

Two Independent Prostate Cancer Risk–Associated Loci at 11q13

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Abstract

Single nucleotide polymorphisms (SNP) at 11q13 were recently implicated in prostate cancer risk by two genome-wide association studies and were consistently replicated in multiple study populations. To explore prostate cancer association in the regions flanking these SNPs, we genotyped 31 tagging SNPs in a ~110 kb region at 11q13 in a Swedish case-control study (Cancer of the Prostate in Sweden), including 2,899 cases and 1,722 controls. We found evidence of prostate cancer association for the previously implicated SNPs including rs10896449, which we termed locus 1. In addition, multiple SNPs on the centromeric side of the region, including rs12418451, were also significantly associated with prostate cancer risk (termed locus 2). The two groups of SNPs were separated by a recombination hotspot. We then evaluated these two representative

SNPs in an additional ~4,000 cases and ~3,000 controls from three study populations and confirmed both loci at 11q13. In the combined allelic test of all four populations, $P = 4.0 \times 10^{-11}$ for rs10896449 at locus 1 and $P = 1.2 \times 10^{-6}$ for rs12418451 at locus 2, and both remained significant after adjusting for the other locus and study population. The prostate cancer association at these two 11q13 loci was unlikely confounded by prostate-specific antigen (PSA) detection bias because neither SNP was associated with PSA levels in controls. Unlike locus 1, in which no known gene is located, several putative mRNAs are in close proximity to locus 2. Additional confirmation studies at locus 2 and functional studies for both loci are needed to advance our knowledge on the etiology of prostate cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1815–20)

Introduction

A prostate cancer risk–associated locus at 11q13 was identified from two genome-wide association studies (GWAS; refs. 1, 2). In a National Cancer Institute Cancer Genetic Markers of Susceptibility GWAS, a single nucleotide polymorphism (SNP) rs10896449 at 11q13 (68,751,243) was found to be significantly associated with prostate cancer risk ($P = 1.8 \times 10^{-9}$) among a total of 4,053 cases and 5,121 controls from five independent study populations (1). Similarly, among a total of 5,122 cases and 5,260 controls from three independent study

populations in a GWAS from the United Kingdom and Australia, rs7931342, a SNP that is only 170 bp away from rs10896449, was significantly associated with prostate cancer risk ($P = 1.7 \times 10^{-12}$; ref. 2). The association between rs7931342 and prostate cancer risk was later confirmed among 13 additional study populations (7,370 cases and 5,742 controls) in the PRACTICAL consortium ($P = 3 \times 10^{-11}$; ref. 3). The prostate cancer association at 11q13 was also confirmed by our group in the Cancer of the Prostate in Sweden (CAPS) study, a Swedish population-based case-control study (4). Both SNPs were significantly associated with prostate cancer risk; $P = 0.002$ for rs10896449 and $P = 0.004$ for rs7931342. SNPs rs10896449 and rs7931342 were in strong linkage disequilibrium among control subjects, with a pairwise r^2 of 0.95. To date, evidence for associations between prostate cancer risk and variants at 11q13 have been consistently found in all of the published studies, although there are no known genes within a 100 kb genomic region flanking these two SNPs.

To assess whether there are additional independent prostate cancer risk variants in the flanking regions, we did a fine-mapping analysis of the 11q13 genomic region. This strategy was motivated by empirical examples

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of prostate cancer associations at 8q24 (5-10) and 17q12 (11, 12), in which additional independent loci were subsequently discovered in the flanking regions at both loci. We describe the results of a fine-mapping study in our Swedish study population and confirmation efforts in three additional study populations.

Materials and Methods

Study Subjects. Four study populations were included in this study. The first was a population-based prostate cancer case-control study in Sweden named CAPS that was used for the fine-mapping study (ref. 13; Supplementary Table 1). Prostate cancer patients in CAPS were identified and recruited from regional cancer registries in Sweden. The inclusion criterion for case subjects was pathologically or cytologically verified adenocarcinoma of the prostate, diagnosed between July 2001 and October 2003. DNA samples from blood and tumor-node-metastasis stage, Gleason grade (biopsy), and prostate-specific antigen (PSA) levels at diagnosis were available for 2,899 patients. Patients who met any of the following criteria were considered as having more aggressive disease: clinical stage T₃/T₄, N+, M+, differential grade 3, Gleason score ≥8, or preoperative serum PSA of ≥50 ng/mL. Control subjects were recruited concurrently with case subjects. They were randomly selected from the Swedish Population Registry, and matched according to the expected age distribution of cases (groups of 5-y intervals) and geographic region. DNA samples from blood were available for 1,722 control subjects.

