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Confirmation of Genetic Variants Associated with Lethal Prostate Cancer in a Cohort of Men from Hereditary Prostate Cancer Families

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Abstract

Germline genetic variants have been suggested as prognostic biomarkers for identifying patients at high risk for lethal prostate cancer (PCa). Validation studies have confirmed the association of several single nucleotide polymorphisms (SNPs) with fatal PCa, but whether these variants affect PCa-specific mortality (PCSM) in patients with an inherited predisposition to PCa, based on familial history, is unknown. For this study, a cohort of 957 PCa patients from 270 hereditary prostate cancer (HPC) families of European ancestry was genotyped for a panel of 22 PCSM-associated SNPs. Death certificates were reviewed to confirm cause of death. Mixed-effect Cox proportional hazards models were used to assess survival according to genotypes, accounting for relatedness and clinicopathological factors. Within this cohort, 98 PCa deaths were confirmed over an average follow-up period of 12.7 years after diagnosis. Variant allele carriers for three SNPs had significantly altered risk for PCSM (rs635261 at *RNASEL*, HR, 0.35, 95% CI, 0.18–0.66; P = 0.002; rs915927 in *XRCC1*, HR, 1.91, 95% CI, 1.21–3.02; P = 0.009; and rs2494750 at *AKT1*, HR, 0.45, 95% CI, 0.23–0.90; P = 0.016). These results confirm the association of genetic variation in three genes with PCa lethality in a cohort of men with an inherited susceptibility to the disease and provide validation evidence that germline SNPs provide prognostic information for PCa patients. Development of a panel of germline biomarkers with clinical utility for distinguishing patients at detection who have an increased risk for fatal PCa is warranted.

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Brief Description: This study evaluated a panel of 22 germline genetic variants previously associated with lethal PCa in a high-risk patient cohort from HPC families. Results validate the association of three SNPs with lethal PCa (rs635261 at *RNASEL*, HR, 0.35, 95% CI, 0.18–0.66; P = 0.002; rs915927 in *XRCC1*, HR, 1.91, 95% CI, 1.21–3.02; P = 0.009; and rs2494750 at *AKT1*, HR, 0.45, 95% CI, 0.23–0.90; P = 0.016). Germline SNPs may provide prognostic information for distinguishing patients at increased risk for progression to fatal PCa.

Keywords

Hereditary prostate cancer; mortality; SNPs; *XRCC1*; *AKT1*

Introduction

Prostate cancer (PCa) ranks second in the United States (U.S.) and fifth internationally as a cause of cancer-related deaths in men, with approximately 30,000 men in the U.S. and over 300,000 men worldwide dying of PCa annually [1, 2]. Recent survival data from the Surveillance, Epidemiology and End Results (SEER) Program indicate that approximately one in 11 (9%) patients diagnosed with PCa will die of their disease [3, 4].

Prostate cancer is a heterogeneous disorder that exhibits a range of clinical behaviors, from indolent, slow-growing to aggressive, rapidly growing tumors with lethal progression. Although a significant number of prostate tumors may not lead to morbidity or mortality, even if left untreated, most clinically localized tumors are treated with curative intent via surgery or radiation [5]. Despite treatment, however, up to one-third of patients will recur during long-term follow-up [6, 7]. Even within those experiencing PCa recurrence, there is heterogeneity in outcomes as only a subset will die of their disease. The standard criteria for risk stratifying clinically localized PCa, namely pre-biopsy PSA level, tumor histologic grade (Gleason score), and tumor stage, do not accurately distinguish patients who will go on to have aggressive as opposed to indolent PCa, especially when evaluating men within a risk stratum. Thus, research focused on discovery and confirmation of biomarkers associated with a high risk for progression to lethal PCa is urgently needed.

