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Evidence for Molecular Differences in Prostate Cancer between African American and Caucasian Men

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Abstract

Purpose: The aim of this study was to compare the frequency of *ERG* rearrangement, *PTEN* deletion, SPINK1 overexpression, and *SPOP* mutation in prostate cancer in African American and Caucasian men.

Experimental design: Dominant tumor nodules from radical prostatectomy specimens of 105 African American men (AAM) were compared with 113 dominant nodules from Caucasian men (CaM). Clinical and pathologic characteristics of the two groups were similar. SPINK1 overexpression was evaluated by immunohistochemistry, *ERG* rearrangement and *PTEN* deletion by FISH, and *SPOP* mutation by Sanger sequencing.

Results: *ERG* rearrangement was identified in 48 of 113 tumors (42.5%) in CaM and 29 of 105 tumors (27.6%) in AAM ($P = 0.024$). *PTEN* deletion was seen in 19 of 96 tumors (19.8%) in CaM and 7 of 101 tumors (6.9%) in AAM ($P = 0.011$). SPINK1 overexpression was present in 9 of 110 tumors (8.2%) in CaM and 25 of 105 tumors (23.4%) in AAM ($P = 0.002$). *SPOP* mutation was identified in 8 of 78 (10.3%) tumors in CaM and 4 of 88 (4.5%) tumors in AAM ($P = 0.230$). When adjusted for age, body mass index, Gleason score, and pathologic stage, *ERG* rearrangement and SPINK1 overexpression remain significantly different ($P = 0.018$ and $P = 0.008$, respectively), and differences in *PTEN* deletion and *SPOP* mutation approach significance ($P = 0.061$ and $P = 0.087$, respectively).

Conclusions: Significant molecular differences exist between prostate cancers in AAM and CaM. SPINK1 overexpression, an alteration associated with more aggressive prostate cancers, was more frequent in AAM, whereas *ERG* rearrangement and *PTEN* deletion were less frequent in this cohort. Further investigation is warranted to determine whether these molecular differences explain some of the disparity in incidence and mortality between these two ethnic groups. *Clin Cancer Res; 20(18); 4925–34. ©2014 AACR.*

Introduction

Prostate cancer is known to exhibit differences among racial/ethnic groups

African American men (AAM) have a higher incidence of and mortality from prostate cancer than those observed in Caucasian men (CaM) as well as other ethnicities (1). Many factors have been postulated to contribute to incidence and/or mortality differences, such as access to care, attitudes toward care, socioeconomic and educational disparities, differences in type and aggressiveness of treatment, and dietary fat intake (2). Some studies have shown that when these factors are controlled, there is no difference in mortality, but the incidence of prostate cancer in AAM has consistently been shown to be higher (3).

Biochemical recurrence has also been demonstrated to be higher in locally advanced disease in AAM, although a difference in biochemical recurrence was not detected between AAM and CaM with organ-confined prostate cancer after radical prostatectomy (4). PSA levels have also been demonstrated to be higher in AAM than CaM with locally advanced prostate cancer (4). Genetic differences in prostate cancer between AAM and CaM are postulated to contribute to these disparities.

Genetic differences in prostate cancer

Differences in genes involved in the androgen signaling pathways have been observed between AAM and CaM, favoring increased androgen activity in AAM (5–7). Also, increased testosterone levels in AAM as compared with CaM

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Translational Relevance

African Americans have a higher incidence of prostate cancer and higher mortality from the disease than those rates observed in Caucasians. Although socioeconomic factors may contribute to these differences, underlying genetic differences are believed to play a role as well. In this study, we highlight significant differences in *ERG* gene rearrangement, *PTEN* deletion, SPINK1 overexpression, and *SPOP* mutation status in prostate cancer of African American men compared with Caucasian men. Our findings suggest biologic differences between prostate cancers from these two ethnic groups, with *ERG* rearrangement, *PTEN* deletion, and *SPOP* mutation less frequent in African American men and SPINK1 overexpression more frequent. In view of forthcoming new molecular diagnostic modalities and targeted therapies for prostate cancer, molecular classification of this disease is germane; understanding ethnic differences in this disease will allow for optimizing screening methods and selecting appropriate treatment plans.

have been shown in some studies (8, 9). Several more recent studies have demonstrated differences in gene methylation and aggressive biomarker expression in prostate cancers between CaM and AAM (10–13), strongly suggesting that genetic differences do exist and at least partially contribute to differences observed in clinical outcomes between these two populations.

