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Circulating Calcium, Phosphorus, and Parathyroid Hormone and Aggressive Prostate Cancer in the North Carolina-Louisiana Prostate Cancer Project

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CIRCULATING CALCIUM, PHOSPHORUS, AND PARATHYROID HORMONE AND AGGRESSIVE PROSTATE CANCER IN THE NORTH CAROLINA-LOUISIANA PROSTATE CANCER PROJECT

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ABSTRACT

Prostate cancer is the most commonly diagnosed cancer among men in the United States. Despite improvements in screening, diagnostic methods, and treatment, African-American men have an increased risk of total and aggressive prostate cancer. In recent years, a growing body of evidence linking biomarkers of calcium and parathyroid hormone (PTH) to prostate cancer development has emerