Analysis of Recently Identified Prostate Cancer Susceptibility Loci in a Population-based Study: Associations with Family History and Clinical Features

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Abstract

Purpose: Two recent genome-wide association studies have highlighted several single nucleotide polymorphisms (SNPs) purported to be associated with prostate cancer risk. We investigated the significance of these SNPs in a population-based study of Caucasian men, testing the effects of each SNP in relation to family history of prostate cancer and the clinicopathologic features of the disease.

Experimental Design: We genotyped 13 SNPs in 1,308 prostate cancer patients and 1,267 unaffected controls frequency matched to cases by five-year age groups. The association of each SNP with disease risk stratified by family history of prostate cancer and clinicopathologic features of the disease was calculated with the use of logistic and polytomous regression.

Results: These results confirm the importance of multiple, previously reported SNPs in relation to prostate cancer susceptibility; 11 of the 13 SNPs were significantly associated with risk of developing prostate cancer. However, none of the SNP associations were of comparable magnitude with that associated with having a first-degree family history of the disease. Risk estimates associated with SNPs rs4242382 and rs2735839 varied by family history, whereas risk estimates for rs10993994 and rs5945619 varied by Gleason score.

Conclusions: Our results confirm that several recently identified SNPs are associated with prostate cancer risk; however, the variant alleles only confer a low to moderate relative risk of disease and are generally not associated with more aggressive disease features.

Recent genome-wide association studies (GWAS) have identified multiple genetic variants associated with prostate cancer risk (1–5). At least four separate regions at 8q24 have been identified and replicated in numerous studies of both Caucasian and African-American men (1, 4–18). Evidence that prostate cancer loci exist on chromosomes 10q11, 17q12, and 17q24 has also been presented and replicated in GWAS (10q11, refs. 1, 4, 19; 17q12, refs. 1, 3, 4, 20; 17q24, refs. 1, 3). In particular, two recent GWAS (1, 4) have each highlighted seven SNPs associated with prostate cancer risk, two of which (one at 10q11.2 and one at 11q13.2) were identified in both studies.

Eeles et al. (2008) conducted a two-stage GWAS that initially analyzed 1,854 cases and 1,894 controls for 541,129 SNPs (1). To increase statistical power, the cases and controls in the 1st stage of the study were selected according to specific criteria. The cases had been diagnosed as a result of clinical symptoms rather than by routine prostate-specific antigen (PSA) screening and were further enriched by only including men diagnosed at ≤60 years of age or who had a family history of prostate cancer. The controls were screened for serum PSA level and were only included if they were ≥50 years of age and had a PSA of <0.5 ng/mL. In stage 2 of the study, 11 SNPs were evaluated in a population-based series of 3,268 cases and 3,366 controls from the United Kingdom and Australia. Although no specific selection criteria were assigned to the stage 2 participants, over half of the control population had a PSA level of <10 ng/mL. Stage 2 analyses provided confirmatory evidence for 8q24, 17q12, and 17q24, and identified seven distinct novel genomic regions on chromosomes 3p12, 6q25, 7q21, 10q11, 11q13, 19q33, and Xp11. The result at Xp11 supports the results from a GWAS published concurrently by Gudmundsson et al. (2008; ref. 2).

Thomas et al. (2008) also conducted a two-stage GWAS with 527,869 SNPs in 1,172 prostate cancer cases and 1,157 controls who volunteered for the Prostate, Lung, Colorectal, and Ovarian cancer screening trial (4). In stage 1, the cases were oversampled to yield 688 aggressive cases (Gleason score ≥7

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Received 8/20/08; revised 1/13/09; accepted 1/25/09; published OnlineFirst 4/14/09.

Grant support: National Cancer Institute, NIH, grants RO1 CA56678, RO1 CA92579, RO1 CA082664, and P50 CA097186, with additional support from the Fred Hutchinson Cancer Research Center and the Intramural Program of the National Human Genome Research Institute.

