

# Genetic Variants and Prostate Cancer Risk: Candidate Replication and Exploration of Viral Restriction Genes

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## Abstract

The genetic variants underlying the strong heritable component of prostate cancer remain largely unknown. Genome-wide association studies of prostate cancer have yielded several variants that have significantly replicated across studies, predominantly in cases unselected for family history of prostate cancer. Additional candidate gene variants have also been proposed, many evaluated within familial prostate cancer study populations. Such variants hold great potential value for risk stratification, particularly for early-onset or aggressive prostate cancer, given the comorbidities associated with current therapies. Here, we investigate a Caucasian study population of 523 independent familial prostate cancer cases and 523 age-matched controls without a personal or family history of prostate cancer. We replicate identified associations

at genome-wide association study loci 8q24, 11q13, and 2p15 ( $P = 2.9 \times 10^{-4}$  to  $P = 4.7 \times 10^{-5}$ ), showing study population power. We also find evidence to support reported associations at candidate genes *RNASEL*, *EZH2*, and *NKX3-1* ( $P = 0.031$  to  $P = 0.0085$ ). We further explore a set of candidate genes related to *RNASEL* and to its role in retroviral restriction, identifying nominal associations at *XPR1* and *RBM9*. The effects at 8q24 seem more pronounced for those diagnosed at an early age, whereas at 2p15 and *RNASEL* the effects were more pronounced at a later age. However, these trends did not reach statistical significance. The effects at 2p15 were statistically significantly more pronounced for those diagnosed with aggressive disease. (Cancer Epidemiol Biomarkers Prev 2009;18(7):2137–44)

## Introduction

Among common cancers, prostate cancer has the greatest heritable risk, estimated to be ~50% (1, 2). Family history remains the best predictor of risk for prostate cancer. Those with a positive family history are likely to have inherited a greater genetic load for the disease than those with a negative family history. The underlying structure of this heritable risk remains largely unknown. Highly penetrant Mendelian mutations have been widely sought through linkage analysis (3). *RNASEL*, *ELAC2*, *MSR1*, and *PODXL* were identified as candidate prostate cancer genes by this approach, and each has been proposed to harbor common low-penetrance variants contributing to population risk of prostate cancer. Given the established role of *RNASEL* and *MSR1* in innate immunity and viral susceptibility, investigators have also proposed additional candidate genes in these pathways as modifiers of prostate cancer risk. These have included genes encoding the Toll-like receptors and the interleukin 1 receptor

antagonist. Additional candidates have been proposed based on genes identified through studies of animal models of prostate cancer (*NKX3-1*), of somatic changes in gene expression in prostate cancer (*PPARG*, *EZH2*), of overlapping hereditary cancer syndromes (*BRCA2*, *CDH1*), and of salient biochemical pathways (*AR*, *SRD5A2*, *CDKN1B*, *TGFB1*, *CYP17A1*, *CYP11A1*; ref. 4). Among all of these candidates, many of the observed associations have replicated inconsistently, suggesting extensive genetic heterogeneity in prostate cancer predisposition, population-specific findings, or type I errors. Recent genome-wide association studies of prostate cancer have identified additional single nucleotide polymorphisms (SNPs) that are significantly associated with prostate cancer and, encouragingly, that have replicated broadly across global study populations (5–7).

In this study, we investigated heritable prostate cancer risk in a study population of familial prostate cancer cases, and controls without a personal or family history of prostate cancer. This design was intended to compare two extremes of the distribution of genetic load for prostate cancer. We first sought to replicate the prostate cancer association of several SNPs that have globally replicated within the prostate cancer genome-wide association studies literature to assess the relative power of the study population. Second, we conducted a replication study of a series of published genes of less certain significance, including: *RNASEL*, *ELAC2*, *PODXL*, *TLR10*, *TLR1*, *TLR6*, *TLR4*, *IL1RN*, *NKX3-1*, *PPARG*, *EZH2*, *CDH1*, *CDKN1B*, *TGFB1*, *CYP17A1*, and *CYP11A1*. Third, we further explored

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potential evidence for the association of prostate cancer with a series of additional genes related to retroviral infection. The impetus for this was the prominence of innate immunity genes among published candidates as well as the recent intriguing discovery of a novel retrovirus of the xenotropic murine leukemia virus family in prostate adenocarcinomas with the risk *RNASEL* genotype (8, 9).

A relatively large number of retroviral restriction genes are known, but few reside near published potential hereditary prostate cancer linkage regions. *RNASEL* was included among the genes of the replication set noted above, and we further explored two potential *RNASEL* regulatory genes near potential linkage areas: the *RNASEL* inhibitor *RNS4I* at 4q31 (10) and *RBM9* at 22q12 (11). *RBM9* encodes a protein predicted to regulate transcript stability by binding 3' UTR AU-rich elements, a mechanism that may regulate *RNASEL* and that is also tied to viral replication (12, 13). *RBM9* is located at the peak of the 22q12 hereditary prostate cancer locus of the International Consortium for Prostate Cancer Genetics (11, 14). *XPR1* is a cell surface receptor with a direct role in resistance of specific mouse strains to murine leukemia viruses (15). The human orthologue of *XPR1* is found 1.6 Mb centromeric to *RNASEL* within the 1q25 *HPC1* prostate cancer locus. Additional retroviral restriction genes near potential linkage regions that were explored include *APOBEC3* genes (16) at 22q13 (11), *APOBEC4* (17) at 1q25 (18), *AICDA* (19) at 12p13 (10, 20, 21), *PIN1* (22, 23) at 19p13 (20, 24-27), and peptidyl-prolyl isomerases (28) *PPID* at 4q32 (18) and *PPIH* at 1p34 (26, 29-31).

