# Common 8q24 Sequence Variations Are Associated with Asian Indian Advanced Prostate Cancer Risk

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## Abstract

**Three sequence variations (rs1447295, rs16901979, and rs6983267) on 8q24 were recently shown to independently affect prostate cancer risk. Asian Indians have a low prostate cancer risk; however, in the absence of screening practices for the disease, most are diagnosed with metastatic prostate cancer. We evaluated the association of these single nucleotide polymorphisms (SNP) with advanced prostate cancer in 153 prostate cancer cases and 227 age-matched controls (northern India). Overall, there was a positive association between carriers of the allele A of rs1447295 and prostate cancer risk [odds ratio (OR), 1.60; 95% confidence interval (95% CI), 1.01-2.52] but no significant association with carriers of alleles A of rs16901979 and allele G of rs6983267. However, significant associations were observed for both SNPs in men with high Gleason scores (≥7) and metastasis. Adjusting for age, the ORs were 1.77 (95% CI, 1.05-2.97) for carriers of rs1447295 A and 1.85 (95% CI, 1.04-3.28) for carriers of the rs16901979 A allele. We also observed significant joint effects among these loci associated with prostate cancer risk and severity, suggestive of additive effects of the independent SNPs. The ORs for the combined effects of rs1447295 A with rs16901979 A or rs6983267 G were 2.61 (95% CI, 1.11-6.12) and 1.84 (95% CI, 1.12-3.06), respectively. There was no joint effect between SNPs rs16901979 A and rs6983267 G. These results confirm the significance of these SNPs in prostate cancer etiology in a previously unstudied population who do not undergo prostate cancer screening and are diagnosed with severe disease.** (Cancer Epidemiol Biomarkers Prev 2008;17(9):2431–5)

## Introduction

Prostate cancer is the second leading cause of cancer death in Americans (1). Incidence, severity, and mortality rates vary widely by geographic location and ethnicity, being more common and aggressive in African Americans (2, 3). In contrast, prostate cancer incidence rates are strikingly low in native Asians (1). This disparity in incidence, one of the largest among all cancers, is likely caused by a combination of environmental and genetic factors (4-6), offering a unique opportunity to compare diverse populations to understand prostate cancer etiology.

Despite large-scale genetic studies, few prostate cancer risk genes have been identified, and confirmatory studies suggest these may have only modest effects (7-12). Recently, a genome-wide linkage study in Iceland (13) and an admixture study among African Americans (14) independently detected single nucleotide polymorphisms (SNPs) associated with prostate cancer risk on chromosome 8q24. Of these, rs1447295 (at-risk allele A) at 8q24.21, denoted region 1, was most strongly associated with prostate cancer risk in pooled case-control studies of Caucasians (13, 15-19). Weaker effects, however, were noted for this SNP among African-American men (15). This SNP was also reported to be associated with prostate cancer aggressiveness, but this effect varied widely by study (18, 19). Moreover, a second region approximately 350 kb upstream to rs1447295 also showed strong association with prostate cancer (15, 16) in both Caucasians and African Americans with rs16901979 (at-risk allele A) showing the strongest effects (15). These effects were independent of those detected for region 1. Finally, a third region located between regions 1 and 2, approximately 70 kb centromeric to rs1447295, was also found to be strongly associated with prostate cancer risk, suggesting the presence of at least three independent loci within 8q24 that may contribute to disease risk (15-17). A common SNP in this region, rs6983267 (at-risk allele G), was shown to be strongly associated with prostate cancer (16, 17).

The incidence of prostate cancer in India is low (20). However, in the absence of screening for prostate cancer, most patients are diagnosed in advanced stage. In this study, we have attempted to evaluate the role of the three principal SNPs at 8q24 with prostate cancer risk in this unique population.

## Materials and Methods

**Study Population and Data Collection.** Study participants were recruited through the Sanjay Gandhi Post

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**Table 1. Asian Indian (north India) sample characteristics**

| Prostate cancer characteristic | Controls ($n$ = 227) | Cases ($n$ = 153) |
|---|---|---|
| Mean (SD) age of diagnosis, y | 63.0 (9.4) | 65.9 (10.1) |
| Median prostate-specific antigen levels (ng/mL) | 0.9 | 31.0 |
| Family history, $n$ (%) | | |
| No | 227 (100) | 149 (97.4) |
| Yes | 0 | 4 (2.6) |
| Tumor stage, $n$ (%) | | |
| $T_1/T_2$ | — | 1 (1) |
| $T_3/T_4$ | — | 112 (99) |
| Tumor grade, $n$ (%) | | |
| <7 | — | 41 (27) |
| ≥7 | — | 112 (73) |
| Bone metastasis, $n$ (%) | | |
| None | — | 32 (21) |
| Any | — | 121 (79) |