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doi:10.1158/1078-0432.CCR-08-2190

Translational Relevance

Currently, clinicians are unable to clearly distinguish between men with less aggressive compared with more aggressive forms of prostate cancer. Finding genetic variants that identify men with aggressive disease, who have a higher risk for prostate cancer–specific mortality, may help clinicians make more informed treatment recommendations. Recent genome-wide association studies have identified a number of single nucleotide polymorphisms (SNPs) associated with prostate cancer risk, but it is unclear whether these particular SNPs uniquely identify the subset of men at higher risk based on the clinical features of the disease or further enhance risk in men with a family history of prostate cancer. The work presented here addresses these issues, and we hope that it will encourage future studies aimed at identifying genetic variants that may distinguish high-risk subsets of men suitable for novel therapeutic trials.

and/or disease stage III-IV) and 484 nonaggressive cases (Gleason score <7 and disease stage I-II). The controls were PSA screened negative at baseline, and all participants were of European ancestry. The 2nd stage involved analyzing the most promising 26,958 SNPs ($P < 0.068$) in 3,941 cases and 3,964 controls from four independent studies of men of European background from the United States, Finland, and France. Seven SNPs achieved or approached genome-wide significance ($P < 10^{-7}$) in the combined analysis of the two stages: three have been previously reported on chromosomes 8q24 (2 independent SNPs) and 17q12, whereas four represent novel SNPs on chromosomes 7p15, 10q11, 10q26, and 11q13.

We were invited to participate in replication efforts for the two above-mentioned studies. In an effort to confirm the above results and determine the significance of these SNPs in a population-based study, we genotyped 13 SNPs in DNA samples from Caucasian men from King County, Washington. The selected SNPs include seven highlighted by Eeles et al. (2008), and seven highlighted by Thomas et al. (2008). One SNP at 10q11 overlapped both studies. We were particularly interested in testing the effects of each SNP in relation to family history of prostate cancer and clinicopathologic features of more aggressive disease.

Materials and Methods

Study population. The study population consists of participants from one of two population-based case-control studies of prostate cancer in Caucasian and African-American residents of King County, Washington (Study I and Study II), whose collection methodologies have been previously described in detail (21, 22). Briefly, Study I cases were diagnosed between January 1, 1993 and December 31, 1996, and were 40 to 64 y of age at diagnosis. Study II cases were diagnosed between January 1, 2002 and December 31, 2005, and were 35 to 74 y of age at diagnosis. Overall, 2,244 eligible prostate cancer patients were identified; 1,754 (78.2%) were interviewed, and blood samples yielding sufficient DNA for genotyping were drawn from 1,457 (83.1%) cases who completed a study interview. A comparison group of controls without a self-reported physician's diagnosis of prostate cancer was identified through random digit dialing. The controls were frequency matched to the cases by 5-y age groups and were recruited evenly throughout each ascertainment period for cases. A total of 2,448 men were identified who met the eligibility criteria; 1,645 (67.2%) were interviewed and, of these men, blood samples were drawn and DNA prepared from 1,352 (82.2%) with the use of standard protocols. For these analyses, only Caucasian participants with DNA were included (1,308 cases and 1,267 controls).

Subjects in both studies completed in-person interviews conducted by trained male interviewers using standardized questionnaires. Information was collected on family structure and cancer history, medical history, and social and demographic factors. Clinical information on cases, including Gleason score, tumor stage, serum PSA level at diagnosis, and primary treatment, was obtained from the Surveillance, Epidemiology and End Results cancer registry. All study procedures were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board and the National Human Genome Research Institute. Written informed consent was obtained from all study participants before participation.

SNP genotyping. A total of 13 SNPs were genotyped with the use of the Applied Biosystems SNPlex Genotyping System. Identification of the specific SNP alleles was carried out with the use of the Applied Biosystems 3730xl DNA Analyzer with GeneMapper software.[5] Quality control included the genotyping of 144 blind duplicate samples distributed across all genotyping batches. There was 100% agreement between blind duplicate samples for all 13 SNPs. Each batch of DNA aliquots genotyped incorporated similar numbers of case and control samples, and laboratory personnel were blinded to the case-control status of the samples.

Statistical analyses. Departure from Hardy-Weinberg equilibrium was assessed for each SNP separately in controls. Unconditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) to measure the association between individual SNP genotypes and prostate cancer risk (23). Differences in risk estimates by first-degree family history of prostate cancer (yes versus no) were tested by including an interaction term in the regression model and comparing the -2 log likelihoods for the full (main effects plus the interaction term) and reduced (main effects only) models. Age at reference date and potential confounding factors, including prostate cancer screening history (never, digital rectal examination only, or PSA tested within the 5 y before the reference date) and first-degree family history of prostate cancer, were examined to see if such factors changed the risk estimates by ≥10%. After these tests, only age at reference date was included in the final models. Differences in mean PSA levels across SNP genotypes in a sample of controls ($n = 383$) were tested with the use of log-transformed PSA in an ANOVA test. Polytomous regression models were used to generate ORs and 95% CIs for the association between SNP genotypes and cases stratified by disease aggressiveness (less versus more), Gleason score [≤7 (3+4) versus ≥7 (4+3)], and tumor stage (local versus regional/distant) compared with controls. More aggressive cases were those with either a Gleason score of ≥7 (4+3), regional or distant stage disease, or a PSA level ≥20 ng/mL at diagnosis. A χ^2 test was used to test for significant differences in risk estimates between lower and higher Gleason scores. Log-additive (trend), recessive, and dominant models were considered for each SNP, and the model with the best fit was chosen for presentation (i.e., the model for which the P value was the smallest). Analyses were done with the use of SAS version 9.1.3.

A permutation procedure was used to account for the effect of multiple testing. Pairs of case-control labels and ages were permuted to approximate the distribution of the age-adjusted P values under the null hypothesis. Five-year age group and case-control labels were permuted together to preserve any relationship that may exist between age and case-control status, and allow age-adjusted P values to be calculated for each permutation that is consistent with the original analysis. For each permutation, log-additive, recessive, and dominant

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Table 1. Association results for 13 susceptibility SNPs and prostate cancer risk in Caucasians, King County, WA

SNP	Alleles*	Chromosome	Model	MAF			
				Cases (n = 1,308)	Controls (n = 1,267)	OR† (95% CI)	P value
rs2660753‡	C/T	3p12.1	Trend	0.12	0.11	1.09 (0.92-1.30)	0.33
rs9364554‡	C/T	6q25.3	Dominant	0.33	0.29	1.39 (1.18-1.62)	4.4 × 10-5
rs10486567§	G/A	7p15.2	Trend	0.2	0.24	0.8 (0.69-0.91)	8.9 × 10-4
rs6465657‡	T/C	7q21.3	Trend	0.49	0.45	1.16 (1.04-1.30)	7.9 × 10-3
rs6983267§	G/T	8q24.21	Trend	0.45	0.5	0.83 (0.74-0.93)	1.0 × 10-3
rs4242382§	G/A	8q24.21	Trend	0.14	0.1	1.39 (1.18-1.66)	1.3 × 10-4
rs10993994‡§	C/T	10q11.23	Trend	0.44	0.38	1.31 (1.17-1.47)	3.7 × 10-6
rs4962416§	T/C	10q26.13	Trend	0.28	0.27	1.06 (0.94-1.20)	0.35
rs7931342‡	G/T	11q13.2	Trend	0.45	0.5	0.82 (0.73-0.91)	4.8 × 10-4
rs10896449§	G/A	11q13.2	Dominant	0.45	0.5	0.7 (0.58-0.83)	8.3 × 10-5
rs4430796§	A/G	17q12	Trend	0.43	0.5	0.77 (0.69-0.86)	3.2 × 10-6
rs2735839‡	G/A	19q33.3	Trend	0.13	0.15	0.84 (0.72-0.99)	0.04
rs5945619‡	T/C	Xp11.22	Trend	0.4	0.33	1.15 (1.06-1.24)	1.1 × 10-3

*Major/minor alleles, based on frequencies in controls.
†Adjusted for age.
‡Eeles et al. (2008).
§Thomas et al. (2008).

models were fit for all SNPs and the minimum of the P values kept for each. The P values were ordered to approximate the null distribution of the order statistics for the P values; i.e., minimum P value, second smallest P value, etc.. The original P values were also ordered and permutation P values were calculated by comparing the ordered P values with the null distribution for the appropriate order statistic. Permutation P values can be interpreted as the probability of observing a P value less than or equal to what was observed for the given order statistic under the null hypothesis of no association with disease for any of the 13 SNPs. For example, the minimum P value was compared with the null distribution for the minimum P value, and the corresponding permuted P value can be interpreted as the probability of the minimum P value being less than or equal to the observed minimum P value under the null hypothesis. The same is true for the second smallest P value, the third smallest P value, etc.. The permutation approach to approximating the null distribution of the order statistics will be valid regardless of any correlation between the SNPs. A SNP was considered to be significantly associated with prostate cancer risk if the nominal P value and the permuted P value were both <0.05.

