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Replication of a Genetic Variant for Prostate Cancer-Specific Mortality

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Abstract

Background—Few genetic variants have been confirmed as being associated with prostate cancer-specific mortality (PCSM). A recent study identified 22 candidate single-nucleotide polymorphisms (SNPs) associated with PCSM in a Seattle-based patient cohort. Five of these associations were replicated in an independent Swedish cohort.

Methods—We genotyped these 22 SNPs in Physicians' Health Study (PHS) participants diagnosed with prostate cancer (PCa). Utilizing the same model found to be most significant in the Seattle cohort, we examined the association of these SNPs with lethal disease with Cox proportional hazards models.

Results—One SNP, rs5993891 in the *ARVCF* gene on chromosome 22q11, which had also replicated in the Swedish cohort, was also significantly associated with PCSM in the PHS cohort (hazard ratio (HR)=0.32; *P*=0.01). When we tested this SNP in an additional cohort (Health Professionals Follow-up Study, HPFS), the association was null (HR=0.95, *P*=0.90); however, a meta-analysis across all studies showed a statistically significant association with a HR of 0.52 (0.29–0.93, *P*=0.03).

Conclusions—The association of rs5993891 with PCSM was further replicated in PHS and remains significant in a meta-analysis, though there was no association in HPFS. This SNP may

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contribute to a genetic panel of SNPs to determine at diagnosis whether a patient is more likely to exhibit an indolent or aggressive form of PCa. This study also emphasizes the importance of multiple rounds of replication.

Introduction

Many genetic variants have been associated with prostate cancer (PCa) risk; however, few have been confirmed as uniquely associated with metastatic or fatal PCa. While clinical factors such as Gleason score, clinical and pathological stage, and prostate-specific antigen level (PSA) at diagnosis help identify which men are more likely to have aggressive disease, additional biomarkers are needed to improve the predictive ability. Lin et al. previously performed a candidate gene study based on biological pathways of interest (e.g., steroid hormones, inflammation, DNA repair), identifying 22 SNPs that were significantly associated ($P<0.01$) with PCSM in a Seattle-based PCa cohort of 1,309 patients with 60 lethal outcomes due to PCa [1]. These SNPs were then genotyped in an independent Swedish cohort, with five SNPs replicating as significantly associated with PCSM (2,875 patients, 501 lethal). However, the clinical and pathological features of the Seattle and Swedish cohorts differed substantially. To further evaluate the potential prognostic ability of these genetic variants, we attempted to replicate these findings, genotyping the 22 SNPs in men with PCa in the Physicians' Health Study.

Materials and Methods

Study Population

The Physicians' Health Study (PHS) began as a randomized, double-blind placebo-controlled trial of aspirin and β-carotene in the prevention of cardiovascular disease and cancer among 22,071 healthy US physicians aged 40–84 years. Men were excluded if they had any serious medical conditions, including cancer (except non-melanoma skin cancer). Blood samples were collected from 68% of the physicians at baseline in 1982–1984. A detailed description of PHS has been published previously [2]. Long-term follow-up is 96% complete for PCa incidence and 100% for mortality. All self-reported PCa cases are verified through medical record and pathology review by the PHS Endpoints Committee. Data were abstracted on PSA at diagnosis, tumor stage, Gleason score, and primary treatment. Death certificates and medical records were reviewed to determine cause of death. Metastases are reported on follow-up questionnaires sent to all men living with PCa and confirmed by additional medical record review.

From a previous nested case-control study [3], we restricted to self-reported Caucasians to reduce potential population stratification. We included the original cases (diagnosed from 1982–2005) and those diagnosed after they were originally matched as controls (total n=1430). PCa deaths are confirmed through a review of death certificates, medical records, and information from the family by a panel of physicians. Metastases are reported on follow-up questionnaires sent to all men living with prostate cancer. During follow-up through March 20, 2012, 194 men died of PCa and an additional 11 men developed distant bone metastases.