## Materials and Methods

**Study Population.** Study subjects were Americans of Northern European descent, ascertained with informed consent between 2002 and 2008 from Vanderbilt University Medical Center and from the VA Tennessee Valley Healthcare System (adjacent hospitals) with Institutional Review Board oversight. Familial prostate cancer cases were ascertained at the time of treatment for the principal diagnosis of prostate cancer, and controls were ascertained at the time of routine preventative screening for prostate cancer. All prostate cancer probands included in the study were from pedigrees with a family history of prostate cancer ( $\geq 2$  affected), and all control probands were from pedigrees without a family history of prostate cancer. Family history included first-degree and second-degree relatives. Controls had a screening prostate-specific antigen test  $< 4$  ng/mL at the time of ascertainment, no personal history of prostate cancer, no record of a prostate-specific antigen test  $\geq 4$  ng/mL, and no record of abnormal digital rectal examination. Controls were individually matched to cases on age in a 1:1 ratio ( $\pm 2.5$  y; age at screen for controls, age at diagnosis for cases). The study included 523 unrelated, independent familial prostate cancer probands and 523 matched control probands. Table 1 provides the characteristics of the study population. Stratification analyses of Gleason score preferentially used the final prostatectomy specimen Gleason score (available for 87% of cases) rather than the initial diagnostic biopsy Gleason score.

**SNP Genotyping.** DNA was extracted from whole blood on an Autopure LS robot with the use of the Puregene DNA Purification System standard protocol (Qiagen). DNA was quantified with the use of the PicoGreen dsDNA Quantitation kit (Invitrogen) and imaged with a Molecular Devices/LJL Analyst HT (Molecular Devices). SNP genotyping was conducted with the use of the Illumina GoldenGate platform (Illumina). SNPs rs10896450, rs6983267, and rs1800470 were exceptions, and were genotyped with the use of the TaqMan platform (Applied Biosystems). The rs10896450 assay was kindly provided by Dr. J. Gudmundsson (deCODE genetics, Reykjavik, Iceland). We obtained 99.7% of the genotypes of SNPs that successfully converted for assay (Fig. 1; Tables 2 and 3; Supplementary Table S1).

SNPs within four regions associated with sporadic prostate cancer in published genome-wide association studies were selected for assay (5-7, 32-34). These included the centromeric 8q24 linkage disequilibrium blocks rs6983267 and rs6998061, the telomeric 8q24 linkage disequilibrium block rs1447295 ( $r^2 = 1.0$  with rs4242382 and rs4242384), 11q13 rs10896450 ( $r^2 = 1.0$  with rs10896449 and  $r^2 = 0.98$  with rs7931342), and 2p15 rs721048.

Tagging SNPs were selected with the use of LDSelect (35) with an  $r^2$  threshold of 0.8 in the CEU subject data of HapMap Release 21 of phase II of the National Center for Biotechnology Information build 35 assembly among SNPs amenable to Illumina assay. CEU subjects were Utah residents of northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain. The minor allele frequency threshold was 0.05, as exceeded by any Caucasian study population of dbSNP, including CEU. Tagging SNPs and candidate functional SNPs are designated within Table 2 and Supplementary Table S1. Additional SNPs that failed assay included: rs1927907 (*TLR4*), rs454078 (*IL1RN*), rs1985604 (*PIN1*), and rs9998052 [*RNS4I* (*ABCE1*)]. At *PPARG* two separate P12A missense SNPs are annotated: rs1805192 in the primary transcript (NM\_005037, NM\_138711, NM\_015869, NM\_138712), and rs1801282 in an alternative transcript (NM\_015869).

**Statistical Analyses.** We used a conditional logistic regression model with two  $\beta$  parameters to estimate odds ratios (OR) and 95% confidence intervals of heterozygous and homozygous states (Intercooled Stata 10; Stata Corporation). The model was adjusted for the matching variable age, and for the number of study subject

**Table 1. Study population characteristics**

	Controls	Cases
No.	523	523
Mean Age*, y	61.3	61.2
Mean no. of brothers	1.8	1.7
Median PSA*	0.9	5.6
Median Gleason sum	—	6
Gleason sum $\leq 6$ , no.	—	275
Gleason sum $\geq 7$ , no.	—	231
Affected in pedigree, no. †	0	—
	2	316
	$\geq 3$	207

\*At diagnosis for cases, at screen for controls.

†Proband plus first-degree and second-degree affected relatives.

