Two Common Chromosome 8q24 Variants Are Associated with Increased Risk for Prostate Cancer

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Abstract

Two variants (rs1447295/DG8S737) of chromosome 8q24 were recently reported to be associated with increased risk of prostate cancer (PC). To confirm this finding, we genotyped and compared the frequencies of these polymorphisms among 1,121 Caucasian men with PC (435 men with familial PC, 491 men with sporadic PC, and 195 men with aggressive PC) to 545 population-based controls. For the single nucleotide polymorphism marker rs1447295, frequencies of the minor allele (A) were 10.3% in controls, 11.9% in sporadic cases, 16.7% in familial cases, and 17.2% in aggressive cases. Compared with controls, the A allele was significantly more common in both familial PC [odds ratios (OR), 1.93; 95% confidence intervals (95% CI), 1.37–2.72; P = 0.0004] and aggressive PC (OR, 1.87; 95% CI, 1.28–2.74; P = 0.0005) but not for sporadic PC (OR, 1.16; 95% CI, 0.85–1.58; P = 0.25). Although the A allele was more frequent in aggressive PC cases when compared with controls, the allele frequencies were similar among cases with high- and low-grade PC (Gleason grades <7 and ≥7, respectively). For the microsatellite marker DG8S737, the −8 allele was significantly more frequent in familial PC (OR, 1.68; 95% CI, 1.09–2.60; P = 0.031), whereas the −10 allele was more frequent in aggressive PC (OR, 2.85; 95% CI, 1.52–5.36; P = 0.0004). Haplotype analysis showed significant differences in haplotype frequencies between the familial PC (P = 0.006) and aggressive PC (P = 0.005) cases versus controls. The −8/A haplotype showed the strongest association with familial PC (P = 0.008), whereas the −10/A haplotype was most strongly associated with aggressive PC (P = 0.00005). These results further confirm the importance of these two polymorphic variants (rs1447295 and DG8S737) as risk factors for PC. However, the mechanism explaining this increased risk has not yet been established. [Cancer Res 2007;67(7):2944–50]

Introduction

It has been known for some time that prostate cancer (PC) tends to cluster in some families (1). Segregation analysis suggests that this familial clustering can best be explained by at least one rare dominant susceptibility gene (2, 3). However, evidence also points to a complex genetic basis, involving multiple susceptibility genes and variable phenotypic expression. Based on early linkage studies of families at high risk for PC, multiple PC susceptibility loci have been postulated to exist: *HPC1* (4), *PCAP* (5), *CAPB* (6), *HPCX* (7), *HPC20* (8), and *HPC2* (9). However, further targeted studies, along with multiple microsatellite-based genome-wide linkage screens have shown the difficulty of replicating linkage findings for PC susceptibility. Thus far, a few of the targeted linkage studies have led to the identification of candidate susceptibility genes including *RNASEL* (*HPC1*) on chromosome 1 (10), *ELAC2* (*HPC2*) on chromosome 17 (9), and *MSR1* on chromosome 8 (11). However, confirmatory studies for these genes have provided mixed results, with a number of studies providing strong support, both functional and epidemiologic, yet other studies suggesting that their roles may be small (1).

A limitation of linkage studies is their weak power to find susceptibility genes of small to moderate effects. Association studies, in contrast, tend to have greater power to detect genes of small risk (12). Recently, Amundadottir et al. (13) identified a region on chromosome 8q24 showing suggestive linkage to PC in Icelandic families. Further analysis of this region led to the identification of several common variants associated with PC in European and African populations. Two representative markers, DG8S737 (microsatellite) and rs1447295 (single nucleotide polymorphism, SNP), showed the strongest association with PC in three case-control series of European ancestry from Iceland, Sweden, and the U.S.

In an attempt to replicate these findings, we compared the genotype and allele frequencies for these two markers (DG8S737 and rs1447295) in 1,121 Caucasian men with PC to 545 population-based controls. The 1,121 men with PC were composed of three groups: cases with familial PC (n = 435), sporadic cases with a negative family history of PC (n = 491), and cases with more aggressive disease (as defined by a Gleason score ≥8) without regard for family history of PC (n = 195).

