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Association of race and socioeconomic status with prostate cancer genomic risk classifier: Implications for precision medicine in prostate cancer

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical and Translational Science

by

Abhinav Grover

Thesis Committee:
Professor Dr. Sheldon Greenfield, MD, Chair
Professor Dr. Sherrie Kaplan, PhD, MPH
Associate Clinical Professor Dr. Edward Uchio, MD

2018

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DEDICATION

To

my parents and friends

In recognition of their support

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ACKNOWLEDGMENTS

I would like to express the deepest appreciation to my committee chair, Professor Dr. Sheldon Greenfield, who has the attitude and the substance of a genius: he continually and convincingly conveyed a spirit of adventure in regard to research and scholarship, and an excitement in regard to teaching. Without his guidance and persistent help this dissertation would not have been possible.

I would like to thank my committee members, Professor Dr. Sherrie Kaplan and Associate Clinical Professor Dr. Edward Uchio, who helped me during the research.

I wanted to especially thank my father, Ashok Grover and mother, Neelam Grover for their remarkable support and encouragement. Last but not the least, I would like to thank Dr. Kanika Kakkar who motivated me to pursue my passion for academic medicine.

ABSTRACT OF THE THESIS

Association of race and socioeconomic status with prostate cancer genomic risk classifier: Implications for precision medicine in prostate cancer

By

Abhinav Grover

Master of Science in Biomedical and Translational Science

University of California, Irvine, 2018

Professor Dr. Sheldon Greenfield, Chair

Background: Africans Americans, low socioeconomic status (SES) and other minority patients have been observed to have higher rates of metastasis and prostate cancer specific mortality. There are several known genetic differences in prostate cancer between Whites and other minority patients which may adversely impact interpretations of validated genetic tests like the Decipher.

Methods: We conducted a cross-sectional analytical study of men with early stage prostate cancer. Mean Decipher scores, Decipher risk categories and gene expression signatures related to molecular pathways and treatment response were analyzed by race/ ethnicity and SES using one way of analysis of variance, linear and logistic regression.

Results: African Americans and other minority patients had non-significantly higher mean Decipher scores (p=0.227). There were non-significant differences in the distribution of Decipher risk categories by race/ethnicity (p=0.167). African American men had slightly lower ETS-related gene (ERG), lower E26 transformation-specific (ETS), higher serine protease inhibitor Kazal-type 1 (SPINK1) and higher Triple Negative molecular subtypes compared to

Whites. African American men had slightly higher post-op radiation response (p=0.207), higher dasatinib sensitivity (p=0.002), but lower docetaxel sensitivity (p=0.007) and lower androgen receptor signaling (p=0.133) compared to Whites.

Conclusions: There were non-significant associations of Decipher scores with race or SES. However, African Americans and other minorities differed in the molecular subtypes and pharmacogenomics of docetaxel and dasatinib compared to Whites. Future efforts to create a precision medicine model for predicting outcomes and personalizing treatments to reduce prostate cancer disparities should include genetic differences in tumor by race/ ethnicity and treatment response.

1. INTRODUCTION

There are roughly 200,000 new cases of prostate cancer diagnosed each year and it leads to 30,000 prostate cancer related deaths annually in the United States.(1) African Americans and other minority races of prostate cancer patients have higher rates of incidence, metastasis and progression as compared to Whites (2) although some of these differences may be explained by quality of and access to healthcare. (3) Their management requires accurate risk stratification. (4) However, there are still several biological and genetic differences by race/ethnicity that have been related to metastatic progression of prostate cancer in these subgroups of patients. (5–7) Such differences may impact the utility of clinically validated genomic prediction models like Decipher as they have not been extensively studied in racially and socioeconomically diverse populations. (8) There are few recent studies which show that race and SES were not associated with Decipher risk scores and also that Africans Americans had different gene expression signatures related to molecular pathways and treatment response as compared to other races but these studies were retrospective with limitations like selection bias, lack of evidence of temporal relationship and a prognostic effect was not examined. (9,10) Therefore, we aimed to understand the association of race and socioeconomic factors on Decipher genomic risk classifiers and gene expression in a prospective study to understand its implications for precision medicine prostate cancer care especially in minority populations.

2. BACKGROUND

2.1 Prostate cancer incidence, prevalence, progression and mortality

There are more than 3 million men living with prostate cancer in the United States and in 2018, it is projected that 200,000 new cases will be diagnosed and around 30,000 will die of the disease. The lifetime risk for developing prostate cancer in men is 11.2%. Around 98.2% of the patients survive at the end of 5 years. (11)

2.2 Risk factors of prostate cancer

There are multiple factors which increase prostate cancer incidence risk including advanced age, family history of prostate cancer, race (Africans Americans), dihydrotestsodterone (DHT), diet (Vitamin E, folic acid, dairy) whereas other factors like folate, finasteride and dutasteride may decrease the risk of prostate cancer. However, studies have shown inconsistent associations between dietary factors like fats, multivitamins etc. and prostate cancer. (12) The risk factors for mortality include age, higher comorbidity burden, race (Africans Americans), clinical stage and lower socioeconomic status. (11) There were reported associations between higher levels of SES and prostate cancer incidence mainly after the introduction of screening patients for high PSA. (13–17) But, according to a prospective study by Rundle et al., differences in screening frequency only partially explained the association between SES and prostate cancer risk and suggested that other health care related factors should also be considered as explanatory factors. There are inconsistent associations of socioeconomic status reported for different races and ethnic groups. (18,19) A large study found association between higher SES and increased incidence of prostate cancer among non-Hispanic Whites, but not among Hispanics or African-Americans (19); while a study in San Francisco reported higher SES to be associated with

prostate cancer among Asian/Pacific Islanders and Hispanics but not among non-Hispanic Whites and African-Americans. (18)

2.3 Screening and diagnosis of prostate cancer

The methods commonly used for screening prostate cancer include digital rectal exam, prostate specific antigen and prostate cancer gene 3 (PCA3) RNA test. PSA test, although sensitive is non-specific and biopsy may help in the diagnosis of prostate cancer. The shortcomings of the non-specific screening tools are high false positive rates leading to over diagnosis and sometimes over treatment. Radical prostatectomy and radiation therapy can have adverse outcomes like erectile dysfunction, urinary incontinence, and bowel problems and may lead to higher risk of suicide post the diagnosis. (12)

2.4 Risk stratification, staging and management of prostate cancer

The management of prostate cancer depends on several factors like life expectancy, family history, pathological features and genetics of the tumor. Therefore, prostate cancer is grouped into various risk groups based on PSA levels, stage and Gleason grade. The risk groups are categorized as very low, low, favorable intermediate, unfavorable intermediate, high, very high, regional and metastatic. The NCCN guidelines recommend different treatment options for different stages of prostate cancer. For most patients with high life expectancy and very low risk, low risk and favorable intermediate risk disease, active surveillance (AS), radiotherapy (RT), high intensity focused ultrasound (HIFU) or radical prostatectomy (RP) are options. (20) However, according to CEASAR (Comparative Effectiveness Analysis of Surgery and Radiation for Localized Prostate Cancer) study, the optimal management for localized prostate cancer should also take into account adverse effects of treatment, competing mortality risks, patient preferences and baseline sexual, bowel and urinary function. The study reported that RP was

associated with higher adverse outcomes related to sexual and urinary function than RT or AS after 3 years; however, there were no meaningful differences in bowel function, hormonal function and quality of life in different treatment arms. These findings may help patients and physicians make informed shared decisions about treatments. (4) Molecular genetic testing of prostate cancer may have additional utility and is recommended for low, favorable intermediate risk groups if life expectancy is more than 10 years. Germline testing is recommended if there is strong family history or greater than high risk disease. (20)

2.5 Prostate cancer genetics and epigenetics

There is well established genetic contribution to risk of prostate cancer which includes inherited germline pathologic variants likely to be found in individuals with strong family history (prostate, breast, ovarian, pancreatic cancer, lynch syndrome) or early onset disease, non-heritable somatic variants/mutations and epigenetics (change in phenotype/gene expression without change in genotype). All of these genetic and epigenetic changes can influence gene expression and thereby, increase prostate cancer risk. (21)