Significant Sliding Windows	Haplotype Heterozygote				Haplotype Homozygote					
	Case Control	OR	95% CI	P	Case Control	OR	95% CI	P		
	<b>XPR1</b>									
	Protective (GTAATGG)									
	99	111	0.83	(0.62 - 1.13)	0.25	1	10	0.10	(0.01 - 0.78)	0.028
	Protective (CTTTG)									
	185	193	0.86	(0.66 - 1.12)	0.25	18	36	0.46	(0.26 - 0.82)	0.0088
	<b>RBM9</b>									
	Risk									
	229	215	1.23	(0.94 - 1.61)	0.12	64	47	1.57	(1.03 - 2.41)	0.037

**Figure 1.** Haplotype association with prostate cancer. To left, sliding haplotype windows of  $P \leq 0.05$  by  $\chi^2$  analysis, graphically ordered from most to least significant (from black to gray). To right, logistic regression analysis for haplotypes designated in black ( $N = 1,046$ ). Among all genes of the study, only *RNASEL*, *XPR1*, and *RBM9* yielded haplotype windows of  $P \leq 0.05$ . The open-boxed haplotype window of *XPR1* is common to both protective haplotypes.

brothers as a potential confounder, because proband selection was based on family history. We added dichotomous interaction terms to our conditional logistic regression models to assess interaction between genotype and age of diagnosis ( $\leq 60$  or  $\geq 61$  y) of cancer risk. Wald  $\chi^2$  tests for interaction were done. A similar approach was used to assess interaction between genotype and prostate cancer aggressiveness (Gleason score  $\leq 6$  or  $\geq 7$ ).

Individual study subject diplotypes were estimated by PHASE v2.1 (36), run for all 1,046 subjects as a group. For a given window of consecutive SNPs at a gene, the diplotype may be assigned with evaluation of the specified window or with evaluation of larger windows encompassing the same markers. The window diplotype of highest probability  $\geq 0.9$  among them was used for sliding window tests of association (37, 38). The sliding window approach evaluated a haplotype window of  $N$  markers, sliding the window along a gene map in single-marker increments. The width of the window was varied from two to the maximum number of SNPs evaluated at a gene. Each  $N$  marker haplotype was compared with the remaining haplotypes of the window as a group among cases and controls. The resulting  $2 \times 2$  contingency table of frequencies was evaluated by a  $\chi^2$  test statistic. A nominally significant haplotype was subsequently modeled by conditional logistic regression as described above. All  $P$  values were calculated with respect to two-sided alternative hypotheses. Because each locus was investigated to replicate a previously published association or, alternatively, to explore a potential association for future replication,  $P$  values were unadjusted for multiple comparisons.

## Results

Four loci that have been significantly associated with prostate cancer in prior genome-wide association studies and that have independently replicated across global study populations were also significantly associated with

familial prostate cancer in our study. Table 2 (top) presents the ORs and significance of each of these. The 2p15 variant rs721048 (32) followed an additive model with a particularly strong effect for homozygous carriers (OR, 4.06;  $P = 4.7 \times 10^{-5}$ ). The 8q24 variant rs1447295 originally identified in the Icelandic study (5) had a relatively low-risk allele frequency (7.1%, case; 4.4%, control), and greatest effect was observed among heterozygous carriers (OR, 1.78;  $P = 2.9 \times 10^{-4}$ ). That variant marks a linkage disequilibrium block that is detected by several additional SNPs that have been observed to be significantly associated with prostate cancer across studies. The adjacent centromeric linkage disequilibrium block is independently associated with prostate cancer risk, detected by rs6983267 (6, 7). We evaluated rs6983267 and the adjacent missense variant rs6998061 (G527E) of the *POU5F1P1* gene, observing moderate pairwise linkage disequilibrium ( $r^2 = 0.69$ ). The association at this linkage disequilibrium block seemed to follow an additive model and was best detected by rs6983267, with a homozygote OR of 0.49 ( $P = 7.5 \times 10^{-5}$ ). The T allele frequency was 0.417 among cases and 0.508 among controls. SNP rs10896450 on 11q13 was originally detected through genome-wide association studies (6, 7)<sup>5</sup> and also confirmed as significantly associated with prostate cancer in our study. It also followed an additive model with greatest effect among homozygotes (OR, 0.51;  $P = 2.2 \times 10^{-4}$ ). The minor allele had a frequency of 20.5% among cases and 24.7% among controls. To facilitate comparisons with other publications, results of an additive model for these variants are given in Supplementary Table S2. Each of these associations indicated good power to detect risk-modifying genetic variants among candidate loci within this familial prostate cancer study population.

Of several candidate genes proposed to be associated with prostate cancer in the published literature and evaluated here, three yield additional evidence to

<sup>5</sup>J. Gudmundsson, personal communication.

