Novel Biomarker Signature That May Predict Aggressive Disease in African American Men With Prostate Cancer

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose

We studied the ethnicity-specific expression of prostate cancer (PC) –associated biomarkers to evaluate whether genetic/biologic factors affect ethnic disparities in PC pathogenesis and disease progression.

Patients and Methods

A total of 154 African American (AA) and 243 European American (EA) patients from four medical centers were matched according to the Cancer of the Prostate Risk Assessment postsurgical score within each institution. The distribution of mRNA expression levels of 20 validated biomarkers reported to be associated with PC initiation and progression was compared with ethnicity using false discovery rate, adjusted Wilcoxon-Mann-Whitney, and logistic regression models. A conditional logistic regression model was used to evaluate the interaction between ethnicity and biomarkers for predicting clinicopathologic outcomes.

Results

Of the 20 biomarkers examined, six showed statistically significant differential expression in AA compared with EA men in one or more statistical models. These include *ERG* ($P < .001$), *AMACR* ($P < .001$), *SPINK1* ($P = .001$), *NKX3-1* ($P = .03$), *GOLM1* ($P = .03$), and androgen receptor ($P = .04$). Dysregulation of *AMACR* ($P = .036$), *ERG* ($P = .036$), *FOXP1* ($P = .041$), and *GSTP1* ($P = .049$) as well as loss-of-function mutations for tumor suppressors *NKX3-1* ($P = .025$) and *RB1* ($P = .037$) predicted risk of pathologic T3 disease in an ethnicity-dependent manner. Dysregulation of *GOLM1* ($P = .037$), *SRD5A2* ($P = .023$), and *MKI67* ($P = .023$) predicted clinical outcomes, including 3-year biochemical recurrence and metastasis at 5 years. A greater proportion of AA men than EA men had triple-negative (*ERG*-negative/*ETS*-negative/*SPINK1*-negative) disease (51% v 35%; $P = .002$).

Conclusion

We have identified a subset of PC biomarkers that predict the risk of clinicopathologic outcomes in an ethnicity-dependent manner. These biomarkers may explain in part the biologic contribution to ethnic disparity in PC outcomes between EA and AA men.

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INTRODUCTION

African American (AA) men experience a higher incidence of and mortality as a result of prostate cancer (PC) than men of other races and ethnicities, including European Americans (EA).^{1,2} This disparity has been attributed partly to socioeconomic factors and inadequate access to health care³ as well as to differences in genetic susceptibility.^{4,5} Although controversy remains about the etiology of disparities in outcomes, differences in disease aggressiveness at presentation suggest a potential role for biologic differences in prostate carcinogenesis between AA and EA men. However, meaningful comparisons of PC

biomarkers associated with PC aggressiveness by race or ethnicity are limited.

Recently, several biomarkers have been correlated with aggressive phenotypes in PC.⁶⁻¹³ These biomarkers show promise as predictors of aggressive disease and have the potential to inform which men may have unfavorable outcomes or may benefit from specific treatments. To date, these predictive biomarkers have been studied primarily in EA men. The relevance of these biomarkers to the observed increased aggressiveness and disease recurrence among AA men is not known.

In an attempt to decipher genetic/biologic differences in prostate tumors by ethnicity, we

Table 1. List of 20 Selected Biomarkers Associated With PC Pathogenesis and a Comparison of Their Expression Levels by Ethnicity Using Two Different Statistical Methods

Biomarker	Function	<i>P</i> *	
		Mann-Whitney	Logit
<i>ERG</i>	Found in 36%-78% of samples; associated with aggressive PC	< .001	< .001
<i>AMACR</i>	Overexpressed in PC relative to benign prostatic tissue	< .001	< .001
<i>SPINK1</i>	Overexpressed in high-grade PC	.001	.028
<i>NKX3-1</i>	Loss associated with advanced-stage PC and CRPC	.029	.064
<i>GOLM1</i>	Upregulated in > 90% of PC tissues (unknown function)	.029	.019
<i>AR</i>	Predictor of decreased biochemical recurrence-free survival	.041	.096
<i>RB1</i>	Loss coincides with emergence of metastatic CRPC	.077	.097
<i>GSTP1</i>	Hypermethylated in 60%-80% of PC; in serum, urine, biopsy tissue	.129	.073
<i>MKI67</i>	Correlates with cancer-specific and overall survival	.129	.115
<i>FOXP1</i>	Negatively regulates <i>AR</i> signaling in PC	.129	.164
<i>EZH2</i>	Implicated in the pathogenesis of metastatic PC	.170	.164
<i>TP53</i>	Exon 6 and 7 mutations correlate with PC tumor progression	.192	.310
<i>MSMB</i>	Independent predictor of recurrence	.280	.192
<i>MYCBP</i>	Transcription factor repressor downregulated in PC	.280	.381
<i>SPOP</i>	Mutations promote <i>AR</i> activity and PC metastatic potential	.280	.381
<i>FOLH1</i>	Associated with PSA recurrence in high-risk cohort	.347	.326
<i>TP63</i>	Downregulated in advanced or malignant CRPC	.374	.453
<i>SRD5A2</i>	A49T, V89L variant correlates with extracapsular disease	.518	.216
<i>PTEN</i>	Most commonly deleted/mutated tumor suppressor in PC	.855	.724
<i>CYP3A4</i>	Associated with PC occurrence and severity	.855	.964

Abbreviations: AR, androgen receptor; CRPC, castration-resistant prostate cancer; Logit, logistic regression; PC, prostate cancer; PSA, prostate-specific antigen.

**P* values were adjusted with the Benjamini-Hochberg false discovery rate method.

performed a comprehensive literature search for biomarkers linked to PC pathogenesis and disease aggressiveness. After the selection of a validated list of biomarkers, we evaluated a matched cohort of AA and EA men for differences in gene expression and determined whether these differences could predict unfavorable pathology or clinical outcomes.¹⁴

PATIENTS AND METHODS

Study Design and Patient Selection

This study employed a matched cohort of AA and EA men identified at four institutions: Thomas Jefferson University, Johns Hopkins University, The Cleveland Clinic Foundation, and the Memorial Sloan Kettering Cancer Center (Data Supplement).^{15,16} We retrospectively included patients from these centers who underwent radical prostatectomy (RP) with bilateral pelvic lymph node dissection for localized PC between 1987 and 2012 and who had been analyzed with the Decipher Prostate Cancer Classifier. Each AA patient was matched to one or two EA patients on the basis of the [Cancer of the Prostate Risk Assessment postsurgical \(CAPRA-S\)](#) score at diagnosis to control for baseline differences in clinicopathologic factors between the comparison groups.¹⁷ AA patients were matched to EA patients within the same institution, and matched patients had CAPRA-S scores that were within 2 points of each other. The CAPRA-S score distribution of matched patients is shown in the Data Supplement. Pathologic staging after prostatectomy was performed according to the 1992 American Joint Committee on Cancer staging system.¹⁸ Information on patient selection from each of the four participating institutions included previously published inclusion and exclusion criteria.¹⁹⁻²² Data from 397 matched patients consisting of 154 AA and 243 EA men were analyzed in this study. Tumor specimen sampling, RNA extraction, and microarray expression data generation were accomplished as previously described.²³

Selection of Biomarkers

A comprehensive literature search was carried out for biomarkers associated with PC pathogenesis and disease aggressiveness. Only biomarkers that

have been reported at least twice in the current literature to be associated with aggressive PC were evaluated in this study. Exploratory PC biomarkers derived from the PC genome-wide association studies alone were excluded from this study. With these criteria, we identified 20 biomarkers associated with PC pathogenesis and disease aggressiveness (Table 1). These include PC-associated factors, PC-specific proteins, androgen pathway factors, tumor suppressor genes, and PC-associated metabolic genes. Molecular subtype expression (ie, *ERG*, *ETS*, and *SPINK1*) was determined by microarray outlier analysis on the Decipher Prostate Cancer Classifier assay (GenomeDx Biosciences, San Diego, CA), as previously described.²⁴ Four prognostic biomarker signatures—md-Penney, md-CCP, Decipher, and md-GPS—also were generated by using the microarray data, as previously described (Ross et al, submitted for publication).^{22,23,25-27}