Germline variants and single nucleotide polymorphisms (SNPs) of BRCA1, BRCA2, mismatch repair genes and HOXB13 have high penetrance and have been associated with moderate to high lifetime risk of developing prostate cancer probably contributing to 5% of disease burden.(22) Additionally, more than 150 SNPs with low penetrance have been identified but their clinical utility is still being investigated either alone or in combinations of SNPs. The low penetrance SNPs and environment interaction results in 30% of prostate cancer cases and the rest 60-80% burden can be attributed to sporadic somatic mutations. (21,22)

There is a need to identify the molecular basis of more aggressive disease at diagnosis and prostate cancer recurrence in African American men and other minorities, who are more likely to

die from prostate cancer than other populations. Epigenetics is one such mechanism which could be a mediator of some of these observed disparities. (23) Epigenetics contributes to gene regulation throughout the whole life course of an organism, regulates gene expression by changing chromatin organization and DNA accessibility and include different processes, such as DNA methylation, histone modifications, and post-transcriptional gene regulation by non-coding RNAs (microRNAs). This process can be influenced by various environmental factors. (23) In tumorigenesis, DNA methylation and demethylation are associated with silencing tumor suppressor genes and activating oncogenes, respectively. Histone post-translational modifications (PTMs) include acetylation, biotinylation, methylation, phosphorylation, ubiquitination, SUMOylation, ADP (adenosine diphosphate) ribosylation, proline isomerization, citrulination, butyrylation, propionylation, and glycosylation, which are known as "the histone code" and strongly contribute to the control of gene expression. (24–33)

There are several epigenetic changes that are common in prostate cancer and important in tumor progression and can aid in accurate risk stratification of patients reducing the risk of over or under treatment especially in African Americans and minorities. There is no one epigenetic marker that has been identified to predict aggressiveness of prostate cancer. However, there are many potential epigenetic markers such as for DNA hypermethylation (RARB, RASSF1A, AOX1, GSTP1, IGF2), DNA hypo-methylation (IGF2), acetylation of HAT, HDAC1, HDAC2 which are histone proteins, histone modifications like increased H3K27 trimethylation (EZH2) detected in prostate cancer tissue and over expression of miRNA-18a, miRNA-129, both micro RNAs being detected in peripheral blood. (23) DNA hypomethylation which happens globally and IGF2 imprinting and hypermethylation of the promoter regions is associated with prostate cancer development and rapid progression. (34–36) Hypermethylation of RASSF1A (Ras

association domain family protein 1, isoform A) which happens more frequently in aggressive tumors. (37,38) There is a gene which is involved in repair of DNA, GSTP1 (Glutathion S-transferase Pi 1) which is hypermethylated in early stages of prostate cancer and helps distinguish benign hyperplasia from metastatic disease. (38–40) The methylation of AOX1 and RARB have been associated with progressive disease. (41,42) Histone modifications like H3K4 methylation analyzed by Ellinger et al in patients with prostate cancer revealed that it could predict PSA recurrence post prostatectomy. (43) The histone methyltransferase EZH2 is responsible for H3K27 trimethylation and its overexpression, found in metastatic castration resistant prostate cancer, correlates with promoter hypermethylation and suppression of few of the tumor suppressor genes. (44,45) Zhao et al. recently reported that a 4-gene methylation classifier panel (APC, CRIP3, GSTP1, and HOXD8) was able to predict patient reclassification on AS. (46) Other biomarkers like TOP2A and EZH2 need further assessment as biomarkers for early identification of patients with increased metastatic potential that may benefit from adjuvant or neo-adjuvant targeted therapy approaches. (47)

2.6 Prostate cancer genetic tests

The various genetic and epigenetic changes related to prostate cancer progression alter the gene expression. This can lead to the observed clinical and pathological features in aggressive tumors. There are various genetic tests that measure this gene expression like Oncotype Dx, Prolaris and Decipher and help in better prognostication of patients. The Decipher® prostate cancer genomic classifier (GC) risk prediction model was developed by investigators at Mayo Clinic and GenomeDx Biosciences. The Decipher test is a 22 gene expression assay which looks at several molecular pathways related to prostate cancer progression and metastasis and has been shown to important for treatment reclassification, both pre and post treatment. (Table 1) The

Decipher biopsy test is used to provide better risk assessment for more individual treatment for all patients diagnosed with localized prostate cancer whereas the Decipher radical prostatectomy (RP) test classifies post-surgery patient with adverse pathology into genomic risk categories for metastasis. (48,49) The Decipher report provides a score ranging from 0 to 1 with higher scores predicting higher risk of metastasis. Scores are used to classify patients into low risk (0-0.45), intermediate risk (0.45-0.6) and high risk (0.6-1) groups. In a recent individual patient level meta-analysis, 855 patients (85% Caucasians) were included. There were 520 classified as low risk, 193 as at intermediate risk and 141 were classified as high risk score patients based on Decipher report. Of these, metastasis was reported for 2.4%, 5.8% and 15.2% of patients in low, intermediate and high risk patients respectively. The false negative rate was 3.3% when cut off for predicting metastasis is taken to be 0.6. (50)

In addition to the risk scores, GenomeDx provides reports for research use from the Decipher genome resource information database (GRID) registry. This registry includes classification of tumors into luminal or basal subtype, molecular subtyping into ERG, ETS, SPINK and triple negative subgroups. The population rank provided in the reports is based on database of 2829 individuals who underwent gene expression testing previously with scores indicating the percentage of individuals below the patient for that characteristic. They are further classified as average if they fall within the average distribution range, or favorable/ unfavorable if they fall outside the average distribution range. Population rank scores of various gene expression signatures related to androgen receptor signaling, tumor proliferation, response to androgen deprivation therapy (ADT) and response to radiation treatment are reported as is lastly information on individual gene over or under-expression of 22-36 genes. (Table 1) The luminal and basal-like prostate cancer subgroups demonstrate divergent clinical behavior. Patients with

luminal B tumors respond better to postoperative androgen deprivation therapy than do patients with non–luminal B tumors according to a retrospective study. (51) The ERG+, ETS+, SPINK+ and TripleNeg are the various known molecular subgroups of prostate cancer. Decipher was shown to be predictive of metastasis independent of molecular subtypes and to be an effective prognostic tool in all subtypes. (52) The system biology landscape of the Decipher biomarkers shows modularity and several gene expression markers related to different biological processes interact with each other, leading to disease progression. The Decipher genes form 10 intertwined modules representing several pathways related to prostate cancer development and progression; mainly cell-cycle, cell-adhesion and cytoskeleton reorganization. Other pathways are related to apoptosis, cell differentiation, immune modularity and inflammation. (53) The molecular pathways underlying progression have been previously shown to be different by race/ ethnicity. (10)

Table 1. Description of different pathways and individual genes related to prostate cancer provided in the Decipher GRID report

Pathways	**ANDROGEN SIGNALING**	**SMALL CELL**	**PROLIFERATION /GROWTH FACTORS**	**DNA REPAIR**	**INVASION/ ANGIOGENESIS**	**IMMUNO-ONCOLOGY**
Description	-interaction with AR -PCa initiation , progression -ADT helpful	-rare -aggressive -resistant to ADT - high CHGA, AURKA, MYCN expression -loss of RB1	-growth factor gene mediated proliferation signals -therapeutic targeting	-genomic instability - determines radiation and PARP inhibitor response -AR involved in DDR activation	-low expression of cell adhesion genes - angiogenesis gene like VEGF/HGF have high expression - therapeutic targets against VEGF/HGF	-expression of genes for ligands on immune cells to bypass immune checkpoint - increased PCSM - drugs against CTLA-4, PD1/PDL1/PDL2 useful
Genes	AR	RB1	Ki67	ATM	SChLAP1	PD1
	KLK2	CCND1	TOP2A**	ATR	EZH2**	PDL1
	PSA (KLK3)	CHGA	EGFR	RAD21	SPARCL1	PDL2
	PCA3*	AURKA	HER2/NEU	DNAPK	GSTP1**	B7H3
	NKX3-1	NEAT1	ERBB3	NBN	VEGFR2 (KDR)	CTLA4
	SRD5A1	MYCN	c-MET	PARP1***	HIF-1α	IDO1