**Table 2. Replication evidence for association with prostate cancer among published loci**

Chr	Gene	Variant		Minor allele heterozygote				Minor allele homozygote				
				Case	Control	OR (95% CI)	P	Case	Control	OR (95% CI)	P	
2p15	(GWAS)	rs721048	T	176	157	1.32 (1.00-1.73)	0.047	41	11	4.06 (2.07-7.98)	$4.7 \times 10^{-5}$	
8q24 cen.	(GWAS)	rs6983267	T	225	252	0.65 (0.49-0.87)	0.0040	100	138	0.49 (0.34-0.70)	$7.5 \times 10^{-5}$	
8q24 cen.	<i>POU5F1P1</i>	rs6998061	A	218	236	0.75 (0.58-0.98)	0.032	79	108	0.56 (0.39-0.80)	0.0015	
8q24 tel.	(GWAS)	rs1447295	A	133	85	1.78 (1.30-2.44)	$2.9 \times 10^{-4}$	7	3	2.36 (0.60-9.21)	0.22	
11q13	(GWAS)	rs10896450	A	247	256	0.72 (0.54-0.96)	0.024	89	128	0.51 (0.35-0.73)	$2.2 \times 10^{-4}$	
1q25	<i>RNASEL</i>	rs11807829	C	240	227	1.06 (0.81-1.37)	0.67	49	60	0.83 (0.54-1.27)	0.39	
		rs533259	T	68	71	0.94 (0.64-1.37)	0.74	3	2	1.48 (0.25-8.90)	0.67	
		rs627928	E541D	T	262	253	0.91 (0.69-1.21)	0.52	81	110	0.64 (0.44-0.92)	0.018
		rs486907	R462Q	A	259	228	1.34 (1.03-1.76)	0.031	76	73	1.22 (0.84-1.78)	0.29
2q13	<i>IL1RN</i>	rs2637988	G	235	248	0.89 (0.68-1.16)	0.37	79	80	0.92 (0.63-1.34)	0.67	
		rs3213448	A	94	94	1.01 (0.74-1.38)	0.94	5	11	0.47 (0.16-1.35)	0.16	
		rs3087263	A	96	93	1.03 (0.75-1.41)	0.87	7	10	0.70 (0.26-1.84)	0.47	
		rs380092	T	208	218	0.95 (0.73-1.22)	0.67	53	48	1.10 (0.72-1.67)	0.67	
		rs315952	C	194	204	0.93 (0.72-1.20)	0.58	44	43	1.00 (0.64-1.57)	0.98	
3p25	<i>PPARG</i> *	rs1805192	P12A	C	0	0		0	0			
		rs1801282	P12A†	G	105	114	0.92 (0.68-1.24)	0.58	8	3	2.66 (0.69-10.17)	0.15
4p14	<i>TLR10</i> *	rs4129009	I775V	G	145	167	0.83 (0.63-1.08)	0.17	22	18	1.15 (0.60-2.21)	0.67
		rs11466658	R525W	T	28	26	1.08 (0.62-1.88)	0.79	0	0		
		rs11096955	I369L	C	214	221	0.93 (0.72-1.21)	0.60	67	71	0.90 (0.62-1.32)	0.60
		rs11096957	N241H	C	214	221	0.93 (0.72-1.20)	0.58	67	71	0.88 (0.60-1.30)	0.53
4p14	<i>TLR1</i>	rs3923647	H305L	T	30	28	1.07 (0.63-1.83)	0.80	0	0		
		rs4833095	N248S	C	181	198	0.88 (0.68-1.13)	0.30	33	31	1.00 (0.59-1.67)	0.98
		rs5743611	R80T	C	69	74	0.93 (0.65-1.32)	0.67	5	7	0.80 (0.25-2.56)	0.71
4p14	<i>TLR6</i>	rs5743815	V427A	C	21	12	1.75 (0.86-3.57)	0.13	0	0		
		rs5743810	P249S	T	264	256	1.08 (0.81-1.44)	0.58	99	101	1.02 (0.71-1.45)	0.91
		rs1039559		C	265	253	1.14 (0.86-1.53)	0.36	125	125	1.10 (0.78-1.54)	0.60
7q32	<i>PODXL</i> *	rs3735035	G112S	T	272	271	0.90 (0.67-1.21)	0.48	109	122	0.80 (0.56-1.14)	0.21
		rs3212298	V358I	A	45	60	0.71 (0.47-1.07)	0.10	2	2	0.94 (0.13-6.84)	0.95
7q36	<i>EZH2</i> *	rs2302427	D185H	C	69	98	0.63 (0.44-0.89)	0.0085	4	5	0.67 (0.18-2.56)	0.56
8p21	<i>NKX3-1</i>	rs1567669		T	201	244	0.71 (0.55-0.92)	0.010	55	51	0.93 (0.61-1.42)	0.74
9q33	<i>TLR4</i>	rs11536869		G	43	41	1.02 (0.66-1.60)	0.91	0	2		
		rs1927911		T	196	212	0.92 (0.71-1.19)	0.52	47	35	1.38 (0.84-2.29)	0.21
		rs11536879		G	45	32	1.47 (0.91-2.38)	0.12	0	1		
		rs5030717		G	110	107	1.02 (0.75-1.38)	0.91	3	4	0.72 (0.12-4.34)	0.72
		rs5030728		A	225	237	0.90 (0.70-1.15)	0.41	44	47	0.86 (0.55-1.36)	0.53
		rs4986791	T399I	T	53	59	0.88 (0.59-1.32)	0.55	2	1	1.74 (0.15-19.63)	0.65
10q24	<i>CYP17A1</i>	rs10883783		A	226	222	1.09 (0.85-1.40)	0.52	55	42	1.36 (0.89-2.09)	0.16
		rs3824755		C	79	95	0.81 (0.58-1.12)	0.19	2	4	0.53 (0.10-2.92)	0.47
		rs6163		A	249	240	1.10 (0.85-1.43)	0.46	85	83	1.08 (0.75-1.56)	0.67
		rs2486758		C	174	161	1.13 (0.87-1.49)	0.36	21	22	1.00 (0.54-1.84)	1.00
12p13	<i>CDKN1B</i>	rs34329		G	225	227	0.99 (0.76-1.28)	0.92	48	46	1.04 (0.67-1.61)	0.85
		rs3093736		A	30	34	0.89 (0.54-1.46)	0.65	0	0		
16q22	<i>CDH1</i> *	rs16260	5'(-160A)	A	201	211	0.97 (0.75-1.25)	0.81	45	37	1.21 (0.77-1.91)	0.41
15q24	<i>CYP11A1</i>	rs4646421		T	96	83	1.21 (0.86-1.70)	0.27	3	6	0.55 (0.14-2.22)	0.40
17p12	<i>ELAC2</i> *	rs4792311	S217L	A	224	216	1.08 (0.84-1.40)	0.56	46	42	1.13 (0.73-1.74)	0.60
19q13	<i>TGFB1</i> *	rs1800470	L10P	C	242	239	0.96 (0.73-1.27)	0.79	66	78	0.76 (0.51-1.12)	0.16

