically graded using the Gleason scoring system [30] and staged using the TMN (tumor-node-metastasis) system [31]. We defined more aggressive and less aggressive disease based on pathologic tumor stage and Gleason score (Table 1). Tumors with pathologic Gleason Scores of 4+3 or higher, or pathologic stage T3b or higher, or N+ (i.e., either high-grade or non-organ-confined disease) were over-sampled from this patient population and defined as more aggressive disease (N=1,253). Tumors with pathologic Gleason score of 3+4 or lower and pathologic stage T2/N0 (i.e., cancer confined to the prostate) were defined as less aggressive disease (N=4,233). Normal seminal vesicle tissue that was obtained and frozen at the time of surgery was used to isolate DNA for genotyping of patients.

Selection of SNPs and SNP genotyping

We selected 20 SNPs that were identified in four GWAS and fine mapping studies and were replicated in at least one independent study population [1-16]. They included three independent SNPs at 8q24, two independent SNPs at 17q12 and 11q13, and one SNP each at 2p15, 3p12, 6q25, 7p15, 7q21, 9q33, 10q11 (*MSMB*), 10q26, 11q13, 17q24.3, 19q13 (*KLK3*), 19q13, 22q13, and Xp11.

These 20 SNPs were genotyped using a MassARRAY QGE iPLEX system (Sequenom, Inc. San Diego, CA). Polymerase chain reaction (PCR) and extension primers for these SNPs were designed using MassARRAY Assay Design 3.0 software (Sequenom, Inc). The primer information is available at <http://www.wfubmc.edu/genomics>. PCR and extension reactions were performed 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 99.2% (range from 98.1%–99.8%). Each of the SNPs in the autosomal chromosomes was in Hardy-Weinberg equilibrium ($P \geq 0.05$).

Statistical analyses

Allele frequency differences between patients with more aggressive disease and less aggressive disease were tested for each SNP using a chi-square test with 1 degree of freedom (df). Allelic odds ratio (OR) and 95% confidence interval (95% CI) were estimated based on a multiplicative model. Associations of Gleason scores (≤ 6 , 3+4, 4+3, or ≥ 8) or tumor stage (T2, T3a, or T3b) with risk allele of each SNP were tested using a chi-square test. A trend test of increasing Gleason scores or tumor stage was also performed for the risk allele of each SNP, using the Cochran-Armitage test.

We also tested the association of each of these 20 SNPs with PSA levels in patients (pre-operative PSA) assuming an additive model, adjusting for age using a multiple linear regression analysis. PSA levels were logarithm-transformed to best approximate the assumption of normality. Association of each of these 20 SNPs with age at diagnosis was also tested assuming a 2-df general model and an additive model.

We tested the cumulative effects of PCa risk SNPs on PCa aggressiveness by counting the number of PCa associated alleles of these 20 SNPs in each subject. We categorized individuals based on quartiles of the number of counts of risk alleles. The Cochran-Armitage test for trend was performed to test increasing risk for aggressive PCa with increasing quartile of number of risk alleles. All reported P-values were based on a two-sided test.

RESULTS AND DISCUSSION

We first tested associations of these 20 PCa risk-associated SNPs with PCa aggressiveness by comparing the genotype frequencies of these SNPs between 1,253 patients with more aggressive disease and 4,233 patients with less aggressive disease using an additive model (Table 2). With the exception of SNPs rs2735839 in the *KLK3* gene ($P = 8.4 \times 10^{-7}$) and rs10993994 in the *MSMB* gene ($P = 0.046$), none of the other 18 SNPs were statistically significantly associated with aggressive disease ($P > 0.05$). For example, the allele frequencies of three 8q24 SNPs (rs1447295 at Region 1, rs16901979 at region 2, and rs6983267 at Region 3) were essentially identical between the more aggressive and less aggressive PCa patients. These null results were similar to our previous findings that were based on 15 of these SNPs in a subset of these patients ($N = 1,563$) [21].