Statistical Analysis

Associations between ethnicity and categorical variables were tested by Fisher's exact test. Differences in the distributions of continuous biomarker expression levels by ethnicity were assessed with the Wilcoxon-Mann-Whitney test. Logistic regression also was used to study the relationship between expression levels and ethnicity. *P* values for these tests were adjusted according to the Benjamini-Hochberg false discovery rate method. Differences in the effect of biomarker expression levels by ethnicity on clinicopathologic outcomes, such as the presence of pathologic T3 (pT3) disease (defined as extraprostatic extension and/or seminal vesicle invasion), pathologic Gleason score (pGS) greater than 7 (3 + 4), 3-year biochemical recurrence (BCR), and metastasis at 5 years, were assessed by testing for an ethnicity-by-biomarker expression interaction in a conditional logistic regression model. An association was determined to be ethnicity dependent when there was a significant ethnicity-by-biomarker interaction odds ratio (OR) that had a *P* value less than .05 in the prediction of at least one clinicopathologic outcome. Alternatively, the ethnicity-by-biomarker relationship was termed ethnicity independent when the interaction OR was not statistically significant. For biomarkers that did not have a significant interaction with ethnicity (*P* > .05), the ethnicity-by-biomarker interaction term was dropped, and the model was fit using only the main effects of ethnicity and the biomarker for predicting clinical outcomes. The ORs for expression levels were reported for increments

Table 2. Clinicopathologic Characteristics of Patients

Patient Characteristic	No. (%) of Total Population (N = 397)	No. (%) of AAM Patients (n = 154)	No. (%) of EAM Patients (n = 243)	Fisher's Exact Test or Mann-Whitney <i>P</i>
Age, years				.031
Median	59	59	60	
Range	37-76	37-73	43-76	
Preoperative prostate-specific antigen, ng/mL				.663
< 10	303 (76)	121 (79)	182 (75)	
10-20	71 (18)	24 (16)	47 (19)	
> 20	23 (6)	9 (6)	14 (6)	
Pathologic Gleason score				.065
≤ 6	135 (34)	59 (38)	76 (31)	
7	192 (48)	76 (49)	116 (48)	
≥ 8	70 (18)	19 (12)	51 (21)	
Extracapsular extension	169 (43)	60 (39)	109 (45)	.254
Seminal vesicle invasion	60 (15)	18 (12)	42 (17)	.277
Positive surgical margin	124 (31)	54 (35)	70 (29)	.222
Lymph node involvement	17 (4)	4 (3)	13 (5)	.214
CAPRA-S score				.302
< 3	175 (44)	75 (49)	100 (41)	
3-5	130 (33)	48 (31)	82 (34)	
> 5	92 (23)	31 (20)	61 (25)	

NOTE. Patients from Thomas Jefferson University, Memorial Sloan Kettering Cancer Center, The Cleveland Clinic Foundation, and Johns Hopkins University were matched in a pooled analysis.

Abbreviations: AAM, African American men; CAPRA-S, Cancer of the Prostate Risk Assessment postsurgical score; EAM, European American men.

of 10% of the expression range for a given biomarker. Discrimination performance of biomarker signatures for binary end points was established using Harrell's concordance statistic (C-index). Statistical analyses were performed in R (version 3; <http://www.r-project.org/>). All statistical tests were two sided and used a 5% significance level.

RESULTS

Clinicopathologic characteristics of the 397 patients are presented in Table 2. AA men presented at an earlier age than EA men (median, 59 v 60 years; $P = .031$). Of the entire cohort, 175 (44%) had a CAPRA-S score of less than 3; 130 (33%) had a CAPRA-S score between 3 and 5; and 92 (23%) had a CAPRA-S score greater than 5.

Biomarker expression patterns were compared between AA and EA men using the Wilcoxon-Mann-Whitney test. Six biomarkers showed statistically significant differential expression by ethnicity: *ERG* ($P < .001$), *AMACR* ($P < .001$), *SPINK1* ($P = .001$), *NKX3-1* ($P = .03$), *GOLM1* ($P = .03$), and androgen receptor (*AR*; $P = .04$; Table 1). The distribution and median expression levels of these are shown in Figure 1A-1F.

We next evaluated the relationship between expression and ethnicity by using a logistic regression model with ethnicity as the end point. Four biomarkers showed significant differential expression: *ERG* ($P < .001$), *AMACR* ($P < .001$), *SPINK1* ($P = .03$), and *GOLM1* ($P = .02$; Table 1; Fig 1G).