More is now known about specific molecular aberrations in prostate cancer, with several new discoveries over the past decade. These include recurrent gene fusions involving androgen-regulated genes (i.e., *TMPRSS2*) and *ETS* family genes (14), *PTEN* genomic deletion (15–18), overexpression of SPINK1 (a low molecular weight trypsin inhibitor; refs. 19, 20), and, more recently, nonsynonymous somatic mutations of *SPOP* (21). Several previous studies have examined the prevalence of *ERG* rearrangement in AAM (22–24), all of which found a lower frequency of *ERG* rearrangement and/or ERG overexpression in AAM. Similarly, another study found increased *ERG* gene expression in prostate cancers among CaM relative to AAM when gene-expression profiling was performed (24). To our knowledge, our study is the first to compare the prevalence of *PTEN* deletion, SPINK1 overexpression, and *SPOP* mutation between AAM and CaM. Furthermore, our study reports on all four of these molecular aberrations in AAM and CaM who were treated at a single academic medical center and demonstrated similar pre- and post-operative clinicopathologic features.

Materials and Methods

Case selection

All parts of this retrospective study were carried out following Institutional Review Board approval. Archival formalin-fixed, paraffin-embedded (FFPE) radical prostatectomy (RP) specimens from 105 consecutive self-identified AAM who underwent RP between 2001 and 2011 were retrieved. Archival FFPE specimens from an existing tissue microarray (TMA) cohort of 113 representative self-identified CaM who underwent RP from 2007 to 2009 were included as controls. Although year of surgery was more variable in the AAM cohort, the remaining clinical and pathologic characteristics of the two groups were similar (Table 1). All patients were treated at our institution, a tertiary care academic medical center, and all patients had preexisting health insurance, suggesting equal access to care. Furthermore, there was no significant difference in the type of primary insurance between the two groups (private vs. government-sponsored) with 81 of 105 AAM (77%) and 81 of 113 CaM (72%) having only private insurance ($P = 0.36$). No patients received hormonal- or radiotherapy before surgery.

Biochemical recurrence information was available for the majority of men; however, these rates were not adjusted for post-RP treatment, as post-RP treatment was administered at the discretion of the treating physicians. Biochemical recurrence was defined as a post-operative PSA value of >0.2 ng/mL on two separate occasions. The median follow-up time in the CaM cohort was 44 months, and the median follow-up time in the AAM cohort was 41 months. There were 3 CaM and 24 AAM who were lost to follow-up.

Pathologic evaluation and TMA construction

Slides of the FFPE tissue from all RP specimens were reviewed by study pathologists to confirm the pathologic characteristics (tumor–node–metastasis stage, Gleason score, margin status). The dominant tumor nodule, defined as the tumor with highest pathologic tumor stage, was selected from each case for construction of TMAs. TMAs were constructed using 0.6-mm cores from the FFPE blocks, with each sample represented in triplicate.

FISH analysis of *ERG* rearrangement and *PTEN* deletion

5-μm-thick tissue sections from the TMA blocks were used for FISH analysis. For detection of *ERG* rearrangement, a dual-color break-apart interphase FISH assay was performed as previously described (14, 25). Briefly, *ERG* rearrangement status was assessed using centromeric (BAC clone RP11–24A11-labeled red) and telomeric (BAC clone RP11–372O17-labeled green) probes (Fig. 1). If >20% of tumor cells were found to have translocation or deletion, the tumor was considered to have an *ERG* rearrangement. For detection of *PTEN* deletion, a gene-specific probe (BAC clone CTD-2047N14) and a reference probe located at 10q25.2 (RP11–431P18) were used (Fig. 1). Deletion of *PTEN* was defined as fewer than two copies of the gene-specific probe in the presence of two reference signals in >20% of the tumor nuclei. For detection of both *ERG* rearrangement and *PTEN* deletion, at least 200 tumor nuclei per case were evaluated using a fluorescence microscope (Olympus BX51; Olympus Optical).

