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14. ABSTRACT

The overall goal of the study is to examine whether altered vitamin D status (as measured by serum metabolites and by functional polymorphisms within genes related to vitamin D transport, metabolism and activity) is associated with increased risk of aggressive prostate cancer, and may explain some of the racial disparity seen in aggressive prostate cancer. All of the project activities as outlined in the Statement of Work Tasks and Milestones are complete. IRB approval was obtained from all local institutions and the DoD HSRRB. The study team participated in regular conference calls to discuss study progress and data collection and analyses throughout the grant period. All assays have been performed and data have been merged and cleaned. Statistical analyses for the main project aims are complete, with additional analyses underway. With the large representation of African Americans in this investigation, the research provides insights into the role of vitamin D in prostate cancer among a chronically underserved population carrying an unequal burden of disease.

15. SUBJECT TERMS
Vitamin D, prostate cancer aggressiveness, polymorphisms, racial disparities

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Table of Contents

Page

INTRODUCTION:

Experimental and ecologic studies support a role of vitamin D in prostate cancer prevention and prognosis; however, epidemiologic study results are inconsistent. Altered vitamin D status (as measured by plasma metabolites and by functional polymorphisms within genes related to vitamin D transport, metabolism and activity) is hypothesized to be associated with increased risk of aggressive prostate cancer, and may explain some of the racial disparity seen in aggressive prostate cancer. It is also hypothesized that plasma parathyroid hormone (PTH), serum calcium and serum phosphorus levels are inversely and directly correlated with plasma 25(OH)D and $1,25(OH)_2D$ levels, respectively, and are positively associated with disease aggressiveness. Polymorphisms within thirteen genes involved in vitamin D transport, metabolism and activity were examined to determine whether 1) allele and genotype frequencies differ by race, 2) plasma vitamin D metabolite concentrations are related to polymorphisms in these genes, 3) allele and genotype/haplotype frequencies are different in more aggressive disease as compared to less aggressive disease, and 4) vitamin D and genetic polymorphisms act synergistically to affect prostate cancer aggressiveness. We examined these associations among vitamin D status, PTH, calcium, phosphorus, polymorphisms in vitamin D-related genes, and prostate cancer aggressiveness in the North Carolina-Louisiana Prostate Cancer Project (PCaP), a previously-conducted case-only study of prostate cancer among equal numbers of African Americans and European Americans. New laboratory data were generated using previously-collected biospecimens from PCaP, and data have been and continue to be analyzed using epidemiologic techniques for estimating odds of high aggressive prostate cancer according to vitamin D metabolites, PTH, calcium, phosphorus and genetic polymorphisms.

The Specific Aims were as follows:

Primary Specific Aim 1 To examine the relationship between 1) circulating vitamin D [as measured by serum vitamin D metabolites 25(OH)D and $1,25(OH)_2D$], and 2) plasma PTH/serum calcium homeostasis and serum phosphorous, and aggressiveness of disease in AA and EA men diagnosed with prostate cancer.

Primary Specific Aim 2 To examine the allele and genotype/haplotype frequencies for polymorphisms in the *VDR*, *CYP24A1*, *CYP27B1,* and *DBP* genes (and their relative gain- or loss-of functional status): 1) by race; 2) in relation to serum vitamin D metabolite concentrations; and 3) in relation to aggressiveness of disease.

Secondary Specific Aim 3 To examine the joint effects of vitamin D status and vitamin D-related polymorphisms on prostate cancer aggressiveness.

BODY:

The project activities, as outlined in the Statement of Work (SOW) Tasks and Milestones, have been completed and ongoing work is focused on continued data analyses and manuscript publication. A 12-month no-cost extension was requested and granted, and the grant ended on September 29, 2015.

Activities in Task #1, the run-in phase of months 1-6, have been accomplished as described in detail below. The activities related to Task #2 (planned to occur in months 7 to 24 of the grant award period) and Task #3 (planned to occur in months 25-36) have been accomplished as outlined below, with continued work on data analyses and manuscript development. The original SOW activities are listed below in the numbered bullet, and the progress and status of those activities listed in the indented lettered bullet underneath each activity.

Task 1: Run-in Phase, Months 1-6:

1. Organize the investigative team and schedule regular conference calls between investigators
    a. Conference calls occurred once per month during Years 1-3 of the grant period.
2. Obtain IRB approval for the study from all institutions and DoD HSRRB
    a. IRB approval was granted by each of the institutions (USC, Roswell Park Cancer Institute, UCLA, and UNC-CH) and by DoD HSRRB.
3. Complete the data acquisition form from the parent PCaP Study
    a. Data was requested and obtained from PCaP.

4. Develop a Manual of Operations (MOP), a detailed document describing data transfer, data merging, and data management systems. The MOP content is based on our successful experience with other large-scale epidemiologic studies.
    a. A system of data transfer has been developed, and the MOP has been assembled.
5. Arrange for shipment of 1,200 serum samples to Roswell Park for vitamin D analyses, 1,200 plasma and 1,200 DNA samples to USC for PTH analyses and genotyping, and serum samples to UCLA for calcium (1,200 samples) and phosphorus (1,200 samples) analyses
    a. It was decided that plasma samples were more appropriate for vitamin D analyses, instead of serum samples, because the plasma samples were originally collected and transported under light-protected conditions.
    b. It was decided that genotyping would be conducted by Roswell Park Cancer Institute Shared Genomic Resources facility due to their having the appropriate technology and experience for the Illumina Goldengate and Sequenom genotyping methodology being used.
    c. Plasma samples and DNA samples were shipped from UNC-CH to Roswell Park, serum samples were shipped to UCLA, and plasma samples were shipped to USC.
6. Drs. Steck and Johnson attend PCRP IMPaCT Annual Meeting or other scientific meeting.
    a. There was not an IMPaCT meeting in Year 1. Dr. Steck attended the American Society of Preventive Oncology meeting in March 2012.

All milestones for Task #1 were met (IRB and HSRRB approval, samples aliquotted and shipped to labs, data systems in place for capture of all data from different sources). No data analyses occurred in Year 1, so no tables or manuscripts containing results are presented for this year.

Task 2: Laboratory Analyses, Interim Data Analyses, Months 7-24:

1. Conduct plasma 25(OH)D and 1,25$(OH)_2$D lab measurements at Roswell Park Cancer Institute
    a. Plasma vitamin D metabolite measurements are complete.
2. Conduct genotyping at Roswell Park Cancer Institute Shared Genomics Resource facility
    a. Genotyping was completed at Roswell Park Cancer Institute using Illumina Goldengate and Sequenom methodology.
3. Conduct plasma PTH measurements at Psychoneuroimmunology Lab at USC
    a. Plasma PTH measurements are complete.
4. Conduct serum calcium and phosphorus measurements at UCLA
    a. Serum calcium and phosphorus measurements are complete.
5. Hire graduate assistant at USC
    a. A senior-level doctoral student, Samuel Antwi, was hired as a graduate assistant. Sam graduated with a PhD in December 2014 and is currently a postdoctoral fellow at the Mayo Clinic in Minnesota.
6. Have all raw data sent to USC and to PCaP parent study
    a. Raw data from Roswell Park and UCLA have been distributed to USC. Data generated from the study will be sent to PCaP upon completion of data analyses and manuscript publication.
7. Manage data, begin cleaning data as it becomes available
    a. Data from PCaP, Roswell Park, UCLA, and USC were merged and cleaned.
8. Drs. Steck and Johnson attend PCRP IMPaCT Annual Meeting or other scientific meeting.
    a. There was no IMPaCT meeting in Year 2. Dr. Steck attended the American Association for Cancer Research Frontiers in Cancer Prevention meeting in November 2012.

All of the milestones for Task #2 (successful completion of lab work and raw data deposit at centralized location) were completed. Data were begun to be merged and cleaned, and preliminary data analyses of the Primary Specific Aim #1 of the study, to examine associations between plasma 25(OH)D and prostate cancer aggressiveness, were conducted. An abstract was submitted in Year 2 to the American Association for Cancer Research Annual Meeting. The results reported in that abstract were later published in the PLoS ONE article appended to this report. These results are described in more detail under Task 3, #3.

Task 3. Final Data Analyses, Months 25-36 plus no-cost extension year, Months 37-48:

1. Clean data, merge all data from different sources by study ID
    a. The genotyping data were cleaned in Year 3. All data have been cleaned and merged by study ID.
2. Perform all exploratory analyses to test for adherence to model assumptions
    a. Preliminary analyses have been performed.
3. Conduct analyses of study data; test study hypotheses
    a. Statistical analyses have been performed examining associations between 25(OH)D, 1,25$(OH)_2$D, genetic polymorphisms and prostate cancer aggressiveness.
    b. **Results are described in the following pages along with reference to the appropriate tables in the PLoS One article:**

To address Primary Specific Aim #1, associations between plasma vitamin D metabolites [25(OH)D3 and 1,25$(OH)_2$D] and aggressive prostate cancer have been examined (Steck et al. 2015). In PCaP, African Americans had lower mean concentrations of 25(OH)D3 compared to European Americans (17.7 ± 7.6 and 24.6 ± 9.6 ng/ml, respectively). As shown in Figure 1 of the PLoS One article in the Appendix, over 60% of African Americans were classified as deficient in vitamin D using the standard cutoff of <20 ng/ml, while only around 30% of European Americans were deficient. As shown in Table 2 of the PLoS One article, the highest tertile and middle tertile when compared to the lowest tertile of plasma 25(OH)D3 were positively associated with high aggressive prostate cancer among African Americans after adjustment for age, African ancestry, body mass index, energy intake, alcohol intake, study site, season, education, physical activity, smoking status, use of non-steroidal anti-inflammatory drugs, and PSA screening history (OR=1.46, 95%CI= 0.89, 2.39 and OR=1.80, 95%CI=1.10, 2.96, respectively). No substantial associations between 25(OH)D3 and prostate cancer aggressiveness were observed in European American men. Also reported in the PLoS One article, we noted a statistically significant interaction between plasma 25(OH)D3 and calcium intake on odds of aggressive prostate cancer among African Americans. As shown in Table 3 of the PLoS One article, among men with high intake of calcium, high plasma 25(OH)D3 was inversely associated with aggressive prostate cancer. In contrast, among men with low intake of calcium, high plasma 25(OH)D3 was positively associated with aggressive prostate cancer. This interaction was not evident among European American men. Other variables, including older age, obesity and study site, did not modify the effect of 25(OH)D3 on prostate cancer aggressiveness.

In a separate analyses also related to Primary Specific Aim #1 (manuscript is in development, Table 1 shown below), plasma 1,25$(OH)_2$D was not associated with odds of aggressive prostate cancer in either African Americans or European Americans in this study (OR=0.83, 95%CI=0.49, 1.41 and OR=0.67, 95%CI=0.40, 1.11, respectively, for highest tertile compared to lowest tertile).

Table 1. Associations between plasma 1,25$(OH)_2$D and prostate cancer aggressiveness by race

Race	1,25(OH)D Tertiles	n (high/low aggressive)	Age-adjusted OR[a] (95% CI)	Adjusted OR[b] (95% CI)
African-Americans	tertile1	69/85	1.00 (Ref)	1.00 (Ref)
	tertile2	50/86	0.73 (0.45-1.18)	0.66 (0.39-1.12)
	tertile3	56/89	0.88 (0.55-1.42)	0.83 (0.49-1.41)
	Cutpoints: T1<23.98, 23.98≤ T2 < 31.18, T3≥31.18 pg/ml			
European-Americans	tertile1	61/141	1.00 (Ref)	1.00 (Ref)
	tertile2	40/140	0.67 (0.42-1.08)	0.68 (0.41-1.11)
	tertile3	38/142	0.65 (0.40-1.06)	0.67 (0.40-1.11)
	Cutpoints: T1<25.40, 25.40≤ T2 < 32.50, T3≥32.50 pg/ml			

[a]Adjusted for age (categorical); [b]Adjusted for age (categorical), education status, alcohol intake, smoking status, season of the blood draw, PSA screening history, physical activity, energy intake, NSAIDs use, study site and BMI.

We created a new variable, called the vitamin D metabolite index, equal to the ratio of $1,25(OH)_2D$ to 25(OH)D3 and categorized research subjects into quartiles of the vitamin D metabolite index. As shown in Table 2 below, higher quartiles (as compared to the lowest quartile) of $1,25(OH)_2D$:25(OH)D3 ratio were associated with reduced odds of high aggressive disease among African Americans after adjustment for age, season, education, alcohol intake, smoking status, PSA screening history, physical activity, energy intake, use of non-steroidal anti-inflammatory drugs, study site and body mass index (OR=0.51, 95%CI=0.28, 0.91; OR=0.41, 95%CI=0.22, 0.76, and OR=0.46, 95%CI=0.25, 0.84 for 2nd, 3rd and 4th quartiles, respectively). Inverse associations were also observed for European Americans, but were not statistically significant (OR=0.64, 95%CI=0.35, 1.17 for 4th compared to 1st quartile). Additional analyses related to Primary Specific Aim #1 are underway to examine associations between serum calcium, phosphorus and parathyroid hormone concentrations and prostate cancer aggressiveness.