One common difficulty in studying PCa aggressiveness is the use of dichotomous classification; patients are grouped into either more aggressive or less aggressive disease. With this dichotomous approach, patients with intermediate Gleason scores and tumor stages may have a higher chance of being misclassified and therefore may reduce the power to detect an association. To overcome this difficulty, we re-analyzed our data by limiting our comparison to two extreme groups: 128 patients with the most aggressive disease (Gleason score ≥ 8 and stage $\geq T3b$) and 2,653 patients with the least aggressive disease (Gleason score ≤ 6 and stage $\leq T2$). However, as with our initial analysis, no statistically different frequencies were found between the two groups, except for the SNPs rs2735839 in the *KLK3* gene ($P = 0.002$) and rs10993994 in the *MSMB* gene ($P = 0.057$) (Table 2).

The null association with PCa aggressiveness for the vast majority of PCa risk-associated SNPs in this study is different from some reported studies. By comparing PCa patients with controls using a case-control analysis, some studies have found associations with PCa risk to be stronger among patients with more aggressive PCa for several SNPs, including those at 8q24 [1,3, 10-11,22-28], 2p15 [7], 6q25 [29], 9q33 [9], 17q12 [5], and 22q13 [13]. However, except for 2p15 [7], none of the other SNPs have been shown to have statistically different genotype frequencies between more or less aggressive PCa patients using a case-case analysis. It is also noted that some of the previous studies, including ours, had several limitations: small sample size, heterogeneous definitions of aggressive PCa in studies where multiple populations were combined, or reliance on clinical, rather than pathological grading and staging. In contrast, the larger number of PCa patients included in our study, along with the uniform pathologic grading and staging of each of these patients, provides convincing evidence that the vast majority of PCa risk-associated SNPs are not associated with these measures of PCa aggressiveness. Our results, to a large degree, clarify the unresolved question in the published studies about associations between the risk-associated SNPs with aggressiveness of PCa.

However, aggressive PCa is a subjective definition, which varies depending on the study. It is typically based on intermediate clinicopathologic variables such as Gleason score, stage, and PSA levels at diagnosis. To reduce the potential impact of subjective and heterogeneous definitions of aggressive PCa, we directly tested associations between these SNPs with Gleason score, stage, PSA levels and age at diagnosis, which can be more objectively measured. Except for SNP rs2735839 in the *KLK3* gene ($P = 7.7 \times 10^{-6}$) and rs10993994 in the *MSMB* gene ($P = 0.02$), none of other SNPs were associated with Gleason score using a 2×4 chi-square test ($P > 0.05$) (Table 3). For tumor stage, only SNP rs2735839 at *KLK3* gene ($P = 1.9 \times 10^{-4}$) was significant ($P > 0.05$). Finally, for PSA levels and age at diagnosis, no association was found for any of these 20 SNPs (Table 4). These results provided further evidence that vast majority of PCa risk-associated SNPs are not associated with clinicopathologic variables and PCa aggressiveness.

The strong statistical associations between SNP rs2735839 in the *KLK3* gene with PCa aggressiveness as well as intermediate clinicopathologic variables are striking. It is important to note that the allele associated with increased PCa risk (G) was, however, more common in patients with more indolent PCa (0.88) than patients with aggressive PCa (0.85), allelic OR = 1.38, 95% confidence interval (CI) = 1.21–1.56, $P = 8.4 \times 10^{-7}$. The difference was more prominent when comparing patients with the most indolent disease (0.89) and the most aggressive disease (0.82), OR = 1.69, 95% CI = 1.22–2.36, $P = 0.002$. A similar trend was found for Gleason score and tumor stage. The frequency of the G allele gradually decreased from 0.89 in patients with Gleason score of ≤ 6 , to 0.87, 0.85, and 0.84, respectively in patients with Gleason score of 3+4, 4+3, and ≥ 8 , P -trend = 3.7×10^{-7} . The frequency of the risk allele of this SNP also gradually decreased from 0.88 in patients with tumor stage $\leq T2$, to 0.86 and 0.84, respectively in patients with tumor stage T3a and T3b, P -trend = 4.6×10^{-5} . A similar finding, that PCa risk associated allele was more common among less aggressive PCa patients

and patients with lower Gleason score, was obtained for SNP rs10993994 in the *MSMB* gene (allele T), although with weaker statistical evidence. It is noted that a common feature for both of these SNPs is that the PCa risk alleles of these two SNPs are known to be strongly associated with higher PSA levels in unaffected controls [8,32-33]. Therefore, the overrepresentation of these two alleles in less aggressive PCa patients may partially represent a PSA detection bias. It is possible that men with these alleles tend to have higher PSA levels and consequently are more likely to have a prostate biopsy resulting in a PCa diagnosis, especially in areas where PSA screening is a common practice. This trend could be more prominent in patients with low Gleason grade or stage (less aggressive disease) because higher PSA level is frequently the only trigger for prostate biopsy and diagnosis of PCa.

