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TITLE: Novel Nomogram That Predicts Aggressive Disease and Treatment Failure Among African-American Men with Prostate Cancer

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14. ABSTRACT: Prostate cancer (PCa) has greatest incidence and mortality among African American (AA) as compared to their European American (EA) counterparts in the US. This disparity has been attributed to a number of factors including access to care, screening patterns, and behavior. More recent data suggest that genetic/biologic factors may at least in part contribute to more aggressive disease in AA men. Using the SCORE database, we studied PCa outcomes in AA vs. EA men after radical prostatectomy. We showed that AA race was a predictor of worse biochemical failure in patients with pathologic Gleason score ≤ 6 or low-grade disease and favorable pathologic features (Yamoah et al., 2014). Next, immunohistochemistry for 20 biomarkers was undertaken on the FFPE tumors of 45 EA and 55 AA men within the SCORE database. To date, 6 biomarkers have been analyzed including TMPRSS2-ERG, AMACR, PSMA, RB, c-Myc, and AR. We observed statistically significant differences in biomarker expression between EA vs AA for AMACR (p=0.004), c-myc (p=0.005), and AR (p=0.002). Furthermore, we demonstrated that there are substantial differences in the distribution of prostate tumor biomarkers between AA and EA men (Yamoah, et al. JCO 2015). The study, which included 154 AAM and 243 EAM samples pulled from the Decipher Genomics Resource Information Database (GRID), evaluated 20 validated biomarkers reported to be associated with PCa initiation and progression. Of 20 biomarkers examined, 6 showed statistically significant differential expression in AAM compared with EAM. These include ERG, AMACR, SPINK1, NKX3-1, GOLM1, and androgen receptor. Dysregulation of AMACR, ERG, FOXP1, and GSTP1 as well as loss-of-function mutations for tumor suppressors NKX3-1 and RB1 predicted risk of pathologic T3 disease in an ethnicity-dependent manner. Furthermore, A greater proportion of AA men than EA men had triple-negative (ERG-negative/ETS-negative/SPINK1-negative) disease (51% v 35%). These data suggest that biomarkers that have been reported to be associated with tumor aggressiveness may in part explain the difference in clinical outcomes between EA and AA men.					
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Introduction:

Globally, men of African descent are known to experience greater incidence of and mortality from prostate cancer (PCa) than their Caucasian or Asian counterparts¹. This observation has been partly attributed to socio-economic factors and inadequate access to healthcare²⁻⁴. However, there is also recent evidence suggesting that genetic differences in susceptibility play a major role in this disparity⁵⁻⁸. Due to the relatively indolent nature of PCa, the decision-making process for determining whether to pursue active surveillance, or to offer different treatment options, is complicated by the balance between life expectancy, comorbidities, clinical benefits, as well as the side effects of treatment⁹. The prediction of clinical outcomes through the use of nomograms is critical in recommending appropriate treatment options for PCa patients. However, it is uncertain whether the current nomograms used to risk-stratify PCa patients for treatment recommendations truly apply to AAM. The reasons include the fact that majority of the clinically useful nomograms were derived using data extrapolated from PCa patients of European ancestry¹⁰. Furthermore, several studies have demonstrated that the current nomograms, which were derived based on limited clinical and pathologic information such as Prostate Specific Antigen (PSA), T-stage and Gleason score, have a predictive accuracy of only 65% to 79%, and thus may be suboptimal^{11, 12}. The inclusion of other potentially informative clinical and pathologic features such as age, AA race, surgical margins, seminal vesicle involvement, lymphovascular involvement or lymph node status have only slightly improved the predictive accuracy of nomograms^{13, 14}. Despite increased disease recurrence and mortality trends among AAM with PCa, these nomograms have been suboptimal in predicting the subset of AAM patients who harbor aggressive disease and are at higher risk for disease recurrence. The genetic contribution to PCa disparity has been well established with the identification of significant racial differences in frequency and expression of various genes and biomarkers. Recently, several biomarkers (Table 1) have been shown to correlate with aggressive phenotypes in prostate cancer¹⁵⁻²². The most notable examples include the TMPRSS2:ERG gene fusion²³, Ki-67 expression¹⁷, and biomarkers involved in androgen metabolism^{8, 18}. The relevance of these biomarkers to the observed increased aggressiveness and disease recurrence among AAM is not known. We hypothesize that addition of these potentially informative biomarkers may significantly improve the predictive capacity of nomograms in predicting aggressive disease measured as the time to PSA failure after treatment among AAM. We propose to evaluate this hypothesis using the following Specific Aims 1) To develop a nomogram that significantly improves the accuracy of distinguishing aggressive from non-aggressive PCa in AAM. 2) To evaluate the incorporation of this novel nomogram into clinical practice.

Keywords: Prostate Cancer, racial disparity, African American Men, Predictive Biomarkers, Nomogram Development

Overall Project Summary:

Prostate cancer (PCa) is the most commonly occurring non-cutaneous malignant cancer in the U.S. African American men (AAM) are known to have the highest rates of PCa. They typically present with advanced disease, and have greater mortality rates than their Caucasian counterparts. Despite the importance of PCa in AAM, we are still unable to make optimal PCa treatment decisions in this group of men. As a result, many clinicians are uncertain about the value of the currently available tools that guide treatment decisions for AAM with PCa. The purpose of this project is to provide insights into the underlying causes of racial disparities in PCa outcomes and ultimately improve the current treatment recommendations in AAM with PCa. This project directly addresses the need to effectively identify aggressive disease in specific individuals or groups based on their unique characteristics. The research portion of this project

will have two basic goals. The first goal will be to develop a new predictive tool, also known as a nomogram, which will improve the ability to predict aggressive disease and make improved treatment decisions among AAM. This nomogram will include biomarkers predictive of aggressive PCa in addition to the predictors currently used in existing nomograms. The new nomogram's predictive accuracy will be evaluated using a large PCa database. The second goal will be to take steps towards the implementation of this new nomogram into decision-making among physicians in the clinical setting. We propose that the use of a novel nomogram that accurately identifies aggressive disease will help reduce prostate cancer health disparity among AAM and will directly impact treatment recommendations among physicians in the clinic setting.

Key Research Accomplishments:

I am particularly fortunate to be a recipient of the CDMRP- DOD Health Disparity Award since September 2013. During the first year of this award I have obtained superb clinical and research mentoring from my mentors; Drs Adam Dicker, Timothy R. Rebbeck and Michael Kattan. My educational experience has been enriched by obtaining two competitive grants: one from the Prostate Cancer Foundation-Young Investigator Award, and another from the NIH LRP program to carry out work on prostate cancer disparity in men of African descent. In my first year working in Dr. Timothy Rebbeck's laboratory, we performed an analysis of outcomes among African-American men with truly low-grade PCa. We showed that African-American race was a predictor of worse biochemical failure in patients with pathologic Gleason score ≤ 6 or low-grade disease and favorable pathologic features. This finding, recently published in *Urologic Oncology* (Yamoah, et, al., 2014), highlights the need for clinically useful biomarkers that will enable us to identify African-American men appropriate for active surveillance vs. those harboring aggressive disease. To this end, I continued to work on prostate cancer related biomarker information during Year 2 to develop a novel biomarker signature that more accurately predicts aggressive disease in men of African descent with prostate cancer.

This work set the platform to begin to address the objective outline in Specific Aim 1 of the proposal. I have made major strides towards the completion the Specific Aim 1, as well as fulfilling the required training component outlined in the SOW.

AIM 1: To develop a nomogram that significantly improves the accuracy of distinguishing aggressive from non-aggressive PCa in AAM. We hypothesized that genetic differences between AAM and men of other racial groups significantly contributes to the aggressiveness of the PCa in AAM. To test this hypothesis:

A) We first performed a comprehensive literature search for biomarkers linked to PCa pathogenesis and disease aggressiveness.

B) Upon selection of a validated list of biomarkers we evaluated for any differences in expression in a matched cohort of AA and EA men.

A) Selection of Biomarkers

A comprehensive literature search was carried out for biomarkers associated with PCa pathogenesis and disease aggressiveness. Only biomarkers that have been reported at least twice in the current literature to be associated with aggressive PCa were selected for this study. Exploratory PCa biomarkers derived from GWAS studies alone were excluded from this study. The list consisting of 20 biomarkers associated with PCa pathogenesis and disease aggressiveness are shown in Table 1. These include PCa-associated factors, PCa-specific proteins, Androgen pathway factors, tumor suppressor genes and PCa-associated metabolic genes.