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Table 1. Clinical and pathologic characteristics of 218 men with prostate cancer treated by radical prostatectomy

	Caucasian	African American	*P*
Number of men	113	105	
Age at surgery			0.148
Mean ± SD	61.2 ± 7.1	59.6 ± 7.6	
Range	45.6–75.5	37.0–73.1	
BMI			0.924
Mean ± SD	26.9 ± 3.2	27.0 ± 5.4	
Range	22.0–38.0	19.1–36.0	
Gleason score [no.(%)]			0.601
6	18 (16)	19 (18)	
7(3+4)	63 (56)	57 (54)	
7(4+3)	18 (16)	21 (20)	
8 and 9	14 (12)	8 (8.0)	
Pathologic stage [no. (%)]			0.160
T2	80 (71)	85 (81)	
T3a	25 (22)	13 (12)	
T3b	8 (7.1)	7 (6.6)	
Margin positivity [no./total (%)]	18/113 (16)	13/105 (12)	0.561
Biochemical recurrence [no./total (%)]	14/110 (12)	12/81 (15)	0.665

Immunohistochemical analysis of ERG and SPINK1 overexpression

Immunohistochemical staining was applied using a commercially available antibody for SPINK1 (clone 4D4, 1:100 dilution; Abnova) and ERG (clone EPR 3864, 1:100 dilution; Epitomics) on the Discovery XT biomarker platform (Ventana Medical Systems, Inc.; Fig. 1). Semiquantitative evaluation of cytoplasmic SPINK1

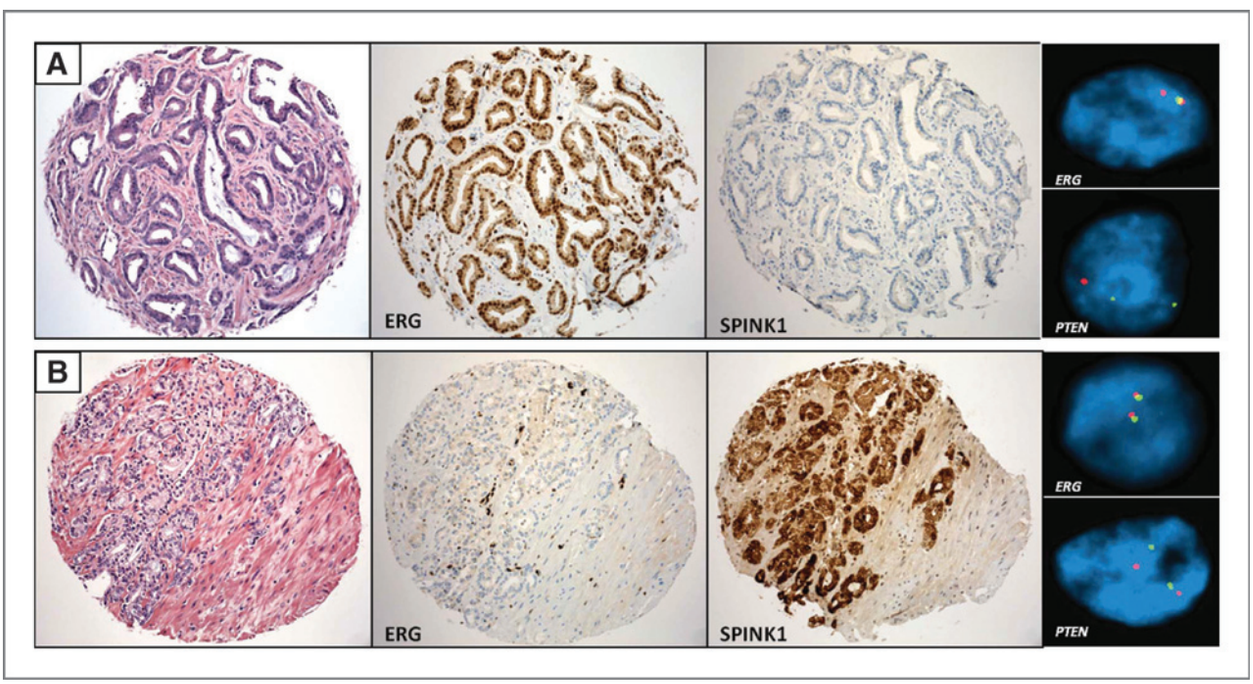

Figure 1 Immunohistochemical staining for ERG and SPINK1 as well as FISH for *ERG* rearrangement and *PTEN* deletion. A, shows a prostatic adenocarcinoma that demonstrates positive *ERG* immunostaining and a corresponding *ERG* rearrangement by FISH. The tumor is negative for SPINK1 overexpression and shows a hemizygous deletion of *PTEN*. B, shows a prostatic adenocarcinoma that demonstrates negative *ERG* immunostaining and no *ERG* rearrangement by FISH. The tumor shows SPINK1 overexpression and no deletion of *PTEN* by FISH.