Finally, we tested the cumulative effect of these 20 PCa risk-associated SNPs on aggressiveness and clinicopathologic variables of PCa. We grouped PCa patients into quartiles based on the total number of these PCa risk-associated alleles they inherited. The frequency of more aggressive PCa, higher Gleason score (≥ 8), and higher tumor stage ($\geq T3b$) among these four groups of patients was compared. No statistically significant trend of higher proportions of more aggressive PCa, or higher tumor stage with increasing quartile was found, $P > 0.05$ (Table 5a). On the contrary, the proportion of men with more aggressive PCa, and the proportion of men with higher Gleason score, was lowest among patients in the highest quartile, and the trend was statistically significant for the proportion of higher Gleason score ($P = 0.036$). To remove the impact of the two SNPs at *KLK3* and *MSMB* genes on the cumulative effect of risk alleles because they may represent PSA detection bias, we performed a similar analysis without these two SNPs. The reverse trend remained but did not reach statistical significance, $P > 0.05$ (Table 5b). It is possible that this opposite trend is influenced by several additional SNPs that may also be susceptible to PSA detection bias because they are associated with PSA levels in controls, including those at 7p15, 10q26, 17q12, and Xp11 [8,33].

The lack of association between PCa risk-associated variants with aggressiveness and clinicopathologic variables of PCa observed in this study has several important implications. From a mechanistic perspective, this lack of association with stage or grade may suggest that the initiating events for both types of PCa (more or less aggressive) may be more similar rather than disparate, and that other factors may be more important in determining the aggressive nature of an individual's PCa. Whether these other factors are genetic, environmental, or stochastic in nature is unclear and 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 PCa, as opposed to a cancer with a predilection for more or less aggressive behavior [19,20].

It is important to note that failure to detect an association between these PCa risk-associated SNPs with PCa aggressiveness and clinicopathologic variables does not imply lack of such genetic variants in the genome. These 20 PCa risk-associated variants were all discovered by comparing all types of prostate cancer cases with controls using a case-control design. Different study designs, such as case-case studies that compare genetic variants among more or less aggressive PCa cases may be more efficient at identifying genetic variants associated with aggressive PCa.

Although only a small percentage of PCa patients (~15%) die of their disease, this amounts to a significant number (~29,000) due to its prevalence in our society [34]. It is thus critical that we continue, through different study designs, to discover markers that identify patients at risk for more aggressive PCa, as they are more likely to benefit from earlier diagnosis and treatment.

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Table 1
Characteristics of Patients in the Johns Hopkins Hospital study

Characteristics	# (%) of cases		
	More aggressive (N=1,253)	Less aggressive (N=4233)	All cases (N=5,749)
<i>Age at enrollment (Year)</i>			
Mean (sd)	59.86(6.83)	57.68(6.47)	58.29(6.68)
<i>Age at diagnosis (Year)</i>			
≤ 65	982(78.43)	3754(88.73)	4935(85.90)
> 65	270(21.57)	477(11.27)	810(14.10)
<i>PSA levels at diagnosis for cases or at enrollment for controls (ng/ml)</i>			
≤ 4	94(12.62)	629(28.45)	746(23.77)
4.01–9.99	374(50.20)	1394(63.05)	1829(58.27)
10–19.99	129(17.32)	168(7.60)	313(9.97)
20–49.99	68(9.13)	19(0.86)	95(3.03)
50–99.99	31(4.16)	1(0.05)	53(1.69)
≤ 100	49(6.58)	0	103(3.28)
Missing	508	2022	2610
<i>T-stage</i>			
T0	NA	NA	NA
T1	NA	NA	NA
T2	308(27.60)	3351(79.16)	3729(67.74)
T3a	467(41.85)	882(20.84)	1386(25.18)
T3b	324(29.03)	0	324(5.89)
T3X	11(0.99)	0	17(0.31)
T4	4(0.36)	0	4(0.07)
TX	2(0.18)	0	45(0.82)
<i>N-stage</i>			
N0	898(87.52)	4233(100)	5212(96.72)
N1	120(11.70)	0	120(2.23)
NX	8(0.78)	0	57(1.06)
<i>M-stage</i>			
M0	NA	NA	NA
M1	NA	NA	NA
MX	1016	4233	5379
<i>Gleason (biopsy)</i>			
≤ 4	0	0	1(0.02)
5	2(0.16)	65(1.54)	72(1.28)
6	19(1.53)	2935(69.34)	3038(54.06)
7(3+4)	93(7.51)	1233(29.13)	1385(24.64)
7(4+3)	569(45.96)	0	569(10.12)
8	297(23.99)	0	297(5.28)
9	241(19.47)	0	241(4.29)

