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Genome-wide association study identifies a genetic variant associated with risk for more aggressive prostate cancer

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Abstract

Background—Of the 200,000 U.S. men annually diagnosed with prostate cancer, approximately 20–30% will have clinically aggressive disease. While factors such as Gleason score and tumor stage are used to assess prognosis, there are no biomarkers to identify men at greater risk for developing aggressive prostate cancer. We therefore undertook a search for genetic variants associated with risk of more aggressive disease.

Methods—A genome-wide scan was conducted in 202 prostate cancer cases with a more aggressive phenotype and 100 randomly sampled, age-matched PSA-screened negative controls. Analysis of 387,384 autosomal SNPs was followed by validation testing in an independent set of 527 cases with more aggressive and 595 cases with less aggressive prostate cancer, and 1,167 age-matched controls.

Results—A variant on 15q13, rs6497287, was confirmed to be most strongly associated with more aggressive ($p_{\text{discovery}}=5.20 \times 10^{-5}$, $p_{\text{validation}}=0.004$) than less aggressive disease ($p=0.14$). Another SNP on 3q26, rs3774315, was found to be associated with prostate cancer risk however the association was not stronger for more aggressive disease.

Conclusions—This study provides suggestive evidence for a genetic predisposition to more aggressive prostate cancer and highlights the fact that larger studies are warranted to confirm this supposition and identify further risk variants.

Impact—These findings raise the possibility that assessment of genetic variation may one day be useful to discern men at higher risk for developing clinically significant prostate cancer.

Keywords

prostate cancer; aggressive prostate cancer; GWAS; genetic variants

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Introduction

Over 217,000 American men were predicted to be diagnosed with prostate cancer last year (1). The majority of patients present with localized tumors that will remain indolent, however 20–30% of patients will have a clinically significant, more aggressive form of the disease and current screening practices cannot predict who these high-risk men will be.

Genome-wide association studies (GWAS) of prostate cancer have identified numerous genetic variants associated with overall risk of disease (2–10) and over 30 variants have subsequently been validated (11–22). Few GWAS have analyzed data according to indices of more aggressive prostate cancer (e.g., advanced stage and/or high tumor grade) (9, 23) and in most instances, risk variants found to be associated with aggressive disease have also been associated with less aggressive prostate cancer (23, 24). However, a recent study by Xu et al. (2010) found that the minor allele of SNP rs4054823, located on 17p12, was present more frequently in cases with aggressive compared to indolent disease ($p=2.1 \times 10^{-6}$) and that the homozygous minor allele genotype was more frequent in cases with poorly differentiated tumors (Gleason score ≥ 8) or higher stage disease (\geq pT3b) (25). Results from linkage analyses have also suggested that risk loci exist for aggressive hereditary prostate cancer (26–31), however the study by Xu et al. (2010) is the first to provide evidence that genetic variants may also increase risk for an aggressive phenotype of the more common sporadic form of the disease.

To search for genetic variants associated with risk of more aggressive prostate cancer, we completed a GWAS on this distinct phenotype and validated our findings in an independent dataset that included both more aggressive and less aggressive prostate cancer cases.

Materials and Methods

Study Subjects

The GWAS and validation study population consists of participants from prior population-based, case-control studies of prostate cancer (Study I and Study II) in residents of King County, Washington, for which methods have been previously described (32, 33). Briefly, Study I cases were diagnosed between January 1, 1993, and December 31, 1996 and were aged 40–64 years at diagnosis. Study II cases were diagnosed between January 1, 2002, and December 31, 2005 and were 35–74 years of age at diagnosis. Overall, 2,244 eligible prostate cancer patients were identified, 1,754 (78.2%) were interviewed, and blood samples yielding sufficient DNA for genotyping were drawn from 1,457 (83.1%) cases who completed a study interview. A comparison group of controls without a self-reported physician's diagnosis of prostate cancer was identified using random digit dialing. Controls were frequency matched to cases by five-year age groups. A total of 2,448 eligible men were identified, 1,645 (67.2%) were interviewed and of these men blood samples were drawn and DNA prepared from 1,352 (82.2%) using standard protocols. For these analyses, only Caucasian participants with DNA were included (1,324 cases and 1,267 controls).