Materials and Methods

Patient samples. For these studies, 435 affected men from 177 families with PC, 491 men with sporadic PC, 195 with aggressive PC, and 545 population-based controls were tested. The methods used to ascertain familial and sporadic PC patients have been described previously (14, 15). Briefly, a total of 12,675 surveys were sent to men who received a radical prostatectomy or radiation therapy at Mayo Clinic from 1966 to 1997. Families having a minimum of three men affected with PC were enrolled for further study. Sporadic PC cases were selected from respondents to our family history survey who reported no family history of PC. Eligible patients with sporadic disease were selected by frequency matching them to the familial index patients according to year of diagnosis, age at diagnosis, and number of brothers. Patients with aggressive disease were specifically collected to enrich for cases with high Gleason score (Gleason grade ≥8). These patients with PC were also identified through the Mayo Clinic radical prostatectomy database, but were selected from those men who did not receive a family history survey and were selected without regard to family

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history. Because of differences in ascertainment criteria, these three case groups were treated separately for subsequent statistical analyses.

The unaffected control group has also been described in our previous publications (14, 15). These men were randomly selected from a sampling frame of Olmsted County, Minnesota provided by the Rochester Epidemiology Project, and has been described previously (16). These men underwent clinical urologic examination that included digital rectal examination, transrectal ultrasound of the prostate, residual urine volume, measurement of serum prostate-specific antigen, physical examination, and cryopreservation of serum for subsequent sex hormone assays. Any samples with abnormal digital rectal examination, elevated prostate-specific antigen, or suspicious lesion by transrectal ultrasound were further evaluated for PC. If digital rectal examination and transrectal ultrasound were unremarkable and serum prostate-specific antigen levels were elevated (>4 ng/mL), a sextant biopsy (three cores each side) of the prostate was done. These men have been followed with biennial examinations. Men without PC on the basis of this work-up and any follow-up exams were used in the control sample.

All individuals included in this report were Caucasians. Because of limited power, all non–Caucasians, including 9 Hispanic, 5 African-American, 1 Native American, 1 Asian, 3 other, and 12 men with unknown race (among 3 familial PC, 8 sporadic PC, 16 aggressive PC, and 4 controls) were excluded. The research protocol and informed consent forms were approved by the Mayo Clinic Institutional Review Board.

Genotyping. For the SNP marker rs1447295, we used a predesigned TaqMan SNP genotyping assay (Assay ID C_2160574_30; Applied Biosystems, Foster City, CA). PCR reactions were carried out in a final reaction volume of 5 μL consisting of 2.5 μL of 2× TaqMan Universal PCR Master Mix, 0.5 μL of 10× Allelic Discrimination Assay Mix, and 2 μL of DNA (10 ng). PCR was done on an ABI PRISM 7900HT SDS with the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles at 92°C for 15 s, and 60°C for 60 s. The genotypes were called automatically and verified manually.

For the microsatellite marker DG8S737, the primers described by Amundadottir et al. (13) were used. The PCR primers are as following (from 5′ to 3′): FAM-TGATGCACCACAGAAACCTG (forward) and CAAGGATGCAGCTCACAACA (reverse). PCR reactions were carried out in a final reaction volume of 12.5 μL consisting of 1× GoTaq reaction buffer with 1.5 mmol/L of $MgCl_2$, 200 μmol/L of each deoxynucleotide triphosphate, 10 pmol of each primer, 0.3 units of GoTaq DNA polymerase (Promega, Madison, WI), and 12.5 ng of template DNA. PCR was done using a Tetrad thermal cycler (MJ Research, Waltham, MA) with the following conditions: initial denaturation at 95°C for 2 min, followed by 30 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. The PCR products were diluted with water at a ratio of 1:10. One microliter of the diluted product was mixed with 9 μL of formamide containing ROX dye size standard (GeneScan 400HD; Applied Biosystems) and was analyzed on an ABI 3100 Genetic Analyzer (Applied Biosystems). The genotype of each sample was called automatically by the instrument, but then evaluated manually for potential misclassification.

Statistical analyses. The association of the SNP genotype with disease status was evaluated by Armitage's test for trend in allele counts. When performing comparisons with familial cases, the variance of the trend test and the variance of the odds ratios (OR) accounted for familial correlations (17).

To analyze the DG8S737 marker, a global χ^2 statistic that generalizes Armitage's test for trend to more than two alleles was used. Each allele was given a "dosage" score of 0, 1, or 2 for each subject, and the averages of these scores were compared between cases and controls. For the comparisons with familial cases, a global test of association was calculated using a robust score statistic which simultaneously considers all alleles and accounts for correlations among family members (18). This global test is a χ^2 statistic with many degrees of freedom due to the large number of DG8S737 alleles, resulting in weak power if just one or two alleles are associated with disease. To increase power for this situation, we also computed Armitage's test for trend for each allele versus the pool of all others, and used the maximum, over all allelic tests, as the summary statistic. Simulations were used to compute P values for this maximum statistic, by randomly permuting case-control status. In addition, we calculated OR and 95% confidence intervals (95% CI) comparing carriers of one allele to all other alleles.

To determine whether specific DG8S737 or rs1447295 genotypes were associated with PC, as opposed to alleles, we also did analyses with genotypes. For marker DG8S737, we constructed a three-allele marker with DG8S737 alleles: −10, −8, and X, where X includes all other DG8S737 alleles. ORs and 95% CIs were calculated comparing subjects with genotypes containing alleles −8 and −10 to subjects with the X/X genotype as baseline. In order to account for familial correlations, we averaged the individual genotype counts for each pedigree, resulting in a single observation per pedigree.

In order to adjust for the potential confounding effect of age, we fit logistic regression models including age as a covariate. For these analyses, age was defined as age at diagnosis for patients and age at blood draw for control subjects. When comparing familial cases to controls, we calculated a robust score statistic which accounts for correlations among patients from the same family and allows for covariate adjustment (18). To adjust for the potential of population stratification, we used 741 SNPs scattered across the genome to create principal components for statistical adjustments, as outlined by Price et al. (19).

Haplotype frequencies were estimated using the expectation/maximization algorithm (20). On the basis of all possible pairs of haplotypes for each subject, given their observed genotypes, posterior probabilities for each individual were used to estimate the expected haplotype counts. These expected results were then used in a haplotype trend regression model (21). All rare haplotypes (frequencies <1%) were collapsed into a single haplotype group, and the most frequent haplotype was considered as the reference in the analyses. Global tests were conducted to assess the significance of all haplotypes, as well as individual tests for each haplotype. Simulated P values are reported.

To account for the correlation among multiple affected men from the same family, we first used the posterior probabilities to estimate the expected haplotype counts per subject. We then averaged these expected haplotype counts for each pedigree, resulting in a single observation per pedigree. P values based on 1,000 simulations are reported.

Results

The characteristics of the three PC patient groups and the control group are shown in Table 1. The age range of all four groups was ~45 to 85 years. Although the distribution of age at diagnosis was similar between the familial and sporadic cases, patients with aggressive PC and the controls tend to be younger than both case groups, because a relatively large part of these groups was <60 years old. Because of this difference, additional analyses were done including age as a covariate to statistically adjust for its potential confounding effects.

For the marker DG8S737, five DNA samples obtained from deCODE (kindly provided by Dr. Amundadottir; deCODE genetics, Reykjavik, Iceland) were genotyped to serve as size standards to ensure comparability between their study and ours. The allele frequencies for DG8S737 and rs1447295 among our four groups are listed in Table 2. The statistical results for testing the association of these markers with our sporadic, familial, and aggressive PC cases (relative to the controls) are presented in Table 3. Because of differences in ascertainment (as outlined in Materials and Methods), these three case groups were treated separately for the statistical analyses.

For the SNP marker rs1447295, the minor allele (A) was significantly more common both among men with familial PC (OR, 1.93; 95% CI, 1.37–2.72; P = 0.0004) and aggressive PC (OR, 1.87; 95% CI, 1.28–2.74; P = 0.0005) compared with controls (Table 3). For the microsatellite marker DG8S737, the global statistic indicated no association of alleles with sporadic PC (global