Pca- prostate cancer, AR-androgen receptor, ADT-androgen deprivation therapy, CHGA- Chromogranin A, CCND1 - Cyclin D1, DDR - DNA damage and repair, ECM-extracellular matrix, PCSM-prostate cancer specific mortality, PARP- poly ADP ribose polymerase

*PCA3 over-expression is associated with advanced pathologic stage. In the early stages of prostate cancer, PCA3 is highly abundant making it a robust diagnostic tumor biomarker. However, patients with advanced-stage prostate cancer and poor outcomes typically have low PCA3 expression

** The genes TOP2A, EZH2 and GSTP1 have previously been associated with epigenetic changes in prostate cancer. TOP2A is a cell proliferation marker and high expression is correlated to poor outcome in prostate cancer. Patients with TOP2A over-expression may be sensitive to etoposide chemotherapy. High expression of EZH2 is associated with invasion, metastatic progression and castrate-resistant prostate cancer (CRPC). Low GSTP1 expression is associated with an increased risk of recurrence. High Expression - High GSTP1 expression may demarcate a basal cell tumor.

***PARP is a key enzyme in DNA damage response and repair pathways. Certain cancers, including ovarian and breast cancer, can become highly dependent on PARP activity making this an attractive therapeutic target.

2.7 Association of race and SES with Decipher genomic risk classifier and GRID reports

There are few studies which report the association of race and SES with Decipher risk scores and genome resource information database reports. Recently, in May 2018, Rayford et al. analyzed Decipher GRID reports of 529 Africans Americans in comparison to 514 patients from other races. The study reported that Africans Americans tended were of younger age at diagnosis ($p<0.004$), had lower Gleason scores ($p=0.04$), had higher prostate specific antigen levels ($p<0.001$), had more Triple Negative (48% vs 39%, $p<0.005$) and SPINK1 (23% vs 11%, $p<0.001$) molecular subtype tumors, had lower Androgen Receptor activity (OR: 2.1, $p=0.01$) and high radiation response (OR: 2.2, $p=0.01$). They also had higher levels of cancer pathway genes sets such as immune response (TNFA signaling via NFKB, interferon alpha, gamma response), apoptosis, hypoxia, reactive oxygen species, K-Ras and p53 signaling, whereas non-African American men tumors were associated with higher levels of fatty acid metabolism, glycolysis, Myc targets, mitotic spindle, DNA repair, PI3K via AKT/mTOR and WNT via beta catenin signaling. However, no difference was reported in Decipher risk categories by race/ ethnicity. (10) In an abstract published by Weiner A. et al., there was no reported association of SES with Decipher scores in 2953 biopsy and 4416 prostatectomy specimens even after stratification of cohort by age, race and tumor characteristics. (9) However, these studies were done retrospectively and have limitations including selection bias, lack of ability to establish causal associations etc. (3)

2.8 Specific aims and objectives

1. What is the association of race with the Decipher risk scores and gene expression signatures?

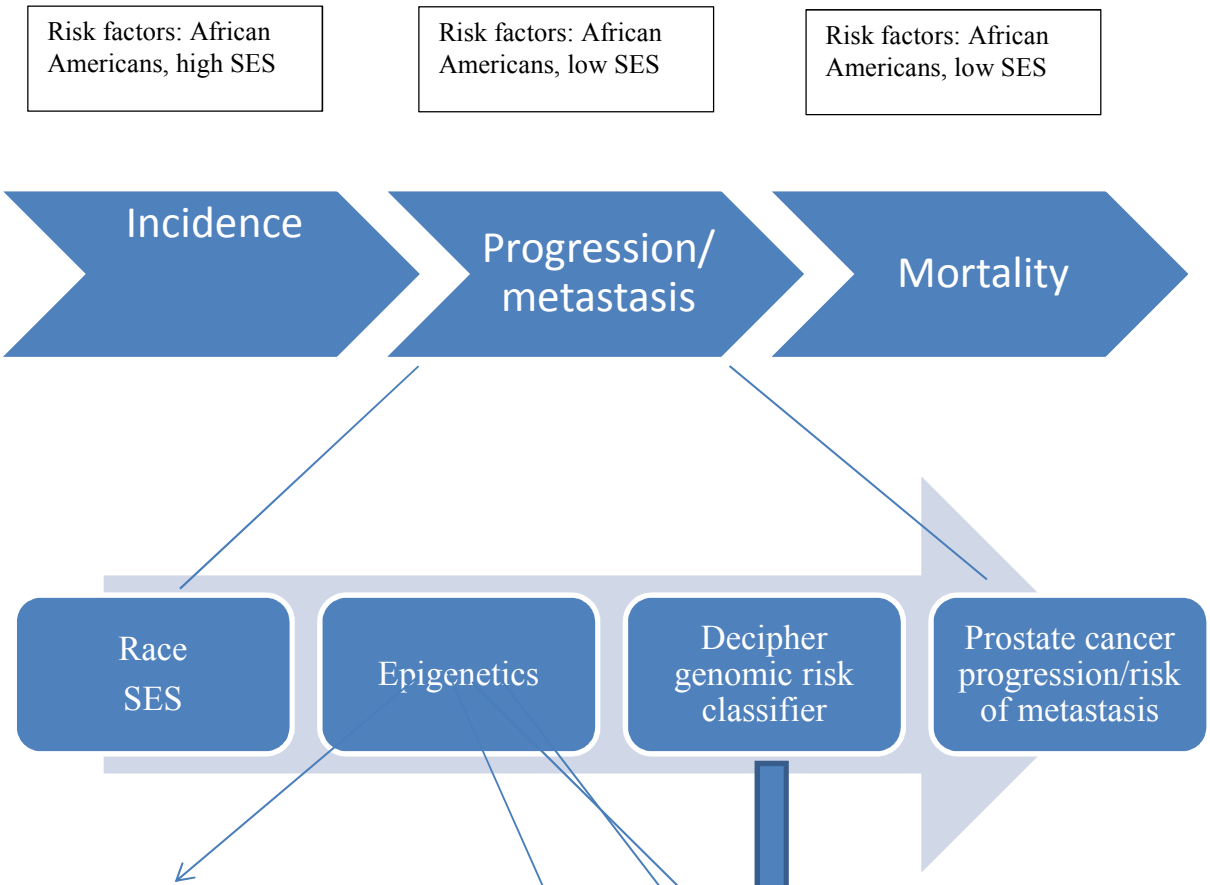
2. What is the association of SES with Decipher risk scores?