Subjects completed in-person questionnaires that collected data on family structure and cancer history, medical history, and social and demographic factors. Clinical information on cases, including Gleason score, tumor stage and PSA level at diagnosis, was obtained from the Seattle-Puget Sound SEER cancer registry. Vital status and underlying cause of death for cases were obtained through the SEER registry. Death certificates were also reviewed to confirm underlying cause of death. For Study I cases, disease recurrence or progression was queried through self-administered surveys and medical record reviews completed in 2005. All cases in the GWAS were from Study I and met one or more of the following criteria: Gleason score $\geq 7(4+3)$; regional or distant stage; diagnostic PSA value ≥ 50 ng/mL;

evidence of progression/recurrence; or prostate cancer-specific death (Table 1). Controls were randomly selected from a subset of Study I controls (n=400) who had a serum PSA \leq 2.0ng/mL and reported a negative PSA and/or DRE screening history within the five-year period prior to study enrolment, and were frequency matched to cases on age (5-year age groups). Cases in the validation phase were defined as having more aggressive disease based on one or more of the following: Gleason score \geq 7(3+4); regional or distant stage; diagnostic PSA value \geq 20ng/mL; evidence of progression/recurrence; and/or prostate cancer-specific death.

All study procedures were approved by the Fred Hutchinson Cancer Research Center institutional review board and genotyping was approved by the National Human Genome Research Institute. Written informed consent was obtained from all study participants prior to participation.

Genotyping

A genome-wide scan was performed using the Affymetrix Human Mapping 500K array set according to the manufacturer's protocol (Affymetrix, Inc, CA, USA). From this GWAS, nine SNPs with a p-value \leq 1×10^{-4} were chosen for validation and genotyped using the Applied Biosystems SNPlex™ Genotyping System (Applied Biosystems, CA, USA). Identification of the specific SNP alleles was carried out using the ABI 3730xl DNA Analyzer with GeneMapper® software. Each batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and laboratory personnel were blinded to the case-control status of samples.

Statistical Analysis

Standard GWAS quality control procedures were performed on the discovery dataset (34). Samples with a missing call rate $>$ 2% were filtered from the analysis. Principle components analysis (35) was used to test for population structure and two samples identified as outliers were removed from the study. Age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated assuming a log-additive genetic model. Likelihood ratio p-values were calculated for each SNP by comparing the full model to the null model (without the SNP). SNPs with likelihood ratio p-values \leq 1×10^{-4} were considered as potential hits and investigated for quality by checking clusters plots, missing call rates, minor allele frequencies and departure from Hardy-Weinberg equilibrium (34). Analyses were performed using R version 2.11.1.

Quality control for the validation dataset included genotyping of 142 blind duplicate samples distributed across all genotyping batches. There was \geq 99.3% agreement between blind duplicate samples for all nine SNPs. Polytomous regression models adjusted for age were used to test a log-additive model of cases stratified by disease aggressiveness (less vs. more) compared to controls. Replication was declared if the direction of the effect was the same as in the initial GWAS and a one-sided p-value \leq 0.0056 (p-value of 0.05/9) was observed. To test for significant differences in risk estimates for more vs. less aggressive disease, a Wald chi-square test was used ($p_{\text{heterogeneity}}$). Analyses were performed using SAS version 9.1.3.

Results

The clinical characteristics of cases included in the GWAS and the validation study are presented in Table 1. Of the 202 cases in the GWAS, selection in order of inclusion criteria yielded 65 (32.2%) who had a Gleason score \geq 7 (4+3), 101 (50%) who had regional or distant stage disease, 1 (0.5%) who had a diagnostic PSA \geq 50ng/mL, and 35 (17.3%) who

had a recurrence/progression event. Of the 527 more aggressive cases in the validation study, 440 (83.5%) had a Gleason score ≥ 7 (3+4), 39 (7.4%) had regional or distant stage disease, 20 (3.8%) had a diagnostic PSA ≥ 20 ng/mL, 25 (4.7%) had a recurrence/progression event, and 3 (0.6%) died of prostate cancer.

In the GWAS, no variants reached genome-wide significance ($p < 1 \times 10^{-7}$) however, nine variants showed some evidence of association with more aggressive disease ($p < 1 \times 10^{-4}$; Table 2). These were then evaluated in an independent population-based dataset. In this validation study of 527 more aggressive cases, 595 less aggressive cases and 1,167 age-matched controls, the risk estimate for rs6497287, on 15q13, was validated and the magnitude of the risk estimate was stronger in cases with aggressive prostate cancer. Under a log-additive model, carriers of the rs6497287 minor allele had an OR of 1.46 (95% CI 1.10–1.94, $p=0.004$) for aggressive disease and an OR of 1.17 (95% CI 0.88–1.56, $p=0.14$) for less aggressive disease, however a test for heterogeneity was not significant ($P_{\text{heterogeneity}}=0.17$). The SNP, rs2341883 on 14q22, was associated with elevated risk estimates of both more and less aggressive prostate cancer (OR 1.20, 95% CI 1.03–1.40, $p=0.01$ and OR 1.19, 95% CI 1.03–1.38, $p=0.01$, respectively), however these results did not remain significant after adjustment for multiple testing. Finally, rs3774315 on 3q26 was more strongly associated with less aggressive disease (OR 1.25, 95% CI 1.08–1.46, $p=0.002$) than more aggressive disease (OR 1.17, 95% CI 0.99–1.38, $p=0.03$; Table 2).