We attempted to replicate any results significant in the PHS in the Health Professionals Follow-up Study (HPFS). The population for genetic studies of PCa has been previously described [4]. There were 1341 total cases included who were diagnosed between 1993 and 2011 and during follow-up through September 30, 2013, 103 men died of PCa. PCa deaths are confirmed with the same methods as those in the PHS.

SNP selection from Lin et al.

We are attempting to validate significant SNP associations from those previously published by Lin et al[1]. This study genotyped 937 SNPs in 156 candidate genes. SNPs were either non-synonymous coding SNPs, tagSNPs, or previously associated with PCa. Six models were run for each SNP – dominant, recessive, and additive; adjusting for age at diagnosis, or age other clinical factors including treatment. In the Seattle cohort, 22 SNPs were significantly associated with PCSM (unadjusted $p<0.01$). In a Swedish replication study, 5 of these SNPs validated ($p<0.05$).

Genotyping

In the PHS, we genotyped all 22 SNPs that reached significance ($P<0.01$) in the Seattle-based PCa cohort, regardless of their significance in the Swedish replication population. In HPFS, we genotyped and analyzed the one SNP that replicated in the PHS. DNA from the PHS and HPFS cases was extracted from whole blood and amplified using Whole Genome Amplification. Genotyping was performed with BioTrove OpenArray Technology [5]. Nine samples had between 3 and 9 replicates performed. Concordance for these samples for all SNPs was 100%. All SNPs had >93% genotyping success rates and no SNPs violated Hardy-Weinberg equilibrium (all SNPs but rs11710277 ($p=0.002$) and rs2839685 ($p=0.004$) had $p\geq0.01$).

Statistical Methods

Analyses were performed with SAS version 9.1 statistical software. All *P* values are two-sided. SNPs were analyzed under the model found to be the best fit in the Seattle cohort in the study by Lin et al [1]. We performed an analysis of time to lethal PCa outcome using a Cox regression model. A lethal PCa outcome was defined as death due to PCa or the development of bone metastases. Follow-up began at the time of PCa diagnosis and individuals were censored at the time of death from another cause or the end of follow-up. We report results from a model adjusted for age at diagnosis (continuous) or a model adjusted for clinicopathologic features, which included age at diagnosis plus PSA at diagnosis (categorical with missing indicator variables), Gleason score (ordinal categories), clinical stage (ordinal categories), and primary treatment (categorical with a missing indicator variable), whichever model was reported as most statistically significant in the original report. We attempted to classify clinicopathologic features as closely as possible to classification used in the Seattle PCa cohort. The definitions of the levels of these covariates can be found in Table 1. Genotype frequencies are reported in Table 2. In models adjusted for clinicopathologic features, the total number of participants may appear lower due to missing clinical stage and Gleason score data. A SNP was considered to validate if the *P* value was ≤0.05 and the effect on mortality risk was in the same direction as in the Seattle

dataset. To create a summary estimate for the SNP that replicated, a meta-analysis was performed using the DerSimonian-Laird random effects model, as there was significant heterogeneity across the four studies (P=0.04).