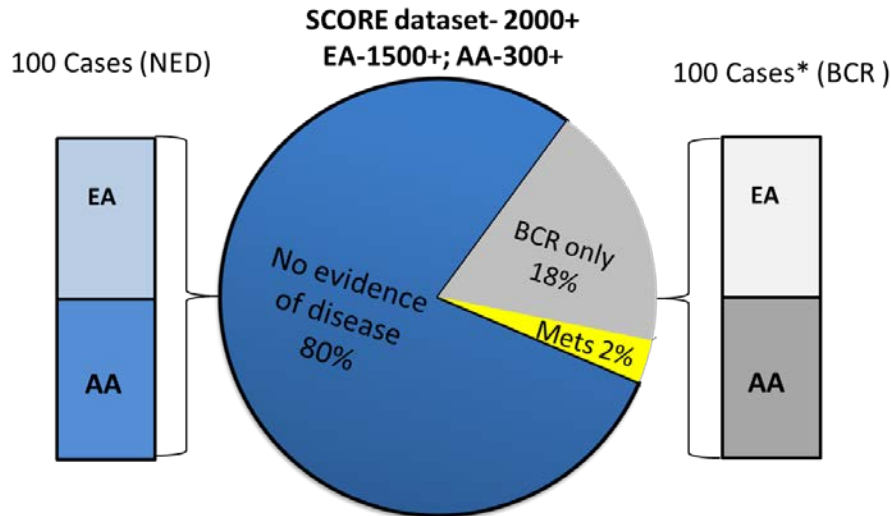
Biomarkers	Description	Function
<i>Pca-associated factors</i>		
TMPRSS2-ERG	Transmembrane protease S2 -(ETS-Related Gene) TF fusion product	Found in 36-78% samples; associated with aggressive Pca
MKI 67	Tumor proliferation rate protein	Correlates with cancer-specific and overall survival
GSTP1	glutathione S-transferase P1 gene	Hypermethylated in 60-80% Pca; in serum, urine, biopsy tissue
SPINK1/TAT1	Serine protease inhibitor Kazaltype/Tumor-associated trypsin inhibitor	Overexpressed in high-grade Pca
MYCBP	c-myc binding protein	Transcription factor repressor downregulated in Pca
EZH2	PcG histone H3 methyltransferase- transcription repressor	Implicated in the pathogenesis of metastatic Pca
<i>Prostate Specific proteins</i>		
MSMB	Prostate specific protein (10q11.2)	Independent predictor of recurrence
FOLH1/PSMA	Cell surface membrane protein	Associated with PSA recurrence in high risk cohort
NKX3-1	Prostate-specific androgen-regulated TF (Chr 8p21)	NKX3.1 loss associated with advanced stage and CRPC
<i>Androgen Pathway</i>		
AR	Intracellular receptor protein	Predictor of decreased biochemical recurrence-free survival
CYP3A4	CY3A4*1B/ CY3A43*3 enzymes (7q21)	Associated with Pca occurrence and severity
SRD5A2	5-alpha reductase II	A49T, V89L variant correlates with extracapsular disease
FOXP1	Novel androgen-responsive forkhead TF	Negatively regulates AR signalling in Pca
SPOP	E3 ubiquitin ligase (Cullin 3) adaptor- Tumor suppressor of AR activity	Mutations promotes AR activity and Pca metastatic potential
<i>Tumor suppressor genes</i>		
TP53	Tumor suppressor gene- TF often mutated in cancer	Exon 6 & 7 mutations correlate with Pca tumor progression
TP63	p53 homologue- Basal cell marker for normal prostate development	Downregulated in advanced or malignant Pca
PTEN	Tumor suppressor lipid phosphatase in the PI3K/AKT/mTOR pathway	Most commonly deleted/mutated tumor suppressor in Pca
RB1	Tumor suppressor gene- inhibits class of E2F TFs	Rb-1 loss coincides with emergence of metastatic CRPC
<i>Pca-Metabolism</i>		
AMACR (Racemase)	Mitochondrial enzyme in bile acid biosynthesis & β-oxidation of FA	Overexpressed in Pca relative to benign prostatic tissue
GOLM1	Gene coding the 73-kDa type II Golgi membrane antigen	Upregulated in >90% of Pca tissues (unknown function)

Abbreviations: TF- Transcription factors, Pca- Prostate cancer, AR- Androgen receptor, FA- Fatty acids, CRPC- Castrate-resistant prostate cancer, Chr- Chromosome

Table 1.

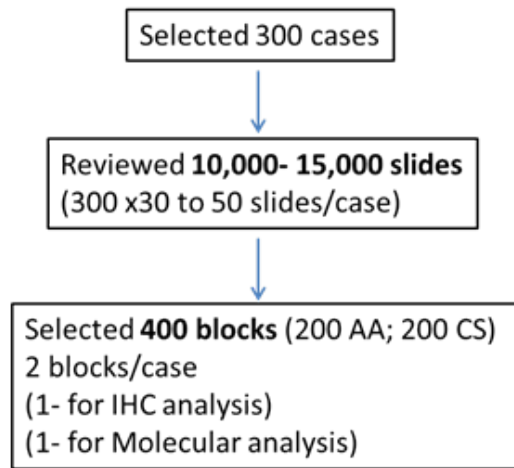
*B-1) Compared biomarker **protein** expression between EA and AA men within SCORE database*

Using the Study of Clinical Outcomes, Risk, and Ethnicity (SCORE) study, we identified prostate tumor tissues from men undergoing prostatectomy at the Hospital of the University of Pennsylvania between 1991-2008.



Pca- Prostate Cancer
 BCR- Biochemical Control Rate
 Mets- Metastasis
 EA- European-American men
 AA- African-American men

We targeted a matched cohort of 100 AA and 100 EA with a roughly equal number of BCR in each group. For this we selected 300 cases from the SCORE database and reviewed H&Es slides for each case and selected slides with dominant tumor lesion for IHC staining as shown below.

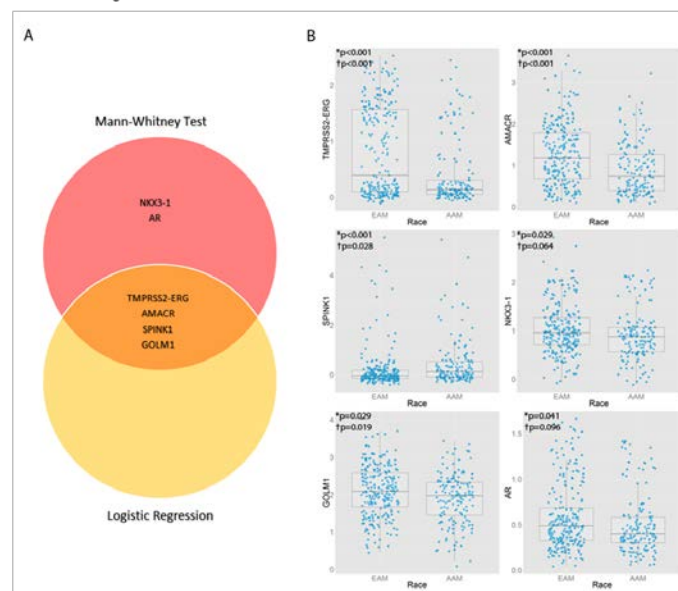


Immunohistochemistry staining for 12 biomarkers have been completed on the FFPE tumors of 45 EA and 55 AA men (mean age: 59.1 years, range: 41-71). Six biomarkers have been analyzed so far including TMPRSS2-ERG, AMACR, PSMA, RB, c-Myc, and AR. In this cohort we observed statistically significant differences in marker phenotype for AMACR (EA Mean: 188.1 vs. AA Mean: 144.7, $p=0.004$), c-myc (EA Mean: 54.7 vs. AA Mean: 21.6, $p=0.005$), and AR (EA Mean: 192.1 vs. AA Mean: 136.5, $p=0.002$).

B-2) Compared biomarker mRNA expression between EA and AA men within Decipher Genomics Resource Information Database (GRID)

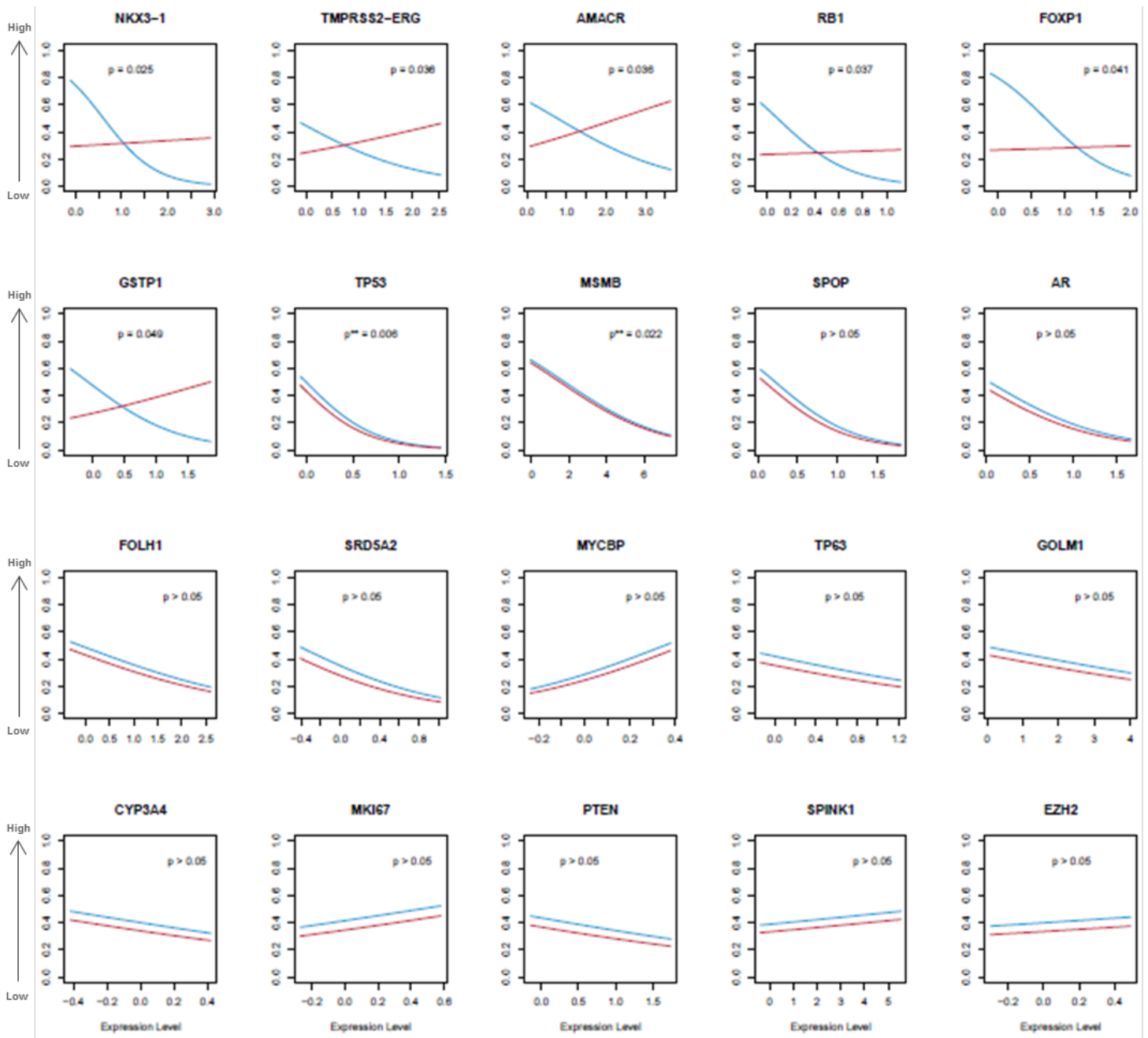
Using the GRID database we obtained a CAPRA-S matched patient cohort of AA and EA men with prostate cancer and compared the biomarker expression at the mRNA level of the 20 selected biomarkers of relevance in PCa aggressive disease. In this analysis we demonstrated that there are substantial differences in the distribution of prostate tumor biomarkers between AA and EA men. Results of this study were of high impact in the field and were subsequently published in the Journal of Clinical Oncology this year (Yamoah, et al. JCO 2015). The study, which included 154 AAM and 243 EAM samples pulled from the GRID, evaluated 20 validated biomarkers reported to be associated with PCa initiation and progression. Of 20 biomarkers examined, 6 showed statistically significant differential expression in AAM compared with EAM. These include ERG, AMACR, SPINK1, NKX3-1, GOLM1, and androgen receptor (Figure 1A below).