Graduate Institute of Medical Sciences Hospital, Lucknow, India, between 2003 and 2006 as part of an ongoing prostate cancer case-control study. In the absence of screening practices and prostate cancer registries in India, patients are mainly diagnosed by appearing in the hospital with symptoms associated with prostate cancer disease. Therefore, the cases in this study represent accurately the cases of prostate cancer diagnosed in this population. Case status and clinical information were obtained by reviewing medical records. Prostate cancer was diagnosed primarily based on lower urinary tract symptoms, clinical impression of the prostate on digital rectal examination, and high prostate-specific antigen level. Patients who had a prior diagnosis of cancer at any other site were excluded. Controls, seen for routine checkups and other acute illnesses at the same institution, were ascertained concurrently with the cases and were matched on age. Controls were specifically screened for prostate cancer by prostate-specific antigen tests and excluded from the study if they had an elevated prostate-specific antigen (≥4 ng/mL), abnormal digital rectal examination, or previous cancer diagnosis. This study has institutional review board approval at Sanjay Gandhi Post Graduate Institute of Medical Sciences and Cornell.

**Genotyping.** DNA was extracted from blood samples using standard methods. Genotyping analyses were done using Assay-by-Design Taqman assays (ABI) and the ABI PRISM 7900 HT System as described previously (8). Call rate for the various SNPs exceeded 99%. To insure the reproducibility of the assays, 5% of all samples were analyzed in duplicates with complete agreement between replicates.

**Statistical Analysis.** Allele and genotype frequencies were computed using gene counting methods. Hardy-Weinberg equilibrium was evaluated in cases and controls assuming random mating. Genotype frequencies were in Hardy-Weinberg equilibrium for all three SNPs. Differences in allele and genotype frequencies were evaluated using Fisher's exact tests. To maximize power, tests for associations between genotype and prostate cancer risk were done based on allele frequencies by combining putative rare risk alleles into binary genotype classes. Although data were explored under different models, the results presented herein are primarily for the dominant model, as this model has the best fit to the data. Genotype-disease associations by Gleason grade and metastasis were estimated by computing odds ratios (OR) adjusted by age using unconditional logistic regression. To fully explore the data, stratified analyses were also undertaken by age of diagnosis, although these analyses were limited by sample size. Because most of the patients were diagnosed with late-stage disease, association studies of genotypes by stage were not possible. Pairwise multigenotype association studies were done using a multiplicative pairwise model of the joint effects of two genes. All statistical analyses were undertaken using STATA version 8.0 (Stata).

## Results

Characteristics of the Asian Indian study sample are shown in Table 1. The median age at diagnosis for the patients was 65.9 years (range, 46-86 years). Median Gleason score at diagnosis was 8.0. Twenty-seven percent of the patients were diagnosed with low-grade disease and 73% with high-grade (Gleason ≥7) disease. The majority of cases had late-stage prostate cancer with 65% of the men having both high-grade disease and bone metastasis.

Genotyping results showed that all at-risk SNP alleles were more common among cases compared with controls. However, of these, only rs16901979 A was significantly more common in prostate cancer cases compared with controls (12.8% vs 8.0%; $P$ = 0.04). The frequencies of rs1447295 A was 12.6% in controls and 17.3% in cases ($P$ = 0.18), whereas the frequency of the more common rs6983267 G allele was 49% in controls and 54% in cases ($P$ = 0.83).

SNP rs1447295 A in carriers was significantly associated with increased risk of prostate cancer. The age-adjusted ORs for men carrying any copy of rs1447295 A, relative to noncarriers of the A allele, were 1.60. This allele was also significantly associated with severe disease (Table 2). The ORs for carriers of any copy of the A allele with high-grade disease or metastasis, compared with controls, were 2.25. There was no significant association between carriers of rs16901979 A and prostate cancer risk overall, but a strong effect was noted in patients with high Gleason score and metastasis (Table 2). The effect of rs1447295 A and rs16901979 A genotypes overall did not differ significantly between individuals above and below age 64 years (data not shown). No significant association was observed for carriers of any copy of rs6983267 G allele with prostate cancer risk.