Characteristics	# (%) of cases		All cases (N=5,749)
	More aggressive (N=1,253)	Less aggressive (N=4,233)	
10	17(1.37)	0	17(0.30)
Missing	15	0	129

263 patients can not be classified as aggressive or indolent cases because of missing phenotypes

Table 2
PCa risk-associated SNPs in more or less aggressive PCa patients at JHH

CHR	SNP	BP	Note	Risk allele	Frequencies (%)		p	OR (95% CI)	Frequencies (%)		p	OR (95% CI)
					Less aggressive N = 4,233	More aggressive N = 1,253			Least aggressive N = 2,674	Most aggressive N = 129		
2	rs721048	63,043,382	2p15	A	0.21	0.21	0.886	0.99(0.89–1.11)	0.20	0.22	0.543	0.91(0.67–1.23)
3	rs2660753	87,193,364	3p12	T	0.14	0.13	0.606	1.04(0.91–1.18)	0.13	0.12	0.419	1.17(0.80–1.73)
6	rs9364554	160,804,075	6q25	T	0.29	0.29	0.611	1.03(0.93–1.13)	0.28	0.28	0.880	1.02(0.77–1.35)
7	rs10486567	27,749,803	7p15	C	0.79	0.77	0.066	1.11(0.99–1.23)	0.79	0.77	0.538	1.10(0.81–1.48)
7	rs6465657	97,460,978	7q21	C	0.52	0.53	0.175	0.94(0.86–1.03)	0.52	0.53	0.643	0.94(0.73–1.21)
8	rs16901979	128,194,098	8q24 (2)	A	0.05	0.06	0.287	0.90(0.74–1.09)	0.05	0.06	0.441	0.82(0.48–1.37)
8	rs6983267	128,482,487	8q24 (3)	G	0.56	0.55	0.233	1.06(0.97–1.16)	0.55	0.58	0.343	0.88(0.69–1.14)
8	rs1447295	128,554,220	8q24 (1)	A	0.13	0.13	0.922	1.01(0.88–1.15)	0.12	0.13	0.748	0.94(0.65–1.37)
9	rs1571801	121,506,927	9q33	T	0.26	0.25	0.898	1.01(0.91–1.12)	0.25	0.22	0.282	1.18(0.87–1.59)
10	rs10993994	51,219,502	10q11	T	0.48	0.46	0.046	1.10(1.00–1.20)	0.49	0.43	0.057	1.28(0.99–1.64)
10	rs4962416	126,686,862	10q26	G	0.31	0.31	0.621	1.02(0.93–1.13)	0.31	0.26	0.111	1.26(0.95–1.68)
11	rs12418451	68,691,995	11q13 (2)	A	0.32	0.32	0.683	1.02(0.93–1.12)	0.32	0.33	0.945	0.99(0.76–1.29)
11	rs10896449	68,751,243	11q13 (1)	G	0.57	0.57	0.936	1.00(0.91–1.09)	0.57	0.55	0.632	1.06(0.83–1.37)
17	rs11649743	33,149,092	17q12 (2)	C	0.84	0.82	0.131	1.09(0.97–1.23)	0.84	0.84	0.817	0.96(0.68–1.36)
17	rs4430796	33,172,153	17q12 (1)	T	0.57	0.57	0.730	1.02(0.93–1.11)	0.57	0.59	0.440	0.90(0.70–1.17)
17	rs1859962	66,620,348	17q24.3	G	0.51	0.51	0.729	1.02(0.93–1.11)	0.52	0.55	0.324	0.88(0.69–1.13)
19	rs887391	46,677,464	19q13	T	0.79	0.79	0.523	1.04(0.93–1.16)	0.79	0.81	0.511	0.90(0.66–1.23)
19	rs2735839	56,056,435	19q13 (KLK3)	G	0.88	0.85	8.4E-07	1.38(1.21–1.56)	0.89	0.82	0.002	1.69(1.22–2.36)
22	rs9623117	38,776,619	New 22q13	C	0.25	0.23	0.242	1.06(0.96–1.18)	0.25	0.20	0.065	1.34(0.98–1.83)
X	rs5945619	51,074,708	Xp11	G	0.40	0.41	0.288	0.95(0.87–1.04)	0.39	0.41	0.651	0.94(0.73–1.22)

P-values and OR(95% CI) were calculated for the allelic test.