The second study population was a hospital-based case-control population at The Johns Hopkins Hospital (JHH) (Supplementary Table 2). Prostate cancer cases were 1,527 men of European descent (by self-report) who underwent radical prostatectomy for treatment of prostate cancer at JHH from January 1, 1999 to December 31, 2006. Each tumor was graded using the Gleason scoring system (14) and staged using the tumor-node-metastasis system (15). Patients who met any of the following criteria were considered as having more aggressive disease: pathologic Gleason score of 7 or higher, stage pT₃ or higher, N+, or M₁. Men undergoing screening for prostate cancer at JHH and in the Baltimore metropolitan area during the same time period were asked to participate as control subjects. A total of 482 men of European descent (by self-report) met our inclusion criteria as control subjects for this study: normal DRE, PSA levels of ≤4.0 ng/mL, and older than 55 years.

The third study population was selected from the American Cancer Society Cancer Prevention Study-II (CPS-II) Nutrition Cohort, a prospective study of cancer incidence (16). Approximately 184,000 adults from the United States between the ages of 50 and 74 were enrolled in 1992 and sent follow-up questionnaires in 1997 and every 2 years afterwards. We identified 1,414 Caucasian men who had been diagnosed with prostate cancer between 1992 and 2003, and had no previous history of cancer. Cancer status was verified through medical records, linkage with state cancer registries, or death certificates. An equal number of controls were matched to the cases on age (±6 mo), race, and date of blood collection (±6 mo) from men who were cancer-free at the time of cancer diagnosis of their matched case using risk set sampling.

The last study population was 1,172 prostate cancer case subjects and 1,157 control subjects who were selected from the Prostate, Lung, Colon, and Ovarian (PLCO) Cancer Screening Trial. This was the study population used for the first stage Cancer Genetic Markers of Susceptibility prostate cancer GWAS (1). Individual genotype data were obtained through an approved data request application. For both CPS-II and PLCO, patients with Gleason score of ≥7 or stage ≥III disease were considered as aggressive prostate cancer cases.

Region of Interest, Tagging SNPs, and SNP Genotyping. We identified a ~110 kb region of interest for the fine-mapping study (68,665,000-68,775,000; build 35) based on the Cancer Genetic Markers of Susceptibility GWAS results at 11q13 in which multiple SNPs in the region had $P < 0.05$ and two recombination hotspots at the boundaries of the region. A total of 31 tagging SNPs were identified to capture ($r^2 > 0.8$) all the SNPs with minor allele frequencies of 1% or higher in the region of interest based on the HapMap phase II data. These SNPs were selected for genotyping in CAPS. PCR and extension primers for these SNPs were designed using MassARRAY Assay Design 3.0 software. PCR and extension reactions were done according to the instructions of the manufacturer, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. Duplicate and water samples, to which the technician was blinded, were included in each 96-well plate as PCR-negative controls. The average genotype call rate for these SNPs was >98% and the average concordance rate was 99.7% among 100 duplicate quality control samples.

Statistical Methods. A Hardy-Weinberg equilibrium test was done using Fisher's exact test. Haplotype blocks were estimated using the HaploView (17) computer program, and a default Gabriel method (18) was used to define each haplotype block; i.e., a region in which all (or nearly all) pairs of markers are in "strong linkage disequilibrium", which is consistent with no historical recombination. SequenceLDhot was used to determine recombination hotspots (19). SequenceLDhot considers a grid of putative hotspot positions, and for each putative hotspot, calculates a likelihood ratio statistic for the presence of a hotspot. Haplotype and background recombination rates generated from PHASE (version 2.1) were used as input files. We assumed that the putative hotspots have a width of 2 kb and the program searches for a new hotspot every 1 kb.