Results

The genotype distributions of all SNPs were consistent with Centre d'Etude du Polymorphisme Humain Hardy-Weinberg equilibrium in the control population. There were no significant differences in the allele frequencies of rs5945619 on the X chromosome in our population compared with those in the control population of Eeles et al. (2008), and the HapMap CEPH population.[6] Of the 13 SNPs investigated, 11 were significantly associated with prostate cancer risk in our population (Table 1), with rs10993994 on 10q11 and rs4430796 on 17q12 showing the strongest associations (OR, 1.31; 95% CI, 1.17-1.47 and OR, 0.77; 95% CI, 0.69-0.86, respectively). All SNPs that were significantly associated with prostate cancer remained significant after adjusting for multiple comparisons.

A first-degree family history of prostate cancer is associated with a 2-fold to 3-fold increase in risk for disease (24, 25) and has been used to identify men who may have an inherited predisposition. In the case-control population studied here, an OR of 2.19 (95% CI, 1.77-2.74; P < 0.0001) was observed for prostate cancer in Caucasian men with a first-degree family history of disease. None of the SNPs tested were associated with risk estimates for prostate cancer that were of comparable magnitude with that associated with having a family history of disease. Nor, for the majority of SNPs tested, was risk modified according to the presence or absence of a family history of prostate cancer (Table 2). Stratifying by family history did, however, reveal altered risk estimates associated with SNPs rs2735839 and rs4242382. The minor allele of rs2735839, located on chromosome 19q33.3, is associated with a decreased risk of prostate cancer in men without a family history of disease (OR, 0.76; 95% CI, 0.64-0.91). However, in men with a family history, there is no association (OR, 1.40; 95% CI, 0.91-2.20; interaction P = 0.01). Because of its proximity to the gene encoding PSA, we also examined the relationship between SNP rs2735839 and PSA levels in a sample of 383 controls. We found a statistically significant association between mean PSA level and the rs2735839 genotype (GG, 1.44; GA, 1.20; AA, 0.78; P = 0.0085). The minor allele of rs4242382, located in region 1 of the risk locus on 8q24, is associated with an increase in risk of prostate cancer in men without a family history (OR, 1.53; 95% CI, 1.27-1.85), but in those men with a family history of the disease, there is no association with risk (OR, 0.80; 95% CI, 0.54-1.21; interaction P = 0.004). However, after accounting for the effects of multiple testing, neither of the above interactions retained significant P values. To investigate these two SNPs further, we generated the risk estimates of prostate cancer in men carrying the minor risk allele of the SNP in addition to having a family history of the disease. For carriers of the rs2735839 risk allele (AA + family history versus GG + no family history), there was an OR of 3.87 (95% CI, 1.71-8.35), which is greater than the risk associated with having a family history alone. By comparison, for carriers of the rs4242382 risk allele (AA + family history

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Table 2. Association between 13 SNPs and risk of prostate cancer stratified by family history in Caucasians, King County, WA

SNP	Chromosome	Model	No family history		OR (95% CI)	Family history		OR (95% CI)	*P* value*
			MAF			MAF			
			Cases	Controls		Cases	Controls		
			n = 1,025	*n* = 1,125		*n* = 283	*n* = 142		
rs2660753	3p12.1	Trend	0.12	0.11	1.09 (0.90-1.32)	0.13	0.12	1.04 (0.67-1.60)	0.84
rs9364554	6q25.3	Dominant	0.33	0.29	1.38 (1.16-1.64)	0.35	0.29	1.38 (0.91-2.09)	0.94
rs10486567	7p15.2	Trend	0.2	0.24	0.8 (0.69-0.93)	0.19	0.23	0.79 (0.56-1.12)	0.95
rs6465657	7q21.3	Trend	0.49	0.45	1.17 (1.03-1.32)	0.49	0.45	1.15 (0.86-1.54)	0.94
rs6983267	8q24.21	Trend	0.45	0.5	0.83 (0.73-0.94)	0.44	0.49	0.84 (0.63-1.11)	0.97
rs4242382	8q24.21	Trend	0.14	0.1	1.53 (1.26-1.85)	0.13	0.16	0.8 (0.53-1.19)	0.004
rs10993994	10q11.23	Trend	0.44	0.38	1.3 (1.15-1.47)	0.44	0.36	1.42 (1.04-1.92)	0.6
rs4962416	10q26.13	Trend	0.28	0.27	1.06 (0.93-1.21)	0.28	0.26	1.12 (0.81-1.54)	0.76
rs7931342	11q13.2	Trend	0.45	0.49	0.85 (0.75-0.97)	0.42	0.51	0.67 (0.49-0.90)	0.14
rs10896449	11q13.2	Dominant	0.46	0.5	0.75 (0.62-0.92)	0.42	0.52	0.49 (0.29-0.79)	0.09
rs4430796	17q12	Trend	0.43	0.49	0.78 (0.69-0.88)	0.43	0.53	0.68 (0.51-0.92)	0.43
rs2735839	19q33.3	Trend	0.12	0.16	0.76 (0.64-0.91)	0.16	0.12	1.41 (0.91-2.17)	0.01
rs5945619	Xp11.22	Trend	0.4	0.32	1.18 (1.08-1.29)	0.38	0.39	0.97 (0.79-1.20)	0.1