Figure 1: A) Venn diagram showing biomarkers with significance racial variation in one or more statistical model. B) Box and Whisker plots showing distribution and median expression levels of biomarkers with significant racial variation.



*p-value using Mann-Whitney U test; †p-value using logistic regression model
All p-values adjusted using Benjamini and Hochberg's false discovery rate method

Dysregulation of AMACR, ERG, FOXP1, and GSTP1 as well as loss-of-function mutations for tumor suppressors NKX3-1 and RB1 predicted risk of pathologic T3 disease in an ethnicity-dependent manner (*Interaction plot below*).

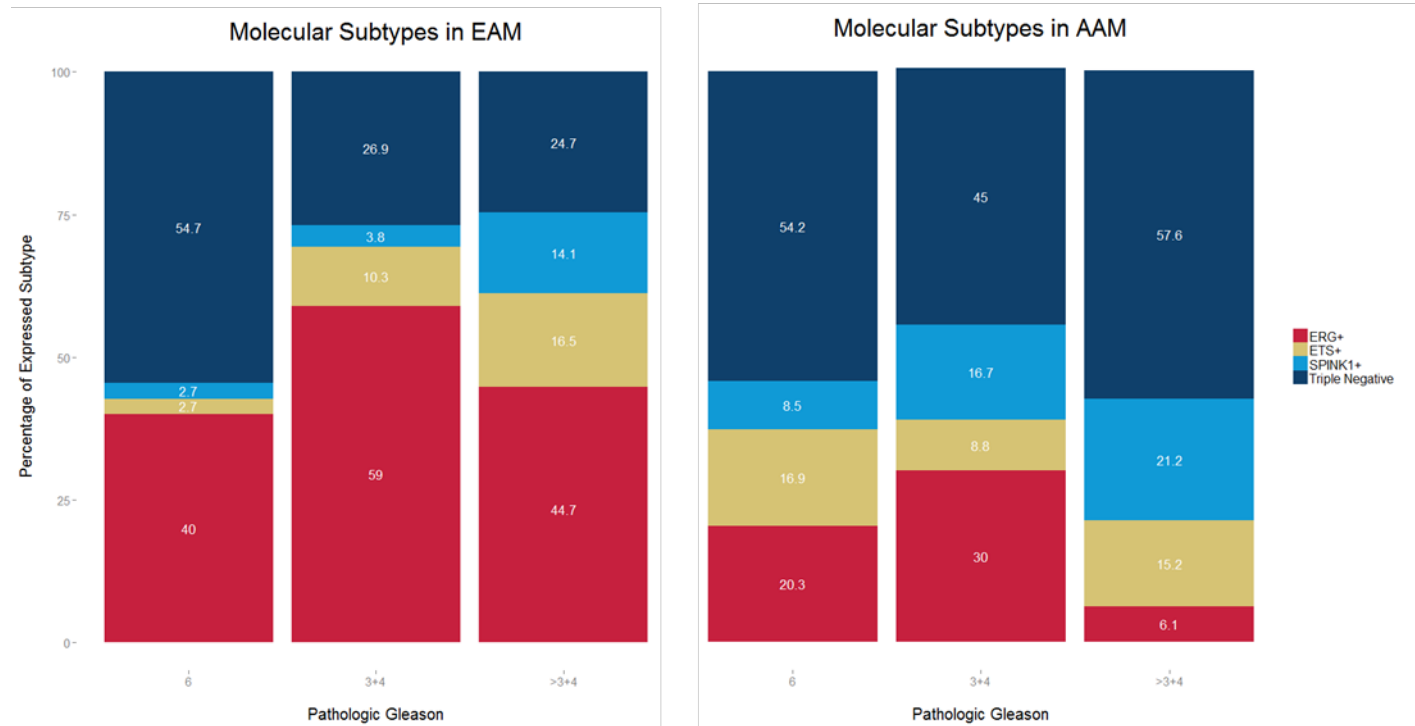


Y-axis: Increasing risk of pT3 disease; X-axis: Increasing biomarker expression

**p-value derived from conditional logistic regression model without Race:Biomarker interaction term ; RED ___ EA ; BLUE ___ AA men

Furthermore, A greater proportion of AA men than EA men had triple-negative (ERG-negative/ETS-negative/SPINK1-negative) disease (51% v 35%). – (*Figure- ETS variants below*)

ETS variants Subtyping by Race and Gleason Score.



Triple negative represent (ERG-, ETS- & SPINK1-);

Year 1 & 2 milestones Achieved:

Based on the SOW submitted, I have successfully completed the projected milestones set up for both research and training-specific tasks for this project. The research-specific task has been addressed above which was to characterization biomarkers that may predict aggressive disease in AA men and to develop a novel nomogram to predict aggressive disease. We have indeed identified a novel biomarker signature that is predictive of adverse outcomes in an ethnic-specific manner. I am in the process of applying for more funding to pursue research on the validity and generalizability of these biomarkers in a more homogenous population of men of African origin living in Africa with PCa using the Men of African Descent Carcinoma of the prostate (MADCaP) consortium.

The Training-specific task was to develop an understanding of biomarker analysis and nomogram development. I have completed a course in Biostatistics at the University of Pennsylvania in 2013. I have also obtained mentorship from Dr. Kattan’s group with the help of Changhong Yu, a biostatistician to work on nomogram development using the R-statistical platform. Through the mentorship of Dr. Rebbeck and Kattan’s group, and relevant course work at UPENN I have achieved the milestone of developing an understanding of biomarker analysis and nomogram development.

Next Steps:

Next, we hope to finish analysis of all 20 biomarkers in the SCORE and MADCaP databases and incorporate the significant biomarkers into existing nomograms. We will then test the novel nomogram to see if it improves the accuracy of predicting aggressive disease, as measured by the time to PSA failure after treatment.

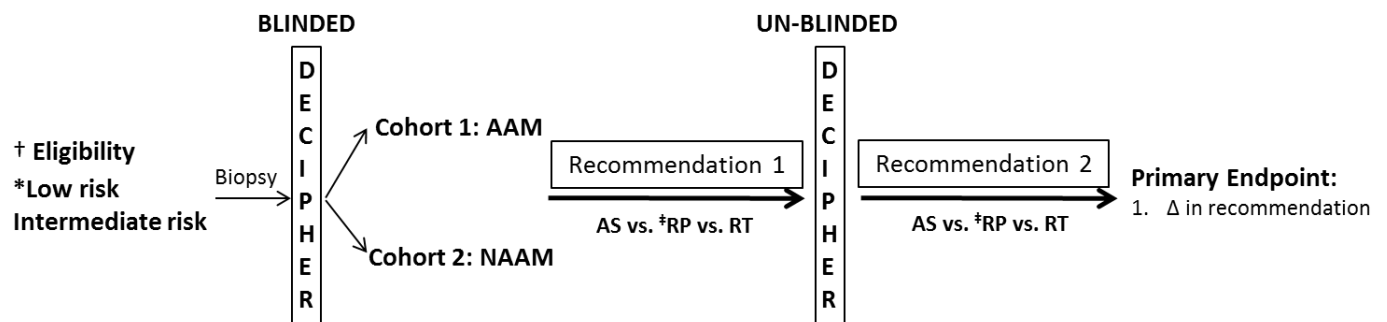
AIM 2: To evaluate the incorporation of a novel biomarker signature into clinical practice.

Currently ongoing- more funding is being pursued for these studies.

The goal of this aim is to compare the impact of genomic test information on physician treatment decision-making between AAM and EAM with PCa. Due to the relatively indolent nature of most PCa diagnosed in the US, the decision-making process for determining whether to pursue active surveillance, or alternative management options, is complicated by the balance between life expectancy, comorbidities, clinical benefits, as well as the side effects of treatment. The ability to predict clinical outcomes is critical in recommending appropriate treatment options for PCa patients. The use of a novel biomarker information that predicts aggressive disease in a subset of patients at risk can aid physicians to make genomic-based informed decision on which PCa patients to or not to offer aggressive treatment. To this end we are currently conducting a prospective validation trial on the use of genomic-based data on physician recommendation to determine it's clinical impact. A secondary endpoint is to study the biomarkers we have identified in our analysis from AIM 1 in a prospectively maintained database of patients undergoing active treatment to guide us in formulating genomic-based intervention studies that will tailor treatment based on genomic risk. A step towards personalized medicine.

Schema of proposed trial below:

VANDAAM trial- A VALIDATION STUDY ON THE IMPACT OF DECIPHER® TEST (GC) ON TREATMENT RECOMMENDATION IN AFRICAN-AMERICAN AND NON-AFRICAN AMERICAN MEN WITH PROSTATE CANCER: (VANDAAM STUDY)



AS- Active Surveillance; RP- Radical prostatectomy; RT- Radiation therapy; AAM- African-American Men; NAAM- non-African-American men; PSA- Prostate Specific Antigen

†- Eligible patients must have enough biopsy sample for Genomic classifier (GC) Decipher test

*- Low risk with high disease burden defined as Gleason score 6 in ≥3 cores involving ≥25% of tissue in at least 2 cores

‡- Decipher (GC) test will be performed on samples from RP cases.

Conclusion:

Of 20 biomarkers examined, 6 showed statistically significant differential expression in AAM compared with EAM. These include ERG, AMACR, SPINK1, NKX3-1, GOLM1, and androgen receptor. Dysregulation of AMACR, ERG, FOXP1, and GSTP1 as well as loss-of-function mutations for tumor suppressors NKX3-1 and RB1 predicted risk of pathologic T3 disease in an ethnicity-dependent manner. Furthermore, A greater proportion of AA men than EA men had triple-negative (ERG-negative/ETS-negative/SPINK1-negative) disease (51% v 35%).