Next, we characterized the molecular subtypes of the *ERG*-family genes and *SPINK1* genes in our cohort as stratified by CAPRA-S risk model and pGS (Fig 2). There was a statistically significant decrease in *ERG*-positive prevalence among AA men compared with EA men in all CAPRA-S risk groups (low, 21.6% v 42% [$P = .006$]; average, 21.3% v 55% [$P < .001$]; high, 19.4% v 47.5% [$P = .012$]; Fig 2A; Data Supplement). AA men were more likely than EA men to be *ETS*

positive within the low-risk CAPRA-S group (17.6% v 5%; $P = .01$). There was no statistically significant difference in *SPINK1*-positive variants by ethnicity, although there was a trend toward increased *SPINK1* expression in AA men than in EA men in all of the CAPRA-S risk groups. Interestingly, AA men were more likely than EA men to be triple negative (ie, *ERG* negative/*ETS* negative/*SPINK1* negative), particularly in the average-risk CAPRA-S group (51.1% v 26.3%; $P = .007$) and the high-risk CAPRA-S group (48.4% v 23.7%; $P = .03$). AA men with a pGS greater than 7 (4 + 3) also were less likely than EA men to be *ERG* positive (6% v 45%; $P < .001$; Fig 2B; Data Supplement) and were more likely to have a triple-negative phenotype (57.6% v 24.7%; $P = .001$).

We next explored the clinical utility of the selected biomarkers in predicting adverse pathologic features and clinical outcomes by using conditional logistic regression. The risk of having pT3 disease had a significant ethnicity-by-biomarker interaction effect for the following biomarkers: *NKX3-1* (interaction OR, 0.57; $P = .025$), *AMACR* (interaction OR, 0.68; $P = .036$), *FOXP1* (interaction OR, 0.66; $P = .041$), *ERG* (interaction OR, 0.72; $P = .036$), *RB1* (interaction OR, 0.67; $P = .037$), and *GSTP1* (interaction OR, 0.65; $P = .049$; Table 3). The corresponding correlation between biomarker expression pattern and the risk of pT3 disease is represented in the interaction plots in Appendix Figure A1 (online only). We observed that the dysregulation of *NKX3-1*, *AMACR*, *ERG*, *FOXP1*, and *GSTP1* and the loss-of-function mutation of tumor suppressor *RB1* decreased the risk of pT3 disease for AA men, whereas the reverse was true for EA men (Table 3; Appendix Fig A1). However, the dysregulation of tumor protein p53 (*TP53*; $P = .006$) and β -microseminoprotein (*MSMB*; $P = .022$) predicted for a decrease in the risk of pT3 disease for both AA and EA men in an ethnicity-independent manner, as depicted by a nonsignificant ethnicity-by-biomarker interaction. (Table 3, panel B; Appendix Fig A1) Similar analyses

Fig 1. (A-F) Box-and-whisker plots showing distribution and median expression levels of biomarkers with significant ethnic variation. (G) Venn diagram showing biomarkers with significant ethnic variation in one or more statistical model. (*) Mann-Whitney *U* test. (†) Logistic regression. All *P* values adjusted using Benjamini and Hochberg's false discovery rate method. AAM, African American men; EAM, European American men.

were conducted for the risk of a pGS greater than 7 (3 + 4), as shown in the Data Supplement. *SPINK1* (interaction OR, 0.52; $P = .049$) emerged as the only biomarker that predicted for risk of a pGS greater than 7 (3 + 4) in an ethnicity-dependent manner. The following biomarkers predicted for risk of a pGS greater than 7 (3 + 4) similarly for both AA and EA men in an ethnicity-independent manner: *MKi67* ($P = .026$), *ERG* ($P = .025$), and *TP63* ($P = .004$).

We then evaluated biomarkers predictive of clinically relevant outcome, such as BCR at 3 years and the risk of metastatic disease at 5 years. Dysregulation of *GOLM1* (interaction OR, 2.05; $P = .037$) predicted for an increased risk of 3-year BCR for AA men, whereas the reverse was true for EA men. *SRDA2* ($P = .013$) and *MKi67* ($P = .023$) predicted the risk of 3-year BCR in an ethnicity-independent manner (Data Supplement). There was no ethnicity-specific correlation between biomarkers and metastasis at 5 years; however, *SRD5A2*

Fig 2. (A) *ETS* variant subtyping by ethnicity and Cancer of the Prostate Risk Assessment postsurgical (CAPRA-S) score. (B) *ETS* variant subtyping by ethnicity and pathologic Gleason score. AAM, African American men; EAM, European American men.