The study was conceptually designed to focus in on the progression of prostate cancer from indolent to aggressive one and the significant socio-demographic and genetic predictors of rapid disease progression. Further, we studied the association of these socio-demographic characteristics with known gene expression markers (Decipher test) of aggressiveness and progression. (Figure 1a)

The biological basis of this association lays in the influence that race, SES, other environmental factors and their interactions have on germline mutations, somatic mutations and epigenetic changes which eventually culminate in gene expression changes causing disease progression. (Figure 1b)

Additionally, the genetic predictors of disease progression include several markers including epigenetic ones, only few of which are included in the Decipher panel of genes. (Figure 1a)

Figure 1. a) Conceptual model for the study

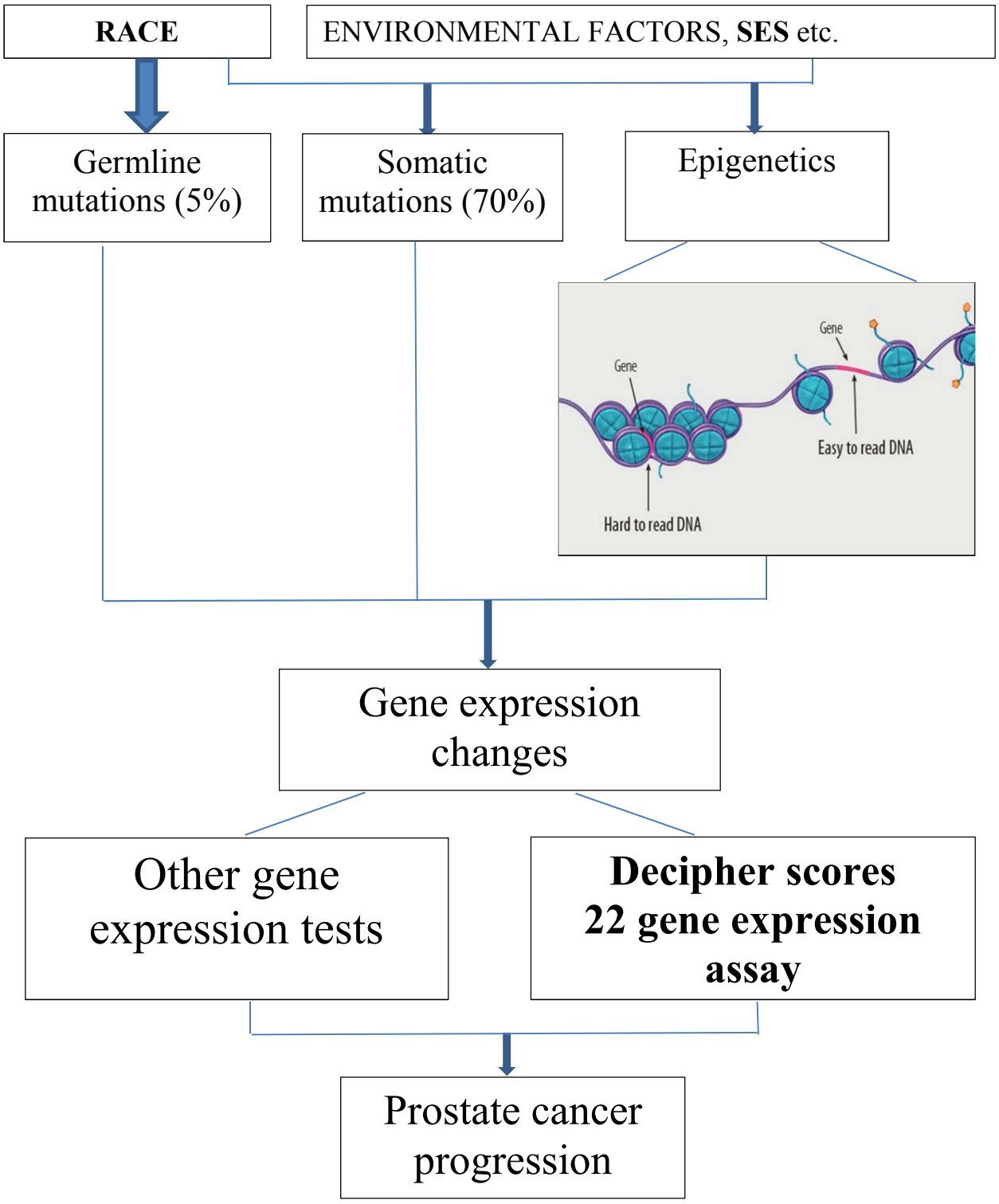


Epigenetic markers of prostate cancer

- IGF2
- **GSTP1**
- RARB
- RASSF1A
- **EZH2**
- **TOP2A**
- IGF2
- HAT
- HDAC1
- HDAC2
- miRNA

ANDROGEN SIGNALING	SMALL CELL	PROLIFE-RATION	DNA REPAIR	INVASION	IMMUNO-ONCOLOGY
AR	RB1	Ki67	ATM	SChLAP1	PD1
KLK2	CCND1	**TOP2A****	ATR	**EZH2****	PDL1
PSA	CHGA	EGFR	RAD21	SPARCL1	PDL2
PCA3	AURKA	HER2/NEU	DNAPK	**GSTP1****	B7H3
NKX3-1	NEAT1	ERBB3	NBN	VEGFR2	CTLA4
SRD5A1	MYCN	c-MET	PARP1	HIF-1α	IDO1

Figure 1. b) Biological model for disease progression



3. METHODS

3.1 Study design

This was a cross-sectional analytical study of data collected from an 18-month longitudinal cohort study with prospective stratification based on risk and treatment of men with early stage prostate cancer. It was conducted at 5 large hospitals (UC Irvine, UCLA, West LA VA, Long Beach VA, and Cedars-Sinai Medical Center (CSMC)). The study was approved by the Institutional Review Board. (HS# 2017-3634) and compliance with Health Insurance Portability and Accountability Act was maintained. An informed consent was obtained from all the patients. Patients with newly diagnosed clinically localized prostate cancer without evidence of nodal or distant metastasis prior to definitive treatment were included in the study. Specific inclusion criteria were: 18 – 79 years of age at the time of enrollment, PSA value <50ng/ml, Clinical stage of T1 or T2, status post prostate biopsy within the last 3 months, and no prior history of prostate cancer treatment and absence of distant metastasis and lymph node involvement. Patients were excluded from the study if they: had a diagnosis of other malignancy (excluding squamous or basal cell carcinoma of the skin) within 3 years of diagnosis of prostate cancer; were age 80 or older, had PSA values equal to or greater than 50ng/ml, or had clinically locally advanced or metastatic disease. An informed consent was obtained from all eligible patients.

A total of 206 patients diagnosed with prostate cancer who either underwent biopsy or radical prostatectomy, and had Decipher molecular genetic testing of the tumor done were included in the analysis. The sample size of 206 was based on the available cohort out of which self-reported race information was available for 175 patients; and SES (education) was reported for 156 of these patients.

3.2 Data collection

Patient reported data - Once enrolled in the study, patients were asked to complete a baseline questionnaire. Patient demographics including age, race, ethnicity, education, employment status, insurance type, and marital status were also collected. (Appendix A)

Tumor-Specific Data - Laboratory parameters like PSA levels, Gleason grade groups, Decipher risk scores and GRID reports (molecular subtype, gene expression signatures) were collected and entered in the electronic database.

Additionally, specific data regarding diagnosis and type of treatment (Surgery, Radiation, Brachytherapy, Cryotherapy, HIFU, Active Surveillance, Watchful Waiting) was also collected.

3.3 Outcomes

Primary outcome - The primary outcome of the study were Decipher genomic risk classifier scores (range 0-1) and risk categories based on the scores (Low (0-0.45), intermediate (0.45-0.6) and high risk (0.6-1)).

Secondary Outcomes- Secondary outcomes of the study were gene expression signatures provided in the GRID reports for molecular subtyping, molecular pathways (AR signaling, tumor proliferation pathway, radiation response signature and drug sensitivity).

3.4 Variables

Dependent variables

1. Decipher scores (range from 0-1)
2. Decipher risk categories

3. Gene signatures' population rank for AR signaling, tumor proliferation pathway, radiation response, ADT response, docetaxel and dasatinib sensitivity. The population rank provided in the reports is based on database of 2829 individuals who underwent gene expression testing previously with scores indicating the percentage of individuals below the patient for that characteristic. They are further classified as average if they fall within the average distribution range, or favorable/ unfavorable if they fall outside the average distribution range.
4. Gene signatures for AR signaling, tumor proliferation pathway, radiation response, ADT response, docetaxel and dasatinib sensitivity categorized as favorable, unfavorable or average response.