Publications, Abstracts, and Presentations:

1. **Yamoah, K.**, Johnson, M., Choerung, V., Yousefi, K., Haddad, Z., Den, R.B., Lal, P., Feldman, M., Dicker, A.P., Klein, E.A, Davicioni, E., Rebbeck, T.R., Schaeffer, E.M., A novel biomarker signature which may predict aggressive disease in African-American men with prostate cancer. (JCO, July 2015) <http://jco.ascopubs.org/cgi/doi/10.1200/JCO.2014.59.8912>

2. **Yamoah, K.**, Deville, C., Vapiwala, N., Malkowicz, B., Spangler, E., Kattan, M., Dicker, A.P., Rebbeck, T. African American men with low-grade prostate cancer exhibit worse outcomes after prostatectomy compared with Caucasian men. Urologic Oncology- (DOI: <http://dx.doi.org/10.1016/j.urolonc.2014.07.005>)

3. **Yamoah, K.**, Walker A, Spangler E, Zeigler-Johnson CM, Malkowicz B, Lee DI, Dicker AP, Rebbeck TR, Lal P. African American race is a predictor of seminal vesicle invasion following radical prostatectomy. Clinical Genitourinary Cancer- 2015 Apr;13(2):e65-72

4. **Yamoah, K.**, Zeigler-Johnson, C., Abra, J., Malkowicz, B., Spangler, E., Dicker, A.P., Whittemore, A., Rebbeck, T. The impact of body mass index on treatment recommendations for patients with low-intermediate risk prostate cancer. (Manuscript submitted)

Abstracts:

Lal, P., **Yamoah, K.**, Ziober, A., Walker, A.H., Zhou, W., Spangler, E., Zeigler-Johnson, C., Feldman, M., Rebbeck, T.R., Racial Differences in the Distribution of Prostate Tumor Biomarkers: The SCORE Study, USCAP

Poster Presentation:

2015 GU- ASCO meeting

Genitourinary Symposium, Orlando, FL

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

2014 AACR-PCF meeting

Advancements in prostate cancer, San Diego, CA

Title: The impact of body mass index on treatment recommendations for patients with low-intermediate risk prostate cancer.

**Oral Presentation:*

2014 ACRO Annual Meeting, Oral presentation, Orlando, FL

Title: African American men with low-grade prostate cancer exhibit worse outcomes after prostatectomy compared with Caucasian men

Lay Press:

<http://www.ncbi.nlm.nih.gov/pubmed/?term=Yamoah+kosj>
<http://www.sciencedaily.com/releases/2015/07/150720161906.htm>
<http://www.renalandurologynews.com/prostate-cancer/biomarkers-predict-aggressive-prostate-cancer-black-patients/article/433386/>
<http://www.cancertodaymag.org/Winter2014/Pages/Personalizing-Prostate-Cancer-Treatment-African-Americans.aspx>
http://www.eurekalert.org/pub_releases/2013-01/tju-ssp012413.php
<http://www.sciencedaily.com/releases/2014/09/140908162119.htm>
<http://www.webmd.com/prostate-cancer/news/20140908/watchful-waiting-may-not-be-best-for-black-men-with-prostate-cancer>

Inventions, Patents and Licenses: None

Reportable Outcomes:

In this final report, we have identified a subset of PCa biomarkers that predict the risk of clinic-pathologic outcomes in an ethnicity-dependent manner. The results suggest that PCa may arise from distinct molecular pathways in EA compared to AA men. Furthermore, these biomarkers may explain in part the biologic contribution to ethnic disparity in PCa outcomes between EA and AA men.

Other Achievements:

I was among the few to be selected to participate in the AACR/ASCO methods in clinical trials workshop in 2014. The training and mentoring I received at this specialized workshop constitutes an important mechanism toward my goal of becoming an independent investigator. I plan to submit the results of my work for presentation at meetings of the American Society of Clinical Oncology (ASCO) and the American Society of Therapeutic Radiation Oncology (ASTRO), and the Prostate Cancer Foundation retreat, to get feedback from experts in the PCa field and to develop and maintain collaborations. The findings and ideas emerging from work supported by the CDMRP- DOD HDR program, along with the continued mentoring, should put me in a strong position towards the path to becoming an independent investigator.

I have begun to mentor a few trainees that have interest in prostate cancer as listed below.

Predoctoral Fellow Trainees:

Summer 2014 Adam Hesney, MD- Radiology Resident, Emory University - Supervised his research on prostate cancer

Summer 2014 Malika Mukhamedova, MS- Medical student, Thomas Jefferson University – Mentored student for summer research project

2014- present Alexandra Thompkins-Johns, Student at Drexel University - Spent a year working under my mentorship in the Rebbeck Lab, University of Pennsylvania

I have obtained a position as a physician scientist at the Moffitt Cancer Center where I will continue to do research in this area.

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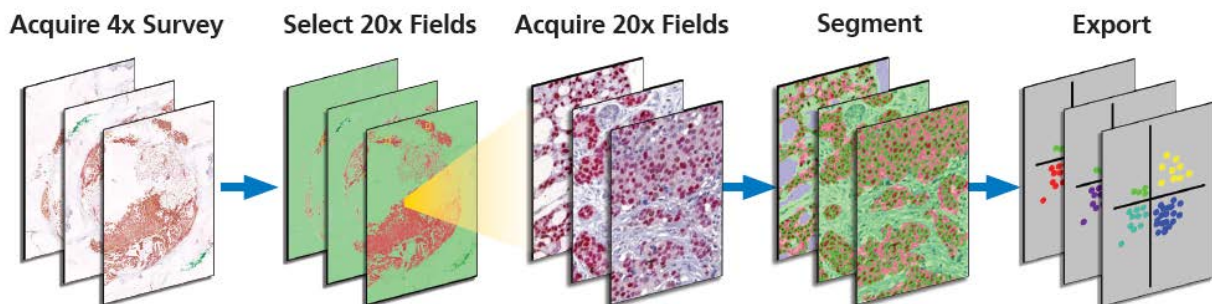
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Appendices:



Only useful for standardized markers or markers that give relative intensity

- **p53, p63, Ki67, PSMA, AR**
- Other 12 markers will be read as present/absent/indeterminate etc.
- **2000+** blocks stained so far
- Results coming in.....



Original article

African American men with low-grade prostate cancer have increased disease recurrence after prostatectomy compared with Caucasian men¹

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Abstract

Purpose: To explore whether disparities in outcomes exist between African American (AA) and Caucasian (CS) men with low-grade prostate cancer and similar cancer of the prostate risk assessment—postsurgery (CAPRA-S) features following prostatectomy (RP).

Methods: The overall cohort consisted of 1,265 men (234 AA and 1,031 CS) who met the National comprehensive cancer network criteria for low- to intermediate-risk prostate cancer and underwent RP between 1990 and 2012. We first evaluated whether clinical factors were associated with adverse pathologic outcomes and freedom from biochemical failure (FFbF) using the entire cohort. Next, we studied a subset of 705 men (112 AA and 593 CS) who had pathologic Gleason score ≤ 6 (low-grade disease). Using this cohort, we determined whether race affected FFbF in men with RP-proven low-grade disease and similar CAPRA-S scores.

Results: With a median follow-up time of 27 months, the overall 7-year FFbF rate was 86% vs. 79% in CS and AA men, respectively ($P = 0.035$). There was no significant difference in one or more adverse pathologic features between CS vs. AA men (27% vs. 31%; $P = 0.35$) or CAPRA-S score ($P = 0.28$). In the subset analysis of patients with low-grade disease, AA race was associated with worse FFbF outcomes ($P = 0.002$). Furthermore, AA race was a significant predictor of FFbF in men with low-grade disease (hazard ratio = 2.01, 95% CI: 1.08–3.72; $P = 0.029$).

Conclusions: AA race is a predictor of worse FFbF outcomes in men with low-grade disease after RP. These results suggest that a subset of AA men with low-grade disease may benefit from more aggressive treatment. © 2014 Elsevier Inc. All rights reserved.

Keywords: African American race; Disparities; Biochemical failure; Adverse pathologic features

1. Introduction

Men of African descent are known to experience greater incidence of and mortality due to prostate cancer (PCa) than men of other races [1]. African American (AA) men have

been shown to experience PCa at an earlier age than Caucasian (CS) men. Furthermore, AA men often present with higher grade and stage of disease at the time of diagnosis [2]. This observation has been partly attributed to socioeconomic factors and inadequate access to health care [3]. However, there is recent evidence suggesting that differences in genetic susceptibility play a major role in this disparity [4,5].

Owing to the relatively indolent nature of most PCAs diagnosed in the United States, the decision-making process for determining whether to pursue active surveillance (AS) or alternative management options is complicated by the

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balance between the life expectancy, comorbidities, clinical benefits and side effects of treatment [6]. The ability to predict clinical outcomes is critical in recommending appropriate treatment options for patients with PCa. Current National Comprehensive Cancer Network (NCCN) guidelines recommend AS as the preferred option for very low-risk PCa in men, defined as prostate-specific antigen (PSA) < 10 ng/ml, clinical stage \leq T1c, Gleason score (GS) \leq 6, positive cores \leq 2, and cancer involvement of \leq 50% per core. The goal of these recommendations is to prevent overtreatment of indolent cancers while identifying patients who develop disease progression and offering treatment with curative intent. However, most predictive tools currently used to risk stratify patients with PCa for treatment recommendations have not been developed or validated in AA men [7]. Furthermore, randomized clinical trials reporting on low-risk PCa treatment outcomes have been unable to effectively address whether interventions depend on race because of the inadequate numbers of AA participants [8].

Whether AA race acts as a prognostic factor for freedom from biochemical failure (FFbF) in patients with pathologic GS \leq 6 disease (referred to here as low-grade disease) and minimal adverse pathologic features after prostatectomy (RP) is poorly understood. The goal of this study is to determine whether disparities in adverse pathologic features and FFbF outcomes exist among an identical cohort of AA and CS men using a prospective cohort of patients with PCa treated with RP.

2. Patients and methods

2.1. Patient selection

The present study is a retrospective analysis of a prospective cohort of 2,012 men (298 AA, 1,673 CS, and 41 other race) with PCa treated with RP at the University of Pennsylvania Health System (UPHS; Philadelphia, PA) recruited to the Study of Clinical Outcomes, Risk and Ethnicity between 1990 and 2012 [9]. Patients without adequate preclinical data including initial PSA or biopsy GS at diagnosis were excluded from the analysis ($n = 457$). Patients of non-CS and non-AA ethnicity were excluded ($n = 41$). Patients with the following criteria were excluded from the study ($n = 249$): tumors > T3 category, GS between 7 (4 + 3) and 10, PSA level \geq 20 ng/ml, or regional lymph node metastasis on imaging or following bilateral pelvic lymph node dissection. We selected the remaining 1,265 patients for this study, which comprised the overall cohort who met the following NCCN criteria for low- to intermediate-risk PCa: biopsy GS \leq 7 (3 + 4), T-stage \leq T2c, PSA \leq 20 ng/ml, and undergoing a RP [10]. Of the 1,265 patients, a subset of 705 men (112 AA and 593 CS) with pathologic GS \leq 6 (low-grade disease determined post-RP) was further analyzed

in this study. We selected low- to intermediate-risk patients in the overall cohort to include patients with biopsy GS 7 (3 + 4) who were downgraded to pathologic GS 6 (3 + 3) following RP.

