ARIC Manuscript Proposal #3518

PC Reviewed: 12/10/19 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Association of polymorphisms in androgen-regulating genes with mortality of prostate cancer patients.

b. Abbreviated Title (Length 26 characters):

2. Writing Group:
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __SM___ **[please confirm with your initials electronically or in writing]**

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3. Timeline:

We are planning to submit an abstract in December 2019 and a paper in a year

4. Rationale:

Prostate cancer is the second most common cancer diagnosed in American men, as well as the second most common cause of cancer death among American men [1]. Screening allows for early detection of most prostate cancer tumors, however 19% of prostate cancer have spread beyond the prostate at the time of diagnosis [2]. The high incidence of prostate cancer in American men and aging of the population, combined with the lack of cure for metastatic prostate cancer patients make this cancer a serious public health concern.

The growth of the prostate and of prostate cancer is, in part, driven by androgens through activation of the androgen receptor and its target genes. Thus, mainstay therapy for patients with metastatic prostate cancer is androgen deprivation therapy (ADT). However, in many of the patients treated with ADT, prostate tumors acquire ADT resistance, a state called castration resistant prostate cancer (CRPC). In CRPC, tumors produce androgens locally, for instance, dihydrotestosterone (DHT) from adrenal precursor steroids such as dehydroepiandrosterone (DHEA), resulting in the stimulation of the androgen receptor and autocrine growth promotion [3]. To treat CRPC, newer oral therapies are being used that modulate and suppress androgen receptor (AR) signaling including enzalutamide and darolutamide (AR receptor antagonists) and abiraterone (CYP17A1 inhibitor, and partial AR antagonist).

Because growth and development of the prostate and of prostate cancer are dependent on the androgen supply, genes involved in androgen regulation, including the production, uptake into prostate cancer cells and conversion into DHT may drive clinical behavior and predict prognosis and other outcomes in prostate cancer patients [4]. Of note, compared to testosterone, DHT has greater AR binding affinity and greater transactivation. Thus we propose to study the association of SNPs in three genes encoding proteins involved in the androgen regulation pathway: hydroxy-delta-5-steroid dehydrogenase, 3 beta-and steroid delta-isomerase 1 (*HSD3B1*), solute carrier organic ion (*SLCO2B1*), and 5α reductase type 2 (*SRD52*A2) (Table 1).

Table 1. SNPs in androgen-regulating genes genotyped in ARIC

SNP	Chr	Gene	Major allele	Minor allele	Frequency of major allele		
					All	Whites	Blacks
rs1047303 (1245C)	1	*HSD3B1*	A	C	0.72	0.68	0.88
rs523349	2	*SRD5A2*	C	G	0.73	0.70	0.76
rs1789693	11	*SLCO2B1*	A	T	0.59	0.65	0.53
rs12422149	11	*SLCO2B1*	G	A	0.90 G	0.90	0.90

The most consistent association [7-11] with poor outcomes in men with prostate cancer treated with ADT has been shown for *HSD3B1* – which encodes an enzyme involved in the de novo synthesis of androgens within prostate cancer tumors [5], and serves as the rate-limiting step in the production of the DHT in tumor [6]. The 1245A→C polymorphism (rs1047303) results in a

change in amino acid 367 from Asn→Thr, leading to increased conversion of DHEA to androstenedione (precursor of DHT) [7]. In a cohort of white prostate cancer patients treated with primary ADT, the C minor allele was associated with a higher risk of progression to CRPC: hazard ratio [HR]=2.34 (95% confidence interval [CI], 1.08-4.49) [8]. This association has been further confirmed in more recent studies conducted in patients treated wth ADT [9-11].