Toward this end, we previously identified a panel of genetic variants that were significantly associated with PCa-specific mortality (PCSM) in a population-based cohort from Seattle-King County, WA, and completed an initial validation effort confirming five genetic variants (one each in the *LEPR*, *CRY1*, *RNASEL*, *IL4*, and *ARVCF* genes) as predictive of lethal PCa in a second large independent population-based cohort of PCa patients from Sweden [8]. Most recently, a confirmation study within the Physician's Health Study cohort further validated the role of one of these SNPs (rs5993891 in the *ARVCF* gene) as being significantly associated with a lower risk of fatal PCa [9]. A meta-analysis revealed a significant 48% reduction in the risk of lethal PCa in cases carrying the variant T allele (hazard ratio, HR = 0.52, 95% CI 0.3–0.9; P = 0.03). The above study cohorts were mainly comprised of PCa patients without a family history of the disease. However, a subset (5%–10%) of PCa patients has the familial form of the disease due to an inherited predisposition, and it is unknown whether genetic biomarkers for lethal PCa are associated with outcomes in these patients. To further evaluate the ability of the SNP panel to distinguish patients at the time of detection who are at high risk for disease lethality, we genotyped a cohort of PCa patients ascertained through a large study of hereditary prostate cancer (HPC) families.

Materials and Methods

Study cohorts

The Seattle-based PCa patient cohort in which these 22 SNP genotypes were first associated with lethal outcomes has been described elsewhere [8]. In brief, 1,309 patients of European ancestry diagnosed with adenocarcinoma of the prostate in 1993–1996 or 2002–2005 were ascertained through the Seattle-Puget Sound population-based SEER cancer registry and provided a blood sample for genetic studies. Over an average follow-up period of 8.5 years, 60 patients were confirmed to have died of PCa based on SEER data and review of death certificates.

The validation patient cohort is comprised of men participating in the Prostate Cancer Genetic Research Study (*PROGRESS*), which was initiated in 1995 and has ascertained and followed over 2,200 members of 307 families with a pattern of familial PCa [10]. Families with two or more PCa survivors who were willing to participate and provide a blood sample were ascertained from across North America by advertising a toll-free number via public media, health-related publications, and the internet as well as communications with urologists, other health-care professionals, and PCa support groups. To be eligible for inclusion, families were required to meet at least one of the following criteria: (1) have three or more first-degree relatives with PCa; (2) have three generations of relatives with PCa; or (3) have two first-degree relatives with PCa diagnosed before age 65. For this analysis, a cohort of 957 men of European ancestry who were diagnosed with histologically confirmed PCa and for whom germline DNA was available for genotyping was included. Medical records were abstracted to obtain clinical and pathological data such as Gleason score (biopsy, surgery), extent of disease (tumor stage), diagnostic level PSA, and primary therapy. Data were coded according to SEER guidelines [11], incorporating the information from surgery for patients whose primary treatment was radical prostatectomy. The ascertainment of information on secondary treatment and vital status was based on two follow-up surveys sent to patients and their relatives, followed by collection and review of death certificates for decedents to determine underlying cause of death (i.e., prostate cancer vs. another cause), which was used to classify PCSM events.

Written informed consent was obtained from all participants in each of the study cohorts. Both studies were reviewed and approved by the Fred Hutchinson Cancer Research Center institutional review board.

Genotyping

Blood samples were collected using ACD tubes and shipped to the Fred Hutchinson Cancer Research Center for processing. Genomic DNA was extracted from peripheral blood lymphocytes using standard techniques[12] and stored at −80°C. For this study, one DNA aliquot per patient was shipped to Dr. Ostrander's laboratory (National Human Genome Research Institute) for genotyping. The MassARRAY iPLEX system (Sequenom, Inc., San Diego, CA) was used to genotype 20 of the 22 SNPs, and the remaining two SNPs (rs2308327 and rs4583514) were genotyped with TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA).