2.2. Preoperative staging

The patients were evaluated at the time of diagnosis by a thorough history and physical examination (including digital rectal examination) followed by routine laboratory studies, including serum PSA levels and GS determined by needle biopsy, and were reviewed at the UPHS. All the patients were staged according to the 1992 American Joint Committee on Cancer staging system [11].

2.3. Treatment

Surgical treatment consisted of a radical retropubic RP or robotic-assisted radical RP and bilateral pelvic lymph node sampling. All pathology slides were prepared as per standard institutional protocol. The RP specimen was initially coated with india ink and fixed in formalin. The whole gland was step-sectioned at 3-mm intervals and the resulting sections were fixed into tissue cassettes. Tissue sections were embedded in paraffin blocks, from which sections were prepared and stained with hematoxylin and eosin for routine histologic analysis by a dedicated genitourinary pathologist. Adverse pathologic features consisting of extraprostatic extension (EPE), seminal vesicle invasion (SVI), and surgical margin status (SM) were noted and recorded. At the discretion of the treating physician, patients with adverse pathologic features including EPE, SVI, or positive surgical margins were treated with adjuvant radiation therapy (RT) or androgen deprivation therapy (ADT) or a combination of both. ADT consisted of a gonadotropin-releasing hormone agonist (leuprolide acetate or goserelin acetate) with or without an antiandrogen (e.g., flutamide and bicalutamide).

2.4. Follow-up and treatment end points

Patient information at each follow-up visit including digital rectal examination and serial PSA values were noted and recorded. PSA failure was defined as a single PSA \geq 0.2 ng/ml along with documentation of failure by a physician or when 2 consecutive PSA values of 0.2 ng/ml were obtained after an undetectable value. Start of the prospective follow-up (i.e., time zero) was defined at the date of surgery for all patients. If PSA was never undetectable postoperatively, then PSA failure was assigned at time zero. Patients with no follow-up PSA measurements ($n = 190$, 14.5%) were included for the evaluation of differences in preoperative and pathologic characteristics but not for the analysis on FFbF outcomes.

2.5. Statistical analysis

Clinical and pathologic variables were compared across the race groups using an analysis of variance model for continuous variables or contingency table chi square test of homogeneity for categorical variables. Predictors of adverse pathologic features were examined using logistic regression models. Age, PSA, and year of surgery were examined as continuous variables. T category (T1a-c vs. T2), biopsy GS, and race were examined as categorical variables. Based on the pathologic findings following surgery, patients were further stratified using cancer of the prostate risk assessment—postsurgery (CAPRA-S), a validated postsurgical score that predicts the risk of cancer recurrence following RP [12]. Variables for determining CAPRA-S score included preoperative PSA, pathologic GS, SM, EPE, and SVI. Patients were categorized as having low (CAPRA-S < 3), intermediate (CAPRA-S: 3–5), and high (CAPRA-S > 5) risk of recurrence.

For survival analysis, the primary event of interest was PSA failure (biochemical disease recurrence). We excluded individuals who did not experience PSA failure at the time of last PSA measurement <0.2 ng/dl or were lost to follow-up. Time to PSA failure was used as a surrogate for FFbF. The FFbF rates were compared across the groups using the log-rank survivorship and the Kaplan-Meier analyses. For multivariate analysis, a forward-stepwise Cox proportional hazards model was used with $P < 0.2$ determining which variables were entered into the model at each step. The variable with the highest P value was successively deleted until only variables with $P < 0.2$ remained. The analyses were conducted using STATA statistical software version 13.0 (STATA Corporation). This study was approved by our Institutional Review Board.

3. Results

The baseline clinical and pathologic characteristics of overall cohort are listed in Table 1. Preoperative factors such as age at RP, PSA at diagnosis, and clinical T category were similar between groups. Compared with CS men, AA men had higher biopsy GS ($P < 0.001$). There was no difference in 1 or more adverse pathologic features among race groups (28% vs. 31%; $P = 0.41$). However, a greater number of AA men had pathologic GS ≥ 7 (52% vs. 43%; $P = 0.01$) and SVI (6% vs. 3%; $P = 0.02$). There was no difference in the use of radiotherapy or ADT between the groups.

Using the Kaplan-Meier survival analysis method, the effect of race on FFbF was evaluated in the overall cohort. The mean and median follow-up time from RP date until last follow-up PSA date was 45 months and 27 (range: 1–207) months, respectively. During this time period, 144 patients (11.5%) experienced biochemical failure. The 7-year FFbF rate between CS men and AA men was

Table 1

Pretreatment and posttreatment characteristics and pathologic outcomes of NCCN low- and intermediate-risk men undergoing radical prostatectomy at the University of Pennsylvania, 1990 to 2012 (overall cohort)

	Caucasian cohort (n = 1,031)	%	African American cohort (n = 234)	%	P value
Age, y					0.48 ^a
Median	60		58		
Mean	59.1		57.8		
IQR	54–64		52–62		
iPSA, ng/ml					0.89 ^a
0–4.0	271	26	59	25	
4.01–10	659	64	150	65	
10.01–20	101	10	25	11	
Median	5.1		5.6		
Mean	5.8		6.2		
IQR	4.1–6.7		4.1–7.8		
Biopsy Gleason score					<0.001 ^b
≤ 6	948	90	162	67	
7 (3 + 4)	103	10	54	23	
Clinical stage					0.63 ^b
T1A–C	583	81	149	85	
T2A	111	16	22	12	
T2B	8	1	4	2	
T2C	12	2	1	1	
Year of prostatectomy					0.006 ^a
Median	2003		2004		
Mean	2002.7		2003.7		
IQR	1999–2007		2000–2008		
Pathologic stage					0.07 ^b
pT2N0	802	77	175	74	
pT3aN0	202	20	44	19	
pT3bN0	23	2	13	6	
pT4aN0	4	1	2	1	
Pathologic Gleason score					<0.001 ^b
≤ 6	596	57	113	46	
7 (3 + 4)	229	22	81	36	
7 (4 + 3)	35	4	14	7	
7 (Unspecified)	145	14	22	9	
8–10	26	3	4	2	
Gleason score upgrading					0.25 ^b
6/7–7/ (8–10)	369	35	72	30	
Adverse pathologic features ^c					0.35 ^b
0	757	73	164	69	
1	147	15	33	15	
≥ 2	127	12	37	16	
Extraprostatic spread	223	22	58	25	0.32 ^b
Seminal vesicle invasion	27	3	13	6	0.02 ^b
Positive surgical margin	162	16	39	17	0.71 ^b
Radiotherapy	11	1	3	1	0.78 ^b
ADT	35	3	8	3	0.5 ^b

Note: Boldfaced values represent statistically significant differences between groups.

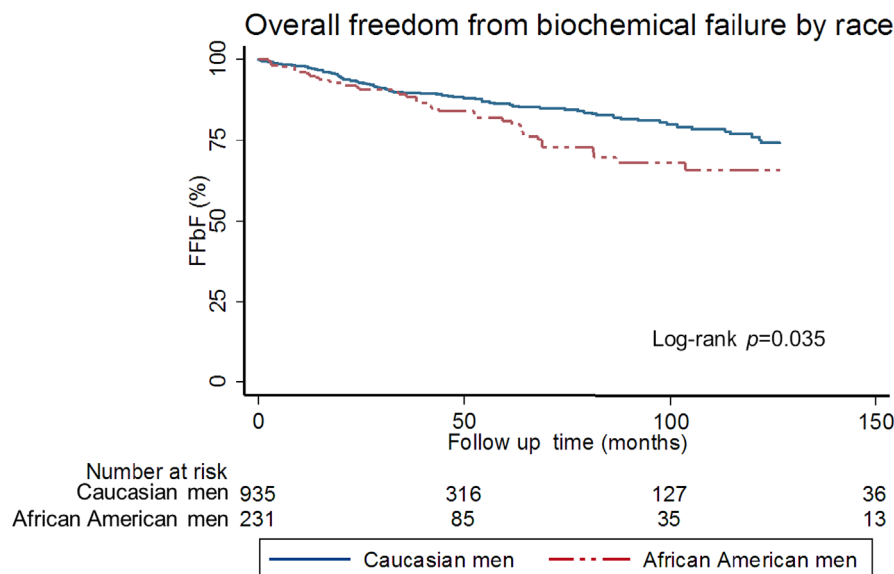
iPSA = initial Prostate-specific antigen, IQR = interquartile range.

^aP value derived from the analysis of variance model.

^bP value derived from Person's chi-square test.

^cAdverse pathologic features: extraprostatic extension, seminal vesicle invasion, and positive surgical margin.

86% vs. 79%, respectively (Fig. 1; $P = 0.035$). There was no difference in adverse pathologic features using the validated CAPRA-S score for risk of recurrence, (Fig. 2A; $P = 0.28$). However, the corresponding Kaplan-Meier estimates of FFbF showed worse outcomes among AA



Abbreviations: FFbF- Freedom From biochemical Failure, NCCN- National Comprehensive Cancer Network
P values derived from the Mantel-Cox log-rank test.

Fig. 1. The Kaplan-Meier curves for FFbF outcomes by race in NCCN low- and intermediate-risk men undergoing radical prostatectomy at the University of Pennsylvania, 1990 to 2012 (overall cohort). (Color version of figure is available online.)

men in the CAPRA-S < 3 group (Fig. 2B; $P = 0.01$). There was no statistically significant difference in the CAPRA-S 3 to 5 and >5 risk groups likely because of the small numbers in both groups (Fig. 2B; $P = 0.67$ and $P = 0.19$), respectively.

