Prostate Cancer Risk Associated Loci in African Americans

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Abstract

Four genome-wide association studies, all in populations of European descent, have identified 20 independent single nucleotide polymorphisms (SNP) in 20 regions that are associated with prostate cancer risk. We evaluated these 20 SNPs in a combined African American (AA) study, with 868 prostate cancer patients and 878 control subjects. For 17 of these 20 SNPs, implicated risk-associated alleles were found to be more common in these AA cases than controls, significantly more than expected under the null hypothesis ($P = 0.03$). Two of these 17 SNPs, located at 3p12, and region 2 at 8q24, were significantly associated with prostate cancer risk ($P < 0.05$), and only SNP rs16901979 at region 2 of 8q24 remained significant after accounting for 20 tests. A multivariate analysis of additional SNPs across the broader 8q24 region revealed three independent prostate cancer risk-associated SNPs, including rs16901979, rs13254738, and rs10086908. The first two SNPs were ~20 kb apart and the last SNP, a novel finding from this study, was ~100 kb centromeric to the first two SNPs. These results suggest that a systematic evaluation of regions harboring known prostate cancer risk SNPs implicated in other races is an efficient approach to identify risk alleles for AA. However, studies with larger numbers of AA subjects are needed, and this will likely require a major collaborative effort to combine multiple AA study populations. (Cancer Epidemiol Biomarkers Prev 2009;18(7):2145–9)

Introduction

Recent genome-wide association studies (GWAS) in four study populations of European descent have revealed more than a dozen prostate cancer risk–associated loci (1-8). Additional independent prostate cancer risk–associated loci were identified in fine mapping studies of these initially discovered loci (9-15). Confirmation of these risk loci among populations of European descent has been reported, but studies examining African American (AA) are limited. No GWAS of prostate cancer in AA has been published to date, and confirmation studies in AA were primarily limited to single nucleotide polymorphisms (SNP) at 8q24 (9, 11, 16). The goal of this study was to systematically evaluate, in an AA population, all reported prostate cancer risk associated loci identified in men of European descent.

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Subjects and Methods

Four independent study populations of AA were included in this combined analysis of 868 cases and 878 controls, including a hospital-based case-control study from Johns Hopkins Hospital (JHH; ref. 2), a population-based case-control study in the western part of North Carolina, a hospital-based case-control study from Wake Forest University School of Medicine (17), and a hospital-based case-control study from Washington University School of Medicine (Supplementary Methods; Table 1).

We selected 20 SNPs that were significantly associated with prostate cancer risk in four previous GWASs (1-8) and follow-up confirmation and fine mapping studies of European descent (9-15). We also selected 14 additional SNPs in the broader 8q24 region that were implicated in several AA populations (9, 11, 16). In addition, a subset ($n = 58$) of ancestry informative markers that have large differences in allele frequencies between the two parental populations of AA (Europeans and Africans) were also included (18). These SNPs were genotyped using a MassARRAY iPLEX system (Sequenom, Inc.). The range of genotyping call rate is from 0.989 to 0.999, with an average of 0.996. The concordance rate was 99.5% among the duplicated samples. Each of the autosomal SNPs was in Hardy-Weinberg equilibrium ($P \geq 0.05$) among control subjects using Fisher's exact test. The proportion of European ancestry was estimated for each subject based on the 58 ancestry informative markers using the computer program, STRUCTURE (19). Subjects with a proportion of European ancestry of >0.8 or with a high proportion of

Table 1. Subjects in four AA study populations

Study population	No. subjects		Mean (SD) age (y)		Mean (SD) proportion of European ancestry	
	Cases	Controls	Cases	Controls	Cases	Controls
JHH	555	353	56.8 (7.40)	54.4 (11.9)	0.22 (0.18)	0.19 (0.14)
Wake	59	66	58.1 (8.39)	58.0 (8.86)	0.21 (0.13)	0.21 (0.18)
NC	173	246	60.7 (7.26)	54.3 (9.54)	0.19 (0.12)	0.18 (0.12)
WashU	81	213	65.9 (10.6)	71.7 (5.69)	0.19 (0.16)	0.22 (0.16)
Total	868	878	58.5 (8.25)	58.9 (12.2)	0.21 (0.17)	0.20 (0.14)

Abbreviations: Wake, Wake Forest University School of Medicine; NC, North Carolina; WashU, Washington University School of Medicine.

missing genotype data (>50%) were excluded. A logistic regression model was used to test for association under an additive model and adjusting for age, ancestry proportion, and study (Supplementary methods).