Blind duplicate DNA samples from 48 patients were distributed evenly across all genotyping batches, and laboratory personnel were blinded to patient outcome status (alive, deceased). The average concordance for the 22 SNP genotypes among the 48 blind duplicates was 99.8%. All 22 SNPs were successfully genotyped in 96% of the cohort. Hardy-Weinberg Equilibrium (HWE) was assessed in the patients who did not die of PCa. For this analysis, we randomly sampled one PCa patient per family to avoid genotype correlations within the family. All 22 SNP genotypes were in HWE (all P-values > 0.05). In addition, the minor allele frequency (MAF) for each SNP was calculated based on the genotype in *PROGRESS* cases who did not die of PCa.

Statistical analysis

The mixed-effect Cox proportional hazards model was used for analysis as it accounts for the correlation of genotypes between family members [13]. For the main models, the best-fitting genetic model (i.e., dominant, recessive, or trend) for each SNP as determined from the Seattle-based cohort that initially reported these SNP-PCSM associations was assumed (i.e., the underlying genetic model was fixed), and two sets of covariates were considered: 1) age at diagnosis only; and 2) age at diagnosis, together with Gleason score, stage, diagnostic PSA level, and primary treatment (categorical with missing indicator variables for clinicopathological covariates). The best-fitting of the two models in the *PROGRESS* cohort was selected based on which model had the lowest P-value. For the correlation structure, we used the pedigree() and kinship() function in the kinship2 library in R to construct the kinship matrix that represents the genetic relationships within families [14]. The mixed-effect Cox model was fitted with the R function coxme() in the coxme library, incorporating the constructed kinship matrix [14]. Follow-up time was calculated from the date of PCa diagnosis to the date of death from PCa, death from another cause or last follow-up.

A genetic variant was considered as validated if one of the two covariate adjusted models had a 1-sided p-value < 0.05 since for validation we required that the effect of the variant allele on PCSM in the *PROGRESS* cohort be in the same direction as that previously observed in the Seattle PCa cohort [8]. Hazard ratios (HR) and associated 95% confidence intervals (CI) were calculated.

Results

Descriptive characteristics of the HPC patient cohort are shown in Table 1. The mean age at diagnosis was 64.5 years and the average length of follow-up was 12.7 years. A total of 98 (10%) deaths were attributed to PCa during the follow-up period. The majority of patients were diagnosed with clinically localized disease and 52.4% underwent radical prostatectomy as primary therapy. At diagnosis, 13.1% of patients had a Gleason score of 7 (4+3) or higher.

The results highlighted three genetic variants for which there was validation evidence for an association with PCSM in the *PROGRESS* cohort (Table 2). The strongest result was for rs635261 downstream of the *RNASEL* gene, with a HR = 0.35 (95% CI, 0.18–0.66; P = 0.002) for carriers of the variant C allele. In addition, rs915927 in the *XRCC1* gene (HR, 1.91, 95% CI, 1.21–3.02; P = 0.009) and rs2494750 at *AKT1* (HR, 0.45, 95% CI, 0.23–0.90;

P = 0.016) were associated with risks for lethal PCa. For comparison, the published results from the previously analyzed Seattle-based PCa cohort are also shown (Table 2). The underlying genetic model for all three of these PCSM-associated SNPs was identical to the earlier Seattle-based model, and the covariates in the model that best fit the *PROGRESS* data for the *RNASEL* variant were also the same as in the Seattle cohort. However, the covariates differed in the best-fitting models for the *XRCC1* and *AKT1* gene variants in the *PROGRESS* cohort.

In further analyses the use of secondary treatment was considered. A covariate was added to the Cox models to assess whether HRs for the three confirmed SNPs remained significant. Data on use of secondary therapy were available for 525 (61%) cases who did not die of PCa and 30 (31%) fatal cases, so we assigned a separate missing category for the remaining cases. The HRs for the three SNPs were not substantially different than those shown in Table 2 (i.e., rs635261, HR = 0.32; rs915927, HR = 1.82; rs2494750, HR = 0.49) and all were statistically significant (all $P \leq 0.035$).