Using a Cox proportional hazard model, the predictors of FFbF following RP were determined (Table 2). In the multivariate model of the overall cohort, T category (hazard ratio [HR] = 2.92; 95% CI: 1.17–7.32; $P = 0.02$) serum PSA (HR = 1.14; 95% CI: 1.09–1.20; $P < 0.001$), clinical GS (HR = 1.51; 95% CI: 1.01–2.27; $P = 0.045$), pathologic GS (HR = 1.59; 95% CI: 1.18–2.15; $P = 0.002$), EPE (HR = 2.01; 95% CI: 1.33–3.04; $P = 0.001$), SVI (HR = 2.47, 95% CI: 1.48–4.12; $P = 0.001$), and SM (HR = 1.7; 95% CI: 1.13–2.56; $P = 0.01$) were predictors of FFbF.

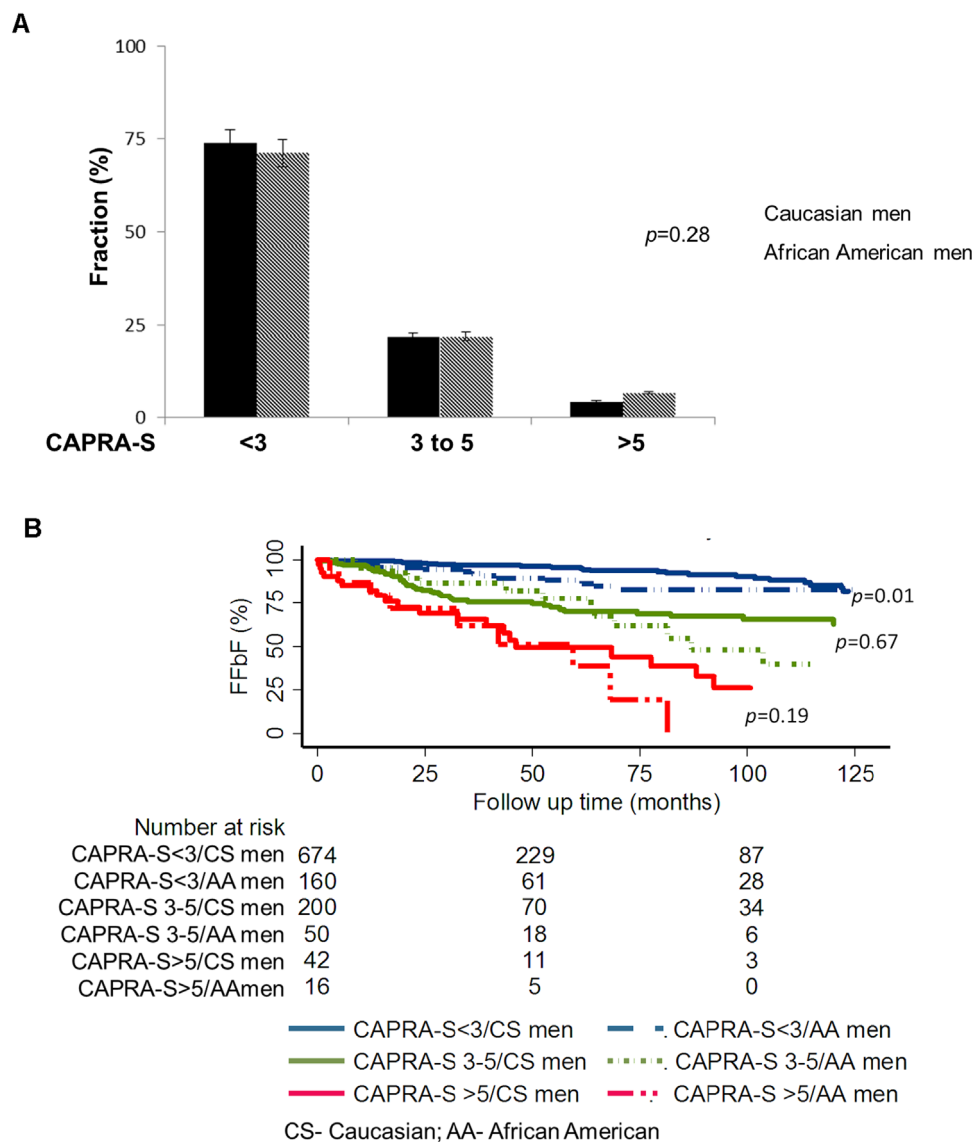
To study the outcomes in men with RP-proven low-grade PCa, we analyzed the characteristics of 705 men (112 AA and 593 CS) who had pathologic GS ≤ 6 (i.e., low-grade disease) following RP, using similar analytic methods employed in the overall cohort. For this analysis, patients who initially had biopsy GS < 7 and then on RP were upgraded to pathologic Gleason grade ≥ 7 were excluded. This represents a true cohort of patients with low-grade disease. In this cohort, there was no difference in any pretreatment and posttreatment characteristics between race groups among patients with low-grade disease (Table 3). To determine the effect of race on FFbF, we analyzed this cohort with low-grade disease with similar CAPRA-S score. This group underwent RP as monotherapy with <5% needing any additional therapy (Table 3). Among patients with low-grade

disease, AA men demonstrated worse 7-year FFbF (Fig. 3A; $P = 0.002$) despite similar CAPRA-S scores in comparison with CS men (Fig. 3B; $P = 0.90$).

Using a multivariate model, the significant predictors of risk for FFbF following RP were determined for patients with low-grade disease (Table 4). Serum PSA (HR = 1.24; 95% CI: 1.15–1.34; $P < 0.001$), EPE (HR = 3.77; 95% CI: 1.79–7.95; $P < 0.001$), and AA race (HR = 2.01, 95% CI: 1.08–3.72; $P = 0.029$) remained predictors of FFbF.

4. Discussion

In this report, we show that AA men with low-grade disease have worse FFbF in comparison with their CS counterparts (Fig. 3A). This observation is not likely because of treatment differences because patient groups had similar adverse pathologic features, as demonstrated by comparable CAPRA-S scores between AA and CS men (Fig. 3B), and there were no differences by race in the utilization of adjuvant radiotherapy or ADT. Additionally, there was no difference in the extent of positive margin status by race to suggest suboptimal surgical technique in AA patients (Table 3). Less than 5% of the entire cohort had documented treatment with additional RT or ADT. These data may reflect the low physician-referral patterns for adjuvant treatment for eligible patients [13,14]. However, these results should be interpreted with caution, as a number of patients may have undergone RP at UPHS and then received RT at another institution.



Abbreviations: FFbF- Freedom From biochemical Failure, NCCN- National Comprehensive Cancer Network, CAPRA-S- Cancer of the Prostate Risk Assessment Post-Surgical scoring system

Fig. 2. (A) Distribution of CAPRA-S score groups by race and (B) the Kaplan-Meier curves for FFbF outcomes by race stratified by CAPRA-S score groups in NCCN low- and intermediate-risk men undergoing radical prostatectomy at the University of Pennsylvania, 1990 to 2012 (overall cohort). (Color version of figure is available online.)

Overtreatment of $GS \leq 6$ PCa diagnosed on biopsies triggered by elevated PSA level remains an ongoing controversy [15]. In fact, a few recent studies have suggested that removing the label “cancer” from biopsy $GS \leq 6$ disease could potentially reduce overtreatment of low-grade disease [16,17]. However, our results suggest caution in applying this to some men and particularly AA men. Biopsy GS alone usually underestimates both grade and extent of disease, thus relabeling of biopsy $GS \leq 6$ disease as noncancer could result in a missed opportunity of curative treatment in some individuals. Consistent with our study (Table 1), the rate of upgrading from biopsy $GS \leq 6$ to pathologic $GS \geq 7$ at RP is estimated at 25% to 35% [18]. A number of studies have shown a suboptimal

correlation between biopsy Gleason scoring and radical RP, despite the migration from sextant biopsies to 12-core sampling. Cookson et al. [19] showed that evaluation of a biopsy GS was identical to that of a specimen core in 31% of cases, whereas it was discrepant by >2 GS in 26%. In more contemporary series using 12 or more biopsy cores, the upgrade rate is approximately 30% [20]. Furthermore, there is evidence to suggest that the zonal distribution of cancer foci within the prostate may differ between AA and CS men, thus influencing the result of evaluation of core biopsies [21]. Therefore, the current practice of recommending no active treatment for patients by relying heavily on parameters such as biopsy grade, number of positive cores on biopsy, and initial PSA may need to be validated in AA men.

Table 2

Univariate and multivariate regression models of factors predicting FFbF in NCCN low- and intermediate-risk men undergoing radical prostatectomy at the University of Pennsylvania, 1990 to 2012 (overall cohort)

	HR	95% CI	P value
Univariate analysis			
Age	0.99	0.96–1.01	0.48
Race	1.43	0.99–2.05	0.05
Serum PSA	1.16	1.11–1.21	<0.001
T-stage	3.79	1.55–9.26	0.003
Clinical Gleason score	2.63	1.80–3.83	<0.001
Year of prostatectomy	1.04	0.99–1.08	0.09
Extraprostatic spread	3.89	2.81–5.38	<0.001
Positive surgical margins	3.72	2.67–5.19	<0.001
Seminal vesicle invasion	5.9	3.71–9.38	<0.001
Pathologic Gleason score	2.63	2.01–3.44	<0.001
Multivariate analysis			
Age	0.99	0.96–1.02	0.50
Race	1.38	0.92–2.07	0.12
Serum PSA	1.13	1.08–1.19	<0.001
T category	2.92	1.17–7.32	0.02
Prostate-specific antigen	1.14	1.09–1.20	<0.001
Extraprostatic spread	2.01	1.33–3.04	0.001
Seminal vesicle invasion	2.47	1.48–4.12	0.001
Positive surgical margins	1.7	1.13–2.56	0.01
Clinical Gleason score	1.11	0.69–1.79	0.67
Pathologic Gleason score	1.59	1.18–2.15	0.009

Note: Boldfaced values represent statistically significant differences between groups.

P values derived from a Cox proportional hazards model.

