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Genetic Ancestry and Prostate Cancer Susceptibility SNPs in Puerto Rican and African American Men

Margarita Irizarry-Ramírez¹, Rick A. Kittles², Xuemei Wang³, Jeannette Salgado-Montilla⁴, Graciela M. Nogueras-González³, Ricardo Sánchez-Ortiz⁵, Lourdes Guerrios⁶, Keila Rivera⁷, Ebony Shah², Ina Prokhorova⁸, Pamela Roberson⁹, Patricia Troncoso⁸, and Curtis A. Pettaway⁹

¹Department of Graduate Studies, Clinical Laboratory Sciences, School of Health Professions, University of Puerto Rico, Medical Sciences Campus, San Juan Puerto Rico, Box 365067, San Juan, Puerto Rico 00936, 787-758-2525 ext 7035 margarita.irizarry@upr.edu ²Department of Surgery, School of Medicine, University of Arizona, rkittles@surgery.arizona.edu; eshah@surgery.arizona.edu ³Department of Biostatistics University of Texas, M.D. Anderson Cancer Center, xuewang@mdanderson.org; gnoguera@mdanderson.org ⁴University of Puerto Rico/MD Anderson Cancer Center Partnership for Excellence in Cancer Research, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico, jeannette.salgado@upr.edu ⁵Urology and Oncology Institute, San Juan Puerto Rico, drsanchezortiz@gmail.com ⁶Veterans Administration Caribbean Health Care System, lourdes.guerrios-rivera@va.gov ⁷Department of Pathology, School of Medicine, University of Puerto Rico, Medical Sciences Campus, keila.rivera11@upr.edu ⁸Department of Pathology, University of Texas M.D. Anderson Cancer Center, ptroncoso@mdanderson.org, iprokhoro@mdanderson.org ⁹Department of Urology, The University of Texas M.D. Anderson Cancer Center, cpettawa@mdanderson.org, psroberso@mdanderson.org

Abstract

Background—The Puerto Rican (PR) population is a racially admixed population that has a high Prostate Cancer (PCa) mortality rate. We hypothesized in this pilot study that West African Ancestry (WAA) was associated with PCa in this heterogeneous (PR) population.

Methods—A case/case and case/control study was performed. Controls, 207 African American (AA) and 133 PR were defined as men with no PCa, a serum PSA < 2.5 ng/ml and a negative rectal examination. Cases were patients with pathological specimens from radical prostatectomies (RP) (291PR and 200AA). DNA was extracted from whole blood of controls and from paraffin embedded normal seminal vesicle from the RPs. We assessed the association of PCa and aggressiveness with genetic ancestry using an Ancestry Informative Marker panel (AIMs) and Wilcoxon rank-sum test and the association of PCa and aggressiveness with 15 previously PCa associated SNPs using Chi square test. Gleason Score(GS) and tumor stage(TS) were used to define low risk (GS 7[3+4]), TS pT2) and high risk (GS 7[4+3], TS > pT2) PCa. Statistical analyses were done using SAS.

Conflict of interests: There are no conflict of interests reported by the author or the coauthors.

Results—No difference in overall percent WAA was found between PR cases and controls. Among PR or AA cases WAA was not associated with disease severity based upon risk group, Gleason score or stage. Among AA controls WAA was significantly higher than in cases. The SNP rs7824364 (chromosome 8q24) PCa risk allele was significantly increased among cases versus controls for both AA ($p<0.0001$) and PR ($p=0.0001$) men. PR men with 1 risk allele exhibited a higher percent of WAA (39% versus 29%, $p=0.034$).

Conclusion—The SNP rs7824364, a local marker of WAA in the 8q24 region was associated with PCa among both AA and PR men and with increased WAA among PR men. This novel relationship of PCA risk loci, WAA with PCa and its phenotype among PR men deserves further study.

Introduction

Prostate cancer (PCa) varies widely among different ethnicities, with African American (AA) men exhibiting an earlier onset of disease and the higher incidence (214.5 per 100,000) and mortality rate (46.3 per 100,000) of any group in the world ¹. In other populations of African descent, there is also a high risk of developing PCa ^{2–6}.

PCa in the Latino population in general and in the Puerto Rican (PR) population in particular has been rarely described. The high mortality (29.0 per 100,000) from PCa observed among Puerto Ricans living in the island, when compared to continental US Hispanic (17.8 per 100,000) and non-Hispanic white (19.8 per 100,000) populations has yet to be addressed ^{1,7}.