Table 1. Characteristics of PC cases and population controls

Characteristic	Familial PC,* N = 435	Sporadic PC, N = 491	Aggressive PC, N = 195	Controls, N = 545
Age,† median (range)	66 (45–84)	65 (46–79)	65 (40–86)	64 (44–91)
Age,† quartiles				
40–60	77 (17.7)	116 (23.6)	63 (32.3)	212 (38.9)
61–65	117 (26.9)	149 (30.4)	40 (20.5)	104 (19.1)
66–70	131 (30.1)	167 (34.0)	48 (24.6)	90 (16.5)
71–91	110 (25.3)	59 (12.0)	44 (22.6)	139 (25.5)
Body mass index (kg/m^2)				
<28	288 (66.5)	308 (62.7)	96 (49.5)	225 (41.3)
≥28	145 (33.5)	183 (37.3)	98 (50.5)	320 (58.7)
Missing	2	0	1	0
Prostate-specific antigen				
<4	40 (12.0)	91 (22.9)	26 (18.3)	503 (92.3)
4–9.9	153 (46.0)	173 (43.6)	68 (47.9)	39 (7.2)
10–19.9	68 (20.4)	72 (18.1)	27 (19.0)	3 (0.6)
≥20	72 (21.6)	61 (15.4)	21 (14.8)	0 (0.0)
Unknown	102	94	53	0
Gleason score				
<7	249 (69.8)	263 (67.4)	0	
7	81 (22.7)	108 (27.7)	0	
≥8	27 (7.6)	19 (4.9)	195 (100.0)	
Unknown	78	101	0	
Nodal status				
+	52 (12.2)	57 (11.6)	27 (15.7)	
−	376 (87.9)	434 (88.4)	145 (84.3)	
Unknown	7	0	23	
Stage				
T_1/T_2	270 (71.8)	287 (67.4)	59 (40.7)	
T_3/T_4	106 (28.2)	139 (32.6)	86 (59.3)	
Unknown	59	65	50	

*One hundred and seventy-seven unique families.
†Defined as age at diagnosis for familial PC and sporadic PC groups, and age at last follow-up for controls.

P = 0.38). However, evidence of an association was observed with aggressive PC (global P = 0.01) and a weak association was observed with familial PC (global P = 0.06). When individual DG8S737 alleles were examined, the −10 allele exhibited the strongest evidence of association with aggressive PC (OR, 2.85; 95% CI, 1.52–5.36; P = 0.0004). However, the −8 allele was also found to be associated with familial PC (OR, 1.68; 95% CI, 1.09–2.60; P = 0.031). Similar results were obtained after adjusting for age (data not shown). Additionally, using the first 10 principal components calculated from 741 SNPs scattered across the genome as adjusting covariates for population stratification, the association of rs1447295 A allele with familial PC remained statistically significant (P = 0.008 versus 0.0004), as did the A allele for aggressive PC (P = 0.0005 versus 0.0005) and the −10 allele for aggressive PC (P = 0.001 versus 0.0004). However, the association of the −8 allele with familial PC was no longer significant after this adjustment (P = 0.11 versus 0.031), with the OR reduced from 1.68 before adjustment to 1.54 after adjustment. These results suggest that population stratification is not likely to be a major factor for most of our results, but the association of the −8 allele with familial PC may not be as robust.

Analysis of rs1447295 genotypes is presented in Table 4. Both heterozygote (A/C) and homozygote (A/A) genotypes were significantly more common among men with aggressive PC (OR, 1.77; 95% CI, 1.19–2.62; P = 0.005; OR, 3.71; 95% CI, 1.11–12.37; P = 0.03). The heterozygote A/C genotype was also more common among men with familial PC (OR, 2.05; 95% CI, 1.36–3.10; P = 0.0007). Although not statistically significant, the homozygote genotype was also more common in this group, with an OR of similar magnitude as that for heterozygotes, although the CIs were quite wide due to the small number of homozygote carriers. For marker DG8S737, genotype X/−8 was significantly more common among men with familial PC, whereas genotype X/−10 was more common among men with aggressive PC (OR, 2.04; 95% CI, 1.22–3.42 and OR, 2.51; 95% CI, 1.30–4.84, respectively).

The association of these markers with Gleason score (as defined by Gleason score ≥7) was further evaluated within both the familial and sporadic PC groups. That is, the allele frequency in cases with a high Gleason score was compared with the allele frequency in cases with a low Gleason score. In both the familial and sporadic PC group, the allele frequencies for both markers were similar among high-grade and low-grade PC (data not shown). No statistically significant differences were observed.