($P = .023$) predicted the risk of metastatic disease at 5 years in both AA and EA men (Data Supplement). Finally, we compared the prognostic performance of four previously validated biomarker signatures for the prediction of metastatic disease after radical prostatectomy. We found similar distributions in signature scores between the ethnic groups (Data Supplement). Although there were relatively few metastatic events as evaluated by receiver operating characteristic (ROC) analysis, we found the following C-indices (95% CIs) for the prediction of metastasis in AA and EA men, respectively: 0.94 (0.87 to 1.00) and 0.81 (0.68 to 0.95) with Penney, 0.78 (0.59 to 0.98) and 0.88 (0.80 to 0.95) with Decipher, 0.89 (0.81 to 0.96) and 0.78 (0.66 to 0.89) with md-GPS, and 0.60 (0.36 to 0.83) and 0.70 (0.55 to 0.84) with md-CCP (Data Supplement).

DISCUSSION

In this report, we explored differences in the expression pattern for selected biomarkers in a matched set of AA and EA men with PC and assessed their performance in predicting the risk of clinicopathologic outcomes. Biomarkers for which this difference was statistically signif-

icant by both the nonparametric Wilcoxon-Mann-Whitney test and the logistic regression model included *AMACR*, *ERG*, *SPINK1*, and *GOLM1*. We observed significant differences in the median expression levels for *NKX3-1* and *AR* by ethnicity with the Wilcoxon-Mann-Whitney test, but the expression patterns were not significantly different by ethnicity in the logistic regression model. This observation may be attributable partly to interinstitutional batch effects and emphasizes the need for careful quality control of assays and an adjustment for the institution when undertaking multicenter studies of this type.

We report that *ERG*, *AMACR*, *RB1*, *FOXP1*, *NKX3-1*, and *GSTP1* predicted risk of pT3 disease in an ethnicity-dependent manner. One of the more striking findings was the ethnic association between pT3 disease and *ERG*. Molecular subtyping of the *ERG*-family genes demonstrated that AA men who had high-risk CAPRA-S scores and an advanced pGS had tumors with relatively lower expressions of *ERG* and were more likely than EA men to have the triple-negative PC subtype. These data suggest that PC may arise from different tumor progenitors and/or distinct molecular pathways in EA men compared with AA men.

Table 3. Differences in the Effect of Biomarker Expression Levels by Ethnicity on the Risk of pT3 Disease

Biomarker	Odds Ratio			P			10% of Range in Expression
	Ethnicity*	Biomarker Expression†	Ethnicity-by-Biomarker Interaction†	Ethnicity*	Biomarker Expression	Ethnicity-by-Biomarker Interaction	
<i>NKX3-1</i>	6.87	1.03	0.57	.015	.869	.026	0.30
<i>ERG</i>	2.43	1.10	0.72	.034	.181	.036	0.26
<i>AMACR</i>	4.18	1.15	0.68	.021	.198	.036	0.36
<i>RB1</i>	4.31	1.02	0.67	.026	.900	.037	0.12
<i>FOXP1</i>	10.71	1.02	0.66	.026	.923	.041	0.21
<i>GSTP1</i>	2.40	1.13	0.65	.037	.410	.049	0.22
<i>TP53</i>	1.30	0.66	NS	.393	.006	NS	0.15
<i>MSMB</i>	1.10	0.76	NS	.762	.022	NS	0.75
<i>SPOP</i>	1.31	0.70	NS	.361	.063	NS	0.18
<i>AR</i>	1.27	0.78	NS	.425	.075	NS	0.16
<i>FOLH1</i>	1.25	0.86	NS	.447	.137	NS	0.29
<i>SRD5A2</i>	1.40	0.82	NS	.258	.221	NS	0.14
<i>MYCBP</i>	1.25	1.17	NS	.455	.235	NS	0.06
<i>TP63</i>	1.33	0.91	NS	.330	.401	NS	0.14
<i>GOLM1</i>	1.27	0.92	NS	.414	.431	NS	0.39
<i>CYP3A4</i>	1.30	0.93	NS	.373	.589	NS	0.08
<i>MKI67</i>	1.34	1.07	NS	.317	.642	NS	0.08
<i>PTEN</i>	1.32	0.93	NS	.332	.739	NS	0.19
<i>SPINK1</i>	1.27	1.04	NS	.432	.751	NS	0.59
<i>EZH2</i>	1.32	1.03	NS	.339	.852	NS	0.08

NOTE. All regression models were adjusted for CAPRA-S score. Boldface indicates statistical significance.