Results

Clinical characteristics of the PHS PCa cases are provided in Table 1. Overall, the PHS had more missing clinical data than the Seattle cohort [1] due to the nature of the trial and retrospective follow-up. The PHS cases were older than the Seattle cohort and had slightly more aggressive disease as defined by later clinical stage and higher Gleason score. Results for the association with PCSM are presented in Table 2. For models adjusted for clinical characteristics, the genotype frequencies in Table 2 excluded those who are missing Gleason score or clinical stage information. While three SNPs were statistically significant at P<0.05 (rs11205, rs228697, and rs5993891), only the association for rs5993891 was in the same direction as the original report. In the Seattle cohort, with a dominant genetic model adjusted for clinical covariates, the hazard ratio (HR) for this SNP was 0.21, 95% Confidence Interval (CI): 0.07–0.61 (P=0.0004) [1]. In the Swedish replication study, the HR for this SNP was 0.72, 95% CI: 0.52–1.01 (one-sided P=0.024) [1]. In the PHS, with the same statistical model as the Seattle PCa cohort (dominant genetic model, adjusting for age at diagnosis, clinical factors, and primary treatment), the result was similar with HR=0.32, 95% CI: 0.14–0.74 (P=0.01). We therefore genotyped rs5993891 in the HPFS cohort (clinical characteristics provided in Supplementary Table 1), using the same adjusted model, and there was no association (HR=0.95, 95% CI: 0.43–2.11, P=0.90). However, in a meta-analysis, the combined HR for these four studies remained statistically significant (HR=0.52, 95% CI: 0.29–0.93, P=0.03).

Discussion

We additionally replicated the previously reported association of rs5993891 in the *ARVCF* gene on chromosome 22q11 with PCSM in the PHS, our well-powered study with long-term follow-up and many PCSM events. However, this SNP association was not replicated further in the HPFS. The HPFS and PHS are very similar cohorts, both in the overall population and the men with PCa in this study (Table 1 and Supp. Table 1). The allele frequency for this SNP was similar across all cohorts. A possibility for the lack of replication is that there was slightly less power in the HPFS analysis for this SNP (1180 participants were not missing Gleason, stage, or genotype information, with only 74 PCSM events). In a meta-analysis of the four cohort studies, however, there was a significant 48% reduction in risk of PCSM in men who carry the minor allele of this SNP.

*ARVCF*a member of the p120-catenin protein family, is involved in adherens junction complexes and may play a role in cell communication [1, 6–7]. Additionally, a study conducted by Reintsch et.al found that inhibition of cell adhesion required recruitment of *ARVCF* to the membrane-bound juxtamembrane domain (JMD) of cadherins [8]. Co-expression of membrane bound JMD with *ARVCF* led to complete loss of cell-cell adhesion, and overexpression of *ARVCF* disrupts cell adhesion, which could play a role in cancer progression [8]. SNP rs5993891 is also in strong linkage disequilibrium with the nearby

COMT gene, which degrades catecholamines. Both of these genes are plausible candidates, and future work should determine if either of these genes is influenced by this SNP. This could potentially lend further evidence to the involvement of this region with PCSM.

A major strength of this study is its ability to test the previous genetic findings in additional cohorts with excellent clinical information that capture the important outcome of interest, PCSM. Ideally, identification of genetic variants or other biomarkers for PCSM could help stratify patients at diagnosis to refine treatment strategies, providing high risk patients with aggressive therapy and allowing low risk patients to avoid unnecessary treatment. The magnitude of the association for this SNP alone is insufficient to influence clinical treatment decisions, but the original finding has now replicated in two additional studies. However, despite the study heterogeneity shown by the lack of association in HPFS, this SNP remains significantly associated with PCSM in a meta-analysis. With additional studies, this SNP or a genetic panel of SNPs may help distinguish at the time of diagnosis those patients likely to exhibit a more indolent or a more aggressive course of PCa.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Clinical characteristics of the Physicians' Health Study prostate cancer cohort