One SNP that was not considered confirmed in the *PROGRESS* cohort because the association was not in the same direction as in the Seattle-based PCa discovery cohort, however, was associated with survival. For rs1137100 in *LEPR*, the variant G allele was associated with a significant increase in the risk of lethal PCa (HR, 1.65, 95% CI, 1.08–2.51), but this risk estimate was in the opposite direction compared to results from both the Seattle and the Swedish population-based PCa cohorts [8].

Discussion

The results from this study provide validation for specific germline alterations that are associated with lethal PCa in men with an inherited predisposition for the disease based on family history. This is the first evidence that several SNPs in our initial panel of 22 PCSM-associated genetic polymorphisms [8] are associated with prognosis in patients from hereditary PCa (HPC) families. We confirmed the association of three genetic variants with the risk of fatal events, one downstream of *RNASEL*, one in *XRCC1* and the third upstream of *AKT1*. There was one other variant (rs1137100 in *LEPR*) that achieved statistical significance in this HPC cohort, but it was not considered as validated because its effect on PCSM was in the opposite direction as compared to results from the Seattle-based cohort that first highlighted this panel of 22 SNPs associated with lethality [8].

The strongest confirmation evidence was for rs635261 downstream of the *RNASEL* gene. Interestingly, another SNP in this gene (rs627839) achieved borderline significance in the prior validation study of the 22 PCSM-associated variants in a Swedish cohort [8]. RNASEL is involved in the interferon-regulated antiviral response and also functions in diverse cellular mechanisms, including cell proliferation, differentiation, apoptosis and tumorigenesis [15]. The *RNASEL* R462Q mutation is a candidate for the hereditary PCa allele HPC1 [16]. In addition, there is evidence that RNASEL can interact with the androgen receptor to promote tumor progression [17]. Recently *RNASEL* variants have also been associated with outcomes after radiation therapy [18], and with increased serum levels of C-reactive protein and interleukin-6 [19], which together suggest a role for inflammation in

response to radiation treatment [18]. Still other studies suggest that a subset of variants within the gene have predictive value for aggressive disease [19, 20]. The gene may not act alone, and at least one study suggests that interactions between variants in *RNASEL* and 8q24 are associated with aggressive prostate cancer [21].

The second strongest validation evidence was for a variant in the *X-ray repair cross-complementing group 1* (*XRCC1*) gene. *XRCC1* is a DNA repair gene whose protein product is involved in a number of DNA repair pathways including base excision repair [22, 23]. XRCC1 is thought to act as a scaffold protein enabling the recruitment of the DNA repair enzymes involved in the insertion of new nucleotides and sealing of the repair break [22, 23]. As such, reduced activity of XRCC1 could lead to an increase in somatic mutations, which in turn might promote disease progression. Multiple studies have evaluated the association between *XRCC1* variants and overall risk of PCa with conflicting results and, in fact, two meta-analyses failed to find any association [24]. However, none of these studies analyzed outcomes such as PCa mortality or focused on HPC families. Interestingly, the *XRCC1* Arg280His variant has been associated with another PCa outcome, radiation induced late stage toxicity [25].

A third variant upstream of *AKT1* was also validated in this study. AKT1 is a member of the AKT/PKB family of kinases. AKT acts as an intermediate signaling molecule in a number of important processes including cell survival, proliferation, tumor invasion, metastasis and angiogenesis [26, 27]. Of particular interest for PCSM is the role of the AKT pathway in epithelial-mesenchymal transition (EMT). EMT is a normal and important developmental mechanism whereby epithelial cells obtain mesenchymal, fibroblast-like properties, including reduced intercellular adhesion and increased motility [28]. Activation of AKT can induce EMT-like events, which in the course of tumor development could give cancer cells invasive and metastatic properties, thus promoting tumor progression and in the long term, PCSM [28].