Results of the haplotype analyses are presented in Table 5. When considering all haplotypes, a significant association was observed with both familial and aggressive PC (global P = 0.006 and 0.005, respectively; Table 5). The haplotype containing both the −8 allele and the A allele showed the strongest association with familial PC

Table 2. Allele frequencies in three PC case groups and population controls

Marker	Allele		Controls	Sporadic PC	Familial PC	Aggressive PC
DG8S737	−20	147	0.001	0.000	0.000	0.000
	−18	151	0.000	0.000	0.003	0.003
	−16	155	0.000	0.000	0.000	0.003
	−14	161	0.000	0.000	0.000	0.003
	−12	163	0.020	0.026	0.023	0.032
	−10	165	0.021	0.024	0.032	0.059
	−8	167	0.057	0.057	0.088	0.067
	−6	169	0.082	0.103	0.090	0.094
	−4	171	0.168	0.130	0.171	0.132
	−2	173	0.225	0.196	0.171	0.202
	0	175	0.099	0.104	0.102	0.094
	2	177	0.117	0.138	0.097	0.102
	4	179	0.077	0.084	0.092	0.075
	6	181	0.059	0.062	0.079	0.073
	8	183	0.059	0.060	0.043	0.048
	10	185	0.010	0.015	0.005	0.013
	12	187	0.004	0.002	0.002	0.000
	14	189	0.000	0.000	0.002	0.000
	16	191	0.001	0.000	0.000	0.000
rs1447295	A		0.103	0.119	0.167	0.172
	C		0.897	0.881	0.833	0.828

(P = 0.008), whereas the haplotype containing the −10 allele and the A allele was most strongly associated with aggressive PC (P = 0.00005).

Discussion

In this study, we replicate the findings reported by Amundadottir et al. (13) showing an association of two polymorphic markers on 8q24 with familial PC and with aggressive PC. Given the historical difficulty in replicating both linkage and gene association studies in PC, these findings provide important clues towards the identification of candidate genetic susceptibility factors for this very common disorder.

Overall, the allele frequencies observed for cases and controls in this current study were quite similar to those reported by Amundadottir et al. (13). The frequency of the −8 allele in our control group was 5.7%, compared with 4.1% observed in the European-American control group of Amundadottir et al. The Icelandic and Swedish controls had frequencies of 7.8% and 7.9%, respectively. The frequency of the SNP marker rs1447295 minor allele (A) among our controls (10.3%) was also similar to the previously reported estimates (8.1%, European-American; 10.6%, Icelandic; and 13.3%, Swedish; ref. 13). For the cases, the frequency of the DG8S737 (−8) allele ranged from 5.7% to 8.8% (OR, 1.05–1.68) for the Mayo groups compared with 8.2% to 13.1% (OR, 1.38–2.10) reported by Amundadottir et al. In the combined samples of Amundadottir et al., the OR for carriers of the −8 allele (1.62) was similar to that observed for our familial PC cases (OR, 1.68), but was larger than what we observed for our aggressive cases (OR, 1.23). These differences, however, are within statistical

Table 3. Association of alleles on chromosome 8q24 with sporadic, familial, and aggressive PC

Comparison	Marker	Allele	Allele frequency		OR (95% CI)*	P
			PC Cases	Controls		
Sporadic PC vs. controls	DG8S737	−8	0.057	0.057	1.05 (0.71–1.57)	0.96
	DG8S737	−10	0.024	0.021	1.11 (0.61–2.04)	0.73
	rs1447295	A	0.119	0.103	1.16 (0.85–1.58)	0.25
Familial PC vs. controls	DG8S737	−8	0.088	0.057	1.68 (1.09–2.60)	0.031
	DG8S737	−10	0.032	0.021	1.55 (0.80–3.02)	0.21
	rs1447295	A	0.167	0.103	1.93 (1.37–2.72)	0.0004
Aggressive PC vs. controls	DG8S737	−8	0.067	0.057	1.23 (0.73–2.05)	0.48
	DG8S737	−10	0.059	0.021	2.85 (1.52–5.36)	0.0004
	rs1447295	A	0.172	0.103	1.87 (1.28–2.74)	0.0005

*OR comparing carriers of the indicated allele to noncarriers.