	All (n=1430)	Non-PCSM (n=1225)	PCSM (n=205)
Age at diagnosis, y			
Mean (sd)	70.5 (7.7)	70.5 (7.6)	70.8 (8.2)
Median	70.5	70.4	70.9
Range	45.5–100.9	50.9–100.9	45.5–92.5
Gleason score, n (%)			
2–4	107 (7.5)	101 (8.2)	6 (2.9)
5, 6	555 (38.8)	519 (42.4)	36 (17.6)
3+4 (or 7)	273 (19.1)	243 (19.8)	30 (14.6)
4+3	151 (10.6)	129 (10.5)	22 (10.7)
8–10	192 (13.4)	127 (10.4)	65 (31.7)
Missing	152 (10.6)	106 (8.7)	46 (22.4)
Clinical stage, n (%)			
T1/T2	1166 (81.5)	1050 (85.7)	116 (56.6)
T3	67 (4.7)	46 (3.8)	21 (10.2)
T4/N1/M1	83 (5.8)	25 (2.0)	58 (28.3)
Missing	114 (8.0)	104 (8.5)	10 (4.9)
PSA at diagnosis, n (%)			
<4	116 (8.1)	112 (9.1)	4 (2.0)
4–9.9	558 (39.0)	531 (43.3)	27 (13.2)
10–19.9	206 (14.4)	184 (15.0)	22 (10.7)
≥20	136 (9.5)	103 (8.4)	33 (16.1)
Missing (pre-PSA era)	217 (15.2)	136 (11.1)	81 (39.5)
Missing	197 (13.8)	159 (13.0)	38 (18.5)
Primary therapy, n (%)			
Radical prostatectomy	576 (40.3)	531 (43.3)	45 (22.0)
Radiation therapy	371 (25.9)	322 (26.3)	49 (23.9)
Androgen deprivation therapy	125 (8.7)	85 (6.9)	40 (19.5)
Other/None	109 (7.6)	96 (7.8)	13 (6.3)
Missing	249 (17.4)	191 (15.6)	58 (28.3)

Table 2

Hazard ratios (HR) for 22 SNPs with prostate cancer-specific mortality in the Physicians' Health Study prostate cancer cohort

SNP	# Minor Alleles	Non-lethal, n (%)*	Lethal, n (%)*	HR (95% CI)**	P**	Seattle HR; Model***
rs1029153	0	593 (50.4)	92 (46.2)	1.05 (0.85–1.30)	0.63	0.22; Tre:A
CXCL12	1	479 (40.7)	92 (46.2)			
	2	105 (8.9)	15 (7.5)			
rs11205	0	372 (36.2)	52 (35.1)	1.73 (1.15–2.61)	0.01	0.21; Rec:ACP
HSD17B4	1	493 (48.0)	65 (43.9)			
	2	162 (15.8)	31 (21.0)			
rs1137100	0	568 (55.7)	86 (58.9)	1.11 (0.78–1.57)	0.57	0.29; Dom:ACP
LEPR	1	380 (37.3)	50 (34.3)			
	2	71 (7.0)	10 (6.9)			
rs11710277	0	850 (84.5)	125 (85.0)	1.32 (0.83–2.10)	0.25	3.71; Dom:ACP
SEMA3F	1	143 (14.2)	21 (14.3)			
	2	13 (1.3)	1 (0.7)			
rs12467911	0	610 (51.1)	95 (47.3)	1.20 (0.91–1.58)	0.21	0.45; Dom:A
SRD5A2	1	479 (40.2)	90 (44.8)			
	2	104 (8.7)	16 (8.0)			
rs1799814	0	935 (90.7)	136 (91.9)	0.83 (0.46–1.50)	0.53	0.13; Tre:ACP
CYP1A1	1	89 (8.6)	12 (8.1)			
	2	7 (0.7)	0 (0.0)			
rs1799964	0	738 (61.9)	119 (58.9)	1.10 (0.83–1.46)	0.49	0.39; Dom:A
TNF/LTA	1	399 (33.5)	74 (36.6)			
	2	55 (4.6)	9 (4.5)			
rs2070874	0	817 (68.5)	134 (66.3)	1.14 (0.85–1.52)	0.4	2.16; Dom:A
IL4	1	339 (28.4)	61 (30.2)			
	2	37 (3.1)	7 (3.5)			
rs228697	0	805 (78.7)	104 (70.3)	1.50 (1.04–2.16)	0.03	0.25; Dom:ACP
PER3	1	207 (20.2)	40 (27.0)			
	2	11 (1.1)	4 (2.7)			
rs2308327	0	865 (72.5)	157 (77.7)	0.79 (0.58–1.06)	0.12	0.32; Tre:A