Independent variables

1. Race was categorized into Whites, Blacks, Hispanics, Asians and all other races were aggregated to a single category.
2. Socioeconomic status was estimated using the number of years of education

Covariates

1. Study site (dummy coded)
2. Age (in years)
3. PSA levels
4. Gleason stage categorized into 5 groups based on previously published classification of tumor grade and dummy coded

3.5 Statistical Analysis

The demographic and clinical characteristics were compared across the different races (mixed and other races were clubbed in one category) using one way analysis of variance and Chi square test for continuous and categorical variables respectively.

The Decipher scores and sociodemographic variables were assessed for their correlation with each other using Pearson's correlation test.

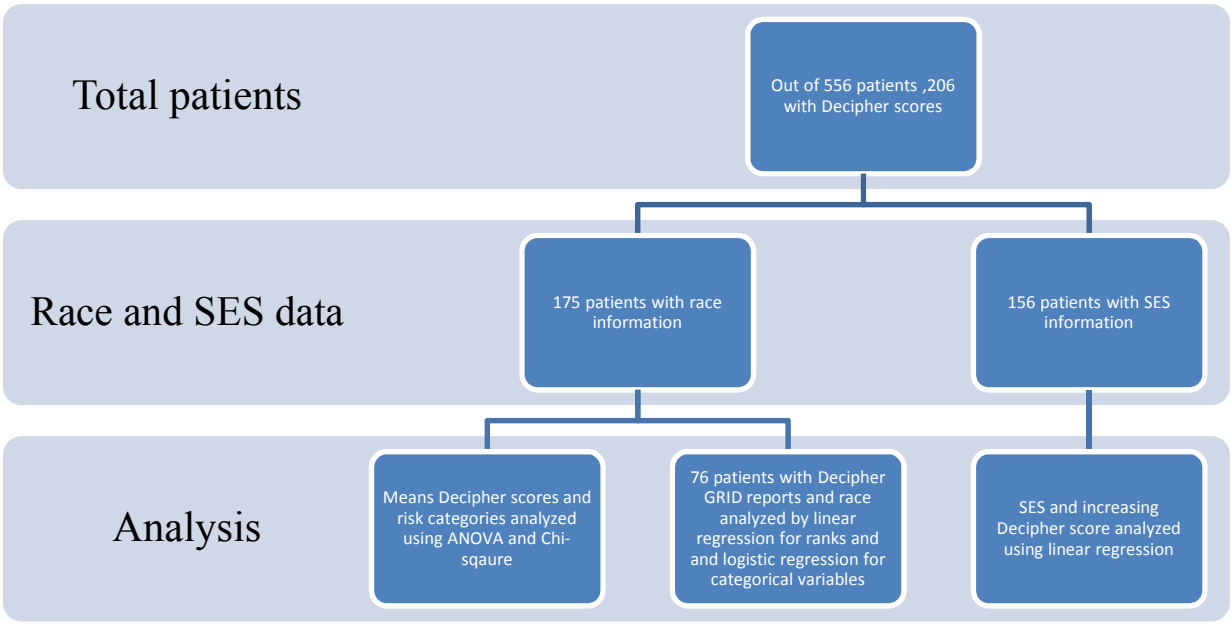
The means of Decipher scores, 5 year risk of metastasis, PCSM and distribution of risk categories were compared across different races and analyzed using one-way analysis of variance and chi-square test respectively.

The population rank for gene expression signatures were compared between Whites and African American patients using linear regression analysis after adjustment for covariates such as PSA levels as they were different amongst the two races.

Multivariable logistic regression odds ratio (OR) analysis after adjustment for PSA levels was used to assess the association of gene expression signatures (signatures of molecular pathways related to progression and signatures of treatment response) comparing African-Americans as compared to Whites.

Linear regressions were used to assess the correlation between SES (based on number of years of education) with increasing Decipher score after adjusting for tumor characteristics, race, and age. Covariates in the model included categorical variables like study site, race and Gleason grade which were dummy coded prior to the analysis.

Figure 2. Study outline



4. RESULTS

We enrolled 556 early stage prostate cancer patients from 5 medical centers in Southern California including UC Irvine, UCLA, Los Angeles Veteran Affairs, Long Beach Veteran Affairs and Cedars-Sinai, out of which Decipher reports were available for 206 patients (20 from radical prostatectomy and 186 from biopsy specimens) whose demographic, clinical and genomic characteristics are described in Table 1. These patients had Decipher scores with a wide range of distribution (0.01- 0.97); 49% were classified as low risk, 23% as intermediate risk and 28% as at high risk for recurrence/ metastasis. (Fig 3, 4)

Table 2. Demographic, clinical and genomic characteristics of patients

Characteristic	**Value**
Age (years)	65.41 ± 7.15
Education (mean, years)	15.6 ± 6.95
Race (n, %)	
Whites	104 (59.4)
Blacks	46 (26.2)
Others	25 (14.2)
Study site (n)	
UC Irvine	53
LB VA	25
UCLA	40
LA VA	51
Cedars	28
PSA (ng/ml)	7.9 ± 6.04
Gleason grade	
Grade 1 (n, %)	27 (17)
Grade 2 (n, %)	65 (41)
Grade 3 (n, %)	42 (26)
Grade 4 (n, %)	16 (10)
Grade 5 (n, %)	9 (6)
Tumor stage (n, %)	
cT1c	128 (62)
cT2a	8 (4)
cT2b	1 (0.5)
cT2c	1 (0.5)
Decipher scores (mean ±S.D.)	0.46 ± 0.23
Decipher categories	
Low risk (n, %)	101 (49)
Intermediate risk (n, %)	46 (22.3)
High risk (n, %)	59 (28.6)

The Decipher risk scores and the calculated 5 year risk of metastasis and calculated 10 year prostate cancer specific mortality follow an exponential curve. (Figure 5, 6)

Figure 3. Distribution of Decipher scores

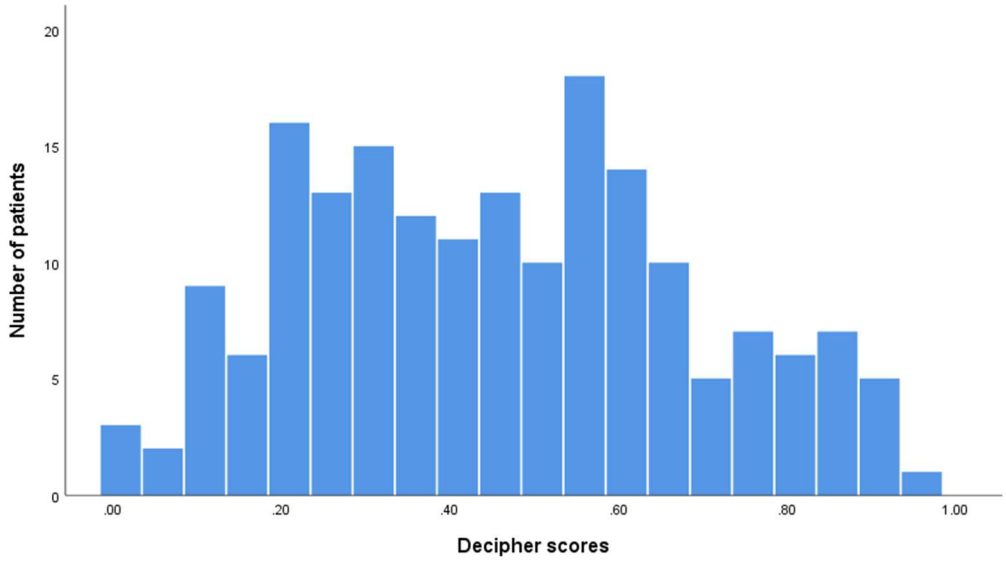


Figure 4. Percentage of patients in low, intermediate and high risk Decipher categories