\*Candidate, rather than tagging SNPs evaluated.

†Different residue variant from an alternative transcript.

support an association with prostate cancer within our study. These data are presented in Table 2 beneath the genome-wide association studies replication set. We observed significant associations at *RNASEL* (39, 40), *EZH2* (41), and *NKX3-1* (42). Our evaluation of the remaining candidates was not significant in either single-allele-based or haplotype-based analyses. At *RNASEL*, homozygotes for the minor allele of rs627928 (E541D) had a significantly reduced risk of prostate cancer (OR, 0.64;  $P = 0.018$ ). That variant marks one of three common haplotypes at the gene on a linkage disequilibrium block that includes tested SNPs rs533529, rs627928, and rs486907 (all pairwise  $D' = 1.0$ ). Another of the three haplotypes is marked by the minor allele of rs486907 (R462Q); heterozygotes for this minor allele had a significantly increased risk of prostate cancer (OR, 1.34;

$P = 0.031$ ). At *EZH2*, rs2302427 (D185H) had a minor allele frequency of 3.7% among cases and 5.2% among controls, conferring significantly reduced risk of prostate cancer among heterozygotes (OR, 0.63;  $P = 0.0085$ ). Tagging SNP rs1567669 at *NKX3-1* also yielded evidence to support an association with prostate cancer among heterozygotes (OR, 0.71;  $P = 0.010$ ).

A third set of candidate loci was also evaluated in this study. These were genes related to *RNASEL* and retroviral restriction, and near genomic regions supported by prior linkage evidence. The impetus for this exploration was the recent discovery of the XMRV  $\gamma$ -retrovirus in prostate cancer tissue, apparently more commonly present among evaluated *RNASEL* 462Q/Q homozygotes (8, 9). Results at these genes are presented in Supplementary Table S1. Only three SNPs yielded

nominal evidence of an association with prostate cancer, one each at *PPIH*, *XPR1*, and *AICDA*. Sliding window haplotype analysis often redundantly identifies an association, but no significant haplotype profile differences between cases and controls were observed at *PPIH* or *AICDA*. Haplotype-based sliding window analyses did, however, yield further evidence to support a protective association at *XPR1* (Fig. 1). *XPR1* is encoded by 15 exons that reside within a region of strong linkage disequilibrium among study subjects. Homozygotes of each of two haplotypes at *XPR1* had a nominally significantly reduced risk of prostate cancer (OR, 0.10;  $P = 0.028$  and OR, 0.46;  $P = 0.009$ ). These two protective haplotypes share alleles that distinguish them from other common *XPR1* haplotypes: rs17373411 A, rs2331886 G, rs3002121 C, rs1533422 T, and rs2271668 T. Haplotype A-G-C-T-T was protective of prostate cancer among homozygotes (OR, 0.31;  $P = 5.4 \times 10^{-4}$ ). Haplotype analysis additionally identified a risk association at *RBM9*, which also resides within a region of strong linkage disequilibrium. Among the seven common haplotypes across *RBM9* in the study population, one was nominally associated with excess risk of prostate cancer among homozygotes (OR, 1.57;  $P = 0.037$ ). *XPR1* and *RBM9* may thus be reasonable candidates for further evaluation in independent study populations.

Finally, we investigated two clinical facets of prostate cancer of importance for prevention and prognosis: age of diagnosis, and Gleason score as an index of disease aggressiveness (Table 3). We first stratified the study population according to cancer diagnosis by age 60 or after age 61 years (two roughly comparably sized groups), and evaluated variants of Table 2 that significantly modified prostate cancer risk. Each of the two independent 8q24 variants had apparently stronger association among those with a younger age of diagnosis (rs1447295 heterozygote OR, 2.11;  $P = 0.0013$  and rs6983267 homozygote OR, 0.39;  $P = 2.7 \times 10^{-4}$ ). In contrast, other variants had apparently greater effects at a later age of diagnosis. 2p15 rs721048 homozygotes had an OR of 6.21 ( $P = 9.3 \times 10^{-4}$ ) among cases diagnosed at age  $\geq 61$  years relative to an OR of 2.77 ( $P = 0.025$ ) among those diagnosed at age  $\leq 60$  years. Our results suggest that heritable variation may modify risk of both early-onset and late-onset prostate cancer. However, tests of interaction between genotype and age stratum on cancer risk were not significant for any of the SNPs of Table 3. The study power to establish different effects for these SNPs across age strata was limited.

