Confirmation of a Positive Association between Prostate Cancer Risk and a Locus at Chromosome 8q24

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Abstract

Background: **Family-based linkage studies, association studies, and studies of tumors have highlighted human chromosome 8q as a genomic region of interest for prostate cancer susceptibility loci. Recently, a locus at 8q24, characterized by both a single nucleotide polymorphism (SNP) and a microsatellite marker, was shown to be associated with prostate cancer risk in Icelandic, Swedish, and U.S. samples. Although the data were provocative, the U.S. samples were not population based, which precludes assessment of the potential contribution of this locus to prostate cancer incidence in the United States.**

Methods: **We analyzed both markers in a population-based, case-control study of middle-aged men from King County, Washington.**

Results: **Overall, there was a significant positive association between the A allele of the SNP rs1447295 and prostate cancer risk [odds ratio, 1.4; 95% confidence interval (95% CI), 1.1-2.0] but no significant association with the microsatellite DG8S737. However, significant associations were observed for both markers in men with high Gleason scores. Adjusting for age, first-degree family history of prostate cancer, and prostate cancer screening history, the adjusted odds ratios were 1.4 (95% CI, 1.1-1.8) for the A allele of the SNP and 1.9 (95% CI, 1.2-2.8) for the −10 allele of the microsatellite.**

Conclusions: **These data suggest that the locus on chromosome 8q24 harbors a genetic variant associated with prostate cancer and that the microsatellite marker is a stronger risk factor for aggressive prostate cancers defined by poorly differentiated tumor morphology.** (Cancer Epidemiol Biomarkers Prev 2007;16(4):809–14)

Introduction

Prostate cancer is the most prevalent noncutaneous cancer diagnosed in males in the developed world. In 2007, ~218,890 men in the United States will be diagnosed with prostate cancer and ~27,050 deaths will be attributed to the disease (1). Although the majority of prostate cancer cases are sporadic, there is also evidence that a subset of cases has an underlying genetic susceptibility to the disease based on epidemiologic studies (2-5), twin studies (6-8), and segregation analyses (7, 9-12).

Considering the older age at diagnosis, lack of distinguishing features between sporadic, familial, and hereditary forms, and locus as well as disease heterogeneity, the identification of prostate cancer susceptibility genes has proven difficult (13-18). Efforts to find susceptibility loci are further complicated by the increased detection of sporadic cases in the United States and a shift toward early-stage disease resulting from extensive use of the serum prostate-specific antigen test for screening (19). It is inescapable that some of the loci originally hypothesized to be associated with prostate cancer may eventually prove to be artifacts. Therefore, at least as important as the initial discovery is the replication of original findings in independent studies.

A recent genome-wide linkage scan of 323 Icelandic high-risk prostate cancer families produced a suggestive linkage signal on chromosome 8q24, with a maximum logarithm of odd score of 2.11 at microsatellite marker D8S529 (located at 148.25 cM; ref. 20). Analysis of several markers in the region identified a single allele, which was described as allele −8, of microsatellite marker DG8S737 that was associated with prostate cancer risk in case-control studies involving Caucasian men from Iceland, Sweden, and the Chicago area of the United States as well as African-American men from the Michigan area of the United States. Overall, the estimated odds ratio (OR) for carriers of the variant −8 allele of microsatellite DG8S737 was 1.62 ($P = 2.7 \times 10^{-11}$). About 13% of the controls carried at least one copy of the variant allele, suggesting a population attributable risk of ~8%. Within the associated haplotype block, 37 single nucleotide polymorphisms (SNP) were identified that were also significantly associated with prostate cancer in men of Caucasian origin, with allele A of SNP rs1447295 showing the strongest association (OR, 1.72; $P = 1.7 \times 10^{-9}$). A subsequent study by Freedman et al. (21), however, suggested that the reported association between marker DG8S737 and prostate cancer in African Americans might simply reflect systematic differences in ancestry between cases and controls across a large region in 8q24. After correcting for admixture, they concluded that the contribution of the −8 allele to the risk of prostate cancer in African Americans was not statistically significant (21). The same group was, however, able to replicate the association of the A allele at rs1447295 with prostate cancer risk in Japanese Americans, Native Hawaiians, Latino Americans, and European Americans. However, the causative genetic mutation at 8q24 has yet to be identified.