The Puerto Rican population, as many others in the Americas, is highly heterogeneous due to the admixture of Native Americans, Europeans and West Africans. Interestingly, the island of Puerto Rico has higher tri-hybrid admixture than other Hispanic populations. In 2011, Via and collaborators estimated genetic admixture on the island, reporting 15.2% Native American, 63.7% European and 14.4% West African ancestries respectively among the population ⁸. Importantly some studies have found disparities in the incidence of diseases or response to treatments in the PR population when compared to other Latino groups ^{9–11}. West Africa is the major ancestral component of AA men as well as of men of African descent in the Caribbean ^{12–14}. It is plausible that genetic susceptibility loci stemming from the West African ancestral (WAA) population may influence the heterogeneity with respect to mortality from PCa in the PR population.

Ricks-Santi and collaborators used Ancestry Informative Markers (AIMs) ^{15–17} to assess the contribution of WAA in risk of PCa in AAs and to detect genetic variants associated with PCa in this population. Recently Fernandez et al, 2015, correlated SNPs associated with PCa susceptibility in men with mixed ancestry and men with European ancestry (EA) in South Africa. The former had an earlier onset of PCa and also had a higher frequency of metastatic disease ¹⁸.

Other studies ^{19–23} have analyzed ancestry and SNPs related to PCa, however none has examined PCa disparities in the Puerto Rican population. In the present pilot study we investigated whether WAA was associated with PCa risk and aggressiveness in this genetically heterogeneous population and compared them to AA men. We assessed WAA

ancestry in PCa cases and healthy controls; we tested the hypothesis that WAA is correlated with aggressive PCa subsequent to radical prostatectomy (RP) and also performed SNP association tests for fifteen GWAS-identified PCa risk related SNPs^{24–26} (Supplemental Table 1). To date no study has correlated WAA using AIMs with prostate cancer aggressiveness in a Hispanic- Caribbean understudied population.

Methods

This study was performed after approval by the Institutional Review Boards of the University of Puerto Rico Medical Sciences Campus and the University of Texas MD Anderson Cancer Center (MDACC), in Houston. Informed consent was obtained prior to sample collection or questionnaire administration.

Subjects

The study population consisted of 424 cases and controls from PR site and 407 cases and controls from the MDACC site. The PR controls consisted of 133 healthy Puerto Rican (defined as having parents and grandparents born in Puerto Rico) men, aged 40–89 years old. Inclusion criteria included having a recent (less than six months old) Prostate Specific Antigen (PSA) test (PSA < 2.5 ng/mL) and a normal Digital Rectal Examination (DRE). Subjects were recruited through advertisement to the general public, the Puerto Rico Clinical and Translational Research Consortium (PRCTRC) and the Puerto Rico Comprehensive Cancer Center (PRCCC) on campus. The PR cases consisted of 291 Puerto Rican previously consented patients who had a RP with available tissue blocks.

The MDACC control subjects consisted of 207 self-identified AA men who were a part of the Prostate Outreach Program (POP) early detection study performed among underserved men with a normal DRE and serum PSA < 2.5 ng/ml²⁷. The MDACC PCa cases consisted of 200 previously consented self-identified AA men who underwent RP with available tissue blocks.

DNA extraction—The sources of genomic DNA for genotyping were either whole blood from the control subjects or paraffin embedded tumor free seminal vesicle tissue from the cases. DNA extraction from paraffin embedded tissue followed standard published methodologies²⁸. Briefly 5–10-micron shavings of non-tumor seminal vesicle tissue were deparaffinized in octane and methanol, lysed and treated with proteinase K. Subsequently DNA was purified by precipitation with isopropanol and 3–5ul of glycogen (Sigma, St. Louis, Mo, USA). DNA was washed in 1ml of 70% ethanol and then set to dry. Dried DNA was dissolved in 25–50ul of DNA hydration buffer (Qiagen INC. Valencia CA, USA) and stored at –20°C. DNA extraction and purification from blood was done following manufacturer's instructions in Gentra Puregene kit from Qiagen (Qiagen INC. Valencia CA, USA).

Quantification of the DNA was done using a NanoDrop 2000 spectrophotometer (Thermo Scientific). DNA quality and integrity was assessed performing a PCR for the housekeeping gene G3PDH (Glyceraldehyde-3-Phosphate Dehydrogenase).

Genotyping of SNPs

Sequenom Mass ARRAY™ was used for genotyping 100 unlinked AIMs²⁹ and SNPs strongly associated with PCa risk in previous GWAS studies^{24–26}. iPLEX™ assays were designed utilizing the Sequenom Assay Design software, allowing for single base extension (SBE) designs used for multiplexing. Multiplex assays were performed to amplify 5–10 ng of genomic DNA by polymerase chain reaction (PCR). Subsequently, a post-PCR single base extension reaction was performed for each multiplex reaction using concentrations of 0.625 μ M for low mass primers and 1.25 μ M for high mass primers. Reactions were dissolved with 16 μ l of H₂O and fragments purified with resin, spotted onto Sequenom SpectroCHIP™ microarrays, and scanned by MALDI-TOF mass spectrometry.