As per the NCCN guidelines, AS is the preferred treatment option for men with very low-risk PCa and life expectancy ≤ 20 years or those with low-risk disease and life expectancy < 10 years [22]. The advantage of AS is to prevent overtreatment of indolent disease while actively monitoring the course of the disease and to intervene only when progression occurs in patients with more aggressive disease [23]. However, evidence for the benefit of AS was based on studies conducted in primarily CS cohorts [24,25]. In studies where race was reported, 5% to 10% of patients enrolled in AS program were AA men [20,26]. One retrospective study evaluated the effect of race on discontinuation of AS for patients with low-risk PCa. Their results showed that AA men had more aggressive disease and were more likely to progress on AS and proceed to treatment faster than CS men were [27]. A large study on pathologic and FFbF outcomes in very low-risk AA men who qualify for AS but underwent immediate RP showed that AA men had significantly higher rates of upgrading, positive surgical margins, and CAPRA-S score than CS men did [28]. However, data from our study showed worse FFbF even in AA patients despite similar CAPRA-S scores and low-grade disease when compared with their CS counterparts (Figs. 2 and 3). The discrepancies in pathologic outcomes between our low-grade study and the prior study are likely due to the fact that, unlike the prior study that evaluated low-risk patients as determined by biopsy Gleason grade, we analyzed a cohort of patients with truly low-grade (pathologic Gleason

Table 3

Pretreatment and posttreatment characteristics and pathologic outcomes of men with pathologic Gleason score ≤ 6 (low-grade disease) following radical prostatectomy at the University of Pennsylvania, 1990 to 2012

	Caucasian cohort (n = 593)	%	African American cohort (n = 112)	%	P value
Age, y					
Median	59		58		0.39 ^a
Mean	58.4		57.8		
IQR	54–63		52–62		
iPSA, ng/ml					
0–4.0	179	30	29	26	0.05 ^a
4.1–10	357	60	79	70	
10.1–20	57	10	4	4	
Median	5		5.4		
Mean	5.6		5.6		
IQR	3.7–6.5		4.1–7.0		
Clinical stage					
T1A–C	357	86	73	91	0.17 ^b
T2A	61	14	7	9	
Pathologic stage					
pT2N0	515	87	96	86	0.45 ^b
pT3aN0	72	12	13	12	
pT3bN0	4	1	2	2	
pT4aN0	2	0	0	0	
Adverse pathologic features ^c					
0	497	84	92	82	0.85 ^b
1	56	9	11	10	
≥ 2	40	7	9	8	
Extraprostatic spread	76	13	16	14	0.64 ^b
Seminal vesicle invasion	6	1	2	2	0.47 ^b
Positive surgical margin	54	9	11	10	0.81 ^b
Radiotherapy	3	0.5	1	1	0.62 ^b
ADT	28	5	5	5	0.94 ^b

iPSA = initial prostate-specific antigen; IQR = interquartile range.

^aP value derived from the analysis of variance model.

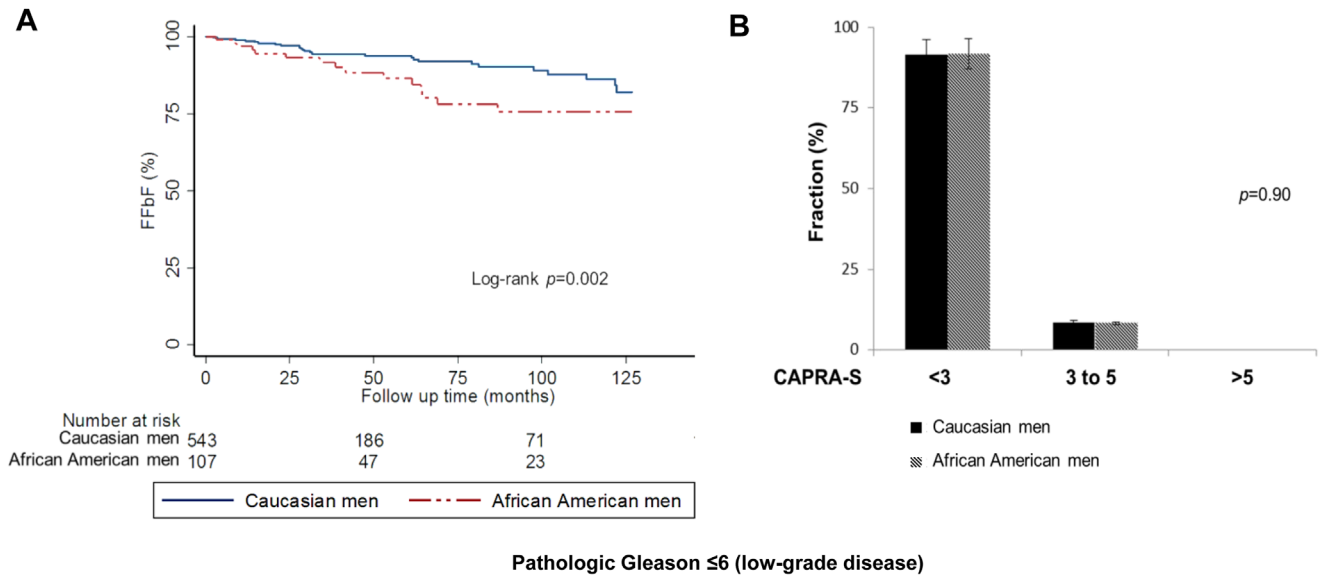
^bP value derived from the Person's chi-square test.

^cAdverse pathologic features: extraprostatic extension, seminal vesicle invasion, and positive surgical margin.

grade ≤ 6) disease. Nonetheless, these emerging data suggest that further study is needed to determine whether some AA men with low-grade disease and CAPRA-S score > 2 may derive benefit from additional/adjuvant therapy such as radiation or ADT. In light of these findings, AA men found to have biopsy GS ≤ 6 with clinically low-risk disease who choose AS should undergo more careful monitoring owing to the possibility of increased oncologic risk.

It is noteworthy that several studies have been conducted regarding the effect of race on FFbF after definitive PCA treatment with radical RP or radiotherapy. However, results from these studies have proven inconclusive [28–30]. These inconsistencies may be partly because of differences in the selection criteria and imbalances in the comparison groups.

The strength of our study is that it provides a stringent analysis of AA and CS men with similar adverse pathologic



Abbreviations: FFbF- Freedom From biochemical Failure, CAPRA-S- Cancer of the Prostate Risk Assessment Post-Surgical scoring system

Fig. 3. (A and B) The Kaplan-Meier curves for FFbF outcomes and CAPRA-S score grouping by race in men with pathologic Gleason ≤ 6 following radical prostatectomy at the University of Pennsylvania, 1990 to 2012. (Color version of figure is available online.)

features. Therefore, known socioeconomic factors such as inaccessibility to health care, late diagnosis, and suboptimal treatment are less likely to account for outcomes disparity in this cohort. Our data have major clinical implications for treatment recommendations, which includes potentially undertreating low-grade disease in AA men. Furthermore,

Table 4

Univariate and multivariate regression models of factors predicting FFbF in men with pathologic Gleason score ≤ 6 (low-grade disease) following radical prostatectomy at the University of Pennsylvania, 1990 to 2012

	HR	95% CI	P value
Univariate analysis			
Age	1.01	0.96–1.05	0.63
African American race	2.02	1.09–3.74	0.025
Serum PSA	1.22	1.06–1.41	0.005
T category	1.37	0.87–2.14	0.17
Clinical Gleason score	2.48	0.76–8.19	0.13
Year of prostatectomy	0.99	0.91–1.06	0.61
Extraprostatic spread	4.05	2.27–7.23	<0.001
Positive surgical margins	3.71	1.94–7.04	<0.001
Seminal vesicle invasion	8.1	2.87–22.8	<0.001
Multivariate analysis			
Age	1.02	0.97–1.06	0.44
Year of prostatectomy	0.99	0.92–1.07	0.81
Clinical Gleason score	1.23	0.35–4.41	0.74
Serum PSA	1.24	1.15–1.34	<0.001
Extraprostatic spread	3.77	1.79–7.95	<0.001
African American race	2.01	1.08–3.72	0.029
Seminal vesicle invasion	2.71	0.89–8.57	0.089
Positive surgical margins	1.83	0.81–4.12	0.15

Note: Boldfaced values represent statistically significant differences between groups.

P values derived from a Cox proportional hazards model.

AA men with low-grade disease need to be enrolled on clinical trials evaluating biomarker-driven risk-adapted treatment options to improve outcomes.

A major limitation to this study is that it has a relatively small number of AA men compared with CS men and represents the experience from a single tertiary center. Though the men in this study had identical adverse pathologic risk features, a randomized controlled trial is required to adequately answer the question of race and FFbF outcomes in men with low-grade disease. The outcomes were not adjusted for socioeconomic factors, diet, obesity, comorbid conditions, and adherence to treatment recommendations. Information on the tumor volume or the percentage of cores positive for tumor were inconsistently reported, and hence we could not adequately investigate outcomes in very low-risk patients who might have been eligible for AS.

5. Conclusion

AA race is a predictor of worse FFbF in patients with pathologic GS ≤ 6 or low-grade disease and favorable pathologic features. This highlights the need for clinically useful biomarkers that will enable us to identify AA men appropriate for AS vs. those harboring aggressive disease that may ultimately benefit from exploration of additional/ adjuvant therapy such as radiation or ADT.