Results and Discussion

Association tests were first done for each of the 20 SNPs that were implicated in populations of European descent in this combined AA study (Table 2A). For 17 of these 20 SNPs, the implicated risk-associated alleles were found to be more common in these AA cases than controls, and the differences were statistically significant for two of these SNPs ($P < 0.05$). The number of SNPs that have the same direction of association as in European populations significantly exceeded the expected number of 10 under the null hypothesis that none of these SNPs are associated with prostate cancer risk in AA ($P = 0.03$, goodness-of-fit test, following a χ^2

Table 2. Summary results of prostate cancer association in AAs

CHR	SNP	BP	Alleles	Risk allele	Allele frequency		Association test	
					Case	Cont	OR (95%CI)	P*
A. SNPs implicated in GWAS in populations of European descent								
2p15	rs721048	63,043,382	A/G	A	0.051	0.041	1.20 (0.86-1.68)	0.29
3p12	rs2660753	87,193,364	T/C	T	0.505	0.483	1.17 (1.02-1.35)	0.029
6q25	rs9364554	160,804,075	T/C	T	0.064	0.064	0.91 (0.68-1.22)	0.52
7p15	rs10486567	27,749,803	T/C	C	0.737	0.714	1.15 (0.99-1.34)	0.075
7q21	rs6465657	97,460,978	T/C	C	0.870	0.870	1.10 (0.89-1.36)	0.38
8q24 (region 2)	rs16901979	128,194,098	A/C	A	0.495	0.431	1.38 (1.19-1.60)	1.7E-05
8q24 (region 3)	rs6983267	128,482,487	T/G	G	0.910	0.899	1.22 (0.96-1.55)	0.10
8q24 (region 1)	rs1447295	128,554,220	A/C	A	0.326	0.325	1.06 (0.91-1.23)	0.46
9q33	rs1571801	121,506,927	T/G	T	0.151	0.138	1.07 (0.88-1.31)	0.48
10q11	rs10993994	51,219,502	C/T	T	0.620	0.604	1.11 (0.96-1.28)	0.15
10q26	rs4962416	126,686,862	G/A	G	0.175	0.174	1.04 (0.86-1.24)	0.72
11q13 (region 2)	rs12418451	68,691,995	A/G	A	0.120	0.127	0.91 (0.74-1.13)	0.39
11q13 (region 1)	rs10896449	68,751,243	A/G	G	0.705	0.690	1.08 (0.92-1.26)	0.35
17q12 (region 2)	rs11649743	33,149,092	T/C	C	0.937	0.931	1.13 (0.85-1.49)	0.40
17q12 (region 1)	rs4430796	33,172,153	T/C	T	0.362	0.325	1.16 (1.00-1.34)	0.056
17q24.3	rs1859962	66,620,348	G/T	G	0.333	0.318	1.05 (0.91-1.22)	0.50
19q13	rs887391	46,677,464	T/C	T	0.503	0.495	1.01 (0.89-1.16)	0.84
19q13 (KLK3)	rs2735839	56,056,435	A/G	G	0.671	0.694	0.86 (0.74-1.00)	0.056
22q13	rs9623117	38,776,619	T/C	C	0.758	0.749	1.09 (0.93-1.29)	0.30
Xp11	rs5945619	51,074,708	G/A	G	0.407	0.364	1.22 (0.99-1.49)	0.058
B. SNPs implicated in previous fine mapping studies of 8q24 among AA populations								
8q24†	rs7008482	126,336,812	T/G	G	0.858	0.842	1.25 (1.02-1.54)	0.034
8q24†	rs2124036	126,717,316	T/C	C	0.795	0.782	1.14 (0.95-1.36)	0.15
8q24†	rs780321	127,152,877	C/T	T	0.730	0.719	1.14 (0.97-1.33)	0.107
8q24‡	rs16901896	128,079,950	G/A	G	0.073	0.057	1.27 (0.95-1.69)	0.10
8q24‡	rs10086908	128,081,119	C/T	T	0.793	0.744	1.31 (1.11-1.55)	1.1E-03
8q24‡	rs4871005	128,082,558	C/A	A	0.714	0.670	1.24 (1.06-1.44)	6.4E-03
8q24§	rs1456310	128,121,615	G/A	G	0.379	0.399	0.91 (0.78-1.04)	0.17
8q24‡	rs1016343	128,162,479	T/C	T	0.245	0.213	1.16 (0.99-1.37)	0.071
8q24‡	rs6981122	128,163,642	A/C	C	0.722	0.690	1.22 (1.04-1.42)	0.013
8q24‡	rs7007694	128,168,348	C/T	T	0.779	0.749	1.15 (0.98-1.35)	0.10
8q24‖	rs13254738	128,173,525	A/C	C	0.681	0.623	1.36 (1.17-1.58)	4.9E-05
8q24‖	rs6983561	128,176,062	C/A	C	0.511	0.457	1.31 (1.13-1.52)	2.8E-04
8q24§	rs16901966	128,179,434	G/A	G	0.339	0.329	1.08 (0.93-1.26)	0.32
8q24‖	rs7000448	128,510,352	C/T	T	0.692	0.652	1.20 (1.03-1.40)	0.016