In the present study, we tested the reported association between prostate cancer and markers DG8S737 and rs1447295 at 8q24 in a population-based, case-control study of Caucasian middle-aged men. We were particularly interested in evaluating the risk of prostate cancer associated with these genetic

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variants according to the clinical features of the disease, family history of prostate cancer, and how much these variants may contribute to prostate cancer incidence in the general U.S. population.

Materials and Methods

Study Population. Study subjects were Caucasian and African-American men, residents of King County, Washington, ages 40 to 64 years, who participated in a population-based, case-control study of risk factors for prostate cancer that has been described previously (22). Briefly, cases were diagnosed with histologically confirmed prostate cancer from January 1, 1993 to December 31, 1996 and identified via the Seattle-Puget Sound Surveillance, Epidemiology, and End Results cancer registry. All males <60 years of age, 100% of African-American men ages 60 to 64 years, and a 75% random sample of Caucasian men ages 60 to 64 years at diagnosis were invited to participate. Of the 917 eligible cases, a total of 753 (82%) was interviewed, and 630 (83.7%) provided a blood sample and were genotyped.

Controls were men without a self-reported history of prostate cancer, identified via random digit dialing, frequency matched to cases by 5-year age groups, and recruited evenly throughout the ascertainment period of the cases. Of the 941 eligible controls that were identified, 703 (74.7%) were interviewed and 564 (80.2%) provided a blood sample and were genotyped. Study subjects completed a structured in-person interview that has been described previously (22) and collected information about demographic and lifestyle characteristics, medical history, prostate cancer screening, and family history of prostate cancer. Men who did versus did not provide a blood sample were not statistically different with respect to demographic or clinical characteristic (data not shown). The institutional review boards of the Fred Hutchinson Cancer Research Center and the National Human Genome Research Institute approved all study procedures and materials. Written informed consent was obtained from all study participants before participation.

Because the locus on chromosome 8q was initially noted in the context of a family-based linkage study, we also examined evidence for this region in 254 hereditary prostate cancer families described previously (23). A genome-wide scan identifying several loci of interest has been recently reported (23, 24).

Genotyping. Genomic DNA was extracted from peripheral blood lymphocytes using standard techniques (25). Genotyping of SNP rs1447295 was carried out using the Taqman SNP Genotyping Assay with unlabeled PCR primers and fluorescently labeled probes for each of the two alleles (Applied Biosystems, Foster City, CA). Genomic DNA (10 ng) was amplified in a reaction volume of 10 μL in the presence of Taqman Universal Master Mix with no AmpErase UNG. The reaction was run on GeneAmp 9700 thermocycler (Applied Biosystems) with a cycling protocol of 95°C for 10 min and then 40 cycles of 15 s at 95°C and 1 min at 60°C. After PCR amplification, an end point plate read was done using 7900HT Fast Real-Time PCR System (Applied Biosystems). Alleles were automatically called using Sequence Detection System software. Twenty-seven of 1,195 (2.2%) samples did not produce genotyping data for SNP rs1447295.

Genotyping of the DG8S737 microsatellite marker was done by using fluorescently labeled M13 sequence antiparallel to the forward primer in the PCR reaction. This will result in labeling of the forward primer and, thus, the microsatellite PCR product. The forward primer was 5′-CACGACGTTGTAAAACGACT-GATGCACCACAGAAACCTG-3′ and the reverse primer was 5′-CAAGGATGCAGCTCACAACA-3′. PCR amplification was carried out in a reaction volume of 10 μL containing 10 ng of genomic DNA, 1 pmol M13 label, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxynucleotide triphosphate, 1 pmol of each primer, and 0.13 unit of AmpliTaq Gold DNA polymerase (buffer supplied by the manufacturer). The reaction was run on GeneAmp 9700 thermocycler using a cycling protocol of 94°C for 4 min and then 35 cycles of 30 s at 95°C, 30 s at 60°C, and 45 s at 72°C followed by an extension step of 7 min at 72°C. An internal size standard was added to the PCR products, and the final product was resolved using an Applied Biosystems 3730 Sequencer. Genotypes were called using GeneMapper software and manually checked by two independent technicians. Samples, whose peak intensities were <50, were not included in the analysis. This resulted in the removal of 46 (3.8%) of the 1,195 DG8S737 genotypes from the data set. A Centre d'Etude du Polymorphisme Humain (CEPH) sample (1347-02) was genotyped and used as a reference for DG8S737 microsatellite genotypes. This allowed unambiguous sizing of the microsatellite alleles.