Genetic Ancestry Estimation

An enriched panel of 100 unlinked AIMs spanning 22 chromosomes was used to provide an estimation of genetic ancestry for each study subject. European Ancestry (EA), WAA, and Native American ancestry (NAA) and was estimated from the genotype data using the Bayesian Markov Chain-Monte Carlo (MCMC) method implemented in the program STRUCTURE version 2.1^{30,31}.

Database

MDACC and PR had a unified data entry form using Research Electronic Data Capture (REDCap), based on agreed variables for both cases and controls. Data collected from the RP cases subsequent to review of the medical record included: demographic information, age, Gleason score (GS), serum PSA, and pathologic stage. Data accrued from the healthy subjects included: demographic information, age, self-reported race, rectal examination findings, and serum PSA level. Data was stored with a unique identifier assigned by the research personnel. All clinical data conflicts were resolved by thorough communication among the database managers and the principal investigators.

Statistical Methods

Low risk PCa aggressiveness was defined by pathologic stage $\leq$ pT2 and [GS 7 = (3+4)] or GS $<$ 7. High risk was defined by pathologic stage $>$ pT2 or GS $>$ 7 or [GS 7 = (4+3)]. To minimize the possible variability on pathology interpretations among the sites, PR and MDACC pathologists reviewed cases of prostatectomy specimens to gain consensus on Gleason scoring of specimens³².

The difference in SNPs presence between groups stratified by study site, case/control status, and disease aggressiveness was assessed using the Chi-squared test. The WAA, EA or NA data were compared between groups using the Wilcoxon rank-sum test. The study was designed to enroll respectively 300 PR and 200 AA cases with $>$ 90% power to detect an association between WAA and PCa aggressiveness, assuming 20% of cases were high grade or stage.

In addition, the study had 86% power to detect a difference in means of 3% ($\mu_1 = 0.82$ and $\mu_2 = 0.79$) with a sample size of 300 cases and controls in the PR group (as observed in AAs by Robbins et al.¹⁷), assuming that the common standard deviation is 0.100 using a two

group t-test with a 0.010 two-sided significance level. We tested for differences in ancestry distributions between cases and controls by fitting logistic regression models with case-control status as the outcome and genetic ancestry as the main covariate, modeling genetic ancestry as either a continuous or categorical variable.

We also used logistic regression models to test the effect of PCa risk SNPs on PCa in our subjects. The MDACC cohort of AA cases and controls served as a positive control for AIM expression as well as for SNPs previously shown to be associated with PCa among AA cohorts. When assessing the statistical significance of the SNPs in association with sites, case/control status or PCa aggressiveness, the Bonferroni correction method was used to adjust for multiple comparisons. All statistical analyses were conducted using SAS (version 9.3).

Results

Recruitment of cases from both cohorts reached the stated goal among the MDACC cohort and missed the goal (n=300) from PR by nine cases (n=291). Control recruitment from the PR site did not reach the goal of 300 subjects achieving 44% (n=133) of the target accrual. Control subject accrual from the MDACC site was successful in matching case recruitment of subjects. The current enrollment showed a higher percent of high grade or stage PCa (28% for PR cohort and 52% for MDACC cohort), than the expected 20%, thus the groups of low vs. high grade or stage cancer, were better balanced in terms of sample size. Therefore, the study still had >80% power to detect an association between WAA and PCa aggressiveness with the current enrollment of 291 patients for the PR cohort and 196 patients for the MDACC cohort.

For the PR cohort, the frequencies of cases : controls respectively by regions in PR were : Metro : 43.49% :41.35%; North 14.73%: 10.53%; Central 10.62%:9.77%; East 21.31%: 38.35%. We did not have controls from the West or the South Region from where, 9.59% of the cases were from. Regions were defined according to Via et al ⁸

Clinico-demographic characteristics of the cohorts (Table 1)

The subject age distribution, serum PSA levels, and body mass index were different among cases and controls from PR and MDACC. PR cases were older, had higher serum PSA levels and lower BMI than MDACC cases. Gleason score and disease severity were higher in cases at MDACC. At both sites the incidence of smoking was higher in cases. Cases from MDACC exhibited a higher incidence of affected first degree relatives than the control group. No such difference was noted for the PR population. The cohort from Puerto Rico exhibited higher levels of educational achievement (68.6–82% >12th grade educational level) when compared to the MDACC cohort (44–62.9% >12th grade educational level).