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Table 2. Prevalence of molecular aberrations in prostate cancer of AAM versus CaM

	African American	Caucasian	*P*	Adjusted *P* value[a]
ERG rearrangement	27.6% (29/105)	42.5% (48/113)	0.024	0.018
PTEN deletion	6.9% (7/101)	19.8% (19/96)	0.011	0.061
SPINK1 overexpression	23.8% (25/105)	8.2% (9/110)	0.002	0.008
SPOP mutation	4.5% (4/88)	10.3% (8/78)	0.230	0.087

[a]Adjusted for age, BMI, Gleason score, and pathologic stage.

expression and nuclear ERG expression was separately performed. Staining of ≥5% of tumor cells was considered positive for each case.

SPOP mutation analysis

Using tissues cores from either fresh-frozen material or archival FFPE blocks, samples of the same tumor nodule used for TMA construction were evaluated for *SPOP* mutations. DNA from fresh-frozen material was extracted using phenol–chloroform and purified by the ethanol precipitation method as previously described (26). DNA from archival FFPE material was extracted using the Qiagen Biorobot Universal system. High-resolution melt analysis followed by direct Sanger sequencing of putative *SPOP* somatic mutations was performed by standard methods following PCR amplification using specific primers. Sequences of the primers used for amplifying and sequencing *SPOP* have been recently described (21).

Statistical analysis

χ^2 or Fisher exact tests were used to evaluate association between categorical variables. The Wilcoxon rank-sum test was performed to compare continuous variables (e.g., age) between groups. For all statistical tests, a *P* value of <0.05 was considered statistically significant.

Results

ERG rearrangement, *PTEN* deletion, SPINK1 overexpression, and *SPOP* mutation in prostate cancer differ in African American versus Caucasian men

ERG rearrangement was identified in 48 of 113 tumors (42.5%) of CaM. In AAM, however, *ERG* rearrangement was found in 29 of 105 tumors (27.6%; $P = 0.024$). There was no significant difference in the mechanism of gene fusion between the two cohorts (translocation vs. translocation with deletion). Of note, *ERG* rearrangement by FISH and protein overexpression by immunohistochemistry were concordant in all cases. Hemizygous deletion of *PTEN* was seen in 19 of 96 tumors (19.8%) in CaM but only 7 of 101 tumors (6.9%) in AAM ($P = 0.011$). SPINK1 overexpression was present in 9 of 110 tumors (8.2%) from CaM in contrast with 25 of 105 tumors (23.8%) from AAM ($P = 0.002$). *SPOP* mutations were present in 8 of 78 (10.3%) prostate cancers in CaM in contrast with 4 of 88 (4.5%) prostate cancers in AAM; however, this difference was not statistically significant ($P = 0.230$). In CaM, *SPOP* mutations involved the F133 (6 cases), F102 (1 case), and K129 (1 case) residues. *SPOP* mutations in AAM involved the F133 (2 cases), F102 (1 case), and Y87 (1 case) residues.

When adjusted for age, body mass index (BMI), Gleason score, and pathologic stage, *ERG* rearrangement and SPINK1 overexpression remained significantly different between the two cohorts ($P = 0.018$ and $P = 0.008$, respectively) and differences in *PTEN* deletion and *SPOP* mutation status approached statistical significance ($P = 0.061$ and $P = 0.087$, respectively). Table 2 summarizes the frequency of all of these molecular findings in the two cohorts.

Association of molecular abnormalities with clinical and pathologic characteristics

When considering both ethnic groups combined, prostate cancers harboring *PTEN* deletions were found to be significantly associated with higher average age ($P = 0.001$), higher Gleason score ($P < 0.001$), higher pathologic stage ($P = 0.003$), and increased rate of biochemical recurrence ($P = 0.024$). All other clinicopathologic parameters were statistically similar with respect to each molecular abnormality. Table 3 summarizes these findings.

Among CaM alone, prostate cancers harboring *PTEN* deletions were found to be significantly associated with higher average age ($P = 0.002$), higher Gleason score ($P = 0.009$), higher pathologic stage ($P = 0.006$), and increased rate of biochemical recurrence ($P = 0.034$; Supplementary Table S1). SPINK1 overexpression was associated with a lower Gleason score in the CaM cohort ($P = 0.016$; Supplementary Table S1). In the AAM cohort, all clinical and pathologic parameters were statistically similar with respect to each molecular abnormality (Supplementary Table S2).