Genotyping accuracy was assessed using 60 blind duplicates that were randomly distributed across all genotyping batches without technician knowledge of location. There was 100% agreement for rs1447295 SNP genotypes and 94% agreement for marker DG8S737 genotypes.

Statistical Analyses. Deviation from Hardy-Weinberg equilibrium for both markers was examined in Caucasian controls using a χ^2 test (26). All subsequent data analyses were limited to Caucasians cases ($n = 597$) and controls ($n = 548$). This group represented 96% of the study population; 33 genotyped African-American cases and 16 controls provided minimal power to evaluate associations in this subgroup and were thus not included. Among Caucasian men, 30 cases and 13 controls had missing data for DG8S737 marker, whereas 15 cases and 10 controls had missing data for the SNP (rs1447295). Subjects with missing data were excluded from the respective statistical analyses.

After examining the frequency of alleles associated with marker DG8S737, two exposure variables were created: one comparing the allele −8 versus all other alleles (defined as X) and a second variable comparing allele −10 versus all other alleles (again, the group of other alleles was defined as X). Unconditional logistic regression was used to examine the associations between alleles of the DG8S737 marker and the SNP (rs1447295) and prostate cancer risk and to compute ORs and 95% confidence intervals (95% CI; ref. 27).

Potential confounding by established and possible risk factors for prostate cancer was assessed for each genotype separately, fitting models using each main effect and then evaluating the change in risk estimates when other variables entered the models one at a time. Covariates that changed the relative risk estimates for the genotypes by ≥10% were retained in the models. Goodness of fit was assessed by likelihood ratio statistics of nested models.

We also examined the association between alleles of marker DG8S737 and SNP rs1447295 with prostate cancer risk according to strata defined by Gleason score. Gleason score was obtained from biopsy reports (29.5%) or from surgical pathology reports (70.5%). For these analyses, prostate cancer cases were grouped into two strata: those with Gleason scores of 2 to 6 or 7 = 3+4 and those with Gleason scores of 7 = 4+3 or 8 to 10. The frequency of alleles in each group of cases was compared with that of controls using polytomous logistic regression (28). Statistical Analysis System version 9.1 was used for statistical analyses.

Results

Table 1 summarizes the distribution of alleles of microsatellite marker DG8S737 in Caucasian cases and controls. The biggest difference in allele frequency between these two groups was

Table 1. Distribution of alleles of microsatellite marker DG8S737 among Caucasian prostate cancer cases and population controls

DG8S737*		Cases (n = 567)	Controls (n = 535)
Allele	deCODE		
177	−14	0.001	0.001
179	−12	0.023	0.017
181	−10	0.030	0.018
182	−8	0.070	0.057
184	−6	0.116	0.110
186	−4	0.146	0.142
188	−2	0.191	0.211
190	0	0.098	0.103
192	2	0.120	0.120
194	4	0.071	0.096
196	6	0.063	0.053
198	8	0.054	0.054
199	10	0.013	0.013
202	12	0.004	0.002
203	14	0.001	0.001
205	16	0.000	0.002
207	18	0.001	0.000

*Thirty cases and 13 controls had missing data for DG8S737 marker (not included in this table).

for allele −10. Frequencies of genotypes for the microsatellite marker DG8S737, as well as genotypes for SNP rs1447295, were consistent with Hardy-Weinberg equilibrium among the controls. Among Caucasian controls, there was evidence for strong linkage disequilibrium between the −10 allele of the DG8S737 marker and the SNP (rs1447295) such that 89.5% of controls who carried at least one −10 allele also carried at least one copy of the A allele: $D' = 0.44$, $\chi^2 = 61.5$, $P < 0.0001$.