Table 2. Crude and adjusted odds ratios for aggressive prostate cancer by quartiles of $1,25(OH)_2D$:25(OH)D3 index by race

Quartiles, Vitamin D metabolite index[a]	n (high/low aggressive)[b]	Age-adjusted OR[c] (95% CI)	Adjusted OR[d] (95% CI)
African-Americans			
Quartile 1	69/64	1.00 (Ref)	1.00(Ref)
Quartile 2	38/64	0.52 (0.31-0.89)	0.51 (0.28-0.91)
Quartile 3	28/65	0.44 (0.25-0.77)	0.41 (0.22-0.76)
Quartile 4	35/64	0.57 (0.33-1.00)	0.46 (0.25-0.84)
European-Americans			
Quartile 1	41/106	1.00 (Ref)	1.00(Ref)
Quartile 2	38/104	1.00 (0.58-1.70)	1.09 (0.63-1.90)
Quartile 3	33/106	0.88 (0.51-1.53)	0.92 (0.53-1.62)
Quartile 4	25/106	0.66 (0.37-1.19)	0.64 (0.35-1.17)

[a]Quartile cutpoints of vitamin D metabolite index:
African-Americans: Q1 < 0.0013449538, 0.0013449538 ≤ Q2 < 0.0018073957; 0.0018073957 ≤ Q3 < 0.0022770399; Q4 ≥ 0.0022770399
European-Americans: Q1 < 0.0010135403, 0.0010135403 ≤ Q2 < 0.001248328; 0.001248328 ≤Q3< 0.0015520382; Q4 ≥ 0.0015520382
[b]Only participants with complete observations for all covariates were included.
[c]Adjusted for age (categorical)
[d]Adjusted for age (categorical), education status, alcohol intake, smoking status, season of the blood draw, PSA screening history, physical activity, energy intake, NSAIDs use, study site and BMI

The goal of Primary Specific Aim #2 was to examine the association between vitamin D-related gene polymorphisms and prostate cancer aggressiveness. TagSNPs (n=315) in 13 genes (*VDR, GC, CYP24A1, CYP27A1, CYP27B1, CYP2R1, CYP3A4, DHCR7, CASR, NADSYN1, RXRA, RXRB, RXRG)* were genotyped using Illumina GoldenGate or Sequenom assays in 524 African-American and 657 European-American men. In Table 3 below, results are presented for the SNPs in which a statistically significant association was observed with aggressive prostate cancer among either African Americans or European Americans. To summarize, among African Americans, 21 SNPs were associated with prostate cancer aggressiveness. The variant allele was associated negatively or positively with high aggressive prostate cancer in eleven and ten SNPs, respectively. For example, two SNPs in the vitamin D binding protein gene known as *GC*, were inversely associated with high aggressive prostate cancer (rs222054: OR, 0.55, 95%CI, 0.38-0.80; and rs16847028: OR, 0.61, 95%CI, 0.39-0.94). Among European Americans, the variant allele was inversely associated with high aggressive prostate cancer for four SNPs in three genes (*CASR*rs3863977; *CYP24A1*rs4809960; *RXRA*rs1007971; and *RXRA*rs3118526), and positively associated with high aggressive prostate cancer for three SNPs in the *CYP27B1* gene (rs703842, rs4646536, and rs10877013). After adjustment for multiple comparisons, none of the SNPs remained statistically significantly associated with aggressive prostate cancer (data not shown).

Table 3. Association between SNPs in genes related to vitamin D and odds of high aggressive prostate cancer by race

		European Americans			African Americans		
Gene* and SNP	**Genotype**	**n, high/low aggressive**	**OR (95%CI)**	**p-value**	**n, high/low aggressive**	**OR (95%CI)**	**p-value**
CASRrs1501898	CC	112/283	1.00 (ref)		190/251	1.00 (ref)	
	AC+AA	75/186	1.02 (0.72-1.46)	0.8914	26/56	**0.59 (0.35-0.99)**	**0.0449**
CASRrs2036399	GG	138/345	1.00 (ref)		151/241	1.00 (ref)	
	AG+AA	49/125	0.97 (0.65-1.43)	0.866	66/66	**1.57 (1.06-2.35)**	**0.0259**
CASRrs3749208	GG	88/219	1.00 (ref)		156/235	1.00 (ref)	
	AG	71/203	0.88 (0.61-1.28)	0.5209	55/71	1.13 (0.75-1.70)	0.5697
	AA	28/48	1.34 (0.78-2.30)	0.2825	6/1	**8.78 (1.03-74.39)**	**0.0462**
CASRrs3863977	GG	101/217	1.00 (ref)		89/124	1.00 (ref)	
	AG+AA	86/253	0.71 (0.50-1.00)	0.0536	127/182	0.97 (0.68-1.39)	0.8678
CASRrs10222633	GG	51/115	1.00 (ref)		84/88	1.00 (ref)	
	AG	93/231	0.97 (0.64-1.47)	0.8871	95/164	**0.61 (0.41-0.91)**	**0.0153**
	AA	43/164	0.84 (0.51-1.36)	0.4761	37/55	0.71 (0.42-1.20)	0.2018
CASRrs12485716	GG	101/238	1.00 (ref)		65/62	1.00 (ref)	
	AG	66/193	0.88 (0.61-1.28)	0.5191	94/156	**0.60 (0.39-0.92)**	**0.0206**
	AA	20/34	1.44 (0.78-2.66)	0.2443	58/89	0.63 (0.39-1.03)	0.0647
CASRrs12486623	AA	101/234	1.00 (ref)		67/69	1.00 (ref)	
	AC+CC	86/236	0.90 (0.64-1.28)	0.5697	150/238	**0.66 (0.45-0.99)**	**0.043**
CASRrs13324814	AA	143/349	1.00 (ref)		139/161	1.00 (ref)	
	AG+GG	44/121	1.01 (0.67-1.52)	0.9593	78/146	**0.63 (0.44-0.91)**	**0.0124**
CYP24A1rs2762929	AA	67/177	1.00 (ref)		25/18	1.00 (ref)	
	AG	94/216	1.16 (0.79-1.69)	0.4424	96/145	**0.48 (0.25-0.94)**	**0.0325**
	GG	26/76	0.92 (0.54-1.58)	0.7651	96/144	**0.50 (0.26-0.98)**	**0.0436**

Table 3. Continued

		European Americans			African Americans		
Gene* and SNP	**Genotype**	**n, high/low aggressive**	**OR (95%CI)**	**p-value**	**n, high/low aggressive**	**OR (95%CI)**	**p-value**
CYP24A1rs4809960	AA	130/259	1.00 (ref)		163/233	1.00 (ref)	
	AG+GG	57/211	**0.57 (0.39-0.82)**	**0.0025**	54/74	1.04 (0.69-1.57)	0.8496
CYP24A1rs13038432	AA	152/407	1.00 (ref)		215/293	1.00 (ref)	
	AG+GG	35/63	1.47 (0.93-2.35)	0.1004	2/14	**0.20 (0.04-0.87)**	**0.0327**
CYP27A1rs691414	GG	180/441	1.00 (ref)		128/203	1.00 (ref)	
	AG+AA	2/7	0.70 (0.14-3.59)	0.6701	81/92	**1.46 (1.01-2.14)**	**0.0465**
CYP27B1rs703842	AA	83/219	1.00 (ref)		90/138	1.00 (ref)	
	AG	79/210	1.04 (0.72-1.51)	0.8336	104/144	1.11 (0.77-1.61)	0.5689
	GG	25/41	**1.82 (1.03-3.23)**	**0.0399**	23/25	1.44 (0.76-2.69)	0.2598
CYP27B1rs4646536	AA	85/220	1.00 (ref)		105/155	1.00 (ref)	
	AG	77/209	1.00 (0.69-1.44)	0.9867	97/136	1.05 (0.73-1.51)	0.7839
	GG	25/41	**1.78 (1.01-3.16**)	0.047	15/16	1.41 (0.66-2.99)	0.37
CYP27B1rs10877013	GG	85/222	1.00 (ref)		103/152	1.00 (ref)	
	AG	77/207	1.02 (0.70-1.47)	0.9285	99/137	1.06 (0.74-1.53)	0.7441
	AA	25/41	**1.80 (1.02-3.19)**	**0.0433**	15/18	1.23 (0.59-2.56)	0.5785
GCrs222054	CC	100/235	1.00 (ref)		156/179	1.00 (ref)	
	CG+GG	87/235	0.92 (0.65-1.30)	0.644	61/128	**0.55 (0.38-0.80)**	**0.0019**
GCrs16847028	GG	155/372	1.00 (ref)		179/227	1.00 (ref)	
	AG+AA	32/98	0.78 (0.50-1.22)		38/80	**0.61 (0.39-0.94)**	**0.0253**
RXRArs1007971	GG	128/285	1.00 (ref)		39/307	1.00 (ref)	
	CG+CC	59/184	**0.65 (0.45-0.95)**	**0.0245**	178/241	1.27 (0.81-1.99)	0.2909

Table 3. Continued

		European Americans			African Americans		
Gene* and SNP	**Genotype**	**n, high/low aggressive**	**OR (95%CI)**	**p-value**	**n, high/low aggressive**	**OR (95%CI)**	**p-value**
RXRArs3118526	GG	147/334	1.00 (ref)		198/288	1.00 (ref)	
	AG+AA	40/136	0.62 (0.41-0.94)	**0.0238**	19/19	1.46 (0.74-2.87)	0.2728
RXRArs7855881	CC	184/467	1.00 (ref)		165/206	1.00 (ref)	
	AC+AA	1/2	1.23 (0.10-14.35)	0.8695	52/99	**0.66 (0.44-0.98)**	**0.0388**
RXRArs7856788	GG	184/467	1.00 (ref)		187/244	1.00 (ref)	
	AG+AA	0/1	NE		30/63	**0.61 (0.38-0.98)**	**0.0421**
RXRArs10776909	GG	112/275	1.00 (ref)		183/281	1.00 (ref)	
	AG+AA	75/194	0.94 (0.66-1.33)	0.718	34/26	**2.01 (1.16-3.50)**	**0.0128**
RXRArs11185659	GG	116/273	1.00 (ref)		126/213	1.00 (ref)	
	AG+AA	71/197	0.84 (0.59-1.19)	0.325	91/94	**1.64 (1.14-2.36)**	**0.0078**
RXRArs11185662	AA	101/257	1.00 (ref)		95/163	1.00 (ref)	
	AG+GG	85/212	1.02 (0.72-1.45)	0.8939	122/144	**1.46 (1.03-2.08)**	**0.0353**
RXRBrs9277935	CC	111/293	1.00 (ref)		102/173	1.00 (ref)	
	AC+AA	76/177	1.11 (0.78-1.58)	0.5713	115/134	**1.46 (1.03-2.08)**	**0.0342**
RXRGrs157880	GG	134/351	1.00 (ref)		77/133	1.00 (ref)	
	AG+AA	53/119	1.18 (0.80-1.74)	0.3948	140/174	1.43 (1.00-2.06)	0.0516
RXRGrs166899	GG	134/352	1.00 (ref)		77/133	1.00 (ref)	
	AG+AA	53/118	1.20 (0.81-1.77)	0.3571	140/174	1.43 (1.00-2.06)	0.0516
RXRGrs517456	GG	134/352	1.00 (ref)		76/133	1.00 (ref)	
	CG+CC	53/118	1.20 (0.81-1.77)	0.3571	141/174	**1.46 (1.02-2.10)**	**0.0409**

*Abbreviations: CASR: Calcium sensing receptor; CYP24A1: Cytochrome P450 24A1; CYP27A1: Cytochrome P450 27A1; CYP27B1: Cytochrome P450 27B1; GC(gene): Vitamin D Binding Protein; RXRA: Retinoid X receptor alpha; RXRB: Retinoid X receptor beta; RXRG: Retinoid X receptor gamma

A polygenic score based on a previous study (Mondul et al. 2013) of SNP predictors of serum 25(OH)D3 levels was calculated utilizing SNPs in the *GC, CYP24A1, CYP2R1,* and *NADSYN1* genes. Mean plasma 25(OH)D3 concentrations were lowest in the group of research subjects with the most 'low vitamin D' alleles, though there was not a linear association between number of 'low vitamin D' alleles and plasma 25(OH)D3 concentration among African Americans (Table 4 below). There was no association between higher number of 'low vitamin D' alleles in the four SNPs that comprised the polygenic score and prostate cancer aggressiveness for either race. Analyses examining interactions between vitamin D metabolites and gene polymorphisms (Secondary Specific Aim #3) are underway.