We imputed all of the known SNPs in the genome based on the genotyped SNPs and haplotype information in the HapMap phase II data (CEU) using a computer program, IMPUTE (20). A posterior probability of 0.9 was used as a threshold to call genotypes.

Allele frequency differences between case patients and control subjects were tested for each SNP, using a χ^2 test with 1 *df*. The allelic odds ratio (OR) and 95% confidence interval (95% CI) were estimated based on a multiplicative model. Results from multiple case-control populations were combined using a Mantel-Haenszel model in which the populations were allowed to have different population frequencies for alleles but were assumed to have a common OR. The homogeneity of ORs among

different study populations was tested using a Breslow-Day χ^2 test and I^2 method statistics by Higgins and Thompson (21). The independence of prostate cancer associations with SNPs at two loci at 11q13 was tested by including both SNPs (assuming an additive model at each SNP) in a logistic regression model among four populations and was adjusted for study population and age. Multiplicative interactions between two SNPs were tested by including both SNPs (assuming a general model) and an interaction term (product of two main effects) in a logistic regression model.

We tested the association of rs7931342 and rs10896449 with PSA levels in controls assuming a 2 *df* general model and adjusting for age using a multiple regression analysis. PSA levels were logarithm-transformed to best approximate the assumption of normality.

Results

We genotyped 31 tagging SNPs in a ~110 kb region of interest at 11q13 in the CAPS study population. The

genotype distributions for all 31 SNPs were consistent with Hardy-Weinberg expectations in control subjects ($P > 0.05$). We also imputed 53 SNPs (call rate $> 90\%$) in the region based on the genotyped SNPs using the computer program IMPUTE (20). Allele frequency differences between cases and controls in CAPS were tested for these 84 SNPs using a χ^2 test (Supplementary Table 3). Multiple SNPs in this 110 kb region were significantly associated with prostate cancer risk in allelic tests (Fig. 1A, *blue diamond*). Specifically, many SNPs within a 37 kb region (68,731,000–68,768,000) flanking rs10896449 (68,751,243) and rs7931342 (68,751,073) were highly significant, and had *P* values similar to that of these two SNPs. However, none of these SNPs was significant after adjusting for rs10896449, suggesting that they are dependent (Fig. 1A, *pink diamond*). These SNPs can be grouped into locus 1 and were in two consecutive haplotype blocks (Fig. 1B).

Several SNPs (68,722,000–68,731) that were immediately centromeric to the haplotype block were not significantly associated with prostate cancer risk.