Table 2 shows the distribution of genotypes and their associations with prostate cancer risk. Only one individual (a case) was homozygous for the microsatellite allele −10, and he was grouped with heterozygotes for analysis. Under a dominant model, we observed a stronger association for the −10 than for the −8 allele in relation to prostate cancer risk (OR, 1.7; 95% CI, 0.9-3.2 versus OR, 1.2; 95% CI, 0.8-1.8, respectively) after adjusting for age, first-degree family history of prostate cancer, and prostate cancer screening history. Men with a −10 allele of DG8S737 who had a first-degree family history of prostate cancer had a higher risk estimate (OR, 3.2; 95% CI, 0.4-27.1) than men with a −10 allele without a family history of prostate cancer (OR, 1.6; 95% CI, 0.9-2.9), although this difference was not statistically significant. For the SNP (rs1447295), the A allele was associated with a 40% increase in the relative risk of prostate cancer (OR, 1.4; 95% CI, 1.1-2.0), and men who had an AA genotype had a 3.4-fold increased risk (OR, 3.4; 95% CI, 1.0-11.7) compared with men with the CC genotype. For the SNP, risk estimates for prostate cancer in strata defined by a first-degree family history of prostate cancer were similar (data not shown). When we considered a combination of the −10 allele of the DG8S737 marker and the A allele of the SNP (rs1447295), men with both had an adjusted OR of 1.8 (95% CI, 0.9-3.5) in comparison with those who carried no −10 allele and no A allele, respectively.

We next examined whether associations between either of the markers differed by Gleason score (Table 3). The association between the DG8S737 allele −10 was stronger in prostate cancer cases diagnosed with higher Gleason scores defined as 7 = 4+3 or 8 to 10 (OR, 1.9; 95% CI, 1.2-2.8). There was, however, no significant association between allele −10 of the microsatellite and prostate cancer risk in men with lower Gleason scores of 2 to 6 or 7 = 3+4 (OR, 1.1; 95% CI, 0.8-1.6). Similar results were observed for the SNP (rs1447295) where men who had a CA or AA genotype and higher Gleason scores (7 = 4+3 or 8-10) had an OR of 1.4 (95% CI, 1.1-1.8) compared with men with the CC genotype. A 20% increase in relative risk was also observed in men with lower Gleason scores (OR, 1.2; 95% CI, 0.98-1.3) who carried one or more A alleles. When we examined associations between the AA genotype of SNP (rs1447295) and Gleason score, there were very few men with the AA genotype in the strata: eight cases had a Gleason score of 2 to 6 or 7 = 3+4, two cases had a high Gleason score of 7 = 4+3 or 8 to 10 (another case had missing information for Gleason score and was not included in the analysis), and there were four controls. Thus, men with the AA genotype or the CA genotype were grouped together to increase power for this analysis. When we considered the combination of both alleles relative to individuals with no −10 alleles and no A alleles, men with at least one −10 allele and at least one A allele (8 cases with high Gleason scores and 17 controls) had an OR of 3.7 (95% CI, 1.5-9.4).

Discussion

We observed increased ORs for prostate cancer among Caucasian men carrying the A allele of the rs1447295 SNP

Table 2. Association of genotypes of chromosome 8q24 markers with prostate cancer risk

Alleles	Cases, n (%)	Controls, n (%)	OR* (95% CI)	OR† (95% CI)
DG8S737				
X/X‡	494 (87.1)	476 (89.0)	1.00 (reference)	1.00 (reference)
−8/X	67 (11.8)	57 (10.7)	1.13 (0.78-1.65)	1.16 (0.77-1.74)
−8/−8	6 (1.1)	2 (0.4)	2.86 (0.57-14.29)	2.77 (0.49-15.68)
−8/X or −8/−8	73 (12.9)	59 (11.0)	1.19 (0.83-1.72)	1.21 (0.81-1.81)
DG8S737				
X/X‡	534 (94.2)	516 (96.4)	1.00 (reference)	1.00 (reference)
−10/X or −10/−10§	33 (5.8)	19 (3.6)	1.66 (0.93-2.96)	1.68 (0.89-3.16)
SNP (rs1447295)				
CC	435 (74.7)	427 (79.4)	1.00 (reference)	1.00 (reference)
CA	136 (23.4)	107 (19.9)	1.24 (0.93-1.65)	1.36 (0.99-1.87)
AA	11 (1.9)	4 (0.7)	2.65 (0.83-8.40)	3.39 (0.98-11.73)
CA or AA	147 (25.3)	111 (20.6)	1.29 (0.97-1.70)	1.43 (1.05-1.95)
DG8S737/SNP (rs1447295)				
XX/CC	415 (74.1)	418 (78.7)	1.00 (reference)	1.00 (reference)
XX/CA or AA	113 (20.2)	94 (17.7)	1.21 (0.89-1.64)	1.36 (0.97-1.91)
−10X or −10−10/CC	2 (0.4)	2 (0.4)	1.28 (0.18-9.22)	1.32 (0.16-11.21)
−10X or −10−10/CA or AA	30 (5.4)	17 (3.2)	1.74 (0.94-3.23)	1.79 (0.92-3.50)

NOTE: Thirty cases/13 controls had missing data for DG8S737; 15 cases/10 controls had missing data for the SNP (rs1447295).
*Models are adjusted for age.
†Models are adjusted for age, first-degree relative with prostate cancer, and prostate cancer screening history.
‡The X defines the group of alleles in DG8S737 other than the allele of interest.
§Only one individual (case) was homozygous for microsatellite allele −10. This individual was grouped with heterozygotes for all further analyses.

Table 3. Association of genotypes of chromosome 8q24 markers with prostate cancer risk stratified by Gleason score

Allele	Controls (n = 535), n (%)	Cases: Gleason 2 - 6 and 7 = 3 + 4 (n = 491)			Cases: Gleason 7 = 4 + 3 and 8 - 10 (n = 73)		
		n (%)	OR* (95% CI)	OR† (95% CI)	n (%)	OR* (95% CI)	OR† (95% CI)
DG8S737							
X/X‡	476 (89.0)	430 (87.6)	1.00 (reference)	1.00 (reference)	62 (84.9)	1.00 (reference)	1.00 (reference)
−8/X or −8/−8	59 (11.0)	61 (12.4)	1.07 (0.88-1.29)	1.08 (0.88-1.33)	11 (15.1)	1.19 (0.84-1.69)	1.22 (0.86-1.73)
DG8S737							
X/X‡	516 (96.4)	467 (95.1)	1.00 (reference)	1.00 (reference)	64 (87.7)	1.00 (reference)	1.00 (reference)
−10/X or −10/−10	19 (3.6)	24 (4.9)	1.17 (0.86-1.60)	1.13 (0.82-1.56)	9 (12.3)	1.93 (1.27-2.93)	1.86 (1.22-2.83)
Allele	Controls (n = 538), n (%)	Cases: Gleason 2 - 6 and 7 = 3 + 4 (n = 504)			Cases: Gleason 7 = 4 + 3 and 8 - 10 (n = 75)		
		n (%)	OR* (95% CI)	OR† (95% CI)	n (%)	OR* (95% CI)	OR† (95% CI)
SNP (rs1447295)							
CC	427 (79.4)	383 (76.0)	1.00 (reference)	1.00 (reference)	50 (66.7)	1.00 (reference)	1.00 (reference)
CA or AA	111 (20.6)	121 (24.0)	1.10 (0.95-1.27)	1.15 (0.98-1.34)	25 (33.3)	1.37 (1.05-1.78)	1.41 (1.08-1.84)
Allele	Controls (n = 531), n (%)	Cases: Gleason 2 - 6 and 7 = 3 + 4 (n = 485)			Cases: Gleason 7 = 4 + 3 and 8 - 10 (n = 72)		
		n (%)	OR* (95% CI)	OR† (95% CI)	n (%)	OR* (95% CI)	OR† (95% CI)
DG8S737/SNP (rs1447295)							
XX/CC	418 (78.7)	366 (75.4)	1.00 (reference)	1.00 (reference)	47 (65.3)	1.00 (reference)	1.00 (reference)
XX/CA or AA	94 (17.7)	95 (19.6)	1.15 (0.84-1.58)	1.30 (0.91-1.85)	17 (23.6)	1.67 (0.93-3.00)	1.74 (0.95-3.19)
−10X or −10−10/CC	2 (0.4)	2 (0.4)	1.28 (0.18-9.17)	1.51 (0.18-12.98)	0 (0.0)	—	—
−10X or −10−10/CA or AA	17 (3.2)	22 (4.5)	1.44 (0.75-2.76)	1.55 (0.75-3.17)	8 (11.1)	3.94 (1.61-9.63)	3.71 (1.46-9.44)

NOTE: Men with missing data for DG8S737 marker (30 cases/13 controls) or the SNP (rs1447295; 15 cases/10 controls) or prostate cancer cases with missing information on Gleason score (n = 3) were excluded from respective analyses.

*Models are adjusted for age.

†Models are adjusted for age, first-degree relative with prostate cancer, and prostate cancer screening history.