*Association was tested under an additive model and adjusted for age, ancestry proportion and study population.
† Reported in Robbins et al. (2007).
‡ Implicated in a subset of JHH study (372 cases and 350 controls).
§Reported in Salinas et al. (2008).
‖Reported in Haiman et al. (2007) and Salinas et al. (2008).

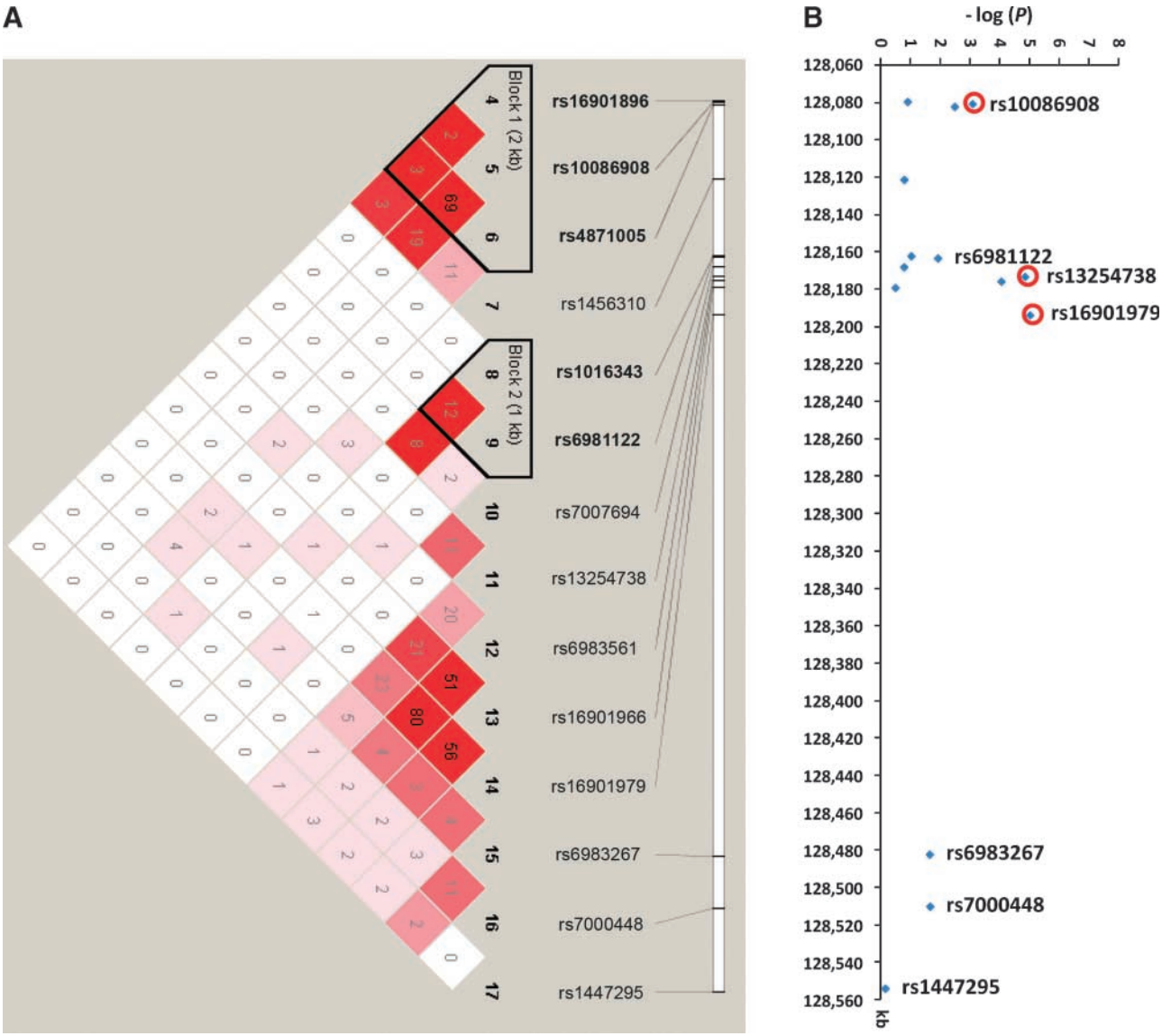

Figure 1. A schematic view of genetic association between SNPs at 8q24 and prostate cancer risk in the combined AA study. **A.** Association test for 14 SNPs at 8q24 (128,060,000-128,560,000) and prostate cancer risk among 853 patients and 874 control subjects assuming an additive model using a logistic regression model and adjusted for age, ancestry proportion, and study. Three SNPs with a red circle were independently associated with prostate cancer risk in a multivariate analysis. SNPs rs7008482, rs2124036, and rs780321 are not included in this figure because they are ~900 kb centromeric to these 14 SNPs. **B.** Inferred haplotype blocks of these 14 SNPs were estimated from the control subjects using the Haploview computer program, color scheme indicates pair-wise D' value with the brightest red for the strongest D' (= 1), and number indicates pair-wise r^2 values.