When we considered pairwise effects of any of the rs1447295 A and rs16901979 A genotypes versus neither or only one genotype on prostate cancer risk, a strong positive association with prostate cancer risk overall was observed with an estimated OR of 2.61 (Table 3). The OR for men with severe disease who had at least one risk allele at both loci, relative to the referent category, was 2.99. The estimated OR for individuals who had at least one at-risk rs1447295 A and rs6983267 G alleles was 1.84, whereas the OR was 3.03 for individuals who were homozygous for rs6983267 G and had at least one copy of

**Table 2. Associations of susceptibility genotypes at 8q24 and prostate cancer in Asian Indians**

| At-risk genotypes | No. (%) individuals and age-adjusted OR (95% confidence interval) compared with controls | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Controls | Cases | Gleason < 7 | Gleason ≥ 7 | No metastasis | Any Metastasis | Gleason <7 and no metastasis | Gleason ≥7 or metastasis | Gleason ≥7 and metastasis |
| Rs1447295 any A | 51 (22.6) | 49 (32.0) | 12 (29.3) | 37 (33.0) | 4 (12.5) | 45 (37.2) | 1 (5.3) | 14 (40.0) | 34 (34.3) |
| | | 1.60 (1.01-2.52) | 1.40 (0.67-2.93) | 1.67 (1.01-2.75) | 0.48 (0.16-1.44) | 2.00 (1.23-3.24) | 0.19 (0.02-1.44) | 2.25 (1.07-4.74) | 1.77 (1.05-2.97) |
| rs16901979 any A | 36 (15.9) | 34 (22.2) | 7 (17.1) | 27 (24.1) | 3 (9.4) | 31 (25.6) | 2 (10.5) | 6 (17.1) | 26 (26.3) |
| | | 1.50 (0.88-2.50) | 1.07 (0.44-2.60) | 1.65 (0.94-2.89) | 0.54 (0.16-1.86) | 1.79 (1.04-3.08) | 0.61 (0.14-2.76) | 1.08 (0.42-2.78) | 1.85 (1.04-3.28) |
| rs6983267 any G | 171 (75.3) | 120 (78.4) | 30 (73.2) | 90 (80.4) | 25 (78.1) | 95 (78.5) | 14 (73.7) | 27 (77.1) | 79 (79.8) |
| | | 1.21 (0.74-1.98) | 0.91 (0.43-1.93) | 1.36 (0.78-2.38) | 1.19 (0.49-2.90) | 1.22 (0.72-2.07) | 0.93 (0.32-2.71) | 1.12 (0.48-2.62) | 1.32 (0.74-2.34) |

NOTE: Deviations from Hardy-Weinberg proportions based on $\chi^2$ test at $P < 0.05$. (none are out of Hardy-Weinberg equilibrium).

**Table 3. Pairwise effects of genotypes at 8q24 on prostate cancer in Asian Indians**

| Genotype combination | No. (%) individuals and age-adjusted OR (95% confidence interval) compared with controls | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Controls | Cases | Gleason < 7 | Gleason ≥ 7 | No Metastasis | Any Metastasis | Gleason <7 and no metastasis | Gleason ≥7 or metastasis | Gleason ≥7 and metastasis |
| rs1447295 any A | 9 (4.0) | 15 (9.80) | 3 (7.3) | 12 (10.7) | 1 (3.1) | 14 (11.6) | 0 | 4 (11.4) | 11 (11.1) |
| rs16901979 any A | | 2.61 (1.11-6.12) | 1.89 (0.49-7.30) | 2.88 (1.17-7.05) | 0.77 (0.95-6.30) | 3.14 (1.32-7.48) | — | 3.09 (0.90-10.65) | 2.99 (1.20-7.48) |
| rs6983267 any G | 29 (12.8) | 22 (14.4) | 6 (14.6) | 16 (14.3) | 3 (9.4) | 19 (15.7) | 2 (10.5) | 5 (14.3) | 15 (15.2) |
| rs16901979 any A | | 1.13 (0.62-2.05) | 1.15 (0.45-2.98) | 1.12 (0.58-2.17) | 0.70 (0.20-2.43) | 1.25 (0.67-2.35) | 0.79 (0.17-3.61) | 1.12 (0.40-3.12) | 1.20 (0.61-2.36) |
| rs1447295 any A | 36 (15.9) | 40 (26.14) | 9 (22.0) | 31 (27.7) | 4 (12.5) | 36 (29.8) | 1 (5.3) | 11 (31.4) | 28 (28.3) |
| rs6983267 any G | | 1.84 (1.12-3.06) | 1.46 (0.64-3.32) | 1.99 (1.15-3.44) | 0.74 (0.25-2.25) | 2.20 (1.30-3.73) | 0.29 (0.04-2.23) | 2.38 (1.07-5.29) | 2.05 (1.17-3.60) |

1447295 A relative to controls. The estimated combined genotype effects of rs1447295 A with either rs16901979 A or rs6983267 G became the strongest for individuals with severe disease. We observed no significant joint effects involving any of the A and G genotypes at rs16901979 and rs6983267, respectively.