The second gene of interest is *SLCO2B1*, which encodes a carrier protein that facilitates the uptake of many drugs and androgens, including testosterone and DHT [12]. A study of prostate cancer patients taking ADT for hormone sensitive prostate cancer reported that the TT genotype for rs1789693 (intronic) in *SLCO2B1* was associated with worse progression-free survival (defined as two consecutive increases in the PSA level meeting the following criteria: >2 ng/mL and >25% increase from the nadir), compared with the AA genotype (HR=1.46 (95% CI; 1.07-1.98) [13]. In contrast, a study of prostate cancer patients receiving arbiraterone for CRPC found that the AA genotype was associated with a ~2-fold higher rate of minimal residual disease (p=0.03) [14]. Further, two studies did not find significant associations for this SNP with cancer progression in patients taking arbiraterone for prostate cancer [15, 16], but overall survival appeared to be better among those with TT allele [15]. The GG genotype of another SNP in the *SLCO2B1* gene, exonic rs12422149, was associated with worse progression (as measured by PSA increase) in patients with metastatic prostate cancer taking ADT: adjusted HRs were 1.65 (95% CI, 1.28-2.12) [15] with comparable associations found in other studies [13, 16]. This functional SNP, involving A→G (Arg312Gln) may influence the efficiency of DHEA transport, thus affecting intracellular levels of androgen and cellular responses to ADT. Similarly, among those on first-line arbiraterone for metastatic CRPC, progression-free survival was worse for the GG genotype: HR=0.46 (95% CI, 0.23–0.94) for GA vs GG (none of the patients had AA genotype) [17].

The third gene of interest is *SRD5A2* with exonic SNP rs523349 associated with missense polymorphism (V89L). Having the G versus C allele in this SNP has been linked to increased 5 alpha reductase type 2 activity and heightened testosterone conversion to DHT, the most potent and strongest binding AR agonist different [6, 8]. In metastatic prostate cancer patients who took ADT, this GG genotype was associated with increased risk of all-cause death HR=2.14 (95% CI; 1.16–3.76) and disease progression: HR=1.93 (95% CI; 1.14-3.14) compared with GC/CC genotypes [18]. This funding was supported by another study that reported an increased risk of biochemical recurrence (defined by PSA increase) in patients after undergoing radical prostatectomy: HR=2.04 (95% CI, 1.16-3.59) for GG/GC vs CC in rs523349 [19].

Thus, we propose focusing on those candidate genes and corresponding SNPs based on biological plausibility and their consistent associations with prostate cancer progression and prostate cancer-specific mortality among patients receiving anti-androgen treatment. Due to limited power, we will not examine all SNPs in those genes or all androgen-regulating genes but focus on four SNPs discussed above. These SNPs may play role in cancer progression because they may affect the metabolism of those drugs [6] or because the genes participate in the different pathways of androgen conversion by which prostate tumors grow. Therefore, the ***main objective*** of our study is to examine whether there is an association of each of these four SNPs with all-cause and prostate cancer mortality in prostate cancer patients irrespective of their stage at diagnosis in the ARIC cohort. The main strength of this study is that we will be able to

examine the association of these SNPs and prostate cancer and all-cause death in a relatively large sample of prostate cancer patients after adjusting for age and stage at diagnosis. All these SNPs have been already genotyped in the previous GWAS (rs1047303, rs523349, rs12422149 were genotyped in the majority of the patients and imputed in the remaining participants) or imputed (rs1789693) in the ARIC study.

The main limitation is the lack of information about treatment. Most likely, prostate cancer patien were diagnosed at higher stage had metastases at diagnosis and died from prostate cancer; however, there is no way of confirming now whether they received ADT or type. Thus, we will have the mixture of treatments with only a small proportion likely having had ADT. Stratifying for stage at diagnosis may be helpful in identifying those with versus without metastatic disease, i.e. isolating those who were more or less likely to have received ADT, but some of the men could have lower stage at diagnosis but developed metastases and get treatment by ADT after diagnosis. An additional limitation is that we will include only those who had information on stage at diagnosis which could bias our findings. To reduce the impact of bias, we will compare the characteristics of those with and without stage.

We will validate these association in the UK biobank in which ~4500 participants (cancer free at baseline) developed prostate cancer during 5.6 years of follow-up [https://www.ukbiobank.ac.uk/about-biobank-uk/] and the genotyping has been already conducted.

Despite the limitations, this study is novel and important because it will allow us to examine the association between SNPs linked to androgen regulation and death in prostate cancer patients irrespective of stage. If our hypotheses are confirmed and replicated in the U.K. Biobank study, we will be able to create risk scores that will combine SNPs associated with higher risk of death. The results from this study will inform the mechanisms underlying the associations with death in prostate cancer patients and may also help physicians to identify high-risk group of patients.