*Comparison of risk estimates for SNP genotypes in the presence and absence of family history.

versus GG + no family history), there was an OR of 1.69 (95% CI, 0.82-3.47), which was similar to the risk associated with having a family history alone.

A major goal of current research is to discover markers that may predict men in whom prostate cancer will recur, progress, and lead to cancer-specific death. We examined whether any of the SNPs recently discovered by the two GWAS (1, 4) were associated with Gleason score, tumor stage, or a composite variable indicating more aggressive disease. The ORs for prostate cancer did not differ significantly by disease aggressiveness or stage (data not shown). However, the association of two SNPs with prostate cancer risk did vary by Gleason score (Table 3). The minor allele of rs10993994 at 10q11.23 was associated with an increased risk of prostate cancer in cases with a lower Gleason score [≤7 (3+4); OR, 1.36; 95% CI, 1.21-1.54]. However, in those cases with a higher Gleason score [≥7 (4+3)] there was no association (OR, 1.04; 95% CI, 0.84-1.30; interaction $P = 0.02$). The minor allele of rs5945619 at Xp11.22 was more strongly associated with risk of prostate cancer in cases with higher (OR, 1.33; 95% CI, 1.14-1.55) than lower Gleason scores (OR, 1.12; 95% CI, 1.02-1.22; interaction $P = 0.03$). After accounting for the effects of multiple testing, neither of the above interaction *P* values was significant.

Table 3. Association between 13 SNPs and risk of prostate cancer stratified by Gleason score in Caucasians, King County, WA

SNP	Chromosome	Model*	Controls	Cases - lower Gleason scores †		Cases – higher Gleason scores ‡		*P* value§
			(*n* = 1,267)	(*n* = 1,102)		MAF	(*n* = 202)	
			MAF	MAF	OR (95% CI)		OR (95% CI)	
rs2660753	3p12.1	Trend	0.11	0.12	1.07 (0.89-1.28)	0.13	1.19 (0.86-1.64)	0.53
rs9364554	6q25.3	Dominant	0.29	0.34	1.43 (1.21-1.68)	0.31	1.18 (0.87-1.60)	0.24
rs10486567	7p15.2	Trend	0.24	0.2	0.79 (0.69-0.91)	0.21	0.81 (0.62-1.06)	0.86
rs6465657	7q21.3	Trend	0.45	0.49	1.2 (1.07-1.35)	0.45	0.98 (0.79-1.22)	0.08
rs6983267	8q24.21	Trend	0.5	0.45	0.82 (0.73-0.92)	0.47	0.9 (0.72-1.12)	0.38
rs4242382	8q24.21	Trend	0.1	0.14	1.37 (1.15-1.64)	0.15	1.53 (1.13-2.08)	0.47
rs10993994	10q11.23	Trend	0.38	0.45	1.36 (1.21-1.54)	0.39	1.04 (0.84-1.30)	0.02
rs4962416	10q26.13	Trend	0.27	0.28	1.06 (0.94-1.21)	0.28	1.04 (0.82-1.31)	0.83
rs7931342	11q13.2	Trend	0.5	0.44	0.81 (0.72-0.91)	0.46	0.85 (0.68-1.06)	0.65
rs10896449	11q13.2	Dominant	0.5	0.45	0.7 (0.58-0.84)	0.46	0.69 (0.49-0.96)	0.94
rs4430796	17q12	Trend	0.5	0.43	0.76 (0.68-0.86)	0.44	0.79 (0.63-0.98)	0.78
rs2735839	19q33.3	Trend	0.15	0.13	0.86 (0.73-1.02)	0.12	0.74 (0.53-1.02)	0.34
rs5945619	Xp11.22	Trend	0.33	0.39	1.12 (1.02-1.22)	0.47	1.33 (1.14-1.55)	0.03

*Model of best fit.
† Gleason score ≤ (3+4).
‡ Gleason score ≥ (4+3).
§ Test for homogeneity of ORs across Gleason score groups.