## Discussion

Our study confirms an association between carriers of the allele A of rs1447295 and prostate cancer and the joint effect of rs1447295 A with either rs16901979A or rs6983267 G alleles on prostate cancer risk compared with controls in a sample of Asian Indian men with advanced prostate cancer from north India. Chromosome 8q24 polymorphisms have not been studied at all in populations with low risk for prostate cancer. Thus, our research is the first to investigate the role of these SNPs in prostate cancer risk focusing on the lowest-risk racial group for prostate cancer, showing a strong association with advanced disease.

Overall, the allele frequencies and ORs we observed in our cases and controls for 8q24 polymorphisms for Asian Indians were similar but not identical to previously reported frequencies and OR values for Caucasians (15, 17, 19) and varied significantly from the values reported for African Americans in whom the risk alleles at these SNPs were considerably more common (15, 16).

The present study has identified significant associations between carriers of rs1447295 A and prostate cancer, consistent with the previous findings reported in Caucasian populations (15-17). In this study, carriage of the rs1447295 A allele was associated with a 60% increased risk for prostate cancer, which is moderately lower than the risk of 87% reported by Wang et al. (19) in aggressive prostate cancer in European Americans. In a combined analysis with four replication studies in Caucasians, Yeager et al. reported ORs of 1.43 and 2.23 for rs1447295 A heterozygotes and homozygotes, respectively (17), whereas combining results from other Caucasian groups gave an estimated OR of 1.53 for this SNP, using an additive genetic model (15). Interestingly, Wang et al. did not find any significant association between this variant and sporadic prostate cancer (19). Also, no significant association was noted in African Americans, where the frequency of the A allele was significantly higher (34%) than in Caucasians or Asian Indians (15).

In this study, we did not observe an association of rs16901979 A or rs6983267 G SNPs with prostate cancer risk overall, although these variants were clearly more common among cases compared with controls. These results are in contrast to findings that noted an association of these genotypes with prostate cancer in Caucasians and African Americans (15-17), perhaps reflecting a lack of statistical power in our sample.

In this study, we also investigated the association of the variants with prostate cancer clinical phenotype. The risk for men carrying rs1447295 A was significantly higher in patients with high-grade disease and/or bone metastasis compared with controls, but no association was observed in the group of cases with less aggressive disease. Whereas some studies did not observe an association with tumor aggressiveness (18, 19, 21), other studies noted an increased risk for high-grade disease, confirming the initial findings of Amundadottir et al. (13, 17, 22). We also observed significant associations of rs16901979 A with severe disease but without differences by age. Gudmundsson et al., however, noted an increased risk of rs16901979 A for early-onset disease in their combined sample of Caucasians and African Americans (15). The effect of rs6983267 was also significantly greater among African Americans diagnosed at younger ages and among those with high-stage disease (16). In contrast, there was no association of rs6983267 with prostate cancer clinicopathologic characteristics in the combined Caucasian sample investigated by Yeager et al. (17). These inconsistent results may be due to racial differences as well as sample size limitations and clinical heterogeneity among studies.

When these SNPs were considered in pairwise association studies to determine the effects of having genotypes with at least one of the at-risk alleles, some effects became considerably stronger, particularly for rs1447295 A/rs16901979 A and rs1447295 A/rs6983267 G and severe disease. These results are suggestive of additive effects of the independent SNPs, because their joint effects are very similar to the sum of the ORs generated by each SNP independently. This is consistent with the results reported by Yeager et al. that noted a strong effect with an OR of 3.13 for individuals homozygous for both risk alleles (17). A positive effect for the joint risk of rs1447295 A with rs16901979 A was also reported by Gudmundsson et al. with an OR of 1.53 in the all-European study group (15), although this effect was not as strong as the one seen in our study. No joint effect was noted for rs16901979 A and rs6983267 G genotypes. These findings are interesting because they suggest associations that may have their greatest effect on severe prostate cancer. In addition, because these variants are considerably more common among African Americans, they may explain, at least in part, the increased prostate cancer risk in this population.

In summary, we have detected significant association of the 8q24 SNPs with advanced prostate cancer. Joint effects among SNPs are likely to have a considerable effect on disease risk and severity. The present results also warrant further studies to better elucidate the role of this chromosomal region in prostate cancer susceptibility.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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