Table 4. Crude and adjusted odds ratios for aggressive prostate cancer by polygenic score index by race

Polygenic Score[a]	n (high/low aggressive)	Adjusted OR[b] (95% CI)	Mean 25(OH)D3 concentration, ng/ml
African-Americans			
0,1,2	35/39	1.00 (Ref)	17.83
3	57/90	0.69 (0.39-1.22)	18.63
4	78/109	0.80 (0.46-1.40)	17.03
5-8	47/69	0.76 (0.42-1.39)	16.84
European-Americans			
0,1,2	75/210	1.00 (Ref)	26.20
3	52/131	1.07 (0.70-1.63)	24.41
4	39/92	1.09 (0.68-1.75)	23.30
5-8	21/37	1.59 (0.87-2.93)	20.96

[a] Polygenic score includes *GCrs2282679, CYP24A1rs6013897, CYP2R1rs10741657*, and *NADSYN1_DHCR7rs12785878* as reported by Mondul et al. 2013 *Cancer Epidemiology Biomarkers and Prevention*; 22(4):688-96.
[b]Adjusted for age and African ancestry

4. Present preliminary results at scientific meetings
    a. Three abstracts were submitted to American Association for Cancer Research meetings as listed below and provided in the Appendix.
5. Prepare and submit manuscripts for publication
    a. One manuscript has been published as listed below. Two other manuscripts are in preparation based on results presented in abstracts, and additional manuscripts are planned.
6. Archive datasets for future analyses
    a. This activity will be accomplished following completion of primary analyses.
7. Plan future studies
    a. Planning for future studies has been ongoing and will continue.
8. Drs. Steck and Johnson attend PCRP IMPaCT Annual Meeting or other scientific meeting
    a. There was no IMPaCT meeting in Years 3 or 4. Dr. Steck attended the AACR Science of Cancer Health Disparities meeting in December 2013 and presented a poster of results related to this project (see abstract provided in the Appendix). Dr. Steck submitted an abstract in November 2015 to the AACR annual meeting to be held in 2016 based on genetic polymorphism results.

All of the Milestones for Task #3 (generate final analytic dataset, perform analyses for Specific Aims, present results at scientific meetings, submit manuscripts for publication) have been completed, and data analyses and manuscript development continue. One abstract was presented at the AACR annual meeting in April 2013, another was presented at the AACR The Science of Cancer Health Disparities meeting in December 2013, and a third will be presented at the AACR Annual Meeting in 2016 (see abstracts provided in the Appendix). A manuscript reporting results for the primary aim, to describe the association between 25(OH)D3 and prostate cancer aggressiveness, was published in PLoS ONE (see attached manuscript in Appendix). Additional manuscripts are in development.

KEY RESEARCH ACCOMPLISHMENTS, ALL YEARS:

YEAR 1:

- Obtained IRB approval for the study from all institutions and DoD HSRRB.
- Developed a system of data transfer.
- Arranged for shipment of plasma samples to Roswell Park for vitamin D analyses and serum samples to UCLA for calcium and phosphorus analyses.
- Conducted plasma 25(OH)D and 1,25$(OH)_2$D lab measurements (by Roswell Park Cancer Institute).
- Conducted serum calcium and phosphorus measurements (by UCLA).
- Hired graduate assistant at USC.
- Obtained data from parent study (PCaP) and began merging and cleaning data.

YEAR 2:

- Arranged for shipment of plasma samples to USC for PTH analyses and DNA samples to Roswell Park for genotyping.
- Conducted plasma PTH measurements (by USC).
- Hired graduate assistant at USC.
- Merged and cleaned data from PCaP, Roswell Park, UCLA and USC.
- Conducted preliminary data analyses of vitamin D metabolites and prostate cancer aggressiveness by race.
- Presented abstract at AACR Annual Meeting in April 2013.
- Submitted abstract to the AACR Science of Cancer Health Disparities 2013 meeting.

YEAR 3:

- Hired graduate assistant at USC.
- Presented abstract at AACR Science of Cancer Health Disparities 2013 meeting.
- Arranged for shipment of DNA samples to Roswell Park Cancer Institute for genotyping.
- Identified tagSNPs and functional SNPs in genes related to vitamin D metabolism or activity.
- Conducted genotyping (by Roswell Park Cancer Institute).
- Merged and cleaned genotyping data.
- Conducted data analyses of association between gene polymorphisms and prostate cancer aggressiveness.
- Submitted manuscript for publication reporting the association between 25(OH)D and prostate cancer aggressiveness.

NO-COST EXTENSION YEAR:

- Continued to supervise graduate assistant at USC until January 2015.
- Revised manuscript reporting the association between 25(OH)D and prostate cancer aggressiveness and published at PLoS ONE (2015).
- Conducted data analyses of association between gene polymorphisms and prostate cancer aggressiveness, and submitted abstract to 2016 AACR Annual Meeting.
- Drafting manuscripts related to abstracts (see manuscripts in preparation listed below).

REPORTABLE OUTCOMES:

Presentations (abstracts are attached in the Appendix):

1. Woloszynska-Read, A., Arab, L., Adams, J., Bensen, J., Fontham, E.T.H., Mohler, J.L., Su, L.J., Tabung, F., Zhang, H., Trump, D., Johnson, C., Steck, S.E. Plasma 25-hydroxyvitamin D Levels are Associated with Aggressive Prostate Cancer among African Americans in the North Carolina-Louisiana Prostate Cancer Project (PCaP). In: Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; 2013 Apr 6-10; Washington, DC. Philadelphia (PA): AACR; Abstract #LB-12, 2013. Poster presentation.
2. Steck, S.E., Woloszynska-Read, A., Arab, L., McMahon, D.M., Bensen, J.T., Adams, J.S., Fontham, E.T.H., Mohler, J.L., Su, L.J., Zhang, H., Trump, D., Johnson, C. Ratio of Plasma 1,25$(OH)_2$D to 25(OH)D is Inversely Associated with Aggressive Prostate Cancer in African Americans in the North Carolina-Louisiana Prostate Cancer Project (PCaP). American Association for Cancer Research The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved. Atlanta, GA. December 2013. Poster presentation.
3. Steck, S.E., Woloszynska-Read, A., Antwi, S.O., Zhang, H., Arab, L., Fontham, E.T.H., Mohler, J.L., Su, L.J., Xiao, F., Smith, G.J., Trump, D., Johnson, C., Bensen, J.T. SNPs in Vitamin D-related Genes are Associated with Prostate Cancer Aggressiveness in the North Carolina-Louisiana Prostate Cancer Project (PCaP). American Association for Cancer Research Annual Meeting. New Orleans, LA. 2016.

Manuscripts published (attached at end of document):

1. Steck SE, Arab L, Zhang H, Bensen JT, Fontham ET, Johnson CS, Mohler JL, Smith GJ, Su JL, Trump DL, Woloszynska-Read A. (2015) Association between Plasma 25-Hydroxyvitamin D, Ancestry and Aggressive Prostate Cancer among African Americans and European Americans in PCaP. PLoS One.10(4):e0125151. PMID: 25919866. PMCID: PMC4412567.

Manuscripts in preparation:

1. Woloszynska-Read A, Arab L, Bensen JT, Fontham ETH., Mohler JL, Su LJ, Zhang H, Trump D, Johnson C, Steck SE. Ratio of Plasma 1,25$(OH)_2$D to 25(OH)D is Inversely Associated with Aggressive Prostate Cancer in African Americans in the North Carolina-Louisiana Prostate Cancer Project (PCaP). Manuscript in preparation.
2. Steck SE, Woloszynska-Read A, Antwi SO, Zhang H, Arab L, Basta P, Fontham ETH, Mohler JL, Su LJ, Xiao F, Smith GJ, Trump D, Johnson C, Bensen JT. SNPs in Vitamin D-related Genes are Associated with Prostate Cancer Aggressiveness in the North Carolina-Louisiana Prostate Cancer Project (PCaP). Manuscript in preparation.

LIST OF PERSONNEL RECEIVING PAY FROM RESEARCH EFFORTS:

Susan Steck, PI, University of South Carolina
Samuel Antwi, Graduate Assistant, University of South Carolina
Hongmei Zhang, Co-investigator, University of Memphis (formerly University of South Carolina)
Candace Johnson, Collaborating PI, Roswell Park Cancer Institute
Lenore Arab, Co-investigator, UCLA David Geffen School of Medicine

CONCLUSION (More detailed results can be found in the Description of Tasks and the Appendix)**:**

In PCaP, plasma 25(OH)D was positively associated with prostate cancer aggressiveness among African Americans but not European Americans, such that subjects with high aggressive prostate cancer had increased odds of having higher plasma 25(OH)D. These findings were unexpected given the original hypothesis that 25(OH)D would be inversely associated with prostate cancer aggressiveness. However, our results support more recent literature which suggests that the relationship between 25(OH)D and prostate cancer is complex, and that 25(OH)D may not be the ideal biomarker for measuring vitamin D status in all populations. In contrast, the ratio of plasma $1,25(OH)_2D$ to 25(OH)D was inversely associated with prostate cancer aggressiveness among African Americans in PCaP. This suggests perhaps a beneficial role of the biologically active metabolite $1,25(OH)_2D$ as a marker of reduced odds of aggressive prostate cancer, particularly when 25(OH)D levels are low. Blood samples were collected after diagnosis, thus it is possible that effects of treatment or extent of disease or associated processes (e.g. weight loss) on plasma 25(OH)D may partially be responsible for the findings. Polymorphisms in genes involved in vitamin D metabolism and activity, the vitamin D binding protein and calcium sensing receptor were associated with prostate cancer aggressiveness, and there was no overlap between race groups in SNPs found to be associated with aggressiveness. Our ongoing work will examine gene-vitamin D interactions to examine whether polymorphisms in genes involved in vitamin D metabolism may explain the unexpected findings related to 25(OH)D. The results add to the growing body of literature on the complex relationship between vitamin D and prostate cancer, and provide unique data on African Americans, a chronically underserved population who are more likely to have low levels of circulating 25(OH)D and who carry an unequal burden of prostate cancer.

REFERENCES:

1. Mondul AM, Shui IM, Yu K, Travis RC, Stevens VL, et al. (2013) Genetic Variation in the Vitamin D Pathway in Relation to Risk of Prostate Cancer—Results from the Breast and Prostate Cancer Cohort Consortium. Cancer *Epidemiology Biomarkers and Prevention* 22(4):688-96.
2. Steck SE, Arab L, Zhang H, Bensen JT, Fontham ET, Johnson CS, Mohler JL, Smith GJ, Su JL, Trump DL, Woloszynska-Read A. (2015) Association between Plasma 25-Hydroxyvitamin D, Ancestry and Aggressive Prostate Cancer among African Americans and European Americans in PCaP. *PLoS One*.10(4):e0125151. PMID: 25919866. PMCID: PMC4412567.

APPENDIX:

Abstract presented at AACR Annual Meeting 2013 (see PLoS One paper for more details)

Title: Plasma 25-hydroxy vitamin D is associated with aggressive prostate cancer among African Americans in North Carolina-Louisiana Prostate Cancer Project (PCaP).

Authors: Anna Woloszynska-Read, Lenore Arab, Jeannette T. Bensen, John Adams, Elizabeth T.H. Fontham, James L. Mohler, L. Joseph Su, Fred Tabung, Hongmei Zhang, Donald Trump, Candace Johnson, Susan E. Steck.

Background: Experimental and ecological studies support links between vitamin D and prostate cancer prevention and prognosis; however, epidemiologic study results are inconsistent. Given the lower levels of circulating 25hydroxyvitamin D [25(OH)D] and higher prostate cancer aggressiveness, incidence, and mortality among African Americans compared to other racial/ethnic groups, the aim of this investigation was to examine the relationship between plasma 25(OH)D and prostate cancer aggressiveness among African Americans and European Americans.

Methods: Plasma 25(OH)D was measured using LC/MS/MS in 537 African-American and 663 European-American newly-diagnosed prostate cancer patients from the North Carolina-Louisiana Prostate Cancer Project (PCaP). Men were classified as cases (high aggressiveness) if Gleason sum ≥8, or PSA >20 ng/ml, or Gleason sum ≥7 AND clinical stage = T3c-T4c, or Gleason sum=7 with a pattern of (4+3). The comparison group (low aggressiveness) included men with Gleason sum <7 AND Stage T1-T2 AND PSA < 9 ng/ml. Plasma 25(OH)D was categorized into tertiles based on distributions among low aggressive cases in each race separately. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated for high aggressive prostate cancer by tertile of plasma 25(OH)D using logistic regression with adjustment for potential confounders.

Results: African Americans had lower mean concentrations of 25(OH)D compared to European Americans (17.7 ± 7.6 and 24.6 ± 9.6 ng/ml, respectively). The highest tertile and middle tertile when compared to the lowest tertile of plasma 25(OH)D were positively associated with highly aggressive prostate cancer among African Americans after adjustment for age, season, education, physical activity, smoking status, and PSA screening history (OR=1.7, 95%CI= 1.0, 2.8 and OR=1.8, 95%CI=1.1, 3.0, respectively). No substantial associations were observed in European American men.