Because the calendar years in which the AAM and CaM cases were accrued were disparate (2001–2011 and 2007–2009, respectively), statistical comparisons of the clinicopathologic characteristics of patients accrued before 2007, from 2007 to 2009, and after 2009, were performed. No significant differences were observed with respect to preoperative PSA, Gleason score, pathologic stage, or the frequency of each of the molecular abnormalities ($P > 0.05$ for all; data not shown).

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Table 3. Association of clinical and pathologic characteristics with molecular abnormalities (combined AAM and CaM cohorts)

	ERG rearrangement			*PTEN* deletion			SPINK1 overexpression			*SPOP* mutation		
	Positive	Negative	*P*	Deleted	Wild-type	*P*	Positive	Negative	*P*	Mutated	Wild-type	*P*
Age at surgery (mean ± SD)	59.9 ± 7.0	60.7 ± 7.6	0.298	64.6 ± 7.4	59.5 ± 7.3	0.001	57.6 ± 8.2	60.9 ± 7.1	0.067	60.8 ± 7.2	60.3 ± 7.4	0.812
BMI (mean ± SD)	26.9 ± 3.2	27.0 ± 4.8	0.622	26.4 ± 2.3	27.1 ± 4.6	0.628	26.0 ± 3.3	27.1 ± 4.5	0.190	27.0 ± 4.7	26.5 ± 3.8	0.899
Gleason score [no./total (%)]			0.285			<0.001			0.163			0.305
6	13/37(35)	24/37(65)		1/35(3)	34/35(97)		9/36(25)	27/36(75)		0/29(0)	29/29(100)	
7(3 + 4)	48/120(40)	72/120(60)		12/109(11)	97/109(89)		20/118(17)	98/118(83)		7/96(7)	89/96(93)	
7(4 + 3)	9/39(23)	30/39(77)		5/35(14)	30/35(66)		4/39(10)	35/39(90)		4/34(12)	30/34(88)	
8 and 9	7/22(32)	15/22(68)		8/18(44)	10/18(56)		1/22(5)	21/22(95)		1/19(5)	18/19(95)	
Pathologic stage [no./total (%)]			0.139			0.003			1.000			0.259
T2	59/165 (35.8)	106/165 (64.2)		13/149 (8.7)	136/149 (91.3)		26/162 (16.0)	136/162 (84.0)		11/123 (8.9)	112/123 (91.1)	
T3a	16/38 (42.1)	22/38 (57.9)		8/33 (24.2)	25/33 (75.8)		6/38 (15.8)	32/38 (84.2)		0/28(0)	28/28 (100)	
T3b	2/15 (13.3)	13/15 (86.7)		5/15 (33.3)	10/15 (66.7)		2/15 (13.3)	13/15 (86.7)		1/14 (7.1)	13/14 (92.9)	
Margin positivity [no./total (%)]	8/218 (3.7)	23/218 (10.5)	0.311	2/197 (1.0)	25/197 (12.7)	0.541	4/215 (1.9)	27/215 (12.6)	0.793	1/166 (0.6)	26/166 (15.7)	0.440
Biochemical recurrence [no./total (%)]	7/191 (3.6)	19/191 (9.9)	0.383	7/171 (4.0)	16/171 (9.3)	0.024	5/188 (2.6)	21/188 (11.1)	0.562	2/141 (1.4)	21/141 (14.9)	0.743

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ERG rearrangements and *SPOP* mutations are mutually exclusive, as are *PTEN* deletions and SPINK1 overexpression

ERG rearrangements and *SPOP* mutations were not seen together in any of the 178 tumors evaluable for both molecular alterations ($P = 0.009$). *PTEN* deletion and SPINK1 overexpression were also mutually exclusive in the 195 tumors evaluable for both events ($P = 0.009$). Furthermore, *ERG* rearrangement and SPINK1 overexpression were mutually exclusive in all but one of the 215 cases ($P < 0.001$). No association was noted between *ERG* rearrangement and *PTEN* deletion, *SPOP* mutation and *PTEN* deletion, or *SPOP* mutation and SPINK1 overexpression ($P > 0.05$). These findings are graphically depicted in Supplementary Fig. S1.

Discussion

We investigated molecular differences in prostate cancer between two clinicopathologically similar cohorts of AAM and CaM treated at our institution. Our findings are concordant with recent studies showing that there is a significantly lower prevalence of *ERG* gene rearrangements in prostate cancers of AAM when compared with CaM (22–24, 27). To our knowledge, this is the first study to evaluate ethnic differences in the prevalence of hemizygous *PTEN* deletions, SPINK1 overexpression, and *SPOP* mutation status in prostate cancer. These findings contribute to our understanding of biologic differences in prostate cancer between AAM and CaM, building essential groundwork for the development of personalized cancer treatment regimens.