Genetic Ancestry—Ancestry analyses were performed to assess the differences between MDACC and PR subjects and differences between cases and controls, and between low and high risk PCa at each site. Table 2 summarizes the ancestry data by study site. As expected there was significantly higher WAA among MDACC subjects (median =77%) as compared

to PR subjects (median = 17%)($p < 0.0001$), while PR subjects had significantly higher levels of both EA and NA than the MDACC cohort.

In figure one (Fig 1) we provide an ancestry distribution plot by disease status, for both sites. The ancestry distribution had a significant difference ($p < 0.05$) between cases and controls only at the MDACC site, with cases exhibiting significantly lower WAA but higher EA and NA. There was no difference in percent WAA between PR cases and controls. (Supplemental Tables 2a and b)

We found no significant difference in ancestral proportions between low and high risk cases (Table 3), or by pathologic stage or GS as single categories, in either the MDACC or PR cohorts. (Supplemental Tables 3–4). In addition when we assessed for an association between serum PSA and WAA, we did not find a significant association in the PR cohort ($p = 0.16$). However among the MDACC cohort serum PSA levels were significantly lower among the AA cases with higher WAA. ($p = 0.03$).

SNPs analysis

Of the fifteen (15) SNPs, ^{21–23} thirteen (13) were in Hardy Weinberg equilibrium. Among each cohort we tested SNP allele frequencies among cases and controls. and for cases, SNP associations with high or low PCa risk groups; Gleason Score ($\geq 4+3$, or 8 vs. $< 4+3$ or 8); and pathologic stage ($\geq pT2$ vs. $< pT2$).

When we compared the SNPs allele frequencies among the PR and MDACC cohorts we noted significant differences in 11 of 13 ($p < 0.0033$) after Bonferroni adjustment for multiple comparisons. (Supplemental Table 5)

In the MDACC cohort ($n = 409$), there were three SNPs that initially exhibited a significant difference in frequencies between cases and controls: rs7824364, rs6983267, and rs7210100 ($p < 0.05$, Table 4). Approximately 82% of cases exhibited 1–2 minor alleles for rs7824364, while 51% of the controls had either one or both alleles; 13% of the cases had one or two minor alleles at rs6983267 compared to 22% of the controls. For rs7210100, approximately 19% of the cases had one or both minor alleles, compared with 10 % of the controls. However after the Bonferroni adjustment for multiple comparisons, only the rs7824364 SNP remained statistically significant (i.e., $p < 0.0033$) in this cohort. None of 13 SNPs correlated with either low or high risk PCa among the MDACC cohort while SNP rs6983267 was initially associated with Gleason score ≥ 8 PCa the association was lost after adjustment for multiple comparisons (data not shown).

In the PR cohort, SNP rs7824364, was also significantly different between the cases and controls ($p = 0.0001$), Table 5, with cases having a higher percentage (41%) of 1 or 2 alleles compared to the controls (19%) respectively. Of note given that rs7824364 was significantly associated with prostate cancer among both cohorts we next examined whether WAA was increased among PR men having one or both of the minor alleles at this locus. The median level of WAA among the PR cohort was 17%. However, among PR having 1–2 minor alleles at the rs7824364 locus, WAA was increased above the median to 39.3% versus 29.2% ($p = 0.034$) in PR men with no minor alleles. SNP rs7210100 was also initially associated with

pathologic stage > pT2 ($p=0.04$). However, after adjustment for multiple comparisons, this difference did not achieve statistical significance (Data not shown).

Multiple logistic regression analysis was performed to determine variables associated with case/control status in the MDACC and PR cohorts separately. The variables considered included the rs7824364 SNP, WAA, age, BMI, PSA, education level, and family history of PCa. Although in both sites the minor allele frequencies for SNP rs7824364 were significantly different between cases and control after univariate analysis, it was no longer significant in the multivariate model for either site after adjusting for serum PSA levels. In the PR cohort, age remained independently associated with case status as did lower WAA among the MDACC cohort (Supplemental Table 6).