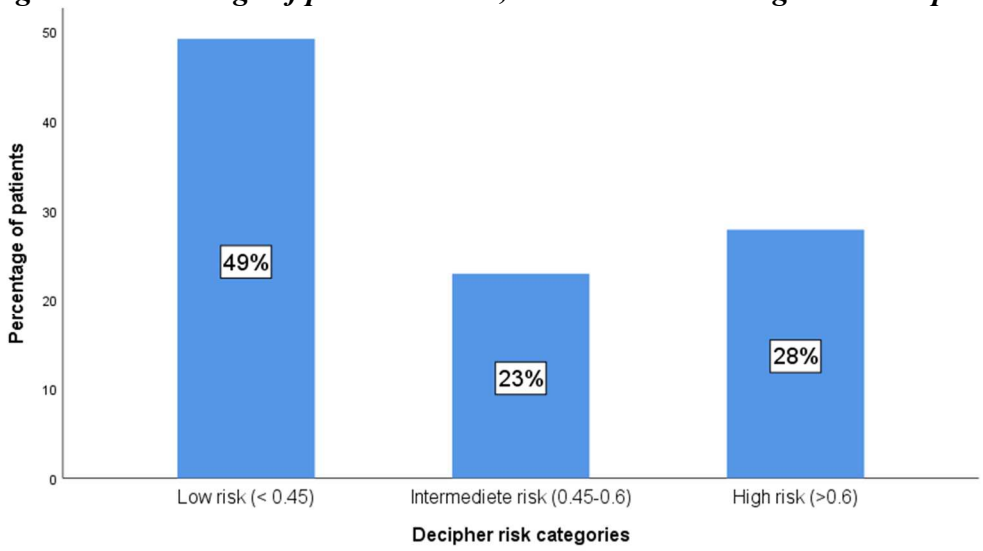


Figure 5. Plot depicting calculated 5 year risk of metastasis against the Decipher scores

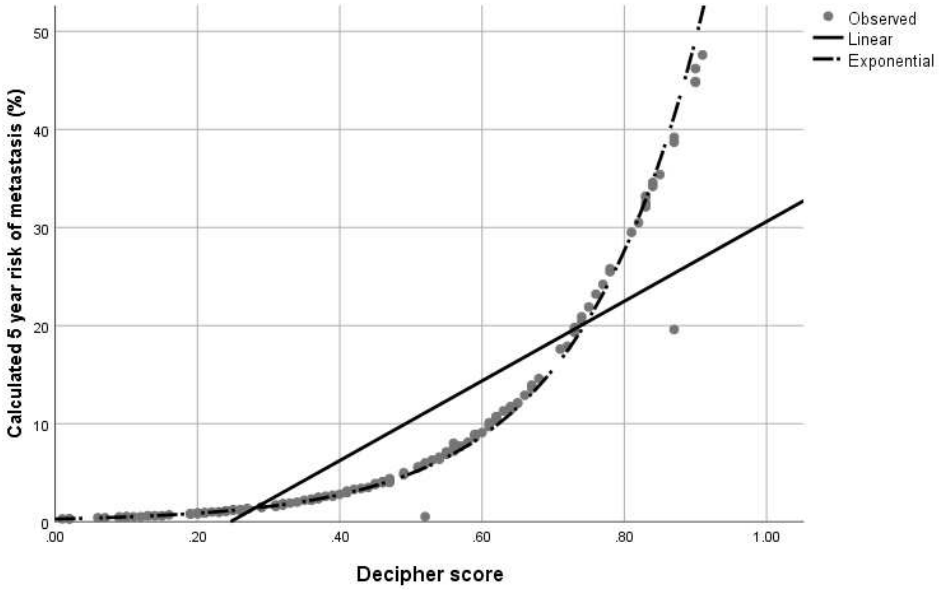
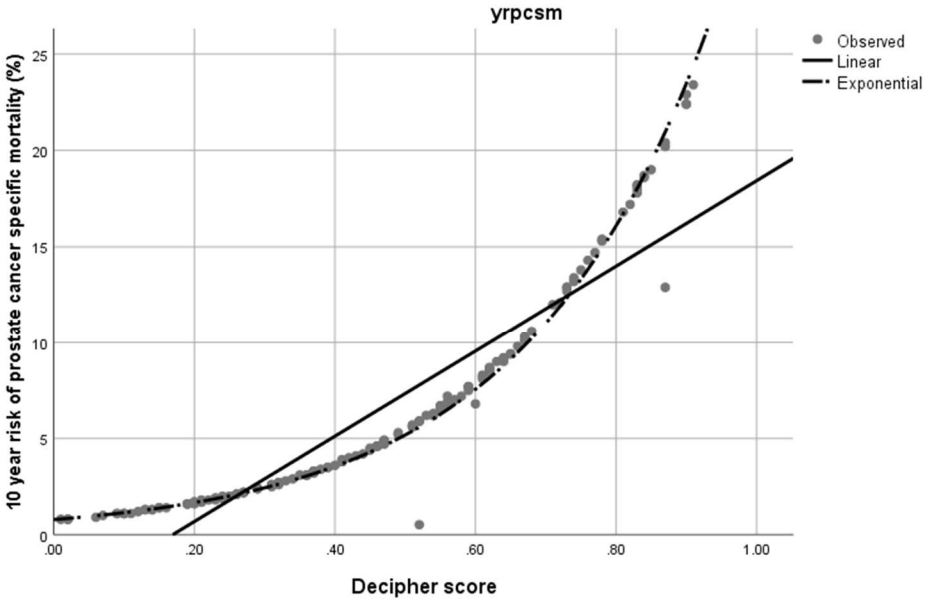


Figure 6. Plot depicting calculated 10 year risk of prostate cancer specific mortality against the Decipher scores



There were 104 Whites, 46 African Americans, 9 Hispanics, 8 Asians, 1 Native American, 3 mixed race and 4 other race patients who had mean Decipher scores of 0.44±0.23, 0.47±0.22, 0.46±0.15, 0.63±0.13, 0.43, 0.41±0.31, and 0.48±0.27 respectively ($p=0.45$), which suggests slightly higher risk of metastasis in non-white minority racial groups. The baseline demographic, clinical, tumor characteristics and Decipher scores with risk categories for different racial groups are described in Table 3. In our study, we had a racially diverse population of patients with 26% African American, 14% other minority races and significant number of individuals with low socioeconomic status who underwent Decipher genetic testing. Also, African American and Hispanic patients had significantly lower education level as compared to Whites, most of whom (>80%) were recruited from the two Veteran Affairs medical centers in our study ($p<0.05$). African Americans and other minority patients had non-significantly higher PSA levels ($p=0.110$) and mean Decipher scores ($p=0.227$) as compared to Whites and consequently, slightly higher predicted risk of metastasis but there was similar distribution of Decipher risk categories across races ($p=0.167$).

Table 3. Baseline demographic, clinical, tumor characteristics and Decipher scores with risk categories in different races

	Whites (n=104)	**African Americans (n=46)**	**Hispanics (n=9)**	**Asians (n=8)**	**Others (n=8)**	P value
Age (years)	65±8	65±6	64±9	68±6	66±6	0.491
Education (mean in yrs)	16.2±6.5	14.1±1.7	13.3±1.9	15.5±1.7	15±1.9	**0.014**
Study site (n)						
UC Irvine	42	1	3	3	4	**0.000**
LB VA	9	8	3	0	2	
UCLA	23	1	0	2	0	
LA VA	14	31	2	2	1	
Cedars	16	5	1	1	1	
PSA values (ng/ml)	7.17±5.13	10.00±13.61	8.84±8.63	8.80±10.08	11.21±8.64	0.110
Gleason grade groups (n, %)						
Group 1	16(20.0%)	9(23.1%)	0(0.0%)	2(28.6%)	2 (22%)	0.636
Group 2	35(43.8%)	15(38.5%)	5(62.5%)	1(14.3%)	4 (44%)	0.418
Group 3	17(21.3%)	9(23.1%)	1(12.5%)	4(57.1%)	3 (33%)	0.230
Group 4	8(10.0%)	3(7.7%)	2(25.0%)	0(0.0%)	0 (0%)	0.377
Group 5	4(5.0%)	3(7.7%)	0(0.0%)	0(0.0%)	0 (0%)	0.755
Tumor stage (n, %)						
cT1c	55(53)	35(76)	7(78)	4(50)	7(87)	0.679
cT2a	6(6)	1(2)	0(0)	0(0)	0(0)	
cT2b	1(1)	0(0)	0(0)	0(0)	0(0)	
cT2c	1(1)	0(0)	0(0)	0(0)	0(0)	

Decipher scores	.44±.23	.47±.22	.46±.15	.63±.13	0.45±0.25	0.227
Decipher risk categories (n, %)						
High	25(24.5%)	14(30.4%)	1(11.1%)	5(62.5%)	2(25%)	0.167
Intermediate	25(24.5%)	9(19.6%)	5(55.6%)	2(25.0%)	2(25%)	
Low	52(51.0%)	23(50.0%)	3(33.3%)	1(12.5%)	4(50%)	
5 year risk of metastasis (%)	8.20±11.47	8.91±11.09	5.49±6.04	14.17±11.02	7.41±7.84	0.616
Prostate cancer specific mortality (%)	6.14±5.68	6.63±5.58	5.13±3.45	9.81±5.17	6.03±4.63	0.819

A subset of 76 men who had known race status and Decipher genome resource information database (GRID) reports included 47 whites, 16 Africans Americans, 6 Hispanics, 4 Asians, 1 mixed and 2 other race patients. African American men had similar age, similar Gleason scores but higher PSA values (p=0.09) when compared with Whites.

African American men had equal ERG (37.5% vs 42.5%, p=0.72), slightly lower ETS (6.25% vs 19.1%, p=0.22), equal SPINK1 (12.5% vs 10%, p=0.8) and slightly higher TripleNeg (43.7% vs 27.6%, p=0.23) expression of molecular subtype when compared with Whites. Additionally, in gene expression score analysis of population rank of African American men compared with Whites, we found that African Americans had equivalent ADT response scores (p=0.405), slightly higher post-op radiation scores (p=0.207), significantly higher dasatinib sensitivity scores (p=0.002), slightly higher tumor cell proliferation (p=0.271), but significantly lower docetaxel sensitivity scores (p=0.007) and slightly lower androgen receptor signaling (p=0.133). (Table 4)

Table 4. Molecular characteristics and gene expression signatures of prostate cancer in White and African American patients

	Whites (n=47)	Blacks (n=16)	Odds ratio*	P value
Tumor type				
Adenocarcinoma	47(100.0%)	16(100.0%)		
Tumor Location				
Basal	3(6.4%)	0(0.0%)		
Luminal	44(93.6%)	16(100.0%)		
Molecular subtype				
ERG	20(42.6%)	6(37.5%)		0.72
ETS	9(19.1%)	1(6.3%)		0.22
SPINK1	5(10.6%)	2(12.5%)		0.8
Triple negative	13(27.7%)	7(43.8%)		0.23
GRID Molecular signatures				
ADT response				
Population rank	38±30	42±25		0.405
ADT response categories				
Average	20(42.6%)	8(50.0%)		
Favorable	10(21.3%)	3(18.8%)	1.22	0.749
Unfavorable	17(36.2%)	5(31.3%)	1.17	0.833
Post op radiation response				
Population rank	26±25	38±28		0.207
Post op radiation response categories				
Average	45(95.7%)	14(87.5%)		
Favorable	2(4.3%)	2(12.5%)	3.07	0.297
Docetaxel sensitivity				
Population rank	69±27	45±25		**0.007**
Docetaxel sensitivity categories				
Average	35(74.5%)	15(93.8%)		
Favorable	12(25.5%)	1(6.3%)	5.43	0.123
Dasatinib sensitivity				
Population rank	23±23	48±28		**0.002**
Dasatinib sensitivity categories				
Average	43(91.5%)	15(93.8%)		
Unfavorable	4(8.5%)	1(6.3%)	1.57	0.695
Tumor proliferation				
Population rank	54±27	62±20		0.271
Tumor proliferation categories				
Average	36(76.6%)	10(62.5%)		
Favorable	0(0.0%)	1(6.3%)	**	**
Unfavorable	11(23.4%)	5(31.3%)	1.45	0.566
AR signaling				

Population rank	65±22	55±15		0.133
AR Signaling categories				
Average	34(72.3%)	16(100.0%)		
Unfavorable	13(27.7%)	0(0.0%)	**	**

*OR calculated after adjustment of PSA
** OR extremely high due to empty cells

The linear regression analysis of the effect of education/socioeconomics on Decipher scores independently and after adjustment for covariates like age, study site, race, PSA, Gleason grade revealed no significant association between SES and Decipher scores. However, the variables which emerged as significant predictors for Decipher scores in the model were PSA (B=0.008, p=0.010), Gleason grade group 3, 4, 5 (B=0.15-0.27, $p<0.05$), and study site of UCLA (B=-0.123, p=0.05) with an overall R square of 29.7%. (Table 5)

Table 5. Linear regression model of education, race and Decipher scores adjusted for covariates (n=114)

Model		Unstandardized Coefficients		t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error			Lower	Upper
1	(Constant)	.430	.045	9.490	.000	.341	.520
	Education	.000	.003	.166	.868	-.005	.006
2	(Constant)	.375	.185	2.027	.045	.008	.742
	Education	.002	.002	.807	.421	-.003	.007
	Age	-.002	.003	-.627	.532	-.007	.004
	LB VA	.038	.066	.573	.568	-.093	.168
	UCLA	-.123	.063	-1.963	**.053**	-.247	.001
	LA VA	.052	.072	.717	.475	-.091	.195
	Cedars	.016	.060	.267	.790	-.104	.136
	PSA	.008	.003	2.404	**.018**	.001	.015
	Gleason2	.043	.058	.746	.457	-.071	.157
	Gleason3	.152	.061	2.496	**.014**	.031	.274
	Gleason4	.270	.080	3.398	**.001**	.112	.428
	Gleason5	.258	.090	2.880	**.005**	.080	.437
	Blacks	-.061	.058	-1.052	.295	-.177	.054
	Hispanics	-.047	.082	-.571	.569	-.210	.116
	Asians	.154	.121	1.268	.208	-.087	.394
	Hawaiian	-.094	.208	-.451	.653	-.505	.318
	Mixed	-.076	.123	-.619	.538	-.321	.168
	Other	-.075	.149	-.505	.615	-.371	.221

Dependent variable = Decipher scores

It is important to study the interactions of these variables with one another. However, we could not conduct such an analysis due to limited sample size.

5. DISCUSSION

Prostate cancer incidence is 200,000 and leads to 30,000 deaths annually in the United States. Africans Americans, low socioeconomic status and other minority patients have been observed to have higher rates of metastasis and prostate cancer specific mortality. (11) In our study, we had a racially diverse population of patients. This is an especially important aspect since there is a deficit in the participation of racial and ethnic minorities in genomic studies despite known biological and genetic differences in prostate cancer. (54–56) This adversely impacts the ability of physicians to make informed decisions utilizing genetic tests, about active surveillance or other treatments in African American men and other minorities. (57) (Table 2, 3)

There were 29% men in high risk Decipher category with greater than 10% risk of metastasis. This is in contrast to a recently conducted meta-analysis of 855 patients, in which 60.9%, 22.6%, and 16.5% of patients were classified by Decipher as low, intermediate, and high risk, respectively. The finding of higher percentage of individuals within high risk category in our study may be explained by the large number of minorities and low socioeconomic individuals in our study who have a higher predisposition to the development of aggressive disease. (50)

African Americans and other minority patients had non-significantly higher PSA levels ($p=0.110$) and mean Decipher scores ($p=0.227$) compared to Whites. There is exponential increase in risk of metastasis and prostate cancer specific mortality with increasing Decipher scores but this association cannot be generalized to the different races since the clinical validation studies of Decipher scores included mostly Caucasians. (Figure 5, 6)

There was similar distribution of Decipher risk categories by race/ ethnicity (p=0.167) but we found some significant and other non-significant differences between the tumor genetics of African Americans and Whites. (Table 3) This finding corroborates with a recent finding by Rayford et al., in which African Americans were found to have higher PSA levels but similar distribution of Decipher risk categories by race/ ethnicity. (10) These results suggest that tumor genetics related to progression might be dependent somewhat on racial ancestry which cannot be completely accounted for Decipher risk categorization. Therefore, additional tumor genetic data needs to be collected for African American and other minorities to accurately estimate the risk of progression. However, these results should be interpreted with caution since the construct of race/ ethnicity has its limitations in that not only was it self-reported but also, the genotypes reflecting phenotypic changes used to identify races might not have perfect linkages with genetic mutations or gene expression changes related to either diseases progression.