The discovery of recurrent gene rearrangements in prostate cancer involving androgen-regulated genes (e.g., *TMPRSS2*) and *ETS* family genes (14) as well as more recent data from whole-genome sequenced localized prostate cancers (26, 28) has increased our understanding of the disease at the molecular level, identifying potentially diagnostic, prognostic, and therapeutic markers. Results from unscreened, population-based cohorts (e.g., Swedish Watchful Waiting Cohort) have suggested that untreated prostate cancer with *ERG* rearrangement runs a more aggressive clinical course than those without *ERG* rearrangement (29). In the setting of surgical or other interventions following diagnosis, the data are insufficient to make any reasonable conclusions. Yoshimoto and colleagues later demonstrated that absence of *ERG* rearrangement and *PTEN* loss in prostate cancer is associated with a favorable outcome (30). Conversely, duplication of *ERG* rearrangement with interstitial deletion of sequences 5′ to *ERG* identified cases of fatal human prostate cancer in patients that had been conservatively managed (15). Regarding distinct molecular characteristics of prostate cancer among different ethnic/racial groups, two recent studies have assessed the difference in prevalence of *ERG* rearrangements between AAM and CaM (22–24). In the study by Magi-Galluzzi and colleagues, *ERG* rearrangements were present in 50% of CaM versus 31% of AAM ($P = 0.07$) and in the study by Rosen and colleagues, *ERG* rearrangements were present in 41.9% of CaM versus 23.9% of AAM ($P < 0.0001$). These findings are in concordance with our study showing that *ERG* rearrangements are less frequent in prostate cancers in AAM (42.5% in CaM vs. 27.6% in AAM, $P = 0.024$). When adjusted for age, BMI, Gleason score, and pathologic stage, the difference remained significant ($P = 0.018$). Similar to the study by Magi-Galluzzi and colleagues, *ERG* rearrangement in our study did not correlate with other clinicopathologic parameters aside from ethnicity (22).

Regardless of *ERG* rearrangement's correlation with clinicopathologic features, ethnic differences in the prevalence of *ERG* rearrangements may have diagnostic implications, with urine-based screening tests currently under investigation (31, 32). A recent review by Truong and colleagues highlights *ERG* rearrangement transcripts as one of the more promising RNA markers for cancer detection in urine samples; a urine-based test that uses a combination of *ERG* rearrangement transcripts and prostate cancer antigen-3 has already been marketed for clinical use (33, 34). Considering that our study and previous ones (22–24, 27) have shown a decreased prevalence of *ERG* rearrangements or *ERG* expression in prostate cancer of AAM, such a urine-based diagnostic test will be less sensitive in this population and may not be as useful of a screening tool as it may be for prostate cancer in CaM.

PTEN, which encodes a phosphoinositide 3-phosphatase that negatively regulates the PI3K and mTOR signaling pathways, is a well-known tumor-suppressor gene in many tumor types; in prostate cancer, mutations in *PTEN* have been found to be associated with higher Gleason score, a higher rate of metastasis, androgen independence, and an overall worse prognosis (15–18). In a more recent, large, nested case–control study, decreased PTEN expression was shown to be associated with an increased risk of biochemical recurrence, independent of other clinicopathologic factors(35). Loss of PTEN results in elevated downstream activity in the PI3K and mTOR pathways, which have known therapeutic targets. Although therapeutic approaches to develop inhibitors targeting the PI3K–AKT pathway have failed in both preclinical and clinical trials for prostate cancer, there are newer AKT pathway inhibitors that show promise, such as AZD5363 (36). Specifically in prostate cancer cell lines, another recent study has shown that loss of PTEN and elevated AKT/mTOR activity is associated with sensitivity to ridaforolimus, a particular mTOR inhibitor under investigation (37). Although population-based mutational analyses on *PTEN* have been performed, there are few studies that have investigated ethnic differences in the prevalence of hemizygous loss of *PTEN* in specific tumors. One study by Winter and colleagues showed no significant racial differences in the expression of PTEN between invasive breast cancers in African American women and those in non-African American women (38). Similar to the racial disparities observed in prostate cancer, breast cancers in African American women are known to have a worse prognosis when compared with those in non-African

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Americans (39), but PTEN does not seem to play a major role in this disparity (38).