Conclusions: Plasma 25(OH)D was positively associated with prostate cancer aggressiveness among African Americans but not European Americans, such that subjects with highly aggressive prostate cancer had increased odds of having higher plasma 25(OH)D. Blood samples were collected after diagnosis, thus it is possible that effects of treatment or extent of disease or associated processes (e.g. weight loss) on plasma 25(OH)D may explain the findings. Our ongoing studies include analysis of vitamin D binding protein (DBP) in the plasma and genotyping of DBP affinity variants in PCaP subjects. This approach may explain the differences seen in AA and EA men with prostate cancer, as DBP has been implicated in modulating the impact of vitamin D status on prostate cancer.

Abstract presented at AACR The Science of Cancer Health Disparities 2013 Meeting (See Tables 1-2 above for results).

Title: Ratio of plasma 1,25$(OH)_2$D to 25(OH)D is inversely associated with aggressive prostate cancer in African Americans in the North Carolina-Louisiana Prostate Cancer Project (PCaP).

Authors: Susan E. Steck, Anna Woloszynska-Read, Lenore Arab, Daria McMahon, Jeannette T. Bensen, John Adams, Elizabeth T.H. Fontham, James L. Mohler, L. Joseph Su, Hongmei Zhang, Donald Trump, Candace Johnson.

Introduction: Epidemiologic studies have reported conflicting results when examining the relationship between circulating vitamin D metabolites and risk of advanced prostate cancer. While 25-hydroxy vitamin D [25(OH)D] is used as a measure of vitamin D status, 1,25-dihydroxy vitamin D [1,25$(OH)_2$D] is the biologically active form and its concentration is tightly regulated. We previously reported increased odds of aggressive prostate cancer among African Americans in the highest tertile of plasma 25(OH)D compared to the lowest, and have now examined plasma 1,25$(OH)_2$D and the ratio of 1,25$(OH)_2$D to 25(OH)D in relation to prostate cancer aggressiveness.

Methods: Plasma 1,25$(OH)_2$D and 25(OH)D were measured using LC/MS/MS in 435 African-American and 563 European-American men with newly-diagnosed prostate cancer from the North Carolina-Louisiana Prostate Cancer Project (PCaP). Men were classified as highly aggressive cases at time of diagnosis if Gleason sum ≥8, or PSA >20 ng/ml, or Gleason sum ≥7 AND clinical stage = T3-T4, or Gleason sum=7 with a pattern of (4+3). The comparison group (low aggressiveness) included men with Gleason sum <7 AND Stage T1-T2 AND PSA < 9 ng/ml. Plasma 1,25$(OH)_2$D and the ratio of plasma 1,25$(OH)_2$D to 25(OH)D were categorized into tertiles and quartiles, respectively, based on distributions among low aggressive research subjects in each race separately. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated for high aggressive prostate cancer by tertiles of 1,25$(OH)_2$D or quartiles of the 1,25$(OH)_2$D:25(OH)D ratio using logistic regression with adjustment for potential confounders.

Results: Plasma 1,25$(OH)_2$D was not associated with odds of aggressive prostate cancer in either African Americans or European Americans in this study (OR=0.83, 95%CI=0.49, 1.41 and OR=0.67, 95%CI=0.40, 1.11, respectively, for highest tertile compared to lowest tertile). However, higher quartiles (as compared to the lowest quartile) of 1,25$(OH)_2$D:25(OH)D ratio were associated with reduced odds of high aggressive disease among African Americans after adjustment for age, season, education, alcohol intake, smoking status, PSA screening history, physical activity, energy intake, use of non-steroidal anti-inflammatory drugs, study site and body mass index (OR=0.51, 95%CI=0.28, 0.91; OR=0.41, 95%CI=0.22, 0.76, and OR=0.46, 95%CI=0.25, 0.84 for 2nd, 3rd and 4th quartiles, respectively). Inverse associations were also observed for European Americans, but were not statistically significant (OR=0.64, 95%CI=0.35, 1.17 for 4th compared to 1st quartile).

Conclusions: The ratio of plasma 1,25$(OH)_2$D to 25(OH)D was inversely associated with prostate cancer aggressiveness among African Americans. Blood samples were collected after diagnosis, thus, it is possible that effects of treatment or extent of disease or associated processes (e.g., weight loss) on plasma vitamin D metabolites may have affected their measurement. Future analyses in PCaP will include examining circulating parathyroid hormone, calcium and phosphorus, as well as genotyping of genes encoding enzymes involved in the vitamin D metabolism and activity, which may help to explain these findings.

Abstract to be presented at AACR Annual Meeting 2016 (see Tables 3-4 above for results)

Title: SNPs in vitamin D-related genes are associated with prostate cancer aggressiveness in the North Carolina-Louisiana Prostate Cancer Project (PCaP).

Authors: Susan E. Steck, Anna Woloszynska-Read, Samuel O. Antwi, Hongmei Zhang, Lenore Arab, Elizabeth T.H. Fontham, James L. Mohler, L. Joseph Su, Feifei Xiao, Gary J. Smith, Donald Trump, Candace Johnson, Jeannette T. Bensen

Introduction: African Americans have higher incidence of, and mortality from, prostate cancer (PCa) compared to other racial/ethnic groups. Frequency of polymorphisms in genes involved in vitamin D metabolism, transport, and activity differ by race/ethnicity. Examining the association between polymorphisms in vitamin D–related genes and PCa aggressiveness may explain differing susceptibility to aggressive PCa among individuals and across different racial/ethnic groups.

Methods: TagSNPs (n=315) in 13 genes (*VDR, GC, CYP24A1, CYP27A1, CYP27B1, CYP2R1, CYP3A4, DHCR7, CASR, NADSYN1, RXRA, RXRB, RXRG)* were genotyped using Illumina GoldenGate or Sequenom assays in 524 African-American and 657 European-American men with newly-diagnosed PCa from PCaP. DNA extracted from blood samples collected at enrollment was stored at -80C prior to analyses. Research subjects were classified as high aggressive if Gleason sum ≥8, or Gleason score (4+3), or PSA >20 ng/ml, or Gleason score (3+4) AND clinical stage = T3-T4. The comparison group (low aggressive) included research subjects with Gleason sum <7 AND Stage T1-T2 AND PSA < 9 ng/ml. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated for high aggressive PCa for each SNP using logistic regression with adjustment for age and proportion of African ancestry. Associations were considered statistically significant at $p<0.05$. A polygenic score based on a previous study of SNP predictors of serum 25-hydroxy-vitamin D levels was calculated utilizing SNPs in the *GC, CYP24A1, CYP2R1,* and *NADSYN1* genes.

Results: Among African Americans, 21 SNPs were associated with PCa aggressiveness. The variant allele was associated negatively or positively with high aggressive PCa in eleven and ten SNPs, respectively. For example, two SNPs in the vitamin D binding protein gene known as *GC*, were inversely associated (rs222054: OR, 0.55, 95%CI, 0.38-0.80; and rs16847028: OR, 0.61, 95%CI, 0.39-0.94). Among European Americans, the variant allele was inversely associated with high aggressive PCa for four SNPs in three genes (*CASR*rs3863977; *CYP24A1*rs4809960; *RXRA*rs1007971; and *RXRA*rs3118526), and positively associated with high aggressive PCa for three SNPs in the *CYP27B1* gene (rs703842, rs4646536, and rs10877013). There was no association between higher number of 'low vitamin D' alleles in the four SNPs that comprised the polygenic score and PCa aggressiveness for either race.

Conclusions: Polymorphisms in genes involved in vitamin D metabolism and activity, the vitamin D binding protein and calcium sensing receptor were associated with PCa aggressiveness, and there was no overlap in SNPs between race groups. Our ongoing work will examine interaction between polymorphisms of vitamin D-related genes and vitamin D metabolite levels on PCa aggressiveness.

Keywords: prostate cancer, vitamin D, genes, polymorphism, health disparities

Conflict of interest: None

Funding: PCaP is carried out as a collaborative study supported by the Department of Defense contract DAMD 17-03-2-0052. The current study was supported by DAMD 11-1-0568.

Publication in PLoS One follows.

Steck SE, Arab L, Zhang H, Bensen JT, Fontham ET, Johnson CS, Mohler JL, Smith GJ, Su JL, Trump DL, Woloszynska-Read A. (2015) Association between Plasma 25-Hydroxyvitamin D, Ancestry and Aggressive Prostate Cancer among African Americans and European Americans in PCaP. PLoS One.10(4):e0125151. PMID: 25919866. PMCID: PMC4412567.

PLOS | ONE

RESEARCH ARTICLE

Association between Plasma 25-Hydroxyvitamin D, Ancestry and Aggressive Prostate Cancer among African Americans and European Americans in PCaP

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Data Availability Statement: All third-party data are available from the PCaP ' For Researchers' website http://ncla-pcap.org/For_Researchers.php. Interested investigators are encouraged to read the 'PCaP snapshot' on the For Researchers website before requesting data. Once the investigator is ready to begin a request, they should use the 'New Project Inquiry" tab on the PCaP For Researchers webpage located on the left in the blue menu box. All data included in our manuscript is publicly available on request directly from PCaP. PCaP data is not open-

Abstract

Background

African Americans (AAs) have lower circulating 25-hydroxyvitamin D3 [25(OH)D3] concentrations and higher prostate cancer (CaP) aggressiveness than other racial/ethnic groups. The purpose of the current study was to examine the relationship between plasma 25(OH) D3, African ancestry and CaP aggressiveness among AAs and European Americans (EAs).

Methods

Plasma 25(OH)D3 was measured using LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) in 537 AA and 663 EA newly-diagnosed CaP patients from the North Carolina-Louisiana Prostate Cancer Project (PCaP) classified as having either 'high' or 'low' aggressive disease based on clinical stage, Gleason grade and prostate specific antigen at diagnosis. Mean plasma 25(OH)D3 concentrations were compared by proportion of African ancestry. Logistic regression was used to calculate multivariable adjusted odds ratios (OR) and 95% confidence intervals (95%CI) for high aggressive CaP by tertile of plasma 25(OH)D3.

access to ensure confidentiality of all study participants (a component of the PCaP Data Sharing Agreement) and to comply with consent limitations provided by each study participant.

Funding: United States Department of Defense 11-1-0568 http://cdmrp.army.mil/default.shtml SES, CSJ; United States Department of Defense W81WH11-1-0308PCa http://cdmrp.army.mil/default.shtml AWR; National Institutes of Health CA067267, CA137650 and CA016056 http://www.nih.gov DLT and CSJ. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Results

AAs with highest percent African ancestry (>95%) had the lowest mean plasma 25(OH)D3 concentrations. Overall, plasma 25(OH)D3 was associated positively with aggressiveness among AA men, an association that was modified by calcium intake ($OR_{T3vs.T1}$: 2.23, 95% CI: 1.26–3.95 among men with low calcium intake, and $OR_{T3vs.T1}$: 0.19, 95%CI: 0.05–0.70 among men with high calcium intake). Among EAs, the point estimates of the ORs were <1.0 for the upper tertiles with CIs that included the null.

Conclusions

Among AAs, plasma 25(OH)D3 was associated positively with CaP aggressiveness among men with low calcium intake and inversely among men with high calcium intake. The clinical significance of circulating concentrations of 25(OH)D3 and interactions with calcium intake in the AA population warrants further study.

Introduction

African Americans (AAs) are diagnosed with aggressive prostate cancer more often and have more than twice the prostate cancer mortality rates as European Americans (EAs) [1,2]. Vitamin D has been hypothesized to play a role in explaining some of the racial disparities in cancer mortality for various cancer types [3–5]. Mean circulating 25-hydroxyvitamin D3 [25(OH)D3, the metabolite measured to assess vitamin D status clinically] is lower among AAs than EAs [5–9]. Vitamin D in humans is derived from cutaneous exposure to ultraviolet (UV)-B rays from sunlight and the conversion of 7-dehydrocholesterol to pre-vitamin D3 with subsequent hepatic and renal conversion of D3 to the most active metabolite 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D3$] [10]. Melanin blocks UV-B, and individuals with darker skin pigmentation require longer time in the sun to produce equivalent amounts of 25(OH)D3 than individuals with less pigmented skin [11]. Dietary and supplemental intake contribute proportionally much less to vitamin D status. Intake of vitamin D is lower in AAs than in EAs which may reflect{16 low intake of milk products among AAs due to a higher prevalence of lactose intolerance [12–14]. African ancestry is inversely associated with serum 25(OH)D3 concentrations [15] and somewhat correlated with skin pigmentation [16,17]. The proportion of African ancestry may be related to genetic differences that affect metabolism, activity or storage of vitamin D.