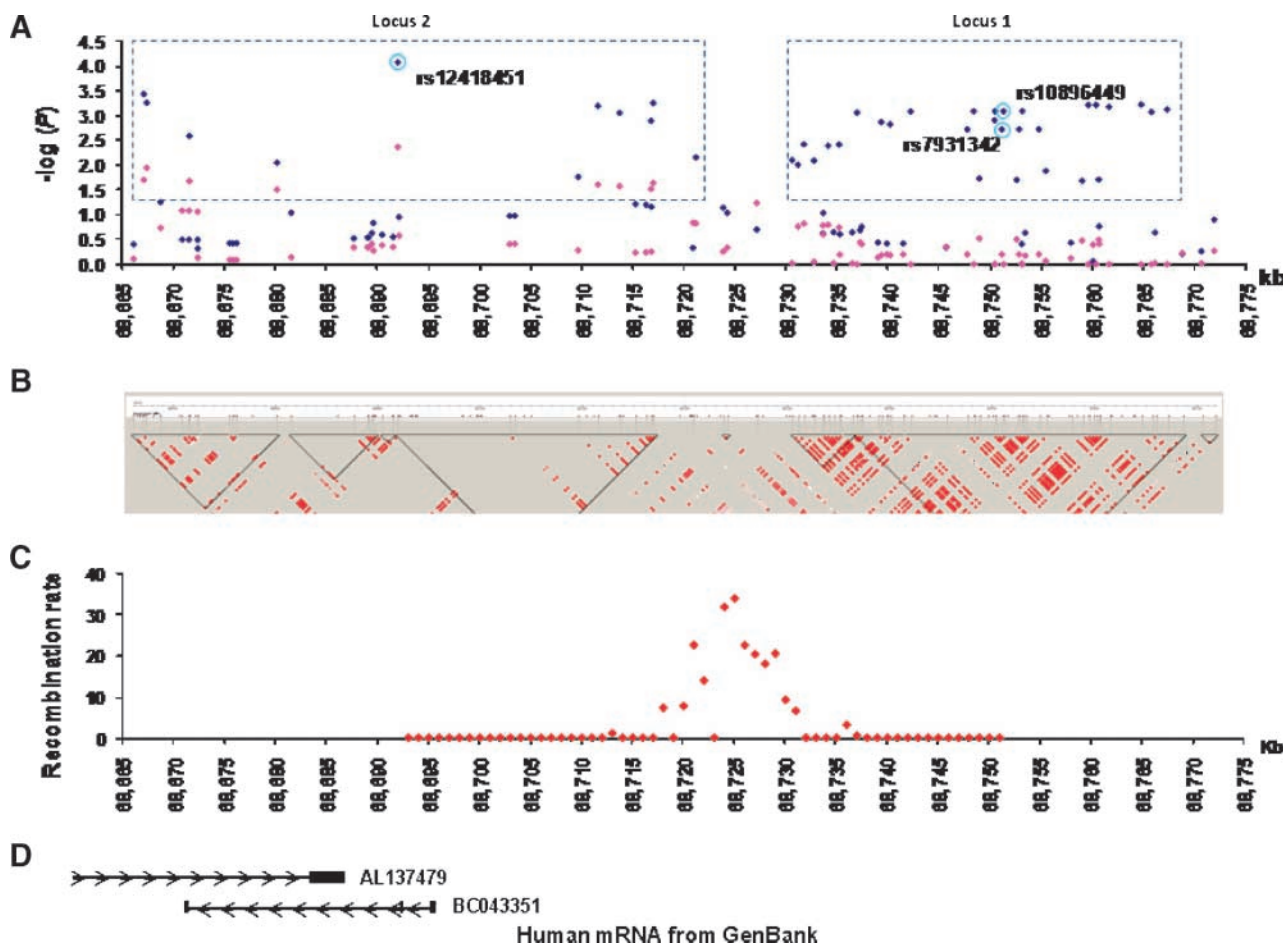


Figure 1. A schematic view of genetic association between SNPs at 11q13 and prostate cancer risk in CAPS. **A.** Allele tests for 84 SNPs at 11q13 and prostate cancer risk in CAPS. Two separate clusters of prostate cancer-associated SNPs were found (*blue dotted boxes*), including the previously reported locus 1 (rs10896449) and a novel locus 2. *Blue diamond*, *P* values of single SNP analysis; *pink diamond*, *P* values after adjusting for rs10896449. **B.** Inferred haplotype blocks of these SNPs were estimated from the control subjects in CAPS using the HaploView computer program. These two loci were in different haplotype blocks. **C.** A recombination hotspot between the two loci using SequenceLDhot software. **D.** mRNA at 11q13.

Table 1. Association of prostate cancer risk with SNPs rs10896449 at locus 1 and rs12418451 at locus 2

Populations	Risk allele	Genotype counts						Heterozygotes		Homozygotes		Allele frequency				
		Cases			Controls			OR		OR		Cases	Control	P*	P [†]	I ^{2‡}
		AA	AG	GG	AA	AG	GG	(95% confidence interval)		(95% confidence interval)						
rs10896449																
CAPS	G	748	1362	710	477	867	342	1.00 (0.87-1.16)		1.32 (1.11-1.57)		0.49	0.46	2.2E-03		
JHH	G	293	706	508	113	233	130	1.17 (0.90-1.52)		1.51 (1.13-2.01)		0.57	0.52	3.8E-03		
CPS-II	G	339	868	553	427	878	469	1.43 (1.16-1.76)		1.48 (1.17-1.86)		0.56	0.51	3.7E-05		
PLCO	G	240	598	328	298	519	276	1.25 (1.05-1.48)		1.49 (1.23-1.79)		0.54	0.49	1.3E-03		
All	G	1620	3534	2099	1315	2497	1217	1.16 (1.06-1.27)		1.42 (1.28-1.58)		0.53	0.49	1.6E-11 0.56 0		
rs12418451																
CAPS	A	1392	1227	262	930	658	126	1.25 (1.10-1.41)		1.39 (1.11-1.75)		0.30	0.27	8.6E-05		
JHH	A	715	644	155	231	215	32	0.97 (0.78-1.2)		1.56 (1.04-2.35)		0.32	0.29	1.8E-01		
CPS-II	A	704	608	124	756	562	116	1.09 (0.91-1.3)		1.47 (1.08-2.01)		0.30	0.28	8.0E-02		
PLCO	A	519	506	113	527	472	78	1.16 (1.00-1.36)		1.15 (0.87-1.51)		0.32	0.29	3.0E-02		
All	A	3330	2985	654	2444	1907	352	1.15 (1.06-1.24)		1.36 (1.18-1.56)		0.31	0.28	1.2E-06 0.68 0		