To search for further evidence that these SNPs were associated with prostate cancer risk, we did a combined analysis of all the more aggressive cases and controls (Supplementary Table 1) and also examined the CGEMS dataset (10, 36). No SNPs achieved genome-wide significance ($p < 1 \times 10^{-7}$) in the combined analysis however, the strongest risk estimate observed was for rs6497287, which is consistent with results from the independent validation component described above. In the CGEMS dataset, SNP rs2341883 (14q22) was directly genotyped and no association was observed for either overall or more aggressive prostate cancer. SNP rs6497287 (15q13) was not genotyped in CGEMS, however rs6497287 is located in *HERC2* and data for 15 SNPs across this gene were available. Upon further investigation it was found that one of these SNPs, rs7183877, is in perfect linkage disequilibrium (LD) with rs6497287, however neither it nor the remaining SNPs showed significant evidence of an association with prostate cancer risk. Variant rs3774315 also was not genotyped in CGEMS, but a SNP in strong LD with this variant, rs3136594 ($r^2=0.85$) was evaluated. In addition, data for six other SNPs located in *TNFSF10*, which rs3774315 tags, were also available. While no variants were more strongly associated with aggressive disease, three were associated with overall prostate cancer risk (rs3815496: dominant OR 1.23, 95% CI 1.05–1.46, $p=0.01$; rs3136597: trend OR 0.79, 95% CI 0.65–0.96, $p=0.02$; rs4894559: dominant OR 1.25, 95% CI 1.06–1.48, $p=0.009$).

Discussion

Over the past few decades PSA screening has not only increased the number of prostate cancer diagnoses, but has also increased the number of patients who are over-treated for indolent disease. Two large, randomized trials recently published interim results on PSA screening in relation to prostate cancer-specific mortality (37, 38). While their main findings were divergent, both studies concluded that screening was associated with substantially increased risks of over-diagnosis and over-treatment. Therefore the ability to target screening to men at higher risk for more aggressive disease may allow for early detection of those at higher risk for developing clinically significant prostate cancer. It may also reduce the number of men at low risk for clinically significant disease whose quality of life and function (e.g., urinary, sexual) may be impacted by intensive treatment that may be unnecessary. Recently, numerous GWAS have discovered genetic variants that are

associated with overall disease risk, however these variants are not uniquely associated with risk of more clinically significant prostate cancer nor have they been shown to be clinically useful for predicting patient outcomes (12, 13, 39–41).

To date, few studies of sporadic prostate cancer have focused on identifying variants associated with more aggressive disease (9, 23–25) and while initial results looked promising, most associations were subsequently shown not to be unique to this phenotype (23, 24). One reason for this could be the inclusion of Gleason score 7 disease in the criteria for defining aggressiveness. Gleason 7 tumors are a heterogeneous group that are less aggressive than Gleason score 8–10 tumors, (42, 43) which may have attenuated associations with aggressiveness. Therefore, defining aggressive disease using stricter phenotype criteria (e.g., Gleason score 8–10, regional/distant stage) may reduce heterogeneity and strengthen results. Lack of statistical power due to limited sample size is also an issue in these studies, especially to detect less common variants or those with weak to modest effects. As the proportion of cases meeting a stricter aggressive disease definition will be small in most studies (e.g., 15–30% of all cases), collaborative efforts will be needed to obtain enough samples to achieve sufficient power.

In the study presented here, we found evidence that SNP rs6497287 is more strongly associated with more aggressive than with less aggressive disease. It must be noted though that a significant difference in risk estimates between the two phenotypes was not observed. Whether this is due to the limited sample size or the fact that this SNP is actually associated with overall disease risk will have to be determined in larger studies. Nonetheless, this variant highlights an interesting prostate cancer candidate gene. SNP rs6497287 is located in *HERC2*, a large 93 exon gene on 15q11–13. This gene functions as a determinant of human iris color (44, 45) and has recently been shown to have a role in the DNA damage response. *HERC2* protein both mediates the specificity of E3 ubiquitin ligase binding (46) and acts as an E3 ligase itself, regulating XPA (47) and BRCA1 protein levels (48). It is interesting to note that *HERC2* translocates from the cytoplasm to the nucleus to participate in the above-described functions and the rs6497287 variant is located close to the nuclear localization signal of *HERC2*. Given the role *HERC2* plays in the DNA damage response, mutations within this gene may compromise genomic stability and thereby increase cancer risk.