The rs635261 SNP is approximately 3,700 bp downstream of *RNASEL*, the *XRCC1* variant, rs915927, is a synonymous change at Pro206, and rs2494750 is approximately 830 bp upstream of the *AKT1* gene. Given the critical role both of the latter proteins perform during development and the activity of XRCC1 in DNA repair, a major disruption to either protein's function is unlikely. The role of these genetic variants is unknown at this time, but they could potentially alter gene expression in a tissue specific manner. Alternatively the variants may tag another mutation that directly affects *RNASEL*, *XRCC1* or *AKT1* function, or that of other critical, nearby genes. Additional studies are needed to resolve these possibilities and to understand the role of each gene in the progression pathway to lethal PCa.

Currently, available clinicopathological characteristics of PCa do not accurately predict an individual patient's outcome. Distinguishing patients at elevated risk for fatal PCa from those with indolent tumors is critical not only to identify high-risk patients who would benefit from early aggressive therapy, but to avoid over-treatment of low-risk patients as well. Our results provide further support that germline biomarkers may enable differentiation between patients with indolent versus aggressive PCa at the time of detection

and have clinical utility for identifying those at increased risk for lethal PCa. Additional studies aimed at the development of a germline biomarkers panel with improved prognostic ability over the current methods are warranted.

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Abbreviations

PCa	prostate cancer
HPC	hereditary prostate cancer
SNP	single nucleotide polymorphism
HR	hazard ratio
CI	confidence interval

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Table 1

Descriptive Characteristics of Prostate Cancer Patients in the *PROGRESS* Cohort

Characteristic	No. = 957	%
Age at diagnosis, years		
Mean	64.5	
Range	40.0–87.0	
Follow-up time, years		
Mean	12.73	
Range	0.28–32.6	
Prostate cancer-specific death		
No[1]	782	81.7
Yes	98	10.2
Unknown[2]	77	8.0
Age at death, years		
Mean	80.5	
Range	54.0–99.0	
Stage at diagnosis		
Local	623	65.1
Regional	240	25.1
Distant	27	2.8
Missing	67	7.0
Gleason score at diagnosis		
≤ 6	561	58.6
7 (3+4)	159	16.6
7 (4+3)	45	4.7
8–10	80	8.4
Missing	112	11.7
Diagnostic PSA level, ng/mL		
< 4.0	77	8.0
4–9.9	360	37.6
10–19.9	157	16.4
≥ 20	130	13.6
Missing	233	24.3
Primary therapy		
Radical prostatectomy	501	52.4
Radiation therapy	256	26.8
Androgen deprivation	75	7.8
Active surveillance	21	2.2
Other	18	1.9
Missing	86	9.0

[1] Includes men who died of other causes (n=258) or were alive at last follow-up (n=524).

[2]Includes men who died but underlying cause of death is unknown.

Table 2

Hazard Ratios (HR) for Prostate Cancer-Specific Mortality Associated with a Panel of 22 SNPs in Two Patient Cohorts