To our knowledge, our study is the first to compare the prevalence of deletions in *PTEN* in prostate cancer between AAM and CaM. We found that hemizygous deletions in *PTEN* were less frequently present in our cohort of AAM compared with that observed in CaM (6.9% vs. 19.8%, $P = 0.011$). However, when adjusted for age, BMI, Gleason score, and pathologic stage, the difference in prevalence was less pronounced and only trended toward significance ($P = 0.061$). Our findings suggest that *PTEN* deletions may not be critical contributors to the increased incidence or mortality of prostate cancer in AAM, but larger studies with more power are warranted to confirm or refute our finding.

As expected, and consistent with prior literature, *PTEN* deletions in our study were significantly associated with a higher average age of patients, higher Gleason score, higher pathologic stage, and increased rate of biochemical recurrence, though our analysis of biochemical recurrence is only a crude estimate that does not adjust for post-RP therapy and is limited by relatively short follow-up time. These associations were statistically significant in CaM when analyzed alone, whereas no significant clinicopathologic associations with *PTEN* deletion were identified in AAM alone, possibly attributable to the low number of *PTEN* deletions in our AAM cohort. Although alterations in the mTOR/AKT and PI3K may still be present in AAM and prove to be therapeutic targets, they may be less frequently due to *PTEN* deletion than in CaM.

SPINK1 has structural similarity to epidermal growth factor (EGF) and has been demonstrated to activate the EGF receptor (EGFR) on the surface of prostate cancer cells, leading to cell growth (20). Using a model of SPINK1-positive prostate cancer (22RV1 cells), Ateeq and colleagues showed that monoclonal antibodies to either SPINK1 or EGFR (cetuximab) could slow the growth of SPINK1-positive tumors by more than 60% and 40%, respectively, suggesting that it may be a reasonable therapeutic target (40). Moreover, SPINK1 overexpression has identified an aggressive subtype of *ETS*-negative prostate cancer, validated in different cohorts (19). SPINK1 overexpression and *ERG* rearrangements have been found to be mutually exclusive in other studies as well (41, 42), similar to our current findings. A study by Leinonen and colleagues found SPINK1 overexpression to be present in 10% of prostate cancers and also found it to be associated with an aggressive form of the disease, although mutual exclusivity with *ERG* rearrangements was not observed in this particular study (43).

Our study is the first to show that SPINK1 overexpression in a particular tumor type correlates with African American ethnicity. In our study, prostate cancers from AAM showed SPINK1 overexpression in 23.8% of cases compared with 8.2% of prostate cancers in CaM ($P = 0.002$), and this difference remained statistically significant after adjusting for age, BMI, Gleason score, and pathologic stage ($P = 0.008$). In the context of the aforementioned literature, which has demonstrated that SPINK1 overexpression is associated with more aggressive prostate cancers, our study suggests that SPINK1 overexpression may be one molecular aberrancy that plays a role in the increased incidence and/or mortality observed in AAM with prostate cancer, although we emphasize that our study was not designed to demonstrate association of these molecular alterations with clinical outcomes. Furthermore, any targeted therapies to SPINK1 that could develop in the future, as proposed by Ateeq and colleagues (40), potentially may benefit more AAM than CaM with prostate cancer.

More recently, whole-genome and -exome sequencing of prostate cancer has elucidated novel recurrent mutations in prostate cancer such as *SPOP*, *MED12*, and *FOXA1* (25, 26). The most common nonsynonymous somatic mutation involves *SPOP*, which encodes the substrate-binding subunit of a cullin-based E3 ubiquitin ligase (44, 45). This recurrent mutation defines a new molecular subtype of *ETS*-negative prostate cancer (21). After having sequenced the *SPOP* gene in more than 300 primary prostate cancers and metastases, all *SPOP* mutations affected conserved residues in the structurally defined substrate-binding cleft (21). Recent work in breast cancer has shown that SPOP directly interacts with a p160 steroid–resistant coactivator, SRC-3, part of a family of proteins that are overexpressed in numerous human cancers; they are associated with poor clinical outcomes and resistance to therapy and are considered to be potential therapeutic targets (45, 46). The interaction of SPOP and SRC-3 in breast cancer promotes cullin 3–dependent ubiquitination and proteolysis, thereby supporting the role of SPOP as a potential tumor suppressor (47).