The epidemiological evidence for an association between circulating 25(OH)D3 and prostate cancer risk has been reviewed [18–20], though few studies have included a large enough sample of AAs to examine intra-racial associations with circulating concentrations of vitamin D [21,22]. The majority of studies have found either null or inverse associations [23–33], but several studies have shown increased risk of prostate cancer at the highest concentrations of 25 (OH)D3 [34–38]. Other studies reported a U- or J-shaped association between 25(OH)D3 and prostate cancer risk [39,40] or overall mortality [41,42]. A recent meta-analysis reported inverse associations with overall cancer mortality for supplementation trials of vitamin D3 but not D2, and no substantial association between circulating 25(OH)D and prostate cancer-specific death [43]. Calcium and vitamin D levels are regulated homeostatically. An interaction between calcium intake and vitamin D status in relation to prostate cancer has been suggested, but rarely reported [34,38].

Given the higher incidence of aggressive prostate cancer among AAs, the conflicting evidence for an association between 25(OH)D3 and prostate cancer, and the underrepresentation of AA in previously published research, the goals of this study were to examine the association between plasma 25(OH)D3 concentrations and prostate cancer aggressiveness in a large, population-based, case-only study of AA and EA men newly diagnosed with prostate cancer, to examine potential interactions with calcium intake, and to examine if African ancestry as measured by ancestry informative markers could shed more light on any such race-specific relationships.

Materials and Methods

Study Population

This study utilizes data from a subset of research subjects in the North Carolina-Louisiana Prostate Cancer Project (PCaP), a population-based, case-only study designed to address racial disparities in prostate cancer. A subset of PCaP participants (n = 1200) who had agreed to future use of their biologic specimens for research were selected *a priori* for inclusion in this study due to resource constraints. The study set consisted of all research subjects diagnosed with high aggressive prostate cancer (n = 302, see aggressiveness definition in Outcomes Assessment section), 112 research subjects diagnosed with Gleason score = 4+3 (all other intermediate aggressive cancer research subjects were excluded), and a random subset (n = 786) of research subjects diagnosed with low aggressive cancer (a random subset was selected because there were many more low aggressive cancer cases than needed for analyses). This research subject selection strategy was implemented prior to any 25(OH)D3 lab measurements or data analyses. PCaP methods have been described in detail [44]. Briefly, residents of the North Carolina (NC) and Louisiana (LA) study areas between the ages of 40–79 years old with an initial diagnosis of histologically-confirmed adenocarcinoma of the prostate between July 2004 and July 2009 were eligible. The sampling frame was weighted to include all eligible AAs, and EAs were under-sampled randomly using randomized recruitment (36). The PCaP protocol was approved by the institutional review boards at all participating sites, which included the two patient recruitment sites [the University of North Carolina at Chapel Hill (UNC) and Louisiana State University Health Sciences Center (LSUHSC)], and the Department of Defense Prostate Cancer Research Program. Prior to their participation in PCaP, all men signed an informed consent and provided signed release for medical records and tumor specimens.

Data Collection

Research subjects were visited in their home by a trained registered nurse who conducted a structured interview, performed anthropometric measurements, and collected biospecimens. The majority of visits were completed on the average within four months of diagnosis. Structured questionnaires were used to collect information about lifestyle factors, family history of prostate cancer, cancer screening history, and prescribed and over-the-counter medications used in the prior two weeks, which included non-steroidal anti-inflammatory drugs (NSAIDs), vitamins and supplements. Men were asked to report usual dietary intake in the year prior to diagnosis using the National Cancer Institute Diet History Questionnaire (DHQ) modified to capture foods common to the geographic areas (e.g., Cajun and creole foods). The modified DHQ inquired about frequency of intake and usual portion size for 124 food items, and food preparation methods. Questionnaire responses were linked to the DHQ Nutrient Database through the Diet*Calc software, and intakes of macronutrients, micronutrients, and minerals, including calcium, were computed. After the in-home visit, medical records and tumor tissue samples were collected for each research subject who provided authorization for release.

Vitamin D Assessment

During in-home visits, study nurses collected 6.5 ml of fasting venous blood into lavender top (EDTA) tubes which were wrapped in foil and transported on ice at 4°C to the Blood and Tissue Procurement Core Laboratory at LSU or the BioSpecimen Processing Facility at UNC. The majority of PCaP blood samples were processed to serum, plasma and DNA on the same or the following day and aliquoted and stored at -80°C. Plasma concentrations of 25(OH)D3 were determined using LC-MS/MS at Heartland Assays, Inc. PCaP plasma samples were stored at -80°C for up to eight years prior to measurement; concentrations of 25(OH)D3 in stored samples has been reported to be quite stable even at -20°C for up to ten years [45].

Ancestry Informative Markers

Ancestry informative markers were genotyped and ancestry estimates (i.e., proportion African descent) were determined as described [46]. Allele frequencies were estimated using maximum likelihood methods. Individual ancestry proportions for self-reporting AA and EA research subjects were estimated using a Bayesian Markov Chain Monte Carlo (MCMC) clustering algorithm implemented in STRUCTURE 2.3.1. Publicly available genotypes were included from YRI, CEU and ASI ancestral populations in the STRUCTURE procedure.

Outcome Assessment

Extensive medical record abstraction was performed in PCaP and data on Gleason grade, clinical stage, and prostate specific antigen (PSA) at diagnosis were used to classify research subjects into three categories of aggressiveness as follows [44]: High aggressive cases: Gleason sum $\geq$ 8, or PSA >20 ng/ml at diagnosis, or Gleason sum = 7 AND stage T3-T4; low aggressive cases: Gleason sum <7 AND diagnosed at stage T1-T2 AND PSA <10 ng/ml at diagnosis; intermediate aggressive cases: all other cases. For the current study, all PCaP research subjects diagnosed with high aggressive cancer and intermediate aggressive cancer research subjects who had Gleason sum = 7 with primary Gleason pattern 4 were combined (this group is referred to as 'high aggressive'). The high aggressive group was compared to a random sample of low aggressive cases with Gleason sum <7, stage T1-T2, and PSA<9 ng/ml (as described in Study Population above).

Statistical analysis

Research subjects were excluded who had incomplete data on confounders or effect modifiers (40 total exclusions). The final study sample consisted of 519 AA and 641 EA men. Descriptive analyses included calculating means and standard deviations for continuous variables and frequencies and percentages for categorical variables, which included frequencies by predetermined categories of 25(OH)D3 based on previous literature (<20ng/ml, 20–30ng/ml, and $\geq$30ng/ml) and by tertiles based on the 25(OH)D3 race-specific (self-reported race) distributions in PCaP. Means and standard deviations of plasma 25(OH)D3 were calculated for EAs and AAs, and by African ancestry, categorized as >95%, 85–95%, <85% based on ancestry informative markers to be comparable to previous literature [15]. Crude and multivariable logistic regressions were used to examine the relationships between plasma 25(OH)D3 concentrations and prostate cancer aggressiveness by comparing high aggressive research subjects to low aggressive research subjects. The distributions of plasma 25(OH)D3 were different between AAs and EAs, so all analyses were stratified by race and plasma 25(OH)D3 concentrations were grouped into tertiles (T) with race-specific cut-off points based on the distribution among low aggressive prostate cancer cases; for AAs: T1<13.30, 13.30 $\leq$ T2 < 18.90,

T3 ≥18.90ng/ml; and for EAs: T1<21.14, 21.14 ≤ T2 < 26.67, T3 ≥26.67 ng/ml. The lowest race-specific tertile was used as the referent in race-stratified analyses. Confounding was assessed using the 10% rule. Final multivariable logistic regression models adjusted for age (years, continuous); African ancestry (%, continuous); body mass index (BMI; kg/m^2, continuous); education (less than 8th grade or some high school; high school graduate or vocational/technical school; some college or college graduate; some graduate training or graduate/professional degree); PSA screening history (0, 1–7, >7 screenings); smoking (non-smoker, former smoker, and current smoker); alcohol intake (g/day, continuous); NSAID use (yes/no); study site (NC, LA); season of blood draw (winter, spring, summer, and fall); physical activity (MET-hrs/wk, continuous); and total energy intake (kcal/day, continuous). Based on 10% rule, marital status and family history of first degree relative with prostate cancer were not confounders and were not included in the final model. Stratified analyses were performed to explore possible effect modifying role of age (< 65, ≥ 65 years), BMI (< 30, ≥30 kg/m^2), study site (NC, LA), and dietary calcium intake (< 1200, ≥ 1200 mg/d) in the relationships between plasma 25(OH)D3 concentrations and prostate cancer aggressiveness, and interactions were tested by including an interaction term in fully adjusted race-stratified models. Sensitivity analyses were performed to examine effects of recent weight change (measured weight at time of interview minus self-report weight one year prior to diagnosis), number of days until processing of blood sample (0–1 day compared to ≥2 days; 10% were processed ≥2 days), or current use of vitamin D supplements at time of blood draw. In addition, research subjects with Gleason score = 4+3 (included in the high risk group in this study, but classified as intermediate aggressive by PCaP) were excluded in sensitivity analyses to restrict the high aggressive group to those subjects with Gleason score of 8 or greater in order to evaluate whether including less aggressive cancers in the high aggressive group may have biased the associations observed. SAS version 9.3 (SAS Institute, Cary, NC) was used for all analyses, and statistical significance was evaluated at $p<0.05$ (two-tailed).

Results

Data from 306 AAs and 456 EAs with low aggressive prostate cancer, and 213 AAs and 185 EAs with high aggressive prostate cancer were included in the analyses. The majority of PCaP research subjects had no family history of prostate cancer, were former smokers, were married or living with a partner, and were not taking vitamin D supplements at the time of interview (Table 1). Almost half of interviews occurred during the Fall (September 21 to December 20). Average age of research subjects was 63 years and average BMI was 29.3 kg/m^2. Low and high aggressive cases were distributed similarly at the NC and LA study sites

The majority of AAs (67% of high aggressive and 70% of low aggressive cases) had 25(OH)D3 concentrations below 20ng/ml, compared to approximately 30% of EAs (31% of high aggressive and 28% of low aggressive) (Fig 1). Research subjects with higher proportions of African ancestry, as indicated by ancestry informative genetic markers, had lower mean concentrations of plasma 25(OH)D3 (17.0 ± 7.2, 18.4 ± 8.3, and 19.1 ± 7.6 ng/ml for AAs with African ancestry of >95%, 85–95%, and <85%, respectively), whereas the mean concentration of 25(OH)D3 among self declared EA men (all with <40% African ancestry) was 24.6 ± 9.7 ng/ml.

Among AAs, higher odds of high aggressive prostate cancer were observed for research subjects in the second and third tertiles of 25(OH)D3 concentration compared to the first, after adjustment for potential confounders ($OR_{T2\ vs\ T1}$: 1.80, 95%CI: 1.10–2.96 and $OR_{T3\ vs.\ T1}$: 1.46, 95%CI: 0.89–2.39, Table 2). In contrast, the point estimates of the ORs for EAs were less than 1.0 but not statistically significantly different from 1.0 ($OR_{T2\ vs\ T1}$: 0.92, 95%CI: 0.59–1.43 and

Table 1. Descriptive statistics by prostate cancer aggressiveness and race.

Characteristics	Low Aggressive				High Aggressive			
	AA		EA		AA		EA	
Continuous variables	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Age, yrs	61	8	63	7	62	8	66	8
Body mass index, kg/m^2	28.8	5.3	29.0	4.8	29.4	6.6	29.8	5.0
Total energy intake, kcal/day	2638.2	1334.3	2355.0	1155.1	3033.0	1607.2	2504.2	1269.6
Alcohol intake, g/day	17.3	45.1	14.3	43.2	20.0	63.0	19.3	49.4
Total vitamin D intake, mcg/d	6.3	7.1	7.7	8.0	7.2	18.9	8.8	9.3
Calcium intake, mg/d	804.3	471.4	923.9	488.4	929.1	516.4	1020.4	585.3
Physical activity, MET-hours/week	3.6	5.1	4.3	5.5	3.3	5.0	4.2	5.6
Plasma 25(OH)D3, ng/ml	17.1	7.4	24.5	8.6	18.4	7.7	25.0	12.0
Categorical variables	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Study Site								
NC	151	49.4	223	51.1	101	47.4	87	47.0
LA	155	50.6	233	48.9	112	52.6	98	53.0
Family History of Prostate Cancer								
No affected 1st degree relative	196	69.5	323	72.9	146	73.4	143	75.7
At least 1 affected 1st degree relative	86	30.5	120	27.1	53	26.6	33	18.7
Education								
Grad/professional degree	24	7.8	98	21.5	7	3.3	41	22.2
Some college or college graduate	95	31.1	189	41.5	60	28.2	77	41.6
High school grad or voc/tech school	107	35.0	136	29.8	68	31.9	41	22.2
Less than high school education	80	26.1	33	7.2	78	36.6	26	14.0
PSA Screening History								
0 screenings	96	31.4	69	15.1	115	54.0	33	17.8
1–7 screenings	137	44.8	206	45.2	60	28.2	79	42.7
> 7 screenings	73	23.8	181	39.7	38	17.8	73	39.5
Smoking Status								
Current smokers	114	37.3	179	39.3	44	20.7	69	37.3
Former smokers	131	42.8	240	52.6	117	54.9	97	52.4
Non-smokers	61	19.9	37	8.1	52	24.4	19	10.3
NSAID Use								
No	147	48.0	150	31.6	82	38.5	65	35.1
Yes	159	52.0	323	68.4	131	61.5	120	64.9
Taking dietary supplement containing vitamin D at the time of the interview								
No	253	81.9	312	70.0	173	79.4	118	63.1
Yes	56	18.1	144	30.0	45	20.6	69	26.9
Season								
Winter (21Dec–22Mar)	55	18.0	81	17.7	26	12.2	31	16.8
Spring (21Mar–20Jun)	54	17.6	88	19.3	46	21.6	35	18.9
Summer (21Jun–20Sep)	53	17.3	71	15.6	34	16.0	32	17.3
Fall (21Sep–20Dec)	144	47.1	216	47.4	107	50.2	87	47.0
African Ancestry (among African Americans)								
High (>0.95)	197	64.4	NA	NA	132	62.0	NA	NA
Medium (0.85–<0.95)	49	16.0	NA	NA	35	16.4	NA	NA
Low (<0.85)	60	19.6	NA	NA	46	21.6	NA	NA
Marital Status								