Similar to previous studies (23, 24), we also identified a SNP rs3774315, that was associated with more aggressive disease in the GWAS, but was subsequently found to be associated with overall prostate cancer risk in the validation dataset. This variant is located on 3q26 in the *TNFSF10* gene, whose protein product TRAIL plays a key role in apoptosis (49). Although the association we observed was not observed in CGEMS, three other *TNFSF10* variants were found to be associated with overall disease risk in CGEMS, providing evidence that this gene may play a role in prostate cancer development and should be investigated further.

There are a number of strengths and limitations of our study that should be noted when interpreting these results. Significant strengths include the focus on aggressive prostate cancer and the population-based resource for confirmation. The genetic heterogeneity of prostate cancer is well known, therefore to reduce heterogeneity and increase the likelihood of identifying aggressive prostate cancer risk variants, cases with clinical features associated with adverse outcomes were chosen for the GWAS. Results from this phase were then validated in a dataset comprising a more widely used definition of aggressive disease, including all cases with a Gleason score ≥ 7 tumor. This definition was used by CGEMS (9) and although Gleason score 7 tumors are heterogeneous and there are differences between the CGEMS study and the one presented here, CGEMS is the only publicly available GWAS dataset that allows assessment of risk for more aggressive prostate cancer to which our

findings can be compared. Another advantage of this study is that over 380,000 SNPs were examined compared to the previous study by Xu and colleagues in which only ~27,000 SNPs were examined for associations with aggressive disease (25). A major limitation of this GWAS is the small sample size due to funding constraints. It should be noted that the small proportion of samples in the GWAS stage and the small proportion of SNPs carried through to the independent replication stage of this study, provided comparable power as would be obtained from jointly analyzing the data from both stages (50). While it is encouraging that both the independent replication and joint analyses highlighted rs6497287, the power of this study is limited and the results here need to be replicated in larger independent datasets.

In summary, we have found evidence that a genetic variant, rs6497287 is more strongly associated with aggressive prostate cancer. This finding lends strength to the hypothesis that there is a genetic susceptibility to clinically significant prostate cancer. As is the prediction for indolent prostate cancer, many low- to moderate-penetrant common risk alleles may contribute to the inherited predisposition for more aggressive disease. Therefore, due to the relatively small proportion of patients with aggressive *versus* indolent disease in most prostate cancer study populations, future collaborative efforts will be needed to attain genome-wide statistical power for finding genetic variants that uniquely distinguish men at higher risk for the more aggressive disease phenotype. Once this goal is realized, genetic variants may be useful for stratification of men who may benefit most from early detection and screening programs. At diagnosis, this information may also be helpful for identifying those patients who, based on their genetic risk profile, may warrant more aggressive initial therapy and also those who can be spared unnecessary therapy, thereby avoiding treatment-related morbidity. In addition to potential public health and clinical benefits, identification of variants associated with more aggressive disease may broaden our knowledge of the underlying biology of prostate cancer, providing insight into possible preventative and therapeutic targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical characteristics of the discovery and validation study participants

Characteristic	Discovery Study		Validation Study	
	More aggressive [†] cases, N=202 (%)	Controls, N=100 (%)	More aggressive [‡] cases, N=527 (%)	Less aggressive cases, N=595 (%)
<u>Age at reference date, y</u>				
35 – 49	17 (8.4)	8 (8.0)	46 (8.7)	40 (6.7)
50 – 54	39 (19.3)	20 (20.0)	68 (12.9)	86 (14.4)
55 – 59	70 (34.7)	34 (34.0)	105 (19.9)	159 (26.7)
60 – 64	76 (37.6)	38 (38.0)	148 (28.1)	172 (28.9)
65 – 69			85 (16.1)	68 (11.4)
70 – 74			75 (14.2)	70 (11.8)
<u>Gleason score</u>				
2 – 4	3 (1.5)		10 (1.9)	57 (9.6)
5 – 6	74 (36.6)		76 (14.4)	536 (90.1)
7 = 3+4	79 (39.1)		300 (56.9)	
7 = 4+3	20 (9.9)		56 (10.6)	
8 – 10	45 (22.3)		84 (15.9)	
Missing	1 (0.5)		1 (0.2)	2 (0.3)
<u>Stage</u>				
Local	65 (32.2)		370 (70.2)	595 (100)
Regional	121 (59.9)		140 (26.6)	
Distant	16 (7.9)		17 (3.2)	
<u>Diagnostic PSA level, ng/mL</u>				
< 4.0	18 (8.9)		47 (8.9)	115 (19.3)
4.0 – 9.9	95 (47.0)		279 (52.9)	354 (59.5)
10.0 – 19.9	30 (14.8)		92 (17.5)	69 (11.6)
20.0 – 49.9	21 (10.4)		51 (9.7)	
≥ 50.0	22 (10.9)		27 (5.1)	
Missing	16 (7.9)		31 (5.9)	57 (9.6)
<u>Progression/Recurrence[§]</u>				