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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 P
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 Figure 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 Figure A1](#)) Similar analyses

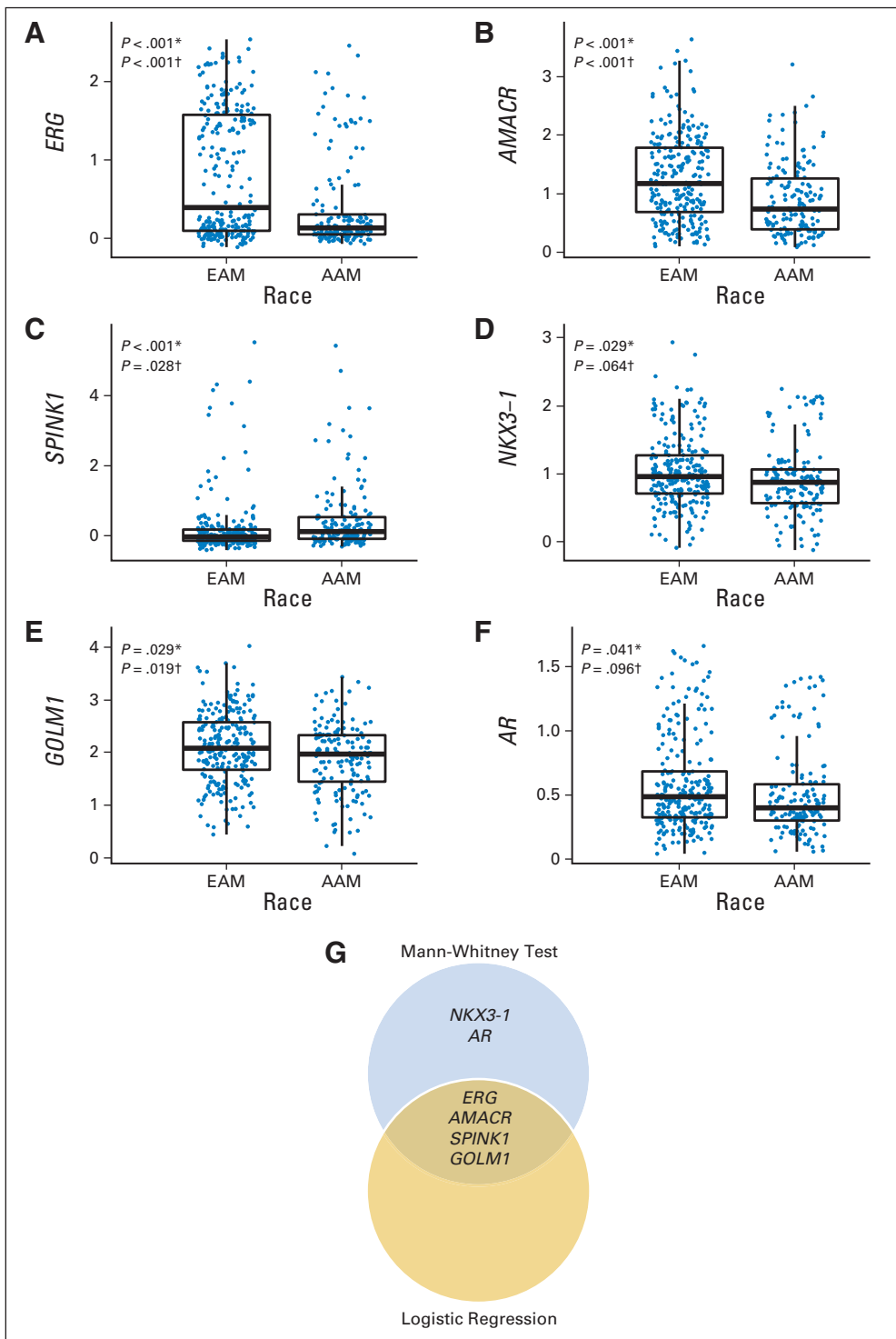


Fig 1. (A-F) Box-and-whisker plots showing distribution and median expression levels of biomarkers with significant ethnic variation. (G) Venn diagram showing biomarkers with significant ethnic variation in one or more statistical model. (*) Mann-Whitney U test. (†) Logistic regression. All P values 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, *SRDA2*

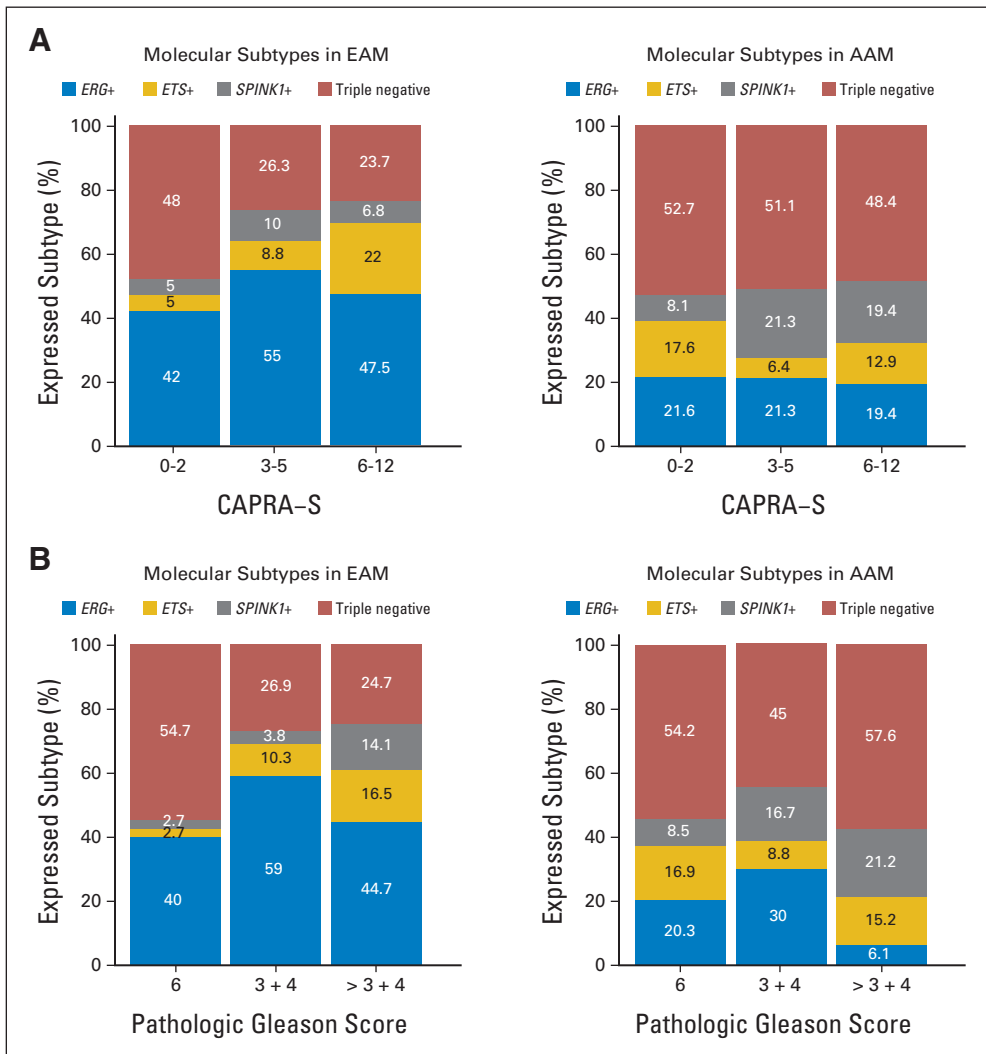


Fig 2. (A) *ETS* variant subtyping by ethnicity and Cancer of the Prostate Risk Assessment postsurgical (CAPRA-S) score. (B) *ETS* variant subtyping by ethnicity and pathologic Gleason score. AAM, African American men; EAM, European American men.

($P = .023$) 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 significant

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*, *RBI*, *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

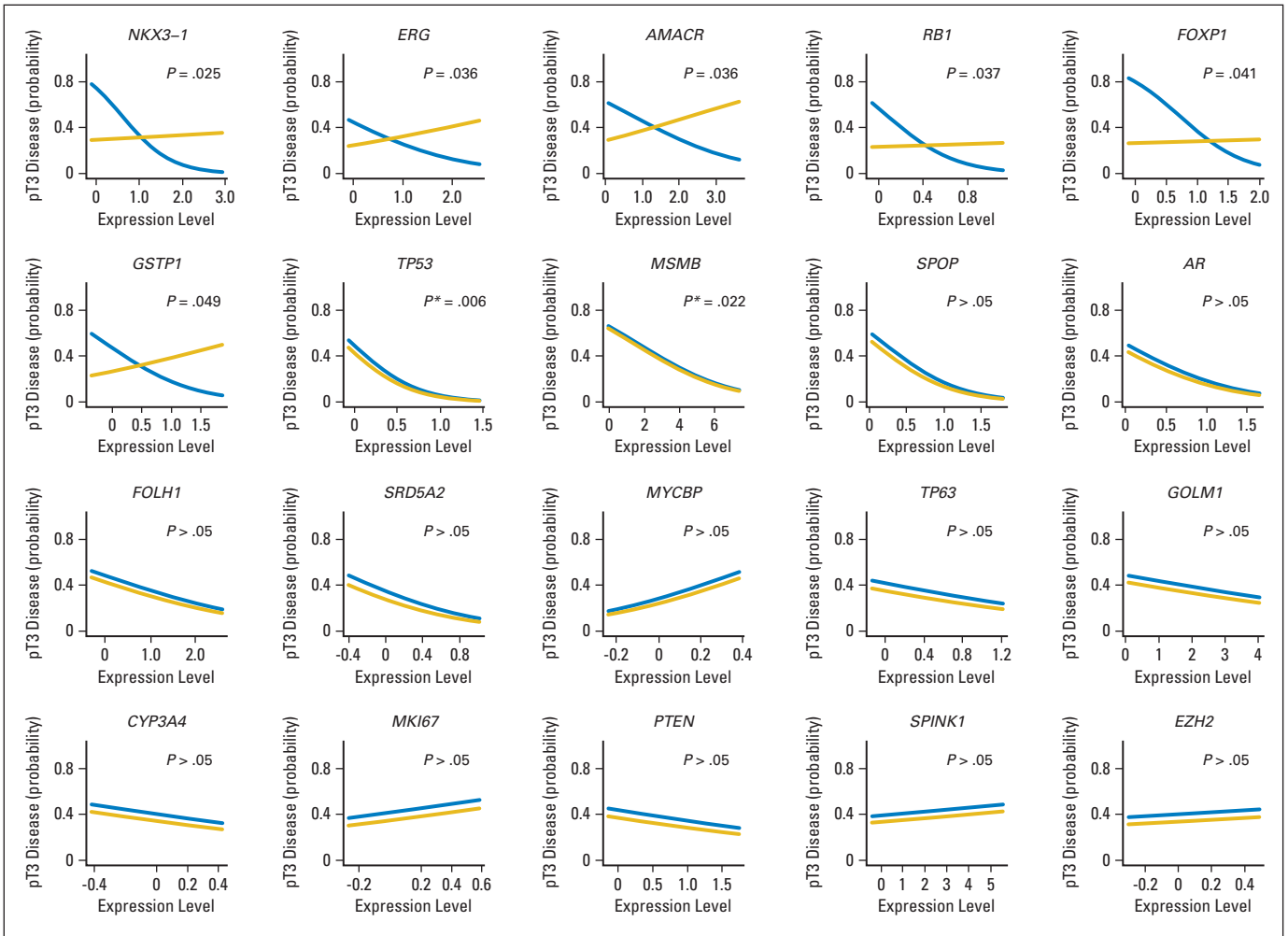


Fig A1. Predicted probability curves showing biomarkers predictive of pathologic T3 (pT3) disease by ethnicity. Blue lines indicate African American men; gold lines indicate European American men.