In prostate cancer cell lines, SPOP mutants have been shown to be unable to interact with SRC-3 protein or promote its ubiquitination and subsequent degradation, suggesting that SPOP plays a critical tumor-suppressor role in prostate cancer and supporting the potential of SRC-3 as a therapeutic target in prostate cancer (48). In our study, *SPOP* mutations were less frequently seen in prostate cancers from AAM than from CaM, although this difference did not reach statistical significance ($P = 0.230$). When adjusted for age, BMI, Gleason score, and pathologic stage, however, this difference approaches statistical significance ($P = 0.087$). Although further work is needed to fully elucidate the biologic and prognostic significance of *SPOP* mutations in prostate cancer *in vivo* as well as the therapeutic potential of SRC-3, our findings suggest that *SPOP* mutations are less likely to play a significant role in prostate cancer in AAM compared with CaM.

Our study certainly is not devoid of limitations. First, our cohorts contained a relatively modest number of patients from a single institution; larger studies are needed to validate our findings. In addition, there were some cases for which there were missing data on the molecular alterations, highlighted in Supplementary Fig. S1, which was attributable to missing or insufficient tissue on the TMAs (for *ERG* rearrangement, SPINK1 overexpression, and *PTEN* deletion) or insufficient tissue for DNA extraction (for *SPOP* mutation analysis). Second, although two techniques were used to assess *ERG* rearrangement and were concordant, only one technique was used for the other molecular

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alterations, limiting our ability to confirm these molecular changes. Third, we also must emphasize that our study was not designed or powered to assess clinical outcomes of our patient cohorts, and, therefore, any conclusions to whether the molecular alterations in these patients have prognostic value would be premature. Assessment of biochemical recurrence in our cohorts was performed only to show rough consistency with prior literature on *PTEN* and its association with worse outcomes (15–18). In addition, the median length of follow-up time in both cohorts was relatively short (41 and 44 months in AAM and CaM, respectively) with a large number of AAM lost to follow-up (24 patients). Finally, we did not obtain socioeconomic data on our patient population, which has been shown to contribute to prostate cancer outcomes (2). However, given that all patients in our study were treated at a single academic tertiary care facility, that all patients had preexisting health insurance, and that >70% of patients in each cohort had private insurance coverage, our patients seem to have had equal access to oncologic care. The comparable (and mostly low) pathologic stages of the tumors between both cohorts at radical prostatectomy also suggest that screening and early diagnosis of prostate cancer occurred in both groups.

In an era in which precision therapy of prostate cancer is rapidly changing, molecular characterization of both localized and metastatic prostate tumors will help stratify which men will benefit from active surveillance, surgery, targeted therapy, and hormonal and/or chemoradiation therapy. Already, recent studies have shown that ethnicity is an important factor in the progression of prostate cancers under active surveillance (12, 49), suggesting perhaps that prostate cancers among different races should be managed differently. It has also been shown previously that prostate cancers with *ERG* rearrangement have a worse outcome under active surveillance (29), highlighting its potential importance in influencing therapeutic management. Furthermore, a study by Bismar and colleagues suggests that molecular aberrancies in *PTEN*, *ERG*, and SPINK1 may be involved in the development of castration-resistant prostate cancer, emphasizing their clinical importance (42). Our study highlights the significant differences that exist at the molecular level when prostate cancers from clinicopathologically similar AAM and CaM are compared. We have demonstrated that *ERG* rearrangement, *PTEN* deletion, and *SPOP* mutation have a lower prevalence in prostate tumors of AAM and likely play a lesser role in incidence or mortality differences. In contrast, SPINK1 overexpression, a molecular aberrancy that has been found to be associated with more aggressive prostate cancer (19, 41, 50), is more prevalent in AAM, suggesting that it plays a more important role in the disease within this ethnic group. As our study was not designed to assess clinical outcomes in association with these molecular alterations, larger studies with more detailed clinical outcome data will be needed to determine whether any of these molecular differences at least partially explain the disparities in incidence and mortality between these two ethnic groups. In addition, future work on whole-genome/exome sequencing of prostate cancer will help us to better characterize potential therapeutic targets in prostate tumors among different ethnic groups.

Disclosure of Potential Conflicts of Interest

A.K. Tewari reports receiving commercial research grants from Boston Scientific and Intuitive Surgical; and is a consultant/advisory board member for Global Prostate Cancer Research Foundation. C.E. Barbieri and M.A. Rubin are co-inventors of a patent on SPOP mutations in prostate cancer issued to Weill Medical College of Cornell University. No potential conflicts of interest were disclosed by the other authors.

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