Discussion

Given the higher PCa mortality among both the PR and AA PCa patients and the heterogeneous ancestral background among the PR population we hypothesized that WAA could be related to PCa mortality among PR prostate cancer patients. In this pilot study an increasing percentage of WAA was not directly associated with either the presence or phenotype of prostate cancer among a select population of men undergoing RP. In fact an enrichment for WAA was found in the healthy control cohort from the MDACC site. This finding has prompted us to study other potential confounders since the AA controls were recruited from community settings in Houston while the cases were AA patients from the MDACC, a tertiary referral center for treatment of cancer. We have previously shown that subjects recruited in the Houston community study exhibited a lower socioeconomic (SES) status in terms of education, insurance, and having a regular physician²⁷. Freeman et al³³ directly correlated low SES with lower PCa survival and found that this was eliminated in equal access care settings³³. Studies done in Latin America and the Caribbean have also shed light into the relationship among environmental and social risk factors with risks for cancers in general and PCa in particular. Romero et al³⁴, suggested that decreased SES, could impact the development of cancers. Allan Patrick analyzing results from The Tobago Prostate Cancer Survey³⁵, also found that within the Afro-Caribbean group, poor social and economic status could impact the development of PCa by factors such as exposure to hazardous substances at work or in the household, poor nutrition and lack of or poor access to health care. Recently Bensen et al³⁶ have characterized genetic ancestry, prostate cancer related SNPs, diet and lifestyle related variables among a well characterized cohort of AA and European men. The African American populations were enriched for WAA (90%) with the EA population exhibiting an average of 97% European ancestry. They found that certain SNPs in either the AA or EA population only correlated with Pca aggressiveness. In addition when evaluating other variables their group found that obesity was a significant predictor of aggressive PCa for the EA population more than the AA cohort³⁷. Saturated fat however was associated with aggressive prostate cancer but only in the EA cohort³⁸. The effect that SES or environmental factors may have on modifying prostate cancer in our cohorts is currently being studied.

When comparing our study with another that suggested an association of increasing WAA and PCa we noted several differences. Our study was a case control and case-case design to

define if increasing WAA was associated with PCa. Controls were pre-selected based upon a normal DRE and serum PSA <2.5ng/ml with the median PSA of 0.7–0.8ng/ml. In contrast, in the longitudinal cohort study by Giri et al³⁹. AA men and men with a family history of PCa were included in the study and underwent biopsy using pre-specified criteria over time. They found a significant increasing prediction of PCa among AA men (versus Caucasian) at lower PSA baseline levels³⁹. This relationship trended toward AA cohorts with an increasing percent of WAA but did not reach significance. The authors noted that their study was relatively small but raised the possibility of a lower risk cohort among AA men that could be defined using WAA. Given the difference in our two study designs our data does not contradict the findings of Giri et al³⁹ in raising the possibility that among heterogeneous populations of men of African descent PCa risk or phenotype may differ. In fact in a follow-up study we will test this hypothesis by evaluating a group of AA men at risk for PCa based upon clinical features to determine if WAA (among other factors) is a risk factor for a positive biopsy, aggressive pathology, or affects the relationship between serum PSA levels and a subsequent cancer diagnosis.

Among cases we did not find enrichment for WAA among aggressive RP cases when compared with less aggressive cancers from either cohort of AA or PR men. These data do not support our hypothesis that increasing WAA itself is associated with PCa aggressiveness in the Puerto Rican population and the mortality disparity noted. However we cannot definitively rule out such a relationship since we did not examine endpoints such as disease recurrence or mortality, nor analyzed WAA among a broader population of PCa patients exhibiting the whole spectrum of the disease to determine its impact on metastatic disease or death in the cohorts. To address this limitation we are currently gathering data on a larger cohort of men from both populations along with clinical follow up to assess disease recurrence and mortality and its relationship with genetic ancestry. A second limitation of our study is that we did not reach the target accrual goal for the PR control cohort and our relatively small overall study size. It was encouraging to note however, that the proportions of WAA among men of African descent in our MDACC cohort (mean = 0.74) was similar to the proportion noted in the study by Giri et al.³⁹ among another African American cohort (mean = 0.75)³⁹. In addition the genetic admixture among PR men reported in our study was virtually identical to that reported by Via et al.⁸

In this pilot study we also wanted to determine in a comparative fashion if there were genetic similarities in alleles associated with prostate cancer or its phenotype among our two cohorts. Of the 15 SNPs in the panel, 9 were in the 8q24 region, an area highly enriched for both African ancestry and prostate cancer risk^{40,41}.