*Based on allelic test assuming multiplicative model. The combined tests are based on Mantel-Haenszel test.

[†]Breslow-Day test for homogeneity.

[‡]Percentage of total variation across studies that is due to heterogeneity rather than chance.

Nevertheless, multiple SNPs further centromeric were found to be associated with prostate cancer risk. More importantly, most of these SNPs remained significant after adjusting for rs10896449 (Fig. 1A, *pink diamond*), suggesting that they are independent from rs10896449 at locus 1. They spanned four consecutive haplotype blocks and can be grouped into locus 2. We estimated the recombination rate across the region among control subjects using SequenceLDhot software (19) and found strong evidence for a recombination hotspot between the two loci at 68,720,000 to 68,730,000 ($P = 1.24 \times 10^{-15}$; Fig. 1C). The recombination hotspot is also reported in the HapMap data (68,720,001-68,728,001; release 21, phases I and II). This recombination hotspot separates these two prostate cancer loci at 11q13. Across the entire 110 kb region of interest at 11q13, rs12418451 (68,691,995) at locus 2 was the most significant SNP ($P = 8.57 \times 10^{-5}$, not adjusted for rs10896449). Unlike locus 1, in which no known gene is located, there are two known mRNAs in locus 2 (AL137479 and BC043531; Fig. 1D).

As a confirmation effort, we examined these two candidate prostate cancer loci at 11q13 in three additional study populations, including the JHH study, the American Cancer Society CPS-II Nutrition Cohort, and the PLCO Cancer Screening Trial. One representative SNP in each locus (rs10896449 at locus 1 and rs12418451 at locus 2) was evaluated. Consistent with previous publications (1-4), a significant association was found for SNP rs10896449 at locus 1 in each population (Table 1). The overall P value of the allelic test in these four populations was 1.57×10^{-11} . Similarly, allele "A" of rs12418451 at locus 2 was also consistently more common in cases than in controls in each population, and was statistically significant in these three independent populations ($P = 0.002$, not adjusted for rs10896449). Together with CAPS, the overall P value of the allelic test in these four populations was 1.2×10^{-6} (not adjusted for rs10896449). For both SNPs, there was no evidence for heterogeneity in allelic associations

among these study populations using a Breslow-Day test for homogeneity and I^2 .

These two SNPs were in moderate linkage disequilibrium in each study population ($P < 0.05$), with a pairwise r^2 of 0.10, 0.16, 0.12, and 0.13, respectively, in CAPS, JHH, CPS-II, and PLCO. However, when the independence of these two SNPs with prostate cancer risk was tested in all four populations by including both SNPs in a logistic regression model adjusted for study population and age, both SNPs remained significant ($P = 0.0001$ for rs10896449 at locus 1 and $P = 0.01$ for rs12418451 at locus 2). No significantly multiplicative interaction between the two SNPs was found ($P = 0.9$).

The frequencies of risk alleles at these two SNPs were not significantly different between aggressive cases and nonaggressive cases in CAPS, JHH, CPS-II, or PLCO (Supplementary Table 4). These two SNPs were not significantly associated with plasma PSA levels among control subjects, using an additive model, $P = 0.11$ and 0.46, respectively, in CAPS and JHH for rs10896449 at locus 1 and $P = 0.24$ and 0.34, respectively, in CAPS and JHH for rs12418451 at locus 2. PSA levels in controls subjects were not available to us for CPS-II and PLCO.

Discussion

Using a fine-mapping approach to study a newly discovered prostate cancer locus at 11q13, we found a second independent locus in the flanking region of the first 11q13 locus in our study populations and in the publicly available Cancer Genetic Markers of Susceptibility (PLCO) study. Among a total of four study populations examined in this study, the overall P value of the allelic test for this novel locus (rs12418451) was 1.2×10^{-6} . Although the nominal P value was highly significant, it did not reach a genome-wide significance level of 2×10^{-8} that accounts for multiple tests of ~ 2 million SNPs in the genome. Evaluation of this novel locus in additional study populations is needed, especially among other Swedish or Scandinavian populations

because the evidence for its association with prostate cancer risk was the strongest in the Swedish population (CAPS). In fact, the evidence for prostate cancer association at locus 2 was stronger than that for locus 1 in CAPS. This study further shows the importance of sharing and using all available data to identify risk factors associated with complex diseases.

If this second locus at 11q13 is confirmed in additional study populations, it would be the third example, following 8q24 (5-10) and 17q12 (11, 12), in which additional independent loci are subsequently discovered in the flanking regions of an initial locus identified from GWAS. The molecular mechanism for this phenomenon is unknown; however, it suggests fine-mapping studies in broad regions surrounding SNPs implicated from GWAS are needed for other prostate cancer risk-associated regions reported in recent GWAS. Considering that the novel loci at 8q24, 17q12, and 11q13 are in different haplotype blocks from their respective initial locus, fine-mapping studies should extend across several haplotype blocks in the flanking region.

The frequencies of risk alleles at these two loci at 11q13 were not significantly different between aggressive cases and nonaggressive cases. This null result with aggressiveness of prostate cancer is similar to the other prostate cancer risk variants recently identified from GWAS, including at 8q24, 17q12, 17q24, 3p12, 7q21, 10q11, and Xp11 (3, 22). Lack of significant differences in risk allele frequencies between aggressive and nonaggressive cases is not unexpected considering that these prostate cancer risk variants were identified by comparing all types of prostate cancer cases with controls. Other study designs, including those comparing aggressive with nonaggressive prostate cancer, may be more appropriate to discover risk variants for aggressive prostate cancer.

A potential confounder of PSA detection bias in genetic association studies of prostate cancer was recently suggested (23). A SNP at 19q13 (rs2735839) near the *KLK3* gene (also known as the *PSA* gene) was found to be associated with prostate cancer risk in a GWAS (2). However, this SNP was also found to be significantly associated with PSA levels in controls (2, 22, 23). The PSA association, when combined with widely used PSA screening in some populations, may lead to the alleles associated with higher PSA levels to be overrepresented in cases and underrepresented in controls. An artifact association with prostate cancer may occur for these SNPs regardless of whether the alleles are truly associated with prostate cancer risk per se. We examined the association of the two SNPs at 11q13 with PSA levels in controls and did not find statistical evidence for the association; (Supplementary Table 5) therefore, the prostate cancer association at 11q13 reported in this study are unlikely confounded by PSA detection bias.

Similar to 8q24 and other newly discovered prostate cancer risk-associated loci, no obvious candidate genes are located within locus 1 at 11q13. However, there are several putative mRNAs that originate from regions in close proximity to locus 2: rs12418451 is within an intron of a spliced expressed sequence tag, *BC043531*, cloned from a human brain cDNA library, and it is within 6 kb of the 3'-end of *AL137479*, a variant transcript of *TPCN2*, a gene with coding SNPs recently associated with pigmentation variation in Europeans (24). The roles of these

genes in the development of prostate cancer remain to be evaluated. However, the discovery of two novel prostate cancer risk-associated variants at 11q13 further shows the advantage of systematic and objective evaluation of data from GWAS and fine-mapping studies, and the potential to uncover new mechanisms for the etiology of prostate cancer.

The finding of additional prostate cancer risk variants in this study provides additional support for the polygenic nature of the disease. As more prostate cancer risk variants are identified, we may gradually improve our ability to predict individual prostate cancer risk using genetic markers (25, 26).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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