distribution with 1 degree of freedom), suggesting that some of these SNPs may also be associated with prostate cancer risk in AA. It is estimated that the study has 80% power to detect SNPs with allelic odds ratio (OR) of ≥1.3 and minor allele frequency of ≥20% at a significance level of 0.05. The limited number of SNPs reaching statistical significance level in this study may reflect the limited statistical power to detect association of moderate effect. We have also compared the association results for the 20 SNPs between this study in AAs and previous published studies in Caucasians (Supplementary Table S1). The strength of associations is similar among AAs and Caucasians, although these SNPs have very different allele frequencies in AAs and Caucasians.

Two of these 17 SNPs, located at 3p12, and region 2 at 8q24, were significantly associated with prostate cancer risk ($P < 0.05$) in this AA study. Of these, SNP rs16901979 at region 2 of 8q24 (nominal $P = 1.9 \times 10^{-5}$) is the only SNP that reached a study-wide significance level of 0.05 after adjustment for 20 independent tests. In addition, considerable but not statistically significant differences in allele frequencies between cases and controls were also found for SNPs at 7p15, 8q24 (region 3), 17q12 (Region 1),

and Xp11 ($P < 0.1$). Notably, SNP rs2735839 at 19q13 (*KLK3*) showed a reversed direction of association compared with that observed in Caucasians ($P = 0.056$).