We noted that three SNP loci trended towards a significant association with PCa, rs7824364 (both cohorts), rs6983267 (MDACC cohort), and rs7210100 (MDACC cohort) ($p < 0.05$, see Tables 4,5). Moreover, in the case only analysis SNP loci rs6983267 among AA men and rs7210100 among PR men were found to be associated with more aggressive prostate cancer with respect to Gleason score and pathologic stage respectively. (Supplemental tables 7,8)

SNPs rs7824364, rs6983267, are located in the chromosome 8q24 region known to be enriched among men of African descent (MAD) with prostate cancer^{40,41}. The third

(rs7210100) located on chromosome 17q21 was also described by Haiman et al⁴² among MAD. This SNP was highly associated with PCa and appeared to be correlated with lower Gleason score and stage PCa⁴². It was interesting that in the PR cohort in this study it trended toward an association with higher pathologic stage. In the above study Haiman et al⁴² noted that the risk allele had a frequency of only about 5% among MAD. The frequency among the PR in our study was even lower at 1.4% while among the MDACC cohort of AA it was substantially higher at 7.5%.

A major finding in the current study was that SNP locus (rs7824364)⁴³ in 8q24 remained significant in both cohorts after multiple comparisons' testing. The rs7824364 SNP is located within the region formerly known as region 2 of 8q24 which spans from 127.894 – 128.233MB. The specific base pair position of rs7824364 is 128135365. At this location the base cytosine is switched for the normally occurring thymine. The 8q24 region contains three independent regions of risk for PCa which have been studied for transcriptional activity in normal prostate tissue or LNCap cell lines by Jia and collaborators⁴⁴. They found that the regions bear epigenomic organization suggesting its possible role as enhancers. Ahmadiyeh and collaborators⁴⁵ demonstrated that these gene free regions interacted with each other but more importantly region 2 interacts with the MYC oncogene in a tissue specific manner potentially as a MYC regulatory unit. Researchers have suggested the possibility that variants in this region modulate the risk for PCa during prostate organogenesis during development, thus many years ahead of the actual tumorigenesis⁴⁶.

Relevant to the present study the rs7824364 SNP was described by Haiman et al⁴² as one of three SNPs achieving genome wide significance in a study among 3425 cases and 3290 controls in an African American Consortium of 11 studies. Recently, Han et al⁴³ mapped this region further, noting genome wide statistical significance for the rs7824364 SNP and three more highly associated PCA loci among MAD with two loci found only in African ancestral populations. Such variants may modulate PCa risk through interacting with long noncoding RNA.

This highlights an important region of ancestry specific racial variation in prostate cancer. We found that among both AA men in the MDACC cohort and the heterogeneous PR population rs7824364 remained significantly associated with PCa with one or both alleles present in 82 and 41% of cases respectively. Furthermore we found WAA was significantly increased among PR men having at least one allele ($p=0.034$) versus those with none present. This was a novel finding and suggests a link between PCa, WAA, and the PR population that clearly deserves further study.

In summary, this study presents the initial results of evolving data on the possible interactions of ancestry, PCa genetic risk loci, and PCa in a comparative fashion among PR and African American men. We found a PCa associated locus at 8q24 region 2 among both cohorts that was associated with increased WAA among the PR PCa cohort. This extends our knowledge with respect to PCa among the understudied PR population. We did not directly demonstrate an association between WAA and disease severity within the confines of a prostatectomy cohort in either group. Whether increased WAA affects PCa recurrence

or mortality is currently under study together with socioeconomic and environmental factors that may influence the onset or progression of the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.
Summary of the ancestral distributions in the MDACC and PR groups for cases and controls at both sites.

Filled - Controls Circles- PR; triangles- MDACC

Open - Cases Circles- PR; triangles- MDACC

Table 1

Summary Statistics of the Clinical and Demographic Characteristics

	PR		p-value	MDACC		p-value
	Control	Case		Control	Case	
Age						0.034
N	133	291	<0.001	208	200	
Mean (SD)	50.5 (8.4)	59.5 (6.6)		55.7 (8.3)	57.3 (6.8)	
Median	49	60		56	58	
BMI			<0.001			0.039
N	133	291		202	159	
Mean (SD)	30.3 (6.1)	28 (4.3)		28.8 (5.6)	29.8 (4.9)	
Median	28.5	27.4		28.3	29	
Pre-operative /control serum PSA level			<0.001			<0.001
N	133	284		209	199	
Mean (SD)	.8 (.5)	6.1 (6.1)		1 (.6)	7.8 (5.5)	
Median	.7	4.8		.8	6	
Age						0.032
Age:35–54	92	57	<0.001	85	59	30.6
Age:55–64	23	156		79	99	51.3
Age>=65	10	69		34	35	18.1
Health Insurance			0.154			<0.001
Public	11	26		28	32	16
Private	120	265		128	168	84
None	2	0		53	0	0
Primary Gleason Grade						<0.001
3	-	246		-	105	52.5
4	-	43		-	93	46.5
5	-	2		-	2	1
Secondary Gleason Grade			-			<0.001