Discussion

In the analyses presented here, we examined the association between prostate cancer risk and 13 SNPs recently highlighted by two GWAS (1, 4). Overall, SNPs on chromosomes 6q25, 7p15, 7q21, 8q24, 10q11, 11q13, 17q12, 19q33, and Xp11 were significantly associated with prostate cancer risk in our population-based study.

Of the seven SNPs described by Eeles et al. (2008), only one, rs2660753 at 3q12, was not significantly associated with prostate cancer risk in our population. This result is consistent with a recent replication study presented by Kote-Jarai et al. (2008) that examined data from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) Consortium and includes studies from 13 centers (19). They observed a weaker association of SNP rs2660753 with prostate cancer risk than previously reported (1). In stage 2 of the initial study by Eeles et al. (2008), a per-allele OR of 1.18 (95% CI, 1.06-1.31) was observed (1), whereas a reduced OR of 1.08 (95% CI, 1.00-1.16) was noted in the PRACTICAL replication study (19). This latter result could be driven in part by the study population presented here, which is a part of PRACTICAL and provided the largest number of cases and controls to that consortium effort (19). Of the seven putatively risk-associated SNPs reported by Thomas et al. (2008; ref. 4), again only one, rs4962416, was not significantly associated with prostate cancer risk in our study. A number of explanations could account for the lack of an association between these SNPs, rs2660753 and rs4962416, in our study. It may be due to different study designs and populations, the reduced sample size of our study compared with the initial GWAS, or, alternatively, these differences may be due to chance. Additional studies are needed to determine the significance of the relationship between rs2660753 and rs4962416, and prostate cancer risk.

We then considered whether a first-degree family history of prostate cancer modified the effect of each SNP investigated. Stratification according to family history did not significantly modify the effect of most SNPs, except two. SNP rs2735839 at 19q33.3 is situated among a cluster of kallikrein genes, including *KLK3*, which encodes PSA (26). The minor allele is associated with a significantly reduced risk of prostate cancer in men without a family history, whereas no association was seen in men with a family history of disease. However, this result could be due to chance, given the limited number of cases and controls with a family history. There is currently a debate on whether the association previously reported between this polymorphism and prostate cancer risk is confounded by PSA screening. The association with rs2735839 was first observed in a GWAS in which controls had a baseline PSA of <0.5ng/mL (1). In the concurrent Cancer Genetic Markers of Susceptibility GWAS, an association between this SNP and prostate cancer risk was not identified (4) and, when rs2735839 was examined among controls only, a strong correlation was found with PSA levels (27). In our study, we found a significant association between prostate cancer risk and rs2735839, and we also found a significant association between PSA level and the rs2735839 genotype (P = 0.0085), in which the minor allele was associated with a decrease in mean PSA levels in controls. As PSA screening was prevalent in men in our study (73% of men with a family history and 58% of men without a family history reported having a PSA test within the past 5 years), it may be that the decrease in risk associated with rs2735839 is due to confounding. Adjusting for prostate cancer screening history did not significantly change our risk estimate, but the adjusted CI includes the null. In addition, the association between rs2735839 and prostate cancer risk has been replicated in other populations in which PSA screening is virtually absent (19). It is clear that the relationship between this SNP and prostate cancer risk needs to be investigated in additional datasets before we can determine whether there is a true association with risk of disease.

The effect of rs4242382 at 8q24 was also modified by family history. The minor allele was associated with a significant increase in risk in men without a family history of prostate cancer but not in those with a family history of the disease. This result seems to reflect the somewhat higher minor allele frequency in controls with a family history than in controls without a family history of prostate cancer. As the control group is population based and family history was not a determining factor for inclusion, this may reflect a true difference in the effect of this SNP on prostate cancer risk according to family history of the disease. However, the result may also be due to the limited number of cases and controls with a family history of prostate cancer in this population. The results from this analysis are in contrast to a previous study by Wang et al. (2007; ref. 17). That study examined SNP rs1447295, which is in complete linkage disequilibrium (r^2 = 1) with rs4242382, and found a stronger association between the rs1447295 SNP genotype and risk in familial prostate cancer cases compared with controls than that observed between sporadic prostate cancer cases versus controls. It has to be noted that for both of the associations described above, the analyses are underpowered due to the limited number of cases and controls with a family history of prostate cancer.

Currently, clinicians are unable to distinguish between those patients with more aggressive forms of prostate cancer that may lead to adverse outcomes and those whose disease will follow a more indolent course. There is a need for markers that can identify men who may benefit most from aggressive treatment regimens or early phase clinical trials. deCODE genetics currently offers an eight-marker deCODE ProCa genetic risk profile for Caucasian males of European ancestry; however, it is not a determinative diagnostic test nor do the SNP genotypes correlate with more aggressive prostate cancer features.[7] Zheng et al. (2008) have also proposed a five-SNP genetic test panel that purports to identify men with an increased risk of disease (28). The relative risk estimate is 4.47 (95% CI, 2.93-6.80) for the small proportion of men having four of the five at-risk alleles. However, this test provides no greater predictive value than having a strong family history defined by having two or more affected first-degree relatives (OR, 4.9; 95% CI, 2.0-12.3; ref. 29 and OR, 5.08; 95% CI, 3.31-7.79; ref. 25). In addition, a recent analysis (30) has shown that these five SNPs do not improve prediction models for risk of developing prostate cancer or of disease-specific mortality in Caucasian men once currently known risk predictors (age, serum PSA, and family history) and outcome predictors (age, diagnostic PSA, Gleason

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score, stage, and primary treatment), respectively, are taken into account.

None of the SNPs examined in our case-control study were associated with a composite measure of disease aggressiveness or tumor stage. However, the effects of rs10993994 and rs5945619 on prostate cancer risk were significantly different by Gleason score. SNP rs10993994, at 10q11, was associated with a significantly increased risk of disease in men with lower Gleason scores but not in men with higher Gleason scores. This SNP lies two base pairs upstream of the transcription start site of *MSMB*, a gene encoding prostatic secretory protein-94, which is secreted into both seminal fluid and blood (31–33). Prostatic secretory protein-94 is a tumor suppressor protein that may impede prostate cancer growth through the promotion of apoptosis (32), the inhibition of the secretion of a matrix metalloproteinase that is implicated in tumor metastasis (31), and by decreasing tumor-associated, vascular endothelial growth factor–mediated vascularization (34). Given the function of this protein, it is not clear why there is an association limited to cases with a lower rather than a higher Gleason score; this result may be due to the smaller number of cases in the higher Gleason score group, or another gene(s) in this region, and not *MSMB*, may be contributing to the deleterious effect.

The effect of rs5945619 at Xp11 on prostate cancer risk was also modified by Gleason score, as there was a significant increase in risk of disease in cases with a higher compared with a lower Gleason score (interaction $P = 0.03$). SNP rs5945619 lies in a 2-Mb LD block between the *NUDT10* and *NUDT11* genes. Although little is known about these particular genes, they belong to a subgroup of proteins that have been functionally linked to vesicle trafficking, stress responses, DNA repair, and apoptosis (35). Although the test for homogeneity between cases with lower versus higher Gleason scores is only nominally significant, the potential role that these genes play in tumor development, and the direction of the association, suggests that this finding warrants replication in a larger data set. Although not statistically significant, the relative risk of prostate cancer was also greater in men with a higher Gleason score than in those with comparatively lower scores in the replication study of Kote-Jarai et al. (2008; ref. 19).

The results presented here confirm the importance of a number of recently highlighted loci in prostate cancer susceptibility even though the variant alleles of these SNPs only confer a low to moderate association with risk of the disease. The effects of most of these polymorphisms do not seem to be modified by a family history of prostate cancer, except for rs4242382 at 8q24 and rs2735839 at 19q33. Two SNPs, rs10993994 at 10q11 and rs5945619 at Xp11, were associated with Gleason score, whereas the remaining SNPs were not associated with indicators of disease aggressiveness or tumor stage. One shortcoming of this study was the limited power due to the smaller numbers in the stratified subgroups. Because of this, it is important that these results be replicated in larger population-based studies. In conclusion, although there has recently been success in identifying prostate cancer susceptibility loci, future studies should also focus on identifying genetic variants that predict risk of more aggressive disease and, thereby, may predict which patients are at higher risk for subsequent metastasis and disease-specific mortality.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank all the men who participated in this study for their time, effort, and cooperation; and Dr. Ziding Feng for his statistical expertise.

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Clin Cancer Res 2009;15:3231-3237.