The distribution of molecular subtypes and treatment responses to radiation and androgen deprivation therapy in our study were similar to the study by Rayford et al who showed significantly higher odds of post op radiation response in African Americans and lower odds of androgen receptor signaling and similar distribution of molecular subtypes. (10) The magnitude of odds ratio and group differences in terms of these variables in our study were similar to the Rayford et al study but lacked significance probably due to the smaller sample size. Therefore, a larger sample size needed to confirm these results. The genetics of treatment response is especially useful in making appropriate decisions about the modality of treatment in both early and late stages of disease.

African American men had higher dasatinib sensitivity scores but lower docetaxel sensitivity as compared to Whites. Although there are some studies comparing pharmacokinetic and pharmacodynamics differences of docetaxel in African Americans and Caucasians, there is no study in the literature to compare our finding. Once the individuals at higher risk of progression have been identified, aggressive treatment modalities like chemotherapy can be guided utilizing these gene signatures. This underscores potential for utilizing pharmacogenomics associations in prostate cancer management in advance stages of disease in different races. However, it needs to be validated in a larger cohort of patients.

The Decipher scores were non-significantly associated with education/ socioeconomic status (Table 5) and this corresponds with recent findings by Weiner et al. (9) This might reflect that SES does not alter tumor biology of prostate cancer unlike other cancers like of the breast. But the interaction of socioeconomic and racial influences on Decipher genomic classifier needs to be investigated further to improve its predictive ability of prostate cancer metastasis and mortality risk in a diverse population. There is a need to look into the addition of epigenetic biomarkers (which are not currently part of Decipher test) which could probably better account for racial and socioeconomic influences on prostate cancer progression.

It is important to note that several chronic stressors like low income, lack of health insurance, inadequate access to healthcare, comorbidities and might flip some of the switches associated with gene expression, including Decipher gene expression assay and influence disease progression and this warrants further investigation.

There were a few peculiar findings in our linear regression model. There was significant association of study site of UCLA with lower Decipher scores. Also, African Americans and Hispanics had lower Decipher scores after adjusting for covariates in our model. However, this association was still non-significant.

There was preliminary evidence of differential expression of various gene markers related to different molecular pathways and gene markers that could be related to epigenetic changes in various subgroups of patients but this aspect needs to be explored further in a larger cohort of patients.

Study Limitations

The sample size of our study was not adequate for the linear regression modelling for the number of variables included and for multiple comparisons of gene signatures across different racial groups.

The utility of construct of self-reported race in genetics research is limited in that the use of these categories creates an impression that health disparities arise because of molecular gneteic differences and independent of social factors. In this way, genomics studies that make population comparisons can inaccurately stereotype racial and ethnic groups, both by implying that such groups are clearly delineated and by associating health outcomes with all individuals in those groups rather than with only those individuals who exhibit the outcome.

Another limitation of our study was the estimation of SES by education alone. Other measures like area deprivation index would more accurately reflect true SES and will be included in future analysis.

Lastly, some of the epigenetic markers that have previously been implicated in understanding prostate cancer progression and disparities were not studied and not part of the Decipher panel of genes. So, these additional markers need to be compared by race/ethnicity for their effect on disease progression outcomes.

CONCLUSIONS

There was non-significant association of Decipher scores with race or SES, but African Americans and other minorities differed in the genetic architecture of prostate cancer as compared to Whites especially in terms of pharmacogenomics of docetaxel and dasatinib. This might warrant a future study to create a precision medicine model for predicting outcomes and personalizing treatments to reduce disparities related to prostate cancer. The study also highlights the need to prospectively validate Decipher scores in diverse population and consider additional biomarkers probably epigenetic markers to better account for racial and socioeconomic influences on prostate cancer progression.

FUTURE DIRECTIONS

The Decipher scores need to be validated against disease progression and metastasis outcomes in a longitudinal prospective cohort study with a racially and socioeconomically diverse population.

The gene signatures related to treatment response need to be validated as well in prospective studies. The patients with favorable gene signatures as discovered in our cohort need to be observed for treatment responses. The multiple site study we are conducting makes it feasible to observe the racial and socioeconomic differences across sites and their association with disease progression in the future.

The individual gene expression markers and involved molecular pathways can be compared across the different races and socioeconomic status patients in a larger cohort of racially and socioeconomically diverse patients to understand the molecular heterogeneity of prostate cancer in these subgroups. This may provide an opportunity to study the molecular basis possibly an epigenetic basis of the interaction between environment, race and prostate cancer progression, metastasis and mortality risk. Additionally, environmental factors other than socioeconomics, including stress, resilience, diet etc. may be studied for their association with Decipher scores and molecular pathways. These associations shall further provide opportunities for creating composite measures for predicting disease progression and improving predictive modelling for treatment outcomes in prostate cancer.

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Appendix A: Demographic data entry form

SECTION 5: ABOUT YOU

The following questions are about what you do to stay healthy and a little bit about yourself.

1. **How would you characterize your usual level of physical activity?**

	(circle one)
Extremely active	1
Very active	2
Active	3
Not very active	4
Not active at all	5

2. **How would you characterize your level of physical activity in the past 4 weeks?**

	(circle one)
A lot higher than usual	1
A bit higher than usual	2
About the same as usual	3
A bit lower than usual	4
A lot lower than usual	5

3. **How would you characterize each of the following?**

	(circle one for each item)				
	Very healthy	Healthy	So-so	Unhealthy	Very unhealthy
a. Your diet	1	2	3	4	5
b. Your sleep habits	1	2	3	4	5
c. Your weight	1	2	3	4	5
d. Your exercise habits	1	2	3	4	5

4. **What is your birthdate?** ___/___/___ (Month) (Day) (Year)

5. **Please circle the highest grade that you completed in school:**

0 K 1 2 3 4 5 6 7 8 — Grade School; 9 10 11 12 — High School; 13 14 15 16 — College; 17+ — Graduate or Professional

6. **What is your racial background?**

	(circle one)
White	1
African American or Black	2
Hispanic	3
Asian or Pacific Islander	4
Native American or Alaskan Native	5
Mixed racial background	6
Other race (please specify): _____	7

7. **Are you of Hispanic origin, such as Mexican-American, Latin American, Puerto Rican, or Cuban?**

(check one) NO ☐ YES ☐ → **What is your ethnic background?**

	(circle one)
Mexican-American	1
Latin-American	2
Puerto Rican	3
Cuban	4

8. **What is your current marital status?**

	(circle one)
Never married	1
Married	2
Separated	3
Divorced	4
Widowed	5

9. **What is your current employment status?**

	(circle one)
Working full time	1
Working part time	2
Retired	3
Unemployed (or looking for work)	4

10. **What type of health insurance do you currently have?**

	(circle one)
Medicare and Medicaid	1
Medicare only	2
Medicaid only	3
Veterans Administration (VA) Healthcare	4
Private/commercial	5
Military healthcare (including CHAMPUS/TriCARE, CHAMP-VA)	6
Other (please specify): _____	7
No health insurance	8

Thank you for participating in this study about the impact of prostate cancer and its treatment on the health and quality of life of men with this disease. We appreciate your help and candidness in answering what are sometimes uncomfortable or awkward questions. Your contribution to this important research is invaluable to us.

END