Genetic Variants				Seattle Cohort				*PROGRESS* Cohort				
SNP	**Chr.**	**Gene**	**Alleles[1]**	**MAF[2]**	**HR**	**95% CI**	**Model[3]**	**MAF[2]**	**HR**	**95% CI**	**P-value[4]**	**Model**
rs1137100	1p31	*LEPR*	A/G	0.27	0.29	0.14–0.60	Dom: ACP	0.26	1.65	1.08–2.51	NC	Dom: ACP
rs228697	1p36	*PER3*	C/G	0.11	0.25	0.10–0.60	Dom: ACP	0.10	1.39	0.84–2.30	NC	Dom: ACP
rs635261	**1q25**	***RNASEL***	**G/C**	**0.36**	**0.22**	**0.07–0.65**	**Rec: ACP**	**0.39**	**0.35**	**0.18–0.66**	**0.002**	**Rec: ACP**
rs627839	1q25	*RNASEL*	G/T	0.47	3.98	1.64–9.65	Dom: ACP	0.48	0.83	0.56–1.21	NC	Dom: ACP
rs4583514	2p21	*MSH2*	G/A	0.38	2.49	1.21–5.10	Dom: ACP	0.38	0.68	0.45–1.03	NC	Dom: ACP
rs4608577	2p21	*MSH2*	T/G	0.17	2.04	1.36–3.07	Tre: A	0.17	1.09	0.77–1.55	0.33	Tre: ACP
rs523349	2p23	*SRD5A2*	C/G	0.29	0.49	0.28–0.86	Dom: A	0.29	1.08	0.71–1.67	NC	Dom: ACP
rs12467911	2p23	*SRD5A2*	C/T	0.28	0.45	0.24–0.81	Dom: A	0.30	1.14	0.75–1.72	NC	Dom: ACP
rs11710277	3p21	*SEMA3F*	A/G	0.09	3.71	1.75–7.90	Dom: ACP	0.06	0.75	0.39–1.42	NC	Dom: ACP
rs11205	5p23	*HSD17B4*	A/G	0.39	0.21	0.06–0.70	Rec: ACP	0.43	1.21	0.79–1.86	NC	Rec: A
rs2070874	5q31	*IL4*	C/T	0.16	2.16	1.27–3.67	Dom: A	0.16	1.17	0.80–1.72	0.25	Dom: A
rs1799964	6p21	*TNF/LTA*	T/C	0.21	0.39	0.20–0.77	Dom: A	0.22	1.19	0.82–1.72	NC	Dom: A
rs4645959	8q24	*C-MYC*	A/G	0.04	0	0.00–inf.	Tre: ACP	0.04	1.14	0.56–2.32	NC	Tre: ACP
rs1029153	10q11	*CXCL12*	T/C	0.31	0.22	0.07–0.75	Tre: A	0.29	1.14	0.87–1.51	NC	Tre: A
rs2839685	10q11	*CXCL12*	C/T	0.15	28.2	7.21–110.2	Rec: ACP	0.14	0.58	0.11–3.10	NC	Rec: A
rs2308327	10q26	*MGMT*	A/G	0.13	0.32	0.13–0.78	Tre: A	0.11	0.85	0.55–1.31	0.26	Tre: ACP
rs10778534	12q23	*CRY1*	T/C	0.36	2.21	1.19–4.12	Dom: A	0.36	0.68	0.45–1.03	NC	Dom: ACP
rs2494750	**14q32**	***AKT1***	**C/G**	**0.07**	**0.22**	**0.07–0.70**	**Tre: ACP**	**0.05**	**0.45**	**0.23–0.90**	**0.016**	**Tre: A**
rs1799814	15q24	*CYP1A1*	C/A	0.05	0.13	0.03–0.57	Tre: ACP	0.05	1.27	0.61–2.64	NC	Tre: ACP
rs25487	19q13	*XRCC1*	G/A	0.36	0.49	0.31–0.77	Tre: A	0.37	0.85	0.65–1.12	0.17	Tre: A
rs915927	**19q13**	***XRCC1***	**A/G**	**0.43**	**2.54**	**1.24–5.18**	**Dom: A**	**0.41**	**1.91**	**1.21–3.02**	**0.009**	**Dom: ACP**
rs5993891	22q11	*ARVCF*	C/T	0.05	0.21	0.07–0.61	Dom: ACP	0.05	1.53	0.91–2.57	NC	Dom: A

[1] Major/minor allele.

[2] MAF: minor allele frequency, calculated from cases who did not die of prostate cancer.

[3] Model: Dom= dominant, Rec= recessive, Tre= trend; A= age at diagnosis, ACP= age plus clinicopathological covariates (i.e., Gleason score, diagnostic PSA, stage, and primary treatment).

[4] NC: not confirmed (i.e., the direction of the HR in the *PROGRESS* confirmation cohort was in the opposite direction compared to the HR in the Seattle discovery cohort).