We have also estimated the ORs for each study population separately and tested homogeneity of the OR among four study populations using a Breslow-Day homogeneity test (Supplementary Table S2). Significant heterogeneity (nominal $P < 0.05$) was found for two SNPs, rs11649743 and rs6981122. However, they were not statistically significant after taking multiple testing into account.

When 14 additional SNPs at 8q24 that were previously implicated in several AA study populations were examined in this combined study, 7 were significantly associated with prostate cancer risk at nominal P value of <0.05 (Table 2B). A multivariate analysis of all nominally significant 8q24 SNPs using a stepwise procedure and adjusted for ancestry proportion, age, and study populations revealed three independent prostate cancer risk–associated SNPs; they were rs13254738 ($P = 0.007$), rs16901979 ($P = 0.013$), and rs10086908 ($P = 0.001$). The first two SNPs have been previously implicated in populations of European descent (2, 3, 6, 8-11) and AA (2, 9, 11, 16). They were ~20 kb apart and were in weak linkage disequilibrium (LD) in the AA control subjects ($r^2 = 0.23$; Fig. 1). The last SNP (rs10086908), ~100 kb centromeric to the first two SNPs ($r^2 = 0$ with each of the two SNPs), was a novel finding. This SNP was evaluated in this study because it was initially implicated in a subset of the JHH AA study (372 cases and 350 controls; $P = 0.003$).[11] The association was replicated in the remaining 481 cases and 524 controls of this study ($P = 0.03$). We did not observe any significant pair-wise interaction among the three independent 8q24 risk SNPs, with P values ranging from 0.34 to 0.65.

Several other 8q24 SNPs, although not statistically significant in the multivariate analysis ($P > 0.05$), had results that were consistent with previous reports and warrant further evaluations in larger studies. Specifically, SNPs rs6983267 (region 3), rs7000448 (region 1), rs7008482, and rs780321 were reported to be associated with prostate cancer risk in at least one previous AA study (9, 11, 16). On the other hand, consistent with the results from two previous AA studies (11, 16), we did not find evidence for prostate cancer association with SNP rs1447295.

We have also tested the association between SNPs and disease aggressiveness. Although the association with prostate cancer was generally stronger among aggressive prostate cancer patients, no statistically significant difference in allele frequencies of these SNPs were found between aggressive and nonaggressive prostate cancer patients (Supplementary Table S3).

Although AAs have the highest incidence and mortality rate of prostate cancer, this group is severely under studied. GWAS of prostate cancer in AA can be a powerful approach to identify genetic risk variants; however, this is difficult to implement at this time because large sample sizes are needed for both the initial discovery and subsequent confirmation stages. A systematic evaluation of risk variants implicated in populations of European descent among AA is a feasible and effective alternative that has been empirically shown in this study. The discovery of three prostate cancer risk variants in AA, if confirmed, may be substantial, especially when considering that only three factors (age, family history, and race) have been documented in the past. In the future, these risk factors may be used to identify men at increased risk to prostate cancer for early screening, prevention, and diagnosis.

Several additional implications are worth noting. Novel prostate cancer risk variants at 8q24 in AA suggest that additional prostate cancer risk variants in AA may exist in the regions flanking other prostate cancer risk–associated SNPs that have been previously implicated by genome-wide studies of populations of European descent. In addition, the generally smaller LD blocks in AA may provide better positional information on causal, functional variants. Fine mapping association studies of each region harboring known prostate cancer risk–associated SNP among AA study populations are warranted. In addition, the substantially different genetic background and environmental exposures between populations of European and African descent may provide a unique opportunity to study gene-gene and gene-environmental interactions in disease risk (20). Finally, our results suggest that studies with larger numbers of AA subjects are needed to detect risk variants with moderate effect, and this will likely require a major collaborative effort to combine multiple AA study populations.

[11] Unpublished data.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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