Abbreviations: AR, androgen receptor; CAPRA-S, Cancer of the Prostate Risk Assessment postsurgical; NS, not significant (regression model was fit without the interaction variable); pT3, pathologic stage T3.

*Reference group is European American men.

†Odds ratios are reported per increase of 10% of the range in observed expression values for given biomarker.

TMPRSS2-ERG fusion results in androgen-regulated overexpression of *ERG*, which is thought to play a critical role in prostate carcinogenesis.²⁸ *TMPRSS2-ERG* fusion has been reported in greater than 50% of EA men and in less than 30% of AA men with PC.²⁸⁻³¹ Our data confirm a predominance of the *ERG*-negative phenotype in AA men and call into question the applicability of the *ERG* gene as a robust biomarker in the AA population.

The other ethnicity-dependent biomarkers—*AMACR*, *RB1*, *FOXP1*, *NKX3-1*, and *GSTP1*—remain interesting areas for discovery. Loss of expression of these biomarkers was associated with an increased risk of pT3 disease in AA men. This was not observed in EA men, nor was it observed in patients who had an advanced pGS, BCR, or risk of metastasis.

AMACR is preferentially overexpressed in approximately 80% of PC tissue biopsies.^{32,33} Contrary to what is observed in EA men, a lower expression level of *AMACR* is associated with a risk of pT3 disease and an aggressive phenotype in AA men (Appendix Fig A1), which implies that *AMACR* may be a biomarker for indolent PC in AA men. Recent data suggest that *RB1* controls PC progression through E2F transcription factor 1-mediated regulation of *AR* expression.³⁴ *FOXP1* functions as an androgen-regulated gene transcription factor that modulates *AR* signaling and contributes to PC pathogenesis.³⁵ *NKX3-1* (8p21) is an androgen-responsive transcription factor that functions as a tumor suppressor gene and has been linked to PC pathogenesis. The *NKX3-1* protein is mostly expressed in primary PC, is downregulated in many high-grade PCs, and is completely lost in the majority of metastatic PC; thus, it provides a correlate to tumor progression.³⁶

Regarding BCR and metastatic progression, *SPINK1* and *GOLM1* predicted for an advanced pGS and 3-year BCR, respectively, in an ethnicity-dependent manner. The serine protease inhibitor Kazaltype 1 (*SPINK1/TATI*) is a prognostic tumor marker overexpressed in high-grade PC.³⁷ Interestingly, *SPINK1* expression is found exclusively in a subset of *ETS* rearrangement-negative tumors.³⁸ Accordingly, our results also showed a greater trend toward increase expression of *SPINK1* in AA men than in EA men. Golgi membrane protein 1 (*GOLM1*) is upregulated in localized PC, and urinary mRNA levels outperform serum prostate-specific antigen in the detection of localized PC.³⁹ Both *SPINK1* and *GOLM1* warrant additional investigation.

Although not predictive of outcomes, the *AR* gene did exhibit differential expression between ethnicities. AA men reportedly have a higher density of *AR* protein expression than EA men who have clinically localized PC, and this density correlates with the mRNA expression from our analysis.⁴⁰ This may be a result of the negative-feedback autoregulation of *AR* gene expression seen in hormone-sensitive PC.

Our results also reveal that a subset of the validated biomarkers perform in an ethnicity-independent manner for predicting at least one of the predefined clinicopathologic outcomes. These include a loss-of-function mutation for tumor suppressors *TP53* and *TP63* and the dysregulation of *MKI67*, *MSMB*, and *SRD5A2*.