(*Continued*)

Table 1. (*Continued*)

Characteristics	Low Aggressive				High Aggressive			
	AA		EA		AA		EA	
Continuous variables	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Single/separated/divorced/widowed	95	31.1	59	12.9	74	34.7	39	21.1
Married/living with partner	211	68.9	397	87.1	139	65.3	146	78.9

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$OR_{T3\ vs.\ T1}$: 0.92, 95%CI: 0.58–1.44). The associations between 25(OH)D3 and prostate cancer aggressiveness were not modified by age, BMI, or study site (Table 3). However, dietary calcium intake appeared to modify the association among AAs ($P_{interaction}$ = 0.001). High aggressive prostate cancer was associated with higher 25(OH)D3 concentrations among men with low calcium intake (<1200mg/d; $OR_{T3\ vs.\ T1}$: 2.23, 95%CI: 1.26–3.95). A decreased odds of high aggressive prostate cancer for higher 25(OH)D3 was observed among men with higher calcium intake (≥ 1200 mg/d; $OR_{T3\ vs.\ T1}$: 0.19, 95%CI: 0.05–0.70). The interaction p-value for EAs was not significant (p = 0.99), though the effect estimates were in a similar direction as those of AAs in men with higher calcium intake.

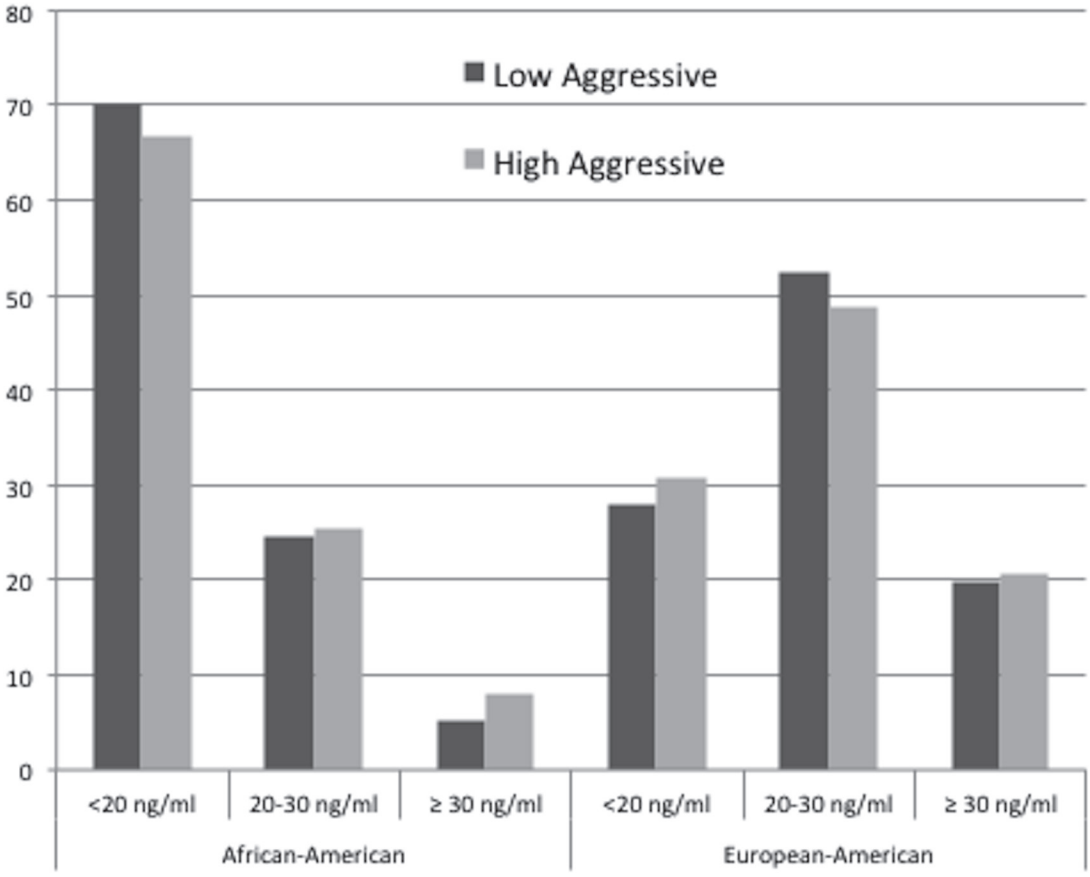

Fig 1. Distribution of plasma 25(OH)D3 by prostate cancer aggressiveness and race.

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Table 2. Age- and multivariable-adjusted odds ratios and 95% confidence intervals for high aggressive prostate cancer by tertiles of plasma 25 (OH)D3.

	25(OH)D3 tertiles, ng/ml	n (high aggressive/ low aggressive)	Age-adjusted odds ratio	Age-adjusted 95% CI	Fully adjusted[a] odds ratio	Fully adjusted[a] 95% CI	Fully adjusted[a] trend test p-value
African Americans	< 13.30	56/102	1.00	referent	1.00	referent	
	13.30 ≤ T2 < 18.90	76/100	1.40	0.89, 2.18	1.80	1.10, 2.96	
	≥ 18.90	81/104	1.36	0.88, 2.11	1.46	0.89, 2.39	0.27
European Americans	< 21.14	67/155	1.00	referent	1.00	referent	
	21.14 ≤ T2 < 26.67	58/150	0.90	0.59, 1.37	0.92	0.59, 1.43	
	≥ 26.67	60/151	0.88	0.58, 1.34	0.92	0.58, 1.44	0.71

[a]Adjusted for age, African ancestry, BMI, total energy intake, alcohol intake, physical activity, smoking status, educational status, PSA screening history, study site, NSAIDs use, and season of blood draw

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In sensitivity analyses, a variable for recent weight change [measured weight in kilograms (kg) at time of interview minus self-reported weight one year prior to diagnosis in kg converted from pounds (lbs)] was included in the models, and there was no substantial change (i.e., >10% change in effect estimates) in the results. Adjusting for number of days to processing the blood sample (blood samples from 126 men were processed two days after collection and from one man five days after collection, whereas all others were processed on the same or next day after collection, with the majority, n = 650 processed on the same day) did not affect the results, neither did adjusting for vitamin D supplement use at the time of the interview. The OR for AA men in the highest tertile of 25(OH)D3 compared to the lowest strengthened to 1.76 (95% CI: 1.02–3.05) after excluding those research subjects with ≥two days between collection and processing. Excluding men who reported taking a vitamin D supplement at the time of interview (19% of AAs and 32% of EAs) or excluding men with Gleason score = 4+3 (originally classified by PCaP as intermediate aggressive) did not change the results of these analyses.

Discussion

This population-based case-only study of AA and EA men with incident prostate cancer confirms the inverse relationship between African ancestry and circulating vitamin D concentrations. A novel finding is that higher total plasma 25(OH)D3 concentrations were associated with increased odds of high aggressive prostate cancer among AAs. This contrasts with the findings among EAs where point estimates suggested a modest protective effect of higher concentrations which was not statistically significant after adjustment for potential confounders. Associations were not modified by age, BMI, or study site. However, a significant interaction between self-reported calcium intake and measured plasma 25(OH)D3 concentration was observed among AAs. The positive association between 25(OH)D3 concentration and high aggressive prostate cancer was observed in men with lower calcium intake (<1200mg/d), whereas higher 25(OH)D3 was associated with reduced odds of high aggressive prostate cancer among men with higher calcium intake (≥1200mg/d).

Similar to previous studies [15,47], mean concentrations of circulating 25(OH)D3 varied by proportion of African ancestry; the lowest mean concentration occurred in men with >95% African ancestry. The distribution of plasma 25(OH)D3 concentration differed between AAs and EAs; race-specific cutpoints had to be used to ensure ample numbers of research subjects in each category and to produce meaningful results when stratified by race. Approximately

Table 3. Association between measured concentrations of plasma 25(OH)D3 and prostate cancer aggressiveness stratified by race and selected demographic, clinical and dietary characteristics.

Race	Characteristic	Tertiles[a]			$P_{interaction}$
		1	2	3	
Age, years					
African Americans	< 65				
	High aggressive/ low aggressive, n	35/65	47/75	44/67	
	OR (95% CI) [b]	1.00 (Ref.)	1.70 (0.90–3.19)	1.52 (0.80–2.86)	
	≥ 65				
	High aggressive/ low aggressive, n	21/37	29/25	37/37	
	OR (95% CI) [b]	1.00 (Ref.)	2.42 (0.97–6.06)	1.72 (0.70–4.22)	0.82
European Americans	< 65				
	High aggressive/ low aggressive, n	28/91	23/87	23/83	
	OR (95% CI) [b]	1.00 (Ref.)	0.85 (0.44–1.65)	0.97 (0.49–1.93)	
	≥ 65				
	High aggressive/ low aggressive, n	39/64	35/63	37/68	
	OR (95% CI) [b]	1.00 (Ref.)	0.89 (0.49–1.64)	0.83 (0.44–1.55)	0.91
BMI (kg/m^2)					
African Americans	< 30				
	High aggressive/ low aggressive, n	33/65	43/57	57/74	
	OR (95% CI) [c]	1.00 (Ref.)	1.87 (0.96–3.65)	1.52 (0.80–2.88)	
	≥ 30				
	High aggressive/ low aggressive, n	23/37	33/43	24/30	
	OR (95% CI) [c]	1.00 (Ref.)	1.71 (0.77–3.84)	1.05 (0.43–2.56)	0.78
European Americans	< 30				
	High aggressive/ low aggressive, n	28/93	37/104	39/103	
	OR (95% CI) [c]	1.00 (Ref.)	1.19 (0.64–2.19)	1.20 (0.65–2.22)	
	≥ 30				
	High aggressive/ low aggressive, n	39/62	21/46	21/48	
	OR (95% CI) [c]	1.00 (Ref.)	0.77 (0.38–1.58)	0.65 (0.31–1.36)	0.25
Study Site					
African Americans	**LA**				
	High aggressive/ low aggressive, n	22/46	40/49	50/60	
	OR (95% CI) [d]	1.00 (Ref.)	2.18 (1.00–4.75)	1.77 (0.83–3.78)	
	NC				
	High aggressive/ low aggressive, n	34/56	36/51	31/44	
	OR (95% CI) [d]	1.00 (Ref.)	1.58 (0.80–3.12)	1.33 (0.66–2.68)	0.78
European Americans	**LA**				
	High aggressive/ low aggressive, n	35/71	28/82	35/80	
	OR (95% CI) [d]	1.0 (Ref.)	0.67 (0.35–1.27)	0.81 (0.43–1.54)	
	NC				
	High aggressive/ low aggressive, n	32/84	30/68	25/71	
	OR (95% CI) [d]	1.00 (Ref.)	1.16 (0.60–2.21)	1.02 (0.51–2.03)	0.57
Dietary Calcium Intake, mg/d					
African Americans	**<**1200				
	High aggressive/ low aggressive, n	39/92	59/87	63/75	
	OR (95% CI) [e]	1.00 (Ref.)	2.07 (1.17–3.66)	2.23 (1.26–3.95)	
	≥ 1200				
	High aggressive/ low aggressive, n	17/10	17/13	18/29	
	OR (95% CI) [e]	1.00 (Ref.)	1.06 (0.28–3.99)	0.19 (0.05–0.70)	0.001

(*Continued*)

Table 3. (*Continued*)

Race	Characteristic	Tertiles[a]			$P_{interaction}$
		1	2	3	
European Americans	< 1200				
	High aggressive/ low aggressive, n	51/126	42/117	38/108	
	OR (95% CI) [e]	1.00 (Ref.)	0.97 (0.58–1.64)	0.97 (0.56–1.69)	
	≥ 1200				
	High aggressive/ low aggressive, n	16/29	16/33	22/43	
	OR (95% CI) [e]	1.0 (Ref.)	0.77 (0.29–2.04)	0.87 (0.35–2.17)	0.99

[a] Cutpoints for tertiles of 25(OH)D3 for African Americans: T1<13.30, 13.30 ≤ T2 < 18.90, T3 ≥18.90 ng/ml; for European Americans: T1<21.14, 21.14 ≤ T2 < 26.67, T3 ≥26.67 ng/ml

[b] Adjusted for African ancestry, BMI, total energy intake, alcohol intake, physical activity, smoking status, educational status, PSA screening history, study site, NSAIDs use, and season of blood draw

[c]Adjusted for age, African ancestry, total energy intake, alcohol intake, physical activity, smoking status, educational status, PSA screening history, study site, NSAIDs use, and season of blood draw

[d]Adjusted for age, African ancestry, BMI, total energy intake, alcohol intake, physical activity, smoking status, educational status, PSA screening history, NSAIDs use, and season of blood draw

[e]Adjusted for age, African ancestry, BMI, total energy intake, alcohol intake, physical activity, smoking status, educational status, PSA screening history, study site, NSAIDs use, and season of blood draw

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70% of AA prostate cancer cases but only approximately 30% of EA cases had 25(OH)D3 concentrations <20ng/ml. While the distribution of African ancestry was not substantially different between NC and LA in this PCaP subset, a higher percentage of AAs from NC versus LA had 25(OH)D3 concentrations <20 ng/ml (74% versus 64%, respectively), which may reflect variations in sunlight exposure and diet between research subjects from the two states.