We separately stratified the study population according to an index of disease aggressiveness: Gleason sum of  $\leq 6$  (well-differentiated or moderately differentiated and

**Table 3. Stratification of prostate cancer cases: by age of diagnosis, by Gleason score**

Variant	Minor allele heterozygote				Minor allele homozygote			
	Case	Control	OR (95% CI)	P	Case	Control	OR (95% CI)	P
Case age $\leq 60$ (n = 508)								
8q24 cen. rs6983267 T	102	108	0.70 (0.46-1.08)	0.11	43	76	0.39 (0.24-0.65)	$2.7 \times 10^{-4}$
8q24 tel. rs1447295 A	71	43	2.11 (1.34-3.32)	0.0013	3	0		
11q13 rs10896450 A	124	132	0.69 (0.45-1.04)	0.08	42	59	0.52 (0.30-0.88)	0.015
2p15 rs721048 T	83	77	1.24 (0.84-1.84)	0.28	19	7	2.77 (1.14-6.74)	0.025
RNASEL rs627928 T	129	124	1.00 (0.68-1.48)	0.99	41	50	0.79 (0.47-1.33)	0.37
RNASEL rs486907 A	114	113	1.08 (0.74-1.58)	0.67	38	33	1.19 (0.70-2.03)	0.53
EZH2 rs2302427 C	32	39	0.76 (0.44-1.29)	0.31	2	3	0.63 (0.10-3.96)	0.63
NKX3-1 rs1567669 T	100	130	0.65 (0.44-0.94)	0.023	31	23	1.13 (0.61-2.10)	0.70
Case age $\geq 61$ (n = 538)								
8q24 cen. rs6983267 T	123	144	0.63 (0.43-0.94)	0.023	57	62	0.62 (0.37-1.05)	0.08
8q24 tel. rs1447295 A	62	42	1.62 (1.04-2.53)	0.033	4	3	1.43 (0.32-6.46)	0.64
11q13 rs10896450 A	123	124	0.77 (0.51-1.14)	0.19	47	69	0.51 (0.31-0.83)	0.0074
2p15 rs721048 T	93	80	1.39 (0.95-2.03)	0.09	22	4	6.21 (2.11-18.29)	$9.3 \times 10^{-4}$
RNASEL rs627928 T	133	129	0.84 (0.56-1.24)	0.37	40	60	0.52 (0.30-0.88)	0.016
RNASEL rs486907 A	145	115	1.72 (1.16-2.53)	0.007	38	40	1.24 (0.73-2.09)	0.43
EZH2 rs2302427 C	37	59	0.57 (0.36-0.89)	0.015	2	2	0.75 (0.10-5.51)	0.78
NKX3-1 rs1567669 T	101	114	0.78 (0.54-1.12)	0.19	24	28	0.78 (0.44-1.38)	0.39
Case Gleason $\leq 6$ (n = 550)								
8q24 cen. rs6983267 T	123	127	0.72 (0.49-1.07)	0.11	50	75	0.46 (0.28-0.76)	0.0021
8q24 tel. rs1447295 A	66	47	1.57 (1.02-2.42)	0.043	3	1	2.66 (0.26-26.65)	0.41
11q13 rs10896450 A	143	128	0.93 (0.63-1.36)	0.70	42	69	0.51 (0.30-0.85)	0.0093
2p15 rs721048 T	89	82	1.22 (0.85-1.75)	0.29	19	9	2.23 (0.99-5.01)	0.053
RNASEL rs627928 T	140	140	0.86 (0.59-1.25)	0.43	37	51	0.60 (0.35-1.03)	0.06
RNASEL rs486907 A	139	127	1.39 (0.95-2.03)	0.09	44	35	1.59 (0.94-2.67)	0.08
EZH2 rs2302427 C	40	57	0.62 (0.40-0.96)	0.034	1	4	0.23 (0.03-2.06)	0.19
NKX3-1 rs1567669 T	112	133	0.72 (0.50-1.02)	0.06	29	29	0.86 (0.48-1.57)	0.63
Case Gleason $\geq 7$ (n = 462)								
8q24 cen. rs6983267 T	92	117	0.54 (0.35-0.84)	0.0063	45	57	0.48 (0.28-0.83)	0.0088
8q24 tel. rs1447295 A	62	34	2.23 (1.37-3.63)	0.0013	4	2	2.24 (0.40-12.50)	0.36
11q13 rs10896450 A	98	122	0.51 (0.32-0.81)	0.0039	43	53	0.49 (0.28-0.84)	0.0099
2p15 rs721048 T	81	68	1.56 (1.01-2.40)	0.046	21	2	12.25 (2.83-52.93)	$7.8 \times 10^{-4}$
RNASEL rs627928 T	111	107	0.93 (0.60-1.42)	0.72	40	52	0.68 (0.40-1.16)	0.16
RNASEL rs486907 A	111	97	1.26 (0.85-1.88)	0.25	31	36	0.92 (0.52-1.63)	0.79
EZH2 rs2302427 C	27	38	0.67 (0.38-1.19)	0.17	3	1	2.23 (0.22-22.73)	0.50
NKX3-1 rs1567669 T	82	103	0.66 (0.44-1.00)	0.051	25	21	1.01 (0.55-1.85)	0.98