SNP	# Minor Alleles	Non-lethal, n (%)*	Lethal, n (%)*	HR (95% CI)**	P**	Seattle HR; Model***
MGMT	1	303 (25.4)	42 (20.8)			
	2	25 (2.1)	3 (1.5)			
rs2494750	0	939 (93.7)	142 (96.6)	0.64 (0.26–1.58)	0.33	0.22; Tre:ACP
AKT1	1	63 (6.3)	5 (3.4)			
rs2839685	0	717 (71.7)	100 (69.0)	0.54 (0.22–1.34)	0.19	28.2; Rec:ACP
CXCL12	1	244 (24.4)	40 (27.6)			
	2	39 (3.9)	5 (3.5)			
rs4583514	0	401 (39.2)	55 (38.5)	1.09 (0.77–1.54)	0.62	2.49; Dom:ACP
MSH2	1	477 (46.7)	73 (51.1)			
	2	144 (14.1)	15 (10.5)			
rs4645959	0	915 (88.8)	139 (93.3)	0.75 (0.39–1.43)	0.38	0; Dom:ACP
c-MYC	1	114 (11.1)	9 (6.0)			
	2	2 (0.2)	1 (0.7)			
rs523349	0	589 (51.6)	93 (49.2)	1.12 (0.84–1.49)	0.44	0.49; Dom:A
SRD5A2	1	463 (40.6)	82 (43.4)			
	2	89 (7.8)	14 (7.4)			
rs5993891	0	884 (86.8)	142 (96.0)	0.32 (0.14–0.74)	0.01	0.21; Dom:ACP
ARVCF	1	131 (12.9)	6 (4.1)			
	2	3 (0.3)	0 (0.0)			
rs627839	0	297 (28.6)	39 (26.2)	1.25 (0.85–1.84)	0.25	3.98; Dom:ACP
RNASEL	1	498 (48.0)	77 (51.7)			
	2	242 (23.3)	33 (22.2)			
rs635261	0	389 (37.8)	52 (34.9)	1.11 (0.72–1.71)	0.64	0.22; Rec:ACP
RNASEL	1	489 (47.5)	70 (47.0)			
	2	152 (14.8)	27 (18.1)			
rs915927	0	384 (32.1)	63 (30.9)	1.03 (0.76–1.39)	0.85	2.54; Dom:A
XRCC1	1	589 (49.2)	96 (47.1)			
	2	225 (18.8)	45 (22.1)			
rs10778534	0	469 (39.2)	76 (37.3)	1.10 (0.82–1.45)	0.53	2.21; Dom:A
CRY1	1	568 (47.5)	100 (49.0)			
	2	159 (13.3)	28 (13.7)			

SNP	# Minor Alleles	Non-lethal, n (%)*	Lethal, n (%)*	HR (95% CI)**	P**	Seattle HR; Model***
rs25487	0	484 (40.4)	90 (45.0)	0.91 (0.74–1.12)	0.38	0.49; Tre:A
XRCC1	1	556 (46.4)	85 (42.5)			
	2	158 (13.2)	25 (12.5)			
rs4608577	0	820 (68.5)	143 (70.1)	0.99 (0.76–1.30)	0.95	2.04; Tre:A
MSH2	1	347 (29.0)	56 (27.5)			
	2	30 (2.5)	5 (2.5)			

* total numbers may be slightly different as SNPs adjusted for clinical factors had some participants excluded for missing Gleason score or clinical stage information

** this study used the genetic model and adjusted for the same covariates (either age or age and clinicopathologic factors [Gleason score, PSA at diagnosis, clinical stage, treatment]) specified in original publication

*** Dom=Dominant, Rec=Recessive, Tre=Additive trend; A=age-adjusted only, ACP=adjusted for age and clinicopathologic factors