‡The X defines the group of alleles in DG8S737 other than the allele of interest; Gleason score was obtained from biopsy pathology reports (29.5% of cases) or from surgical pathology reports for cases who had a radical prostatectomy (70.5%).

and among those carrying the −10 allele of microsatellite marker DG8S737 on chromosome 8q24, although the latter result was not statistically significant. Given that 20.6% of the population controls carry at least one copy of the A allele of SNP rs1447295, the population attributable risk is 6.3% among Caucasian men ages 40 to 64 years. This estimate is similar to the population attributable risk of 8% reported previously (20). However, estimates of the proportion of prostate cancer incidence in the population attributable to this SNP are likely inflated because the OR used in the calculation does not take into account potential interactions between the SNP and other unknown genetic variants or environmental exposures, and the disease is not rare.

This SNP result confirms the previous findings reported in populations of Western European descent (20, 21). The OR for the A allele of the SNP was 1.4 (95% CI, 1.1-2.0) in this study compared with 1.7 ($P = 1.7 \times 10^{-9}$), 1.3 ($P = 4.5 \times 10^{-3}$), and 1.7 ($P = 6.7 \times 10^{-3}$) in Caucasian case-control populations from Iceland, Sweden, and Chicago, respectively (20). Amundadottir et al. (20) reported that this SNP is not associated with risk in African-American populations and that the increased risk of prostate cancer associated with the variant is confined to populations of European ancestry. They did, however, report an increased relative risk of prostate cancer in African-American men associated with the −8 allele of microsatellite marker DG8S737 (OR, 1.6; $P = 2.2 \times 10^{-3}$) and suggested a population attributable risk of 16%. Due to the population-based nature of our study, and hence the small number of African-American men in our population from western Washington, we were unable to address this issue.

Interestingly, we did not observe a significant association between prostate cancer risk and the −8 allele of the DG8S737 microsatellite marker. Rather, we observed a greater difference in allele frequency between cases and controls for the −10 allele (7.0% versus 5.7% for the −8 allele versus 3.0% versus 1.8% for the −10 allele). Wang et al. (29) in a study from the Mayo Clinic also note a difference in allele frequency over that reported by Amundadottir (20). They report that the −8 allele is significantly more frequent in familial prostate cancer (OR, 1.7; 95% CI, 1.1-2.6; $P = 0.031$), whereas the −10 allele is more frequent in aggressive prostate cancer (OR, 2.5; 95% CI, 1.3-4.7; $P = 0.002$). We note that the frequency of the −8 allele in Caucasians within our population-based study is similar to that reported for the Chicago population (0.057 in our Caucasian controls versus 0.04 in Chicago controls) but is slightly lower than those reported for Swedish (0.08 in controls) and Icelandic (0.08 in controls) populations (20).

The population-based controls used in this study were men without a self-reported history of prostate cancer who were recruited via random digit dialing and age matched to cases. By comparison, the European-American study from Chicago used controls recruited for studies of asthma and diabetes mellitus as well as healthy subjects from the University of Chicago recruited by ''word of mouth'' (20). There are obvious disadvantages to that scheme, including biases that may be introduced based on the men who chose to participate versus those who did not. In addition, cases from our study were population based. The Chicago cases recruited from the Pathology Core of Northwestern University's Prostate Cancer Specialized Program of Research Excellence may reflect a skewed set of cases in terms of tumor stage or grade compared with our population-based sample. Another difference may be the ages at diagnosis for cases. Our study included men diagnosed with prostate cancer at middle age, whereas other studies include men with a wider range of ages at diagnosis. Finally, a more trivial explanation for the contrasting findings about the DG8S737 microsatellite −8 allele may reflect subtle differences in binning the microsatellite alleles between the

Icelandic and U.S. laboratories at both National Human Genome Research Institute and the Mayo Clinic (29), although given the common use of the CEPH reference sample we believe that is unlikely.