Table 4. Association of genotypes on chromosome 8q24 with sporadic, familial, and aggressive PC

Comparison	Marker	Genotype	Genotype frequency		OR (95% CI)*	*P*
			PC Cases	Controls		
Sporadic PC vs. controls		X/X	0.846	0.850	1.00 (reference)	
		X/−8	0.105	0.102	1.04 (0.69–1.56)	0.87
	DG8S737	X/−10	0.041	0.043	0.97 (0.52–1.81)	0.91
		−8/−8	0.002	0.006	0.34 (0.04–3.27)	0.35
		−8/−10	0.006	0.0	—	0.92
		−10/−10	0.0	0.0	—	—
		C/C	0.780	0.805	1.00 (reference)	
	rs1447295	A/C	0.201	0.185	1.12 (0.82–1.54)	0.48
		A/A	0.018	0.010	1.88 (0.62–5.65)	0.26
Familial PC vs. controls		X/X	0.767	0.850	1.00 (reference)	
		X/−8	0.162	0.102	2.04 (1.22–3.42)	0.007
	DG8S737	X/−10	0.065	0.043	1.91 (0.89–4.13)	0.10
		−8/−8	0.007	0.006	0.91 (0.07–11.03)	0.94
		−8/−10	0.0	0.0	—	—
		−10/−10	0.0	0.0	—	—
		C/C	0.681	0.805	1.00 (reference)	
	rs1447295	A/C	0.303	0.185	2.05 (1.36–3.10)	0.0007
		A/A	0.016	0.010	1.83 (0.40–8.44)	0.44
Aggressive PC vs. controls		X/X	0.769	0.850	1.00 (reference)	
		X/−8	0.113	0.102	1.23 (0.71–2.12)	0.46
	DG8S737	X/−10	0.097	0.043	2.51 (1.30–4.84)	0.006
		−8/−8	0.005	0.006	0.97 (0.10–9.44)	0.98
		−8/−10	0.011	0.0	—	0.95
		−10/−10	0.005	0.0	—	0.97
		C/C	0.688	0.805	1.00 (reference)	
	rs1447295	A/C	0.280	0.185	1.77 (1.19–2.62)	0.005
		A/A	0.032	0.010	3.71 (1.11–12.37)	0.03

*OR comparing subjects with the indicated genotype to subjects with genotype X/X, where X = all DG8S737 alleles other than −8 and −10.

variability of the ORs. For rs1447295 (A), the frequency among cases ranged from 11.9% to 17.2% (OR, 1.16–1.93) for the Mayo groups compared with 12.7% to 16.9% (OR of 1.29–1.72) in Amundadottir et al. As before, the differences observed are within statistical variability of the ORs.

Amundadottir et al. postulated that either the −8 allele confers risk, or it is closely correlated through linkage disequilibrium with a risk variant. Our findings that the −10 allele had a larger OR (2.85) than the −8 allele (1.23) for aggressive PC cases suggests that the causative variant may not reside at DG8S737, but rather with a variant in high linkage disequilibrium near this marker. Additionally, our findings of a stronger association of the −10/A haplotype with aggressive PC compared with that of the −8/A haplotype further supports this notion. Finally, although there is compelling evidence that these variants increase the risk for PC, our data does not support a difference in risk between cases with low Gleason score and those with a high Gleason score.

These general findings have also now been recently replicated by several other groups. Freedman et al. (22) used admixture mapping in 1,597 African-Americans to identify a locus at 8q24 that substantially affects risk for PC in African-Americans, the same region reported by Amundadottir et al. (13). In additional analyses, however, the admixture signal detected in this region could not be explained by the SNP alleles identified by Amundadottir et al. (13), suggesting the presence of other unmapped risk alleles at this locus for African-Americans. Freedman et al. (22) extended their replication analysis of the rs1447295 SNP association identified by Amundadottir et al. (13) to four other ethnic groups. In each group, the previously described association was replicated; Japanese-Americans ($P < 0.00034$), Native Hawaiians ($P < 0.00015$), Latino-Americans ($P < 0.0014$), European-Americans ($P < 0.022$), and all four groups together ($P < 4.2 \times 10^{-9}$). Finally, the Cancer Genetic Markers of Susceptibility project (http://cgems.cancer.gov) released results from the first phase of the PC genome-wide association scan. In this study, >500,000 SNPs were genotyped in 1,177 cases and 1,105 controls. In this data set, the rs1447295 SNP showed a significant association ($P = 0.000408$) and was ranked among the top 315 most significant SNP's genome-wide. Of note, other SNPs within this region also showed significant associations, again suggesting the presence of unmapped risk alleles.