relatively indolent) and  $\geq 7$  (poorly differentiated and relatively aggressive). Again, this resulted in the division of the study population into two roughly comparably sized groups. More pronounced effects were observed among those with more aggressive cancer (Gleason  $\geq 7$ ) relative to those with less aggressive cancer (Gleason  $\leq 6$ ) for variants of 8q24 (rs1447295) and of 2p15 (rs721048). However, among the SNPs of Table 3, the test for interaction between genotype and Gleason score stratum on cancer risk was significant only for 2p15 rs721048 (homozygote interaction  $P = 0.024$ ). A further test of heterogeneity across age of diagnosis and Gleason score revealed that these facets are not independent among the familial cases. Those in the high-Gleason score group tended to have an older mean age of diagnosis (on average 2.3 years older;  $t$  test  $P = 0.0016$ ).

## Discussion

Our study strongly replicated observed associations between prostate cancer and genetic variants that have been recently uncovered by three genome-wide association studies. For all four genome-wide association study loci that we investigated in our familial prostate cancer study, we observed greater effect sizes than have been observed in prior studies unselected for family history. A study design sampling case and control probands based on family history may improve the power to detect disease loci. SNPs detecting the association of adjacent linkage disequilibrium blocks of 8q24 have been validated broadly (43). The risk-modifying allele of the telomeric linkage disequilibrium block at rs1447295 nicely replicated within our study. The minor risk allele was sufficiently infrequent that nearly all carriers were heterozygotes. Our stratification results were concordant with published findings. We observed greater risk among those with aggressive prostate cancer or with an early age of diagnosis. Amundadottir et al. (5), Helfand et al. (44), Wang et al. (45), Schumacher et al. (46), Severi et al. (47), and Suuriniemi et al. (48) each observed a greater effect at rs1447295 in more aggressive disease. The genome-wide association study of Thomas et al. (7) was also concordant at rs4242382 ( $r^2 = 1.0$  with rs1447295), with a greater effect among aggressive cases. Schumacher et al. additionally evaluated age of diagnosis, finding a more pronounced effect among those with an early age of diagnosis. The observation of a stronger association for rs1447295 with aggressive disease and early age of diagnosis is particularly notable, given that our familial cases with more aggressive disease had a significantly *later* rather than a younger age of diagnosis (by 2.3 years on average).

Our investigation of the adjacent centromeric 8q24 block included a candidate SNP in moderate linkage disequilibrium with the published SNP rs6983267. *POU5F1P1* at that locus is a retrotransposed pseudogene, with a fully intact open reading frame that has been shown to be expressed in cancerous tissue (49). In contrast, the parent gene *POU5F1* (*OCT4*) on 6p21 is not expressed in some transformed cells (50). The SNP that we selected for assay is the missense variant G527E (rs6998061). We show that it, too, detects an association with prostate cancer, with a protective minor allele that acts in an additive fashion. However, the missense SNP

did not improve upon the strong association signal of rs6983267 and, thus, does not supplant it as the best candidate of the linkage disequilibrium block.

Three separate genome-wide association studies have identified the association of 11q13 with prostate cancer although at different SNPs. These SNPs are rs7931342 of Thomas et al. (7), rs10896449 of Eeles et al. (6), and rs10896450 of the Icelandic genome-wide association study.<sup>6</sup> All three SNPs are in linkage disequilibrium and equivalently detect the association. Within our data, the minor allele of 11q13 rs10896450 was significantly protective and acted additively. Our stratified analyses at 11q13 also indicated a slightly greater effect among aggressive cases relative to indolent cases but similar effects within older and younger age-of-diagnosis groups. A trend toward a greater effect among those with aggressive disease was not evident in the data of Thomas et al. However, only 11.6% of cases of the initial phase of that study had a family history of prostate cancer, and the proportion of replication phase subjects with a family history was not reported. Eeles et al. had noted that the association at 11q13 was stronger among those with a positive family history.

A fourth variant originally detected in the Icelandic genome-wide association study on 2p15 and subsequently validated across multiple study populations (32) (including an overlapping subject group of this study) was reinvestigated here to enable cross-comparisons with other variants. Among the original replication study groups, the two with the greatest proportion of familial cases had yielded strongest evidence for the association. In our study of familial cases, homozygotes for the minor risk allele had a 4-fold increased risk for prostate cancer. The association at 2p15 seemed stronger among both older and more aggressive cases, subgroups that were significantly correlated. The Icelandic study of the 2p15 variant had also noted a greater effect among more aggressive cases.

Among candidate genes that have been proposed to be associated with risk of prostate cancer within the published literature, our investigation replicated associations at *RNASEL*, *NKX3-1*, and *EZH2*. Our study failed to support an association of prostate cancer with variants of *PPARG*, *TLR10*, *PODXL*, *CDH1*, *ELAC2*, *IL1RN*, *TLR1*, *TLR6*, *TLR4*, *CYP17A1*, *CDKN1B*, and *CYP11A1*.