In this study, we also investigated whether either of the variants was more strongly associated with more aggressive prostate cancer based on Gleason score. This is the first study of these genetic variants to analyze cases stratified by tumor differentiation, in which cases with a Gleason score of 7 were subdivided into those with 3+4 versus 4+3 patterns. Previous studies have shown that Gleason score 4+3 tumors were more aggressive and were associated with a higher frequency of biochemical failure, systemic recurrence, and cancer-specific death than Gleason 3+4 tumors (30, 31). We observed a statistically significant association in cases with higher Gleason scores of 7 = 4+3 or 8 to 10 (OR, 1.9; 95% CI, 1.2-2.8) compared with controls, but no association was observed in the group of cases with lower Gleason scores of 2 to 6 or 7 = 3+4 compared with controls for the microsatellite −10 allele. We also observed that the A allele of the SNP rs1447295 had a higher relative risk estimate for prostate cancer among men with higher Gleason scores (OR, 1.4; 95% CI, 1.1-1.8), confirming the findings of Amundadottir et al. (20). By comparison, Freedman et al. (21) did not find an association between tumor grade and the SNP rs1447295 in a multiethnic cohort composed of Japanese Americans, Native Hawaiians, Latino Americans, and European Americans, perhaps reflecting a lack of statistical power. Although Wang et al. (29) reported an overall association of the A allele with prostate cancer risk, they found no difference in risk estimates according to Gleason grade (i.e., Gleason score <7 versus ≥7). We speculate that the different classification of Gleason 7 tumors into low- and high-grade tumors between the different studies may have contributed to the contrasting results. Wang et al., however, did show an association with both the A allele of SNP rs1447295 (OR, 2.0; 95% CI, 1.4-2.9; $P = 0.0001$) and the −10 allele of microsatellite DG8S737 (OR, 2.5; 95% CI, 1.3-4.7; $P = 0.002$) in men with aggressive prostate cancer, defined as having a Gleason score ≥8.

Gleason score has been studied as a marker for aggressive disease in linkage studies aimed at finding loci for more aggressive forms of familial prostate cancer, leading to suggestions of loci on chromosomes 5q31-33, 7q32, 19q12-13.11, 1q24-25, 1q42.2-43, Xq12-13, 4q, and 22q11.1 (24, 32-39). The strongest data to date highlight a locus on chromosome 19q12-13.11; none have pinpointed chromosome 8q24.

Interestingly, the study of Wang et al. did find an association with both the A allele of the SNP (OR, 1.9; 95% CI, 1.4-2.7; $P = 0.0004$) and the −8 allele of the microsatellite (OR, 1.7; 95% CI, 1.1-2.6; $P = 0.031$) and familial prostate cancer in a study of 438 affected men from 178 prostate cancer families. These families had an average of 4.4 men affected by family history and an average of 2.4 men affected with genotype data available (29). However, this analysis involved a comparison between familial cases ascertained from prostate cancer families and a set of 549 community controls recruited for a separate study of lower urinary tract symptoms. Results from that study are difficult to interpret because it is unknown whether the genotype distributions of the controls represent those of the underlying population from which the familial cases were ascertained.

Using another approach, we investigated whether either the SNP or microsatellite was associated with hereditary prostate cancer using data from 254 hereditary prostate cancer families for which a genome-wide scan for susceptibility loci had recently been completed (23). Families have been described previously; all met the previously stated criteria of hereditary prostate cancer families by the presence of multiple first-degree relatives affected with prostate cancer, often at an early age (23). Analyses using the computer program LAMP allowed us to assess if either one of the markers was in linkage disequilibrium, either complete or partial, with a putative causative allele (40). These results were uniformly negative, providing no evidence that either variant could explain linkage in this region through association with hereditary prostate cancer.

The contrasting findings between the case-control and family-based analyses suggest that this locus on 8q24 may be more important for enhancing risk of sporadic than hereditary prostate cancer. Although sporadic cancer has been thought to result from a serial accumulation of acquired and uncorrected somatic mutations, germ-line mutations in low penetrance genes may confer an increased risk for heterogeneous diseases, such as prostate cancer, particularly in the presence or absence of environmental exposures or other genetic variants. In the future, it may be possible to determine whether a specific genetic variant leads to different clinical forms of prostate cancer and, if so, whether specific treatments are more or less likely to be successful.

In conclusion, these population-based data confirm an association between the A allele of the rs1447295 SNP on chromosome 8q24 and prostate cancer risk in Caucasian middle-aged men. The DG8S737 microsatellite marker was also found to be associated with prostate cancer but only in men with more aggressive tumors defined by higher Gleason scores. Both of these variants on chromosome 8q24 may increase the risk of more aggressive forms of prostate cancer. The identification of a specific disease-causing variant is likely to further unravel the role of chromosome 8q24 in prostate cancer susceptibility.

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