The functional significance of the two polymorphic variants reported by Amundadottir et al. (13) is, at this point, unclear. The two markers are 8.4 kb apart from each other and are located in an intronic region of a putative gene, which seems to have at least four alternative transcripts (ranging from 8 to 2 exons). However, only the short forms seem to be expressed in normal prostate tissue (13). Surprisingly, the putative gene does not possess a long stretch of open reading frames and the predicted short protein sequences do not match any existing protein domain. In addition to the

Table 5. Association of haplotypes on chromosome 8q24 with sporadic, familial, and aggressive PC

Haplotype*			Controls	Sporadic PC			Familial PC			Aggressive PC		
			Frequency (%)	Frequency (%)	Simulated *P*		Frequency (%)	Simulated *P*		Frequency (%)	Simulated *P*	
					Global	Haplotype		Global	Haplotype		Global	Haplotype
	DG8S737	rs1447295			0.267			0.006			0.005	
−12	163	C	0.020	0.022		0.782	0.023		0.415	0.027		0.485
−10	165	A	0.015	0.021		0.313	0.031		0.019	0.059		0.00005
−8	167	A	0.046	0.046		0.949	0.082		0.008	0.067		0.138
−8	167	C	0.011	0.012		0.821	0.006		—	—		—
−6	169	A	0.030	0.046		0.095	0.051		0.115	0.032		0.869
−6	169	C	0.052	0.057		0.562	0.039		0.421	0.062		0.443
−4	171	C	0.166	0.130		0.036	0.168		0.716	0.132		0.130
−2	173	C	0.221	0.196		0.140	0.171		0.081	0.202		0.440
0	175	C	0.097	0.104		0.707	0.102		0.980	0.094		0.863
2	177	C	0.117	0.135		0.181	0.096		0.306	0.102		0.443
4	179	C	0.075	0.084		0.507	0.092		0.135	0.072		0.869
6	181	C	0.059	0.062		0.803	0.079		0.305	0.073		0.356
8	183	C	0.057	0.060		0.869	0.043		0.331	0.048		0.494
10	185	C	0.010	0.015		0.309	0.005		—	0.013		0.755

*All haplotypes with frequencies <1% were collapsed into one group for the analysis but are not listed in the table. The most common haplotype was used as the baseline reference in the haplotype global test.

putative gene, however, there are several other transcripts with unknown function in this chromosomal region.

The closest characterized gene is the proto-oncogene *c-MYC*, approximately 264 kb from rs1447295. It has been known for some time that amplification, or gain, of chromosome 8q24 (including the *c-MYC* region) is a frequent event in PC (23). Using array-CGH, Saramaki et al. recently reported four segments of 8q amplification showing frequent gain in >30% of PC cases (24). One of these segments includes these two markers as well as the *c-MYC* gene. This coamplification may indicate some functional relevance of these markers or of this region. Whether the risk alleles are preferentially amplified in these cases remains to be established. In another recent report using 50 patients with histologically different prostate tissues, gain at 8q24 was present in 10% of normal tissues, 19% of atrophy lesions, 21% of prostatic intraepithelial neoplasia, and 27% of cancer tissues (25). These results suggest that gain of chromosome 8q24 is very common in normal tissues and may predispose to the development of PC. Thus, it will be important to determine if the polymorphic risk alleles identified by Amundadottir et al. (or other alterations in linkage disequilibrium with these markers) are involved in the regulation of *c-MYC* expression or if they are involved in promoting the amplification, or gain, of the 8q24/*c-MYC* region. Clearly, further analysis of this genomic region will be necessary to clarify the functional consequences of these genetic markers.

It is worthwhile to highlight the strategy used by Amundadottir et al. to identify these two significant markers (13). Given that the identification of susceptibility genes by linkage analysis alone has proven to be extremely difficult for all completed PC genome scans (1), it is clear that additional approaches are required. Targeting candidate regions identified by linkage for high-density SNP association studies provides an alternative strategy to identify candidate susceptibility genes. The study by Amundadottir et al. (13) provides an example of how association studies can be used in combination with linkage analysis to successfully identify candidate genes/regions in a complex disease.

In summary, we have detected significant association of two genetic markers (DG8S737 and rs1447295) with PC. This result suggests a role for these polymorphisms or other linked variants in the development of PC, and supports the original finding reported by Amundadottir et al. (13). Although more studies are needed to elucidate the mechanism responsible for this association, our results provide strong guidance for taking the next steps.

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