	PR		p-value	MDACC		p-value
	Control	Case		Control	Case	
3	-	173	59.5	-	83	41.5
4	-	109	37.5	-	99	49.5
5	-	9	3.1	-	18	9
Gleason Score						<0.001
6	-	143	49.1	-	13	6.5
3-4	-	103	35.4	-	86	43
4-3	-	29	10	-	69	34.5
8	-	7	2.4	-	19	9.5
9	-	8	2.7	-	13	6.5
10	-	1	.3	-	0	
BMI			0.002			0.005
Normal	16	12	69	49	23.4	18
Overweight	63	47.4	145	71	35.1	73
Obese	54	40.6	77	81	40.6	68
Missing	0	0	0	8		41
Stage			-			0.093
pT2	-	-	234	-	-	148
pT3	-	-	57	-	-	52
Do you smoke cigarettes?			0.023			<0.001
Current smoker	20	15	37	36	17.4	28
Previous smoker	105	78.9	250	76	36.7	151
Non-smoker (never smoked)	8	6	4	95	45.9	0
Missing	0	0	0	2		21
Family history of prostate cancer			0.482			0.337
No	99	78	210	75	66.4	129
Yes	28	22	71	38	33.6	51
Unknown/Missing	6		10	96		20
Brother and/or father and/or son affected			0.211			0.001
No	109	83.8	216	186	89.4	136
Yes	21	16.2	59	22	10.6	41

	PR		MDACC		p-value
	Control	Case	Control	Case	
Unknown/Missing	3	16	1	23	
Family history of other cancer					
No	67	51.5	0	0	0.758
Yes	63	48.5	0	0	
Unknown/Missing	3	7	209	25	
Surgical Margin Status					
Negative	-	-	-	-	0.770
Positive	-	-	-	-	
Education					
12 grade	24	18	108	52.2	0.017
>12 grade	109	82	93	44	
Unknown/Missing	0	0	8	76	0.004
Severity					
Low Risk	-	-	-	-	<0.001
High Risk	-	-	-	-	

Table 2

Subject ancestry proportions by study site.

Ancestry	Site	N	Mean $\pm$ SD	Median (range)	p-value
West African Ancestry	MDACC	392	0.74 $\pm$ 0.16	0.77 (0.02 – 0.98)	<0.0001
	PR	415	0.21 $\pm$ 0.14	0.17 (0.01 – 0.78)	.
European Ancestry	MDACC	392	0.19 $\pm$ 0.14	0.16 (0.01 – 0.86)	<0.0001
	PR	415	0.64 $\pm$ 0.15	0.65 (0.05 – 0.95)	.
Native Ancestry	MDACC	392	0.07 $\pm$ 0.06	0.06 (0.01 – 0.53)	<0.0001
	PR	415	0.15 $\pm$ 0.09	0.14 (0.01 – 0.85)	.

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Table 3

Association of ancestral proportions with pCa severity

MDACC					
Ancestry	Severity	N	Mean +/- SD	Median (range)	P-value
West African Ancestry	High	101	0.69 +/- 0.2	0.75 (0.08 – 0.93)	0.87
	Low	95	0.72 +/- 0.12	0.74 (0.3 – 0.95)	.
European Ancestry	High	101	0.23 +/- 0.18	0.19 (0.02 – 0.86)	0.88
	Low	95	0.19 +/- 0.12	0.18 (0.02 – 0.66)	.
Native Ancestry	High	101	0.08 +/- 0.06	0.06 (0.02 – 0.28)	0.30
	Low	95	0.08 +/- 0.05	0.07 (0.02 – 0.26)	.
PR					
Ancestry	Severity	N	Mean +/- SD	Median (range)	p-value
West African Ancestry	High	82	0.21 +/- 0.15	0.16 (0.01 – 0.68)	0.85
	Low	209	0.2 +/- 0.12	0.17 (0.01 – 0.73)	.
European Ancestry	High	82	0.62 +/- 0.15	0.63 (0.21 – 0.95)	0.12
	Low	209	0.65 +/- 0.13	0.65 (0.16 – 0.9)	.
Native Ancestry	High	82	0.16 +/- 0.09	0.15 (0.01 – 0.57)	0.12
	Low	209	0.15 +/- 0.08	0.13 (0.01 – 0.38)	.