Table 3
Association of PCa risk-associated SNPs with clinicopathologic variables

CHR	SNP	BP	Note	Risk allele	Frequencies (%) in patients with Gleason grade					Frequencies (%) inpatients with T-stage				
					% N = 3,111	3+4 N = 1,385	4+3 N = 569	% N = 555	P-3 d.f.	P-trend	T2 N = 3,729	T3a N = 1,386	T3b N = 328	P-2 d.f.
2	rs721048	63,043,382	2p15	A	0.20	0.21	0.20	0.21	0.820	0.577	0.20	0.21	0.19	0.397
3	rs2660753	87,193,364	3p12	T	0.13	0.14	0.13	0.14	0.901	0.723	0.14	0.13	0.13	0.655
6	rs9364554	160,804,075	6q25	T	0.29	0.30	0.30	0.27	0.124	0.870	0.29	0.29	0.30	0.513
7	rs10486567	27,749,803	7p15	C	0.79	0.79	0.77	0.77	0.252	0.064	0.79	0.78	0.78	0.222
7	rs6465657	97,460,978	7q21	C	0.52	0.53	0.54	0.53	0.474	0.213	0.52	0.52	0.55	0.407
8	rs16901979	128,194,098	8q24 (2)	A	0.05	0.05	0.04	0.06	0.227	0.332	0.05	0.05	0.06	0.133
8	rs6983267	128,482,487	8q24 (3)	G	0.56	0.56	0.53	0.56	0.242	0.707	0.55	0.56	0.56	0.811
8	rs1447295	128,554,220	8q24 (1)	A	0.13	0.14	0.13	0.12	0.358	0.629	0.13	0.14	0.11	0.205
9	rs1571801	121,506,927	9q33	T	0.25	0.26	0.24	0.26	0.432	0.579	0.26	0.25	0.22	0.127
10	rs10993994	51,219,502	10q11	T	0.49	0.48	0.45	0.45	0.023	0.004	0.49	0.47	0.49	0.192
10	rs4962416	126,686,862	10q26	G	0.31	0.31	0.31	0.30	0.797	0.585	0.31	0.32	0.29	0.251
11	rs12418451	68,691,995	11q13 (2)	A	0.32	0.33	0.30	0.33	0.518	0.768	0.32	0.32	0.33	0.737
11	rs10896449	68,751,243	11q13 (1)	G	0.57	0.58	0.57	0.57	0.827	0.823	0.57	0.58	0.58	0.738
17	rs11649743	33,149,092	17q12 (2)	C	0.84	0.82	0.82	0.83	0.067	0.102	0.83	0.83	0.83	0.923
17	rs4430796	33,172,153	17q12 (1)	T	0.57	0.57	0.56	0.56	0.733	0.416	0.57	0.57	0.60	0.306
17	rs1859962	66,620,348	17q24.3	G	0.52	0.50	0.52	0.50	0.498	0.258	0.51	0.53	0.52	0.179
19	rs887391	46,677,464	19q13	T	0.79	0.79	0.79	0.79	0.958	0.771	0.79	0.80	0.78	0.535
19	rs2735839	56,056,435	19q13 (KLK3)	G	0.89	0.87	0.85	0.84	7.7E-06	3.7E-07	0.88	0.86	0.83	1.9E-04
22	rs9623117	38,776,619	New 22q13	C	0.25	0.25	0.24	0.23	0.460	0.191	0.25	0.24	0.24	0.428
X	rs5945619	51,074,708	Xp11	G	0.39	0.42	0.42	0.39	0.166	0.331	0.40	0.41	0.44	0.096

Table 4
Association of PCa risk-associated SNPs with clinicopathologic variables in JHH PCa patients