5. Main Hypothesis/Study Questions:

Hypothesis: Polymorphisms in rs1047303 (*HSD3B1*); rs1789693 and rs12422149 (both in *SLCO2B1*); and rs523349 (*SRD5A2*) associated with increased androgen production and transport are associated with the increased prostate cancer-specific mortality and all-cause mortality in prostate cancer patients. We hypothesize that C in rs1047303, T in 1789693, G in rs1242214 and G in rs523349 are associated with increased risk of death.

Question 1: Is there an association between each SNP and all-cause mortality in prostate cancer patients after adjusting for age at diagnosis and stage?

Question 2: Is there an association between each SNP and prostate cancer-specific mortality in prostate cancer patients after adjusting for age at diagnosis and stage?

Question 3: Are there associations between each SNP and all-cause and prostate cancer-specific mortality in prostate cancer patients after strtification by stage?

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

All participants who developed prostate cancer during follow-up up to 2015, but without prevalent cancer at baseline, who had information on the stage of diagnosis, and who consented to participating in the studies on cancer and genetics will be included into the analysis. Kaplan-Meier curves (with log-rank tests) and Cox proportional hazards regression will be used to assess associations of the four SNPs individually with all-cause and prostate cancer-specific mortality. Proportional hazards assumptions will be tested for each model using an interaction term between exposure and follow-up time. Person-years of follow-up will be calculated as the difference between the end of follow-up and diagnosis date. The follow-up will end at the time of death or the end of 2015, whichever occurred first. For prostate cancer-specific mortality, the participants will be censored at date of prostate cancer death or death due to other causes. The multivariable models will be adjusted for age at diagnosis, center, stage and grade at diagnosis. If the grade of diagnosis is missing, it will be included as a separate category. The main analysis will include Black and Whites if the estimates for a given candidate SNP and outcome are comparable for the whole cohort and Whites only (the sample size will be too low to examine associations in Blacks only). Although confounding is not expected in sutying gene-outcome associations, we will examine whether adjusting for BMI, smoking, education, and physical activity change the associations, and if there is a change, the variable will be included into the models.

We will conduct several exploratory analyses stratified by stage at diagnosis and year of diagnosis although the sample size will be limited. Year of diagnosis after 2010 may be a surrogate for those treated with novel medications since those novel medications have become available in the last decade. Also, we will conduct a sensitivity analysis in which deaths with lower level of confirmation will be censored at the time of diagnosis, but not considered a death (i.e. competing analysis). In addition, we will examine the associations in those for whom treatment information exists but these data will be available for limited number of people and will only include information about first course treatment and characterized only as radiation, surgery and chemotherapy as recorded in the cancer registries. Finally, we will compare the characteristics (such as age, BMI and grade at diagnosis) of prostate cancer patients who did and did not have information about stage and treatment.

Power calculation. Assuming 600 prostate cancer patients and 250 all-cause deaths, and two-sided alpha=0.05, there will be 80% power to detect RR=1.6 in the model comparing homozygous genotype for major allele to heterozygous + homozygous genotype for minor allele, as was performed in previous studies (two-sided alpha=0.05). For prostate cancer deaths, we will be be able to detecte RR=2.0 with 80% power.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes ____ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ___X_ Yes ____ No

(This file ICTDER has been distributed to ARIC PIs, and contains
the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ___X_ Yes ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? __X__ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

__X____ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?
2693. Winchester et al, Association of SNPs in genes involved the inflammatory pathway with prostate cancer incidence and mortality in the Atherosclerosis Risk in Communities (ARIC) Study
2763. Winchester et al, A pathway-based analysis of germline variants in DNA-repair genes and prostate cancer mortality in the Atherosclerosis Risk in Communities (ARIC) Cancer Study
3482. Platz et al. Plasma Proteins and All-Cause Mortality in Cancer Survivors in ARIC

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? __X__ Yes ____ No

11.b. If yes, is the proposal
___ A. primarily the result of an ancillary study (list number* _2011.07, 1995.04 GWAS?_____)
_X__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at https://www2.cscc.unc.edu/aric/approved-ancillary-studies

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this

policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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