Studies have shown that the tumor suppressor gene *TP53* may offer prognostic value in PC after different treatments.⁴¹ The *TP63* gene, a homolog of the tumor suppressor gene p53 family, is downregulated in PC.^{42,43} In our study, both *TP53* and *TP63* varied in a

similar manner. The *MKi67* protein serves as a tumor proliferation index marker and as a marker for treatment outcomes in patients with PC, although the biologic relevance remains poorly understood.^{8,44} MSMB is one of the most abundant proteins in human seminal fluid and reportedly is an important biomarker for PC susceptibility.^{45,46} MSMB has been an independent predictor of recurrence after radical prostatectomy, although it has not been shown to improve the predictive performance of existing models.¹² Finally, genome-wide association studies have shown that 5- α reductase type 2 (ie, *SRD5A2*) allelic variants occur at the highest frequency in AA men and are associated with increased PC risk.⁴⁷ Although this protein did not show any ethnicity dependence, it represents the only individual biomarker that provided a significant correlation with risk of metastatic disease at 5 years. The lack of ethnicity dependence might be the result of low numbers of metastatic occurrences.

Finally, we performed an exploratory analysis of four prognostic biomarker signatures, including three that are commercially available in the United States and are entering routine clinical use. For these prognostic biomarker signatures, we did not find differences in the distribution of score values between EA and AA men. In addition, each of the four models had similar C-indices for predicting metastatic onset in AA men and EA men.

This report constitutes, to our knowledge, the largest and only study that describes a set of biomarkers that have the ability to predict for risk of adverse clinicopathologic outcomes in an ethnicity-dependent manner. These biomarkers provide a source of relevant knowledge in developing a signature that may be unique to AA men with aggressive PC. The findings in this study also constitute an important step in elucidating the contribution of tumor biology in the ethnic disparity of PC outcomes.

Although our sample size is relatively small, this is, to our knowledge, the largest study to date on predictive biomarkers for AA population. Despite its size, the statistical significances identified here attest to the ability of this sample set to identify relevant effects, even after correction for multiple-hypothesis testing. A major strength of our study is that the cohort of AA men was selected from a random sample of AA men in a multi-institutional cohort of patients with PC across major cities in the United States. Furthermore, this study provides clinically relevant biomarkers that are useful in predicting adverse outcomes in the at-risk AA population. Nonetheless, the validity of these biomarkers and prognostic signatures will need to be tested in a prospective study.

This study is not without limitations. We did not have enough events to evaluate PC-specific or overall survival in our data set. The median follow-up time for the overall cohort was 39 months. Longer-term data is required to evaluate such end points. Data derived from

this study were based on men who self-identified as AA. Indeed, within the AA population, there are emerging data to suggest marked genetic heterogeneity, which could weaken this study's ability to detect significant mRNA changes.⁴⁸ We are currently validating these biomarkers in men of African descent within an international consortium. Furthermore, because of the matched nature of the data, we were unable to study survival end points with typical survival analysis methods. End points, such as BCR and metastasis, instead were converted into binary end points. Patients whose data were censored before these chosen end points were dropped from the analysis. Censored patients did not account for a large proportion of the study population ($n = 23$ for BCR; $n = 25$ for metastasis), but the omission of their data could potentially introduce bias. As a result, in studying the metastatic end point, data from all patients at the Memorial Sloan Kettering Cancer Center were dropped, because follow-up times were unavailable.

In conclusion, we have identified a set of biomarkers that demonstrate ethnic dependence in predicting the risk of one or more adverse clinicopathologic outcomes in AA men. These results show that there are differences in the biology and pathogenesis of PC in AA men compared with EA men that affect applications in both diagnostics and therapeutics. Additional validation is warranted for applicability in PC diagnosis and treatment. The ability to identify a subset of AA men who harbor aggressive disease will enable clinicians to more accurately risk stratify these patients for appropriate treatment recommendations that improve disease control and ultimately reduce the disparities in outcomes in this patient population.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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GLOSSARY TERMS

CAPRA (Cancer of the Prostate Risk Assessment)

score: A 0 to 10 score on the basis of a multivariable Cox model that predicts biochemical and clinical (metastasis and mortality) end points after primary treatment for prostate cancer. A post-surgical version (CAPRA-S) offers improved prediction of the same end points after radical prostatectomy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Novel Biomarker Signature That May Predict Aggressive Disease in African American Men With Prostate Cancer

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Appendix

Fig A1. Predicted probability curves showing biomarkers predictive of pathologic T3 (pT3) disease by ethnicity. Blue lines indicate African American men; gold lines indicate European American men.