The findings of increased odds of high aggressive prostate cancer at higher plasma 25(OH)D3 concentrations among AAs is counter to the original hypothesis that higher vitamin D may be protective against aggressive prostate cancer and contrasted to some epidemiological studies supporting the original hypothesis [21,22,30,48,49]. However, several nested case-control studies have reported increased risk of aggressive prostate cancer [34,38] or total prostate cancer [35,37] for men with higher concentrations of 25(OH)D3. The majority of these studies were in European or EA populations (or did not report results separately by race), with 25(OH)D3 concentrations greater than approximately 32ng/ml associated with increased risk. These concentrations are much higher than those observed in PCaP AA research subjects at higher odds of aggressive prostate cancer (second tertile cutpoint >13.3 ng/ml). The differences in study populations, choice of control groups, and distribution of plasma 25(OH)D3 concentrations limit ability to compare results across studies. Only 5% of AAs had 25(OH)D3 concentrations above 32ng/ml in PCaP. Thus, it is unclear what the effect of higher concentrations of 25(OH)D3 would be in this population.

In contrast with our findings, Murphy et al. reported that AA (and EA) men with low serum 25(OH)D concentrations (<12 ng/ml compared to >12ng/ml) had higher odds of higher Gleason grade and higher tumor stage in a cross-sectional study performed in Chicago, IL [21]. Kristal et al. recently reported reduced risk of Gleason 7–10 prostate cancer among AAs in the upper three quintiles of serum 25(OH)D compared to the lower two quintiles in the Selenium and Vitamin E Cancer Prevention Trial [22]. In a recent randomized, placebo-controlled clinical trial, varying doses of vitamin D supplementation had no effect on PSA levels in healthy AA men without prostate cancer [50]. The current study results, along with these

recent reports underscore the need for more research on vitamin D and prostate health among AAs who have been underrepresented in previous research studies.

Experimental models support a role of vitamin D in halting prostate cancer progression which contrasts with inconsistent findings in the epidemiologic literature. Vitamin D has been reported to inhibit proliferation in prostate cancer cell lines and inhibit invasion and metastases in some prostate cancer animal models, such as the Dunning rat model [51–55]. The balance of 25(OH)D3 concentrations and 24-hydroxylation that inactivates $1,25(OH)_2D3$ at the target tissue may affect the response to circulating 25(OH)D3 [39]. Clinical cutpoints for assessing vitamin D status remain unstandardized, but the Institute of Medicine recommended a 25(OH)D3 cutpoint of >20ng/ml as sufficient for skeletal health outcomes [56]. Recent research calls into question the uniform application of cutpoints across all racial groups [57]. The majority of 25(OH)D3 is bound to vitamin D binding protein in circulation [58]. Genotypes of the gene encoding the vitamin D binding protein (*GC*) are distributed differentially across races; AAs have a higher prevalence of genotypes associated with low levels of vitamin D binding protein [59–62]. A recent study reported that AAs and EAs had similar concentrations of free 25(OH)D [considered to be more bioavailable than bound 25(OH)D], after *GC* genotypes were accounted for, which may explain the paradox of higher bone mineral density among AAs than EAs even in the presence of lower total 25(OH)D concentrations [57]. Levels of vitamin D binding protein were not available in the current study, though genotyping of genes involved in vitamin D metabolism and activity (including the vitamin D binding protein, *GC*) are underway.

Plasma samples were collected after diagnosis, so reverse causality is a possible explanation for the findings; disease status may have affected plasma 25(OH)D3 concentrations. Vitamin D is fat-soluble and stored in adipose tissue. One possible explanation for the unexpected findings may be that men with high aggressive disease were more likely to have lost weight recently than those with low aggressive disease, which would release 25(OH)D3 into circulation from adipose tissue and increase plasma concentrations of 25(OH)D3. However, adjustment for recent weight change in the year prior to diagnosis did not change the association between 25 (OH)D3 concentrations and aggressiveness. Systematic differences in the way blood samples were collected and processed were investigated, but adjustment for time to blood processing and exclusion of those research subjects with two or five days before processing also did not substantially affect results. Another potential explanation for the unexpected finding was the possibility that research subjects diagnosed with prostate cancer may have started supplementation with vitamin D after diagnosis but before the interview, and that this would have been done differentially by AA research subjects with high versus low aggressive prostate cancer either due to physician recommendation or of their own volition. Nurse interviewers conducted an inventory of all medications and supplements used by research subjects at the time of the home visit. A slightly higher percentage of AA men with high versus low aggressive prostate cancer reported taking vitamin D supplements (21% versus 18%, respectively), but adjustment for recent use of vitamin D supplements or exclusion of those subjects reporting vitamin D supplement use did not change the results.

The current study found an interaction between calcium intake and plasma 25(OH)D3 concentrations among AAs. The positive association between 25(OH)D3 and high aggressive prostate cancer was observed in men with low calcium intakes (below the Recommended Daily Allowance of 1200 mg/d for men 71 years and older), whereas reduced odds of high aggressive prostate cancer for high concentrations of 25(OH)D3 was observed among AA men with higher calcium intakes. These results contrast with the Albanes et al. report of the Alpha-tocopherol, Beta-carotene Cancer Prevention Study in Finnish men that increased risk of prostate cancer for higher concentrations of serum 25(OH)D was more prominent among men

with higher intakes of calcium (≥1338 mg/d) [38]. The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial reported no difference in effect of 25(OH)D3 on prostate cancer across all levels of calcium intake [34]. It is not clear if the finding in PCaP of an interaction with calcium represents a true biologic interaction perhaps related to genetic predisposition to lactose intolerance or may be due to chance given the small number of research subjects with calcium intake ≥1200 mg/d. Further research should evaluate the interaction between calcium and vitamin D in prostate cancer.

Strengths of the study include the use of rapid case ascertainment in identification of a population-based sample of a large number of clinically and epidemiologically well-characterized AA and EA men with incident prostate cancer from two study sites in the United States. Additionally, all men were genotyped for ancestry informative markers and thus proportion of African ancestry was available and used to reduce confounding by population stratification. Sunlight exposure produces as much as 90% of the vitamin D requirement for humans [63]. Plasma concentrations as the exposure variable provide a better indicator of vitamin D status than dietary intake or supplement data alone. Plasma 25(OH)D3 concentrations can vary by season, so season of blood collection was controlled for in the analyses. Nutrient biomarkers, such as plasma 25(OH)D3, provide more objective markers of exposure than participant recall of diet and supplement use. However, one measurement of plasma reflects only recent exposure, though 25(OH)D3 concentrations and skin color, the major determinant of 25(OH)D3, are relatively stable over time [28,64]. Other limitations of this study include those inherent to any observational epidemiological investigation, such as non-participation and the possibility of selection bias or recall bias for self-reported covariates.

In conclusion, an interaction between plasma concentrations of 25(OH)D3 and calcium intake was observed in relation to odds of high aggressive prostate cancer among AAs but not EAs. The study results add to the growing literature [22,40] suggesting that the relationship between 25(OH)D3 and disease is complex particularly in the context of cancer-related racial disparities. Recommendations for universal cutpoints of vitamin D status and increasing 25(OH)D3 concentrations in all populations may not be ideal. Other factors may need to be considered which include calcium intake, genetic ancestry, background variation, and concentrations of bioavailable vitamin D.

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Author Contributions

Conceived and designed the experiments: AWR SES LA CSJ. Performed the experiments: AWR SES LA CSJ HZ JTB. Analyzed the data: AWR SES LA HZ. Contributed reagents/materials/analysis tools: AWR SES LA CSJ HZ JTB JLM GJS. Wrote the paper: SES AWR LA HZ ETHF CSJ LJS DLT.

References

1. Hebert JR, Daguise VG, Hurley DM, Wilkerson RC, Mosley CM, et al. (2009) Mapping cancer mortality-to-incidence ratios to illustrate racial and sex disparities in a high-risk population. Cancer 115: 2539–2552. doi: 10.1002/cncr.24270 PMID: 19296515

2. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014. CA Cancer J Clin 64: 9–29. doi: 10.3322/caac.21208 PMID: 24399786
3. Giovannucci E (2009) Vitamin D and cancer incidence in the Harvard cohorts. Ann Epidemiol 19: 84–88. doi: 10.1016/j.annepidem.2007.12.002 PMID: 18291673
4. Grant WB (2008) Differences in vitamin-D status may explain black-white differences in breast cancer survival rates. J Natl Med Assoc 100: 1040. PMID: 18807432
5. Egan KM, Signorello LB, Munro HM, Hargreaves MK, Hollis BW, et al. (2008) Vitamin D insufficiency among African-Americans in the southeastern United States: implications for cancer disparities (United States). Cancer Causes Control 19: 527–535. doi: 10.1007/s10552-008-9115-z PMID: 18219582
6. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR (2002) Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone 30: 771–777. PMID: 11996918
7. Zadshir A, Tareen N, Pan D, Norris K, Martins D (2005) The prevalence of hypovitaminosis D among US adults: data from the NHANES III. Ethn Dis 15: S5-97-101.
8. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, et al. (2007) High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr 137: 447–452. PMID: 17237325
9. Freedman DM, Cahoon EK, Rajaraman P, Major JM, Doody MM, et al. (2013) Sunlight and other determinants of circulating 25-hydroxyvitamin D levels in black and white participants in a nationwide U.S. study. Am J Epidemiol 177: 180–192. doi: 10.1093/aje/kws223 PMID: 23292956
10. Deeb KK, Trump DL, Johnson CS (2007) Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 7: 684–700. PMID: 17721433
11. Taksler GB, Cutler DM, Giovannucci E, Smith MR, Keating NL (2013) Ultraviolet index and racial differences in prostate cancer incidence and mortality. Cancer 119: 3195–3203. doi: 10.1002/cncr.28127 PMID: 23744754
12. Arab L, Carriquiry A, Steck-Scott S, Gaudet MM (2003) Ethnic differences in the nutrient intake adequacy of premenopausal US women: results from the Third National Health Examination Survey. J Am Diet Assoc 103: 1008–1014. PMID: 12891149
13. Arab L, Nelson C, Cambou MC, Kim H, Horst R, et al. (2010) Contributions of food intake to serum 25 OH vitamin D levels in healthy African American and Caucasian Los Angelinos. FASEB J 24: 325.322.
14. Moore CE, Murphy MM, Holick MF (2005) Vitamin D Intakes by Children and Adults in the United States Differ among Ethnic Groups. J Nutr 135: 2478–2485. PMID: 16177216
15. Signorello LB, Williams SM, Zheng W, Smith JR, Long J, et al. (2010) Blood vitamin d levels in relation to genetic estimation of African ancestry. Cancer Epidemiol Biomarkers Prev 19: 2325–2331. doi: 10.1158/1055-9965.EPI-10-0482 PMID: 20647395
16. Shriver MD, Parra EJ, Dios S, Bonilla C, Norton H, et al. (2003) Skin pigmentation, biogeographical ancestry and admixture mapping. Hum Genet 112: 387–399. PMID: 12579416
17. Parra EJ, Kittles RA, Shriver MD (2004) Implications of correlations between skin color and genetic ancestry for biomedical research. Nat Genet 36: S54–60. PMID: 15508005
18. Yin L, Raum E, Haug U, Arndt V, Brenner H (2009) Meta-analysis of longitudinal studies: Serum vitamin D and prostate cancer risk. Cancer Epidemiol 33: 435–445. doi: 10.1016/j.canep.2009.10.014 PMID: 19939760
19. Gilbert R, Martin RM, Beynon R, Harris R, Savovic J, et al. (2011) Associations of circulating and dietary vitamin D with prostate cancer risk: a systematic review and dose-response meta-analysis. Cancer Causes Control 22: 319–340. doi: 10.1007/s10552-010-9706-3 PMID: 21203822
20. Gupta D, Lammersfeld CA, Trukova K, Lis CG (2009) Vitamin D and prostate cancer risk: a review of the epidemiological literature. Prostate Cancer Prostatic Dis 12: 215–226. doi: 10.1038/pcan.2009.7 PMID: 19350051
21. Murphy AB, Nyame Y, Martin IK, Catalona WJ, Hollowell CM, et al. (2014) Vitamin d deficiency predicts prostate biopsy outcomes. Clin Cancer Res 20: 2289–2299. doi: 10.1158/1078-0432.CCR-13-3085 PMID: 24789033
22. Kristal AR, Till C, Song X, Tangen CM, Goodman PJ, et al. (2014) Plasma vitamin D and prostate cancer risk: results from the Selenium and Vitamin E Cancer Prevention Trial. Cancer Epidemiol Biomarkers Prev 23: 1494–1504. doi: 10.1158/1055-9965.EPI-14-0115 PMID: 24732629
23. Corder EH, Friedman GD, Vogelman JH, Orentreich N (1995) Seasonal variation in vitamin D, vitamin D-binding protein, and dehydroepiandrosterone: risk of prostate cancer in black and white men. Cancer Epidemiol Biomarkers Prev 4: 655–659. PMID: 8547833

24. Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW (1995) Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States). Cancer Causes Control 6: 235–239. PMID: 7612803

25. Gann PH, Ma J, Hennekens CH, Hollis BW, Haddad JG, et al. (1996) Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev 5: 121–126. PMID: 8850273

26. Nomura AM, Stemmermann GN, Lee J, Kolonel LN, Chen TC, et al. (1998) Serum vitamin D metabolite levels and the subsequent development of prostate cancer (Hawaii, United States). Cancer Causes Control 9: 425–432. PMID: 9794175

27. Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P (2000) Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control 11: 847–852. PMID: 11075874

28. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E (2004) Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 15: 255–265. PMID: 15090720

29. Jacobs ET, Giuliano AR, Martinez ME, Hollis BW, Reid ME, et al. (2004) Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer. J Steroid Biochem Mol Biol 89–90: 533–537.

30. Li H, Stampfer MJ, Hollis JB, Mucci LA, Gaziano JM, et al. (2007) A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. PLoS Med 4: e103. PMID: 17388667

31. Faupel-Badger JM, Diaw L, Albanes D, Virtamo J, Woodson K, et al. (2007) Lack of association between serum levels of 25-hydroxyvitamin D and the subsequent risk of prostate cancer in Finnish men. Cancer Epidemiol Biomarkers Prev 16: 2784–2786. PMID: 18086789

32. Travis RC, Crowe FL, Allen NE, Appleby PN, Roddam AW, et al. (2009) Serum vitamin D and risk of prostate cancer in a case-control analysis nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Epidemiol 169: 1223–1232. doi: 10.1093/aje/kwp022 PMID: 19359375

33. Ordonez-Mena JM, Schottker B, Haug U, Muller H, Kohrle J, et al. (2013) Serum 25-hydroxyvitamin d and cancer risk in older adults: results from a large German prospective cohort study. Cancer Epidemiol Biomarkers Prev 22: 905–916. doi: 10.1158/1055-9965.EPI-12-1332 PMID: 23462913

34. Ahn J, Peters U, Albanes D, Purdue MP, Abnet CC, et al. (2008) Serum vitamin D concentration and prostate cancer risk: a nested case-control study. J Natl Cancer Inst 100: 796–804. doi: 10.1093/jnci/djn152 PMID: 18505967

35. Park SY, Cooney RV, Wilkens LR, Murphy SP, Henderson BE, et al. (2010) Plasma 25-hydroxyvitamin D and prostate cancer risk: the multiethnic cohort. Eur J Cancer 46: 932–936. doi: 10.1016/j.ejca.2009.12.030 PMID: 20064705

36. Barnett CM, Nielson CM, Shannon J, Chan JM, Shikany JM, et al. (2010) Serum 25-OH vitamin D levels and risk of developing prostate cancer in older men. Cancer Causes Control.

37. Brandstedt J, Almquist M, Manjer J, Malm J (2012) Vitamin D, PTH, and calcium and the risk of prostate cancer: a prospective nested case-control study. Cancer Causes Control 23: 1377–1385. doi: 10.1007/s10552-012-9948-3 PMID: 22706676

38. Albanes D, Mondul AM, Yu K, Parisi D, Horst RL, et al. (2011) Serum 25-hydroxy vitamin D and prostate cancer risk in a large nested case-control study. Cancer Epidemiol Biomarkers Prev 20: 1850–1860. doi: 10.1158/1055-9965.EPI-11-0403 PMID: 21784952

39. Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, et al. (2004) Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer 108: 104–108. PMID: 14618623

40. Schenk JM, Till CA, Tangen CM, Goodman PJ, Song X, et al. (2014) Serum 25-hydroxyvitamin d concentrations and risk of prostate cancer: results from the Prostate Cancer Prevention Trial. Cancer Epidemiol Biomarkers Prev 23: 1484–1493. doi: 10.1158/1055-9965.EPI-13-1340 PMID: 25085836

41. Michaelsson K, Baron JA, Snellman G, Gedeborg R, Byberg L, et al. (2010) Plasma vitamin D and mortality in older men: a community-based prospective cohort study. Am J Clin Nutr 92: 841–848. doi: 10.3945/ajcn.2010.29749 PMID: 20720256

42. Amrein K, Quraishi SA, Litonjua AA, Gibbons FK, Pieber TR, et al. (2014) Evidence for a U-shaped relationship between pre-hospital vitamin D status and mortality: a cohort study. J Clin Endocrinol Metab: jc20133481.

43. Chowdhury R, Kunutsor S, Vitezova A, Oliver-Williams C, Chowdhury S, et al. (2014) Vitamin D and risk of cause specific death: systematic review and meta-analysis of observational cohort and randomised intervention studies. BMJ 348: g1903. doi: 10.1136/bmj.g1903 PMID: 24690623

44. Schroeder JC, Bensen JT, Su LJ, Mishel M, Ivanova A, et al. (2006) The North Carolina-Louisiana Prostate Cancer Project (PCaP): methods and design of a multidisciplinary population-based cohort study of racial differences in prostate cancer outcomes. Prostate 66: 1162–1176. PMID: 16676364

45. Hollis BW (2008) Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr 88: 507S–510S. PMID: 18689391

46. Sucheston LE, Bensen JT, Xu Z, Singh PK, Preus L, et al. (2012) Genetic ancestry, self-reported race and ethnicity in African Americans and European Americans in the PCaP cohort. PLoS One 7: e30950. doi: 10.1371/journal.pone.0030950 PMID: 22479307

47. Yao S, Zirpoli G, Bovbjerg DH, Jandorf L, Hong CC, et al. (2012) Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. Breast Cancer Res 14: R58. PMID: 22480149

48. Gilbert R, Metcalfe C, Fraser WD, Donovan J, Hamdy F, et al. (2012) Associations of circulating 25-hydroxyvitamin D with prostate cancer diagnosis, stage and grade. Int J Cancer 131: 1187–1196. doi: 10.1002/ijc.27327 PMID: 22033893

49. Shui IM, Mucci LA, Kraft P, Tamimi RM, Lindstrom S, et al. (2012) Vitamin D-related genetic variation, plasma vitamin D, and risk of lethal prostate cancer: a prospective nested case-control study. J Natl Cancer Inst 104: 690–699. doi: 10.1093/jnci/djs189 PMID: 22499501

50. Chandler PD, Giovannucci EL, Scott JB, Bennett GG, Ng K, et al. (2014) Null Association Between Vitamin D and PSA Levels Among Black Men in a Vitamin D Supplementation Trial. Cancer Epidemiol Biomarkers Prev.

51. Chen TC, Holick MF (2003) Vitamin D and prostate cancer prevention and treatment. Trends Endocrinol Metab 14: 423–430. PMID: 14580762

52. Moreno J, Krishnan AV, Feldman D (2005) Molecular mechanisms mediating the anti-proliferative effects of Vitamin D in prostate cancer. J Steroid Biochem Mol Biol 97: 31–36. PMID: 16024246

53. Krishnan AV, Peehl DM, Feldman D (2003) Inhibition of prostate cancer growth by vitamin D: Regulation of target gene expression. J Cell Biochem 88: 363–371. PMID: 12520538

54. Johnson CS, Hershberger PA, Trump DL (2002) Vitamin D-related therapies in prostate cancer. Cancer Metastasis Rev 21: 147–158. PMID: 12465754

55. Getzenberg RH, Light BW, Lapco PE, Konety BR, Nangia AK, et al. (1997) Vitamin D inhibition of prostate adenocarcinoma growth and metastasis in the Dunning rat prostate model system. Urology 50: 999–1006. PMID: 9426741

56. Medicine Io (2011) Dietary Reference Intakes for Calcium and Vitamin D; Ross AC, Taylor CL, Yaktine AL, Valle HBD, editors: The National Academies Press.

57. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, et al. (2013) Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 369: 1991–2000. doi: 10.1056/NEJMoa1306357 PMID: 24256378

58. Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, et al. (1986) Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. J Clin Endocrinol Metab 63: 954–959. PMID: 3745408

59. Arnaud J, Constans J (1993) Affinity differences for vitamin D metabolites associated with the genetic isoforms of the human serum carrier protein (DBP). Hum Genet 92: 183–188. PMID: 8370586

60. Braun A, Bichlmaier R, Cleve H (1992) Molecular analysis of the gene for the human vitamin-D-binding protein (group-specific component): allelic differences of the common genetic GC types. Hum Genet 89: 401–406. PMID: 1352271

61. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, et al. (2008) Genetic and environmental determinants of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels in Hispanic and African Americans. J Clin Endocrinol Metab 93: 3381–3388. doi: 10.1210/jc.2007-2702 PMID: 18593774

62. Constans J, Hazout S, Garruto RM, Gajdusek DC, Spees EK (1985) Population distribution of the human vitamin D binding protein: anthropological considerations. Am J Phys Anthropol 68: 107–122. PMID: 4061596

63. Holick MF (2004) Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 80: 1678S–1688. PMID: 15585788

64. Murphy AB, Kelley B, Nyame YA, Martin IK, Smith DJ, et al. (2012) Predictors of serum vitamin D levels in African American and European American men in Chicago. Am J Mens Health 6: 420–426. doi: 10.1177/1557988312437240 PMID: 22398989

FEDERAL FINANCIAL REPORT

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1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned by Federal Agency (To report multiple grants, use FFR Attachment)	Page	of
USA Medical Research ACQ Activity/DOD	W81XWH-11-1-0568	1	1 pages

3. Recipient Organization (Name and complete address including Zip code)

UNIVERSITY OF SOUTH CAROLINA RESEARCH FOUNDATION, 901 SUMTER STREET, COLUMBIA, SC 29208

4a. DUNS Number	4b. EIN	5. Recipient Account Number or Identifying Number (To report multiple grants, use FFR Attachment)	6. Report Type	7. Basis of Accounting
11-131-0249	57-0967350	11590 FA46	☐ Quarterly ☐ Semi-Annual ☐ Annual ■ Final	☐ Cash ■ Accrual

8. Project/Grant Period From: (Month, Day, Year)	To: (Month, Day, Year)	9. Reporting Period End Date (Month, Day, Year)
09/30/2011	9/29/2015	9/29/2015

10. **Transactions**	Cumulative
(Use lines a-c for single or multiple grant reporting)	
Federal Cash (To report multiple grants, also use FFR Attachment):	
a. Cash Receipts	706,958.00
b. Cash Disbursements	699,304.95
c. Cash on Hand (line a minus b)	7,653.05
(Use lines d-o for single grant reporting)	
Federal Expenditures and Unobligated Balance:	
d. Total Federal funds authorized	706,958.00
e. Federal share of expenditures	699,304.95
f. Federal share of unliquidated obligations	
g. Total Federal share (sum of lines e and f)	699,304.95
h. Unobligated balance of Federal funds (line d minus g)	7,653.05
Recipient Share:	
i. Total recipient share required	0.00
j. Recipient share of expenditures	0.00
k. Remaining recipient share to be provided (line i minus j)	0.00
Program Income:	
l. Total Federal program income earned	0.00
m. Program income expended in accordance with the deduction alternative	0.00
n. Program income expended in accordance with the addition alternative	0.00
o. Unexpended program income (line l minus line m or line n)	0.00

	a. Type	b. Rate	c. Period From	Period To	d. Base	e. Amount Charged	f. Federal Share
11. Indirect Expense	PREDETERMINED	45.0%	09/30/11	9/29/2015	242,086.01	108,938.70	108,938.70
				g. Totals:	242,086.01	108,938.70	108,938.70

12. *Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation:*

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