CHR	SNP	BP	Note	Risk allele	PSA levels at diagnosis (ng/mL)			Age at diagnosis (year)				
					WW	WR	RR	P-additive	WW	WR	RR	P-additive
2	rs721048	63,043,382	2p15	A	6.34	6.71	6.75	0.156	58.24	58.41	58.12	0.611
3	rs2660753	87,193,364	3p12	T	6.47	6.38	7.12	0.937	58.33	58.20	57.41	0.222
6	rs9364554	160,804,075	6q25	T	6.42	6.41	6.87	0.499	58.45	58.10	58.16	0.099
7	rs10486567	27,749,803	7p15	C	7.36	6.38	6.47	0.582	58.51	58.51	58.15	0.063
7	rs6465657	97,460,978	7q21	C	6.63	6.50	6.25	0.265	58.00	58.37	58.37	0.168
8	rs16901979	128,194,098	8q24 (2)	A	6.39	6.55	14.02	0.154	58.30	58.13	57.86	0.516
8	rs6983267	128,482,487	8q24 (3)	G	6.04	6.50	6.53	0.183	58.46	58.34	58.07	0.103
8	rs1447295	128,554,220	8q24 (1)	A	6.42	6.51	5.89	0.911	58.30	58.22	58.54	0.899
9	rs1571801	121,506,927	9q33	T	6.45	6.47	6.06	0.593	58.24	58.32	58.50	0.478
10	rs10993994	51,219,502	10q11	T	6.76	6.32	6.45	0.331	58.40	58.38	57.95	0.080
10	rs4962416	126,686,862	10q26	G	6.61	6.26	6.62	0.467	58.37	58.23	58.04	0.249
11	rs12418451	68,691,995	11q13 (2)	A	6.57	6.48	6.09	0.288	58.40	58.25	57.93	0.127
11	rs10896449	68,751,243	11q13 (1)	G	6.95	6.29	6.47	0.310	58.38	58.43	58.06	0.134
17	rs11649743	33,149,092	17q12 (2)	C	6.74	6.72	6.35	0.174	57.57	58.61	58.19	0.287
17	rs4430796	33,172,153	17q12 (1)	T	6.35	6.59	6.38	0.924	58.56	58.20	58.28	0.406
17	rs1859962	66,620,348	17q24.3	G	6.54	6.40	6.58	0.884	58.44	58.19	58.36	0.811
19	rs887391	46,677,464	19q13	T	6.08	6.28	6.61	0.146	58.48	58.52	58.15	0.070
19	rs2735839	56,056,435	19q13 (KLRK3)	G	5.73	6.03	6.60	0.031	59.01	58.47	58.22	0.125
22	rs9623117	38,776,619	New 22q13	C	6.44	6.57	6.22	0.960	58.31	58.21	58.55	0.952
X	rs5945619	51,074,708	Xp11	G	6.43	.	6.53	0.664	58.44	.	58.06	0.034

Table 5
Cumulative effect of PCa risk-associated alleles on PCa aggressiveness and clinicopathologic variables

# of risk alleles	# (%) of subjects	# (%) of subjects with aggressive disease	# (%) of subjects with T-stage $\geq$ T3b	# (%) of subjects with Gleason score $\geq$ 4+3
<i>a. All 20 PCa risk-associated SNPs</i>				
≤ 15	1,435(25.0%)	324(22.6%)	89(6.2%)	297(20.7%)
16–17	1,395(24.3%)	307(22.0%)	85(6.1%)	269(19.3%)
18–19	1,468(25.5%)	336(22.9%)	66(4.5%)	318(21.7%)
≥ 20	1,450(25.2%)	286(19.7%)	88(6.1%)	240(16.6%)
<i>P-trend</i>		0.153	0.465	0.036
<i>b. 18 SNPs (rs10993994 at the KLK3 gene and rs2735839 at the MSMB gene are excluded)</i>				
≤ 12	1,175(20.4%)	262(22.3%)	69(5.9%)	238(20.3%)
13–14	1,425(24.8%)	302(21.2%)	79(5.5%)	273(19.2%)
15–16	1,553(27.0%)	371(23.9%)	86(5.5%)	338(21.8%)
≥ 17	1,595(27.8%)	318(19.9%)	94(5.9%)	275(17.2%)
<i>P-trend</i>		0.406	0.930	0.158