Association results across published studies have been inconsistent at *RNASEL*. Our result for E541D is consistent with the meta-analysis result of Li et al., who showed a significant OR of 1.37 for major allele (Glu) homozygotes or heterozygotes relative to minor allele (Asp) homozygotes among familial Caucasian cases (39). Our investigation concordantly found that minor allele (Asp) homozygotes had a significant OR of 0.64 relative to major allele homozygotes. Converting our analysis of the full study population to the more comparable major allele as dominant model, we observe an OR of 1.47 ( $P = 0.018$ ). This effect was most prominent among subjects diagnosed beyond age 61 years. The meta-analysis of R462Q among Caucasians by Rennert et al. found that minor allele (Gln) heterozygotes and homozygotes each had a significantly elevated risk of prostate

<sup>6</sup>J. Gudmundsson, personal communication.

cancer (40). Our data for all cases support a risk effect and, as at E541D, it was most apparent in those diagnosed beyond age 61 years. Caucasian study populations segregate three major haplotypes for the *RNASEL* linkage disequilibrium block harboring these risk-modifying variants. Study subjects may inherit minor allele diplotypes: null/null, null/risk (462Q), null/protective (541D), protective/protective, protective/risk, and risk/risk. The estimated effects of *RNASEL* variants on prostate cancer risk generally seem modest.

Linkage, association, and mouse model evidence have been published supporting a role for *NKX3-1* in prostate cancer. Zheng et al. identified a peak heterogeneity lod score of 2.04 at *NKX3-1* (51). Gelmann et al. found that the minor allele of rs2228013 (C154T, R52C, MAF 0.04) was associated with prostate cancer risk among subjects of the Physician Health Study with more aggressive disease (stage C or D, or Gleason score  $\geq 7$ ; ref. 42). Three SNPs at the 4kb *NKX3-1* gene were genotyped within HapMap phase II and did not include rs2228013. Among them, rs1567669 has a pairwise  $r^2$  of 0.57 with rs4872176 and of 0.69 with rs11781886 in CEU subjects. Only rs1567669 within the 3' UTR successfully converted for genotyping assay in our study. We observed a significant protective effect among heterozygotes for the minor allele of rs1567669. Collectively, linkage and association results support further investigation of the role of *NKX3-1* in familial prostate cancer.

Only a single prior study has investigated the potential role of *EZH2* in prostate cancer predisposition despite its established role in the progression of aggressive prostate cancer. Bachmann et al. conducted a mutation screen at *EZH2* among hereditary prostate cancer pedigrees linked to 7q35 and evaluated the discovered variants for evidence of association with sporadic and familial prostate cancer (41). They observed eight haplotypes of frequency  $>0.01$ , one of which was significantly less frequent among familial cases than among controls. That haplotype was distinguished from all other haplotypes by the minor allele of rs2302427 (185H). The minor allele frequency was 0.10 among controls and 0.06 among familial cases. Concordantly, we observed a minor allele frequency of 0.10 among controls and 0.07 among familial cases, a significant difference within our study. Additional investigation of the role of this gene in familial prostate cancer seems to be warranted.

Our further exploration of several genes related to *RNASEL* and viral restriction identifies nominally significant associations with familial prostate cancer. Given the number of multiple comparisons made across these novel candidates, none remain significant upon Bonferroni correction. However, candidates *XPR1* and *RBM9* may remain of interest for subsequent replication efforts. Haplotype-based sliding window tests at these two genes redundantly identified nominal associations with familial prostate cancer in our study. *XPR1* is within the *HPC1* linkage interval 1.6 Mb centromeric to *RNASEL*, and *RBM9* is within the 882 kb one recombinant consensus region of the International Consortium for Prostate Cancer Genetics hereditary prostate cancer locus on 22q12.3 (11, 14, 52). Within HapMap CEU data, each gene resides alone within a linkage disequilibrium block, suggesting that an association is unlikely to be attributable to an adjacent gene in linkage disequilibrium with either candidate.

In summary, our investigation has strongly replicated associations at 8q24, 11q13, and 2p15 in a familial prostate cancer study population. The ORs that we observed at these loci were generally stronger than those described within study populations unselected for family history of prostate cancer, suggesting that greater power for discovery may be afforded by study of familial cases. Our data are also concordant with prior published prostate cancer associations at *RNASEL*, *NKX3-1*, and *EZH2*. Overall, the level of significance that we observed for these was not as great as that observed for the genome-wide association studies replication set. In subsequent stratification analyses of age of diagnosis, our data further suggest that the heritable component of prostate cancer may predispose to late-onset as well as to early-onset disease. This observation has potentially important implications for genome-wide association study strategies. We failed to support the association of a set of additional genes with familial prostate cancer. This latter group included several genes associated with prostate cancer within published literature, and additional candidates potentially related to retroviral restriction and located near hereditary prostate cancer linkage regions. Haplotype-based analysis supported an association at only two of these candidates, *XPR1* and *RBM9*. Both of these are intriguing candidates, based on genomic position and function, and may be worthy of further investigation.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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# BLOOD CANCER DISCOVERY

## Genetic Variants and Prostate Cancer Risk: Candidate Replication and Exploration of Viral Restriction Genes

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