Characteristic	Discovery Study		Validation Study	
	More aggressive [†] cases, N=202 (%)	Controls, N=100 (%)	More aggressive [‡] cases, N=527 (%)	Less aggressive cases, N=595 (%)
No	69 (34.2)		71 (46.1)	192 (74.7)
Yes	97 (48.0)		48 (31.2)	
Missing	36 (17.8)		35 (22.7)	65 (25.3)
<u>Prostate cancer-specific death</u>				
No	164 (81.2)		496 (94.1)	595 (100.0)
Yes	38 (18.8)		31 (5.9)	

[†] More aggressive: <65 years at diagnosis, diagnostic PSA level \geq 50ng/mL, regional/distant stage, Gleason score \geq 7(4+3), progression/recurrence event, and/or prostate cancer-specific death;

[‡] More aggressive: diagnostic PSA level \geq 20ng/mL, regional/distant stage, Gleason score \geq 7, progression/recurrence event, and/or prostate cancer-specific death;

[§] Data available for Study I participants only;

^{||} Includes patients who are alive, died of other causes or their vital status is unknown.

Table 2
Results of nine SNPs selected for validation of an association with more aggressive prostate cancer

SNP	Chr	Gene	500 K GWAS			Validation Study								
			Controls (N=100)	More Aggressive† (N=202)	Controls (N=1167)	More Aggressive# (N=527)	Less Aggressive (N=595)	MAF†	OR	95% CI	p‡	MAF	OR	95% CI
rs3774315	3q26	TNFSF10	0.15	1.55 – 4.03	7.34×10 ⁻⁵	0.25	1.17	0.99 – 1.38	0.03	1.25	1.08 – 1.46	0.002	0.45	
rs8015211	14q22	FBXO34	0.25	1.53 – 3.32	1.64×10 ⁻⁵	0.33	1.12	0.96 – 1.30	0.08	0.99	0.85 – 1.15	0.56	0.17	
rs12433615	14q22		0.23	1.47 – 3.18	4.06×10 ⁻⁵	0.31	1.10	0.94 – 1.28	0.12	1.10	0.95 – 1.28	0.10	0.97	
rs1188069	14q22		0.13	1.55 – 4.11	6.38×10 ⁻⁵	0.20	1.12	0.94 – 1.34	0.11	1.02	0.86 – 1.22	0.40	0.39	
rs2341883	14q22		0.24	1.41 – 3.02	1.01×10 ⁻⁴	0.32	1.20	1.03 – 1.40	0.01	1.19	1.03 – 1.38	0.01	0.96	
rs6497287	15q13	HERC2	0.01	8.92	2.10 – 37.9	5.20×10 ⁻⁵	0.06	1.46	1.10 – 1.94	0.004	1.17	0.88 – 1.56	0.14	0.17
rs11150069	16q23	WWOX	0.41	0.42	0.28 – 0.62	9.43×10 ⁻⁶	0.30	1.01	0.85 – 1.19	0.54	1.11	0.95 – 1.29	0.90	0.34
rs4628973	16q24		0.38	0.35	0.24 – 0.54	4.62×10 ⁻⁷	0.30	1.02	0.86 – 1.20	0.58	0.89	0.76 – 1.04	0.07	0.16
rs225061	Xp21	IL1RAPLI	0.28	0.59	0.44 – 0.81	9.71×10 ⁻⁴	0.22	0.95	0.73 – 1.23	0.34	0.81	0.63 – 1.05	0.06	0.32

† Minor Allele Frequency;

‡ More aggressive: diagnostic PSA ≥50ng/mL, regional/distant stage, Gleason Score ≥7(4+3), recurrence/progression event and/or prostate cancer-specific death;

§ Two-sided p-value;

|| More aggressive: diagnostic PSA ≥20ng/mL, regional/distant stage, Gleason score ≥7 (3+4), recurrence/progression event and/or prostate cancer-specific death;

†† One-sided p-value;

††† Heterogeneity p-value for the difference in ORs for more vs. less aggressive prostate cancer.