Table 4

SNP data by case and control status within MDACC subjects.

covariate	levels	Control	Case	P-value
rs16900305	0	154(78.6%)	160(82.1%)	0.38
	1	42(21.4%)	34(17.4%)	.
	2	.(%)	1(0.5%)	.
rs7008482	0	131(68.2%)	140(72.9%)	0.56
	1	55(28.6%)	48(25%)	.
	2	6(3.1%)	4(2.1%)	.
rs6983561	0	54(31.4%)	54(34%)	0.54
	1	88(51.2%)	72(45.3%)	.
	2	30(17.4%)	33(20.8%)	.
rs16901979	0	64(33.7%)	54(30.7%)	0.07
	1	97(51.1%)	78(44.3%)	.
	2	29(15.3%)	44(25%)	.
rs10505483	0	65(33.2%)	58(30.5%)	0.11
	1	101(51.5%)	87(45.8%)	.
	2	30(15.3%)	45(23.7%)	.
rs7824364	0	88(49.4%)	31(17.5%)	<0.0001
	1	68(38.2%)	113(63.8%)	.
	2	22(12.4%)	33(18.6%)	.
rs6983267	0	150(77.7%)	171(87.2%)	0.03
	1	38(19.7%)	24(12.2%)	.
	2	5(2.6%)	1(0.5%)	.
rs7130881	0	130(67%)	116(60.4%)	0.27
	1	60(30.9%)	68(35.4%)	.
	2	4(2.1%)	8(4.2%)	.
rs7175701	0	42(21.4%)	44(22.9%)	0.78
	1	101(51.5%)	92(47.9%)	.
	2	53(27%)	56(29.2%)	.
rs11556218	0	132(67.3%)	129(66.5%)	0.98
	1	56(28.6%)	57(29.4%)	.
	2	8(4.1%)	8(4.1%)	.
rs4616256	0	131(66.8%)	121(62.7%)	0.27
	1	60(30.6%)	61(31.6%)	.
	2	5(2.6%)	11(5.7%)	.
rs11325	0	65(33.3%)	63(33.3%)	0.52
	1	99(50.8%)	88(46.6%)	.
	2	31(15.9%)	38(20.1%)	.
rs7210100	0	173(89.6%)	159(81.1%)	0.04

covariate	levels	Control	Case	P-value
	1	20(10.4%)	35(17.9%)	.
	2	.(%)	2(1%)	.

(Levels: 0 = Not present; 1 = 1 allele; 2= 2 alleles)

Table 5

SNP data by case and control status within PR subjects.

SNPs	levels	Control	Case	P-value
rs16900305	0	110(88.7%)	265(91.1%)	.76
	1	5(4%)	9(3.1%)	.
	2	9(7.3%)	17(5.8%)	.
rs7008482	0	24(19.4%)	61(21.6%)	.37
	1	61(49.2%)	118(41.7%)	.
	2	39(31.5%)	104(36.7%)	.
rs6983561	0	97(78.2%)	178(71.5%)	.34
	1	23(18.5%)	63(25.3%)	.
	2	4(3.2%)	8(3.2%)	.
rs16901979	0	89(71.8%)	179(67.5%)	.35
	1	25(20.2%)	70(26.4%)	.
	2	10(8.1%)	16(6%)	.
rs10505483	0	90(72.6%)	195(67.2%)	.18
	1	24(19.4%)	79(27.2%)	.
	2	10(8.1%)	16(5.5%)	.
rs7824364	0	100(80.6%)	162(59.1%)	.0001
	1	13(10.5%)	70(25.5%)	.
	2	11(8.9%)	42(15.3%)	.
rs6983267	0	52(41.9%)	132(47%)	.62
	1	54(43.5%)	114(40.6%)	.
	2	18(14.5%)	35(12.5%)	.
rs7130881	0	82(66.7%)	201(69.3%)	.35
	1	39(31.7%)	78(26.9%)	.
	2	2(1.6%)	11(3.8%)	.
rs7175701	0	60(48.4%)	141(49.1%)	.35
	1	46(37.1%)	118(41.1%)	.
	2	18(14.5%)	28(9.8%)	.
rs11556218	0	101(81.5%)	213(73.2%)	.06
	1	23(18.5%)	69(23.7%)	.
	2	.(%)	9(3.1%)	.
rs4616256	0	72(58.1%)	185(63.8%)	.50
	1	42(33.9%)	82(28.3%)	.
	2	10(8.1%)	23(7.9%)	.
rs11325	0	68(54.8%)	158(54.5%)	.99
	1	44(35.5%)	105(36.2%)	.
	2	12(9.7%)	27(9.3%)	.
rs7210100	0	123(99.2%)	272(96.1%)	.24

SNPs	levels	Control	Case	P-value
	1	1(0.8%)	10(3.5%)	.
	2	.(%)	1(0.4%)	.

(Levels: 0 = Not present; 1 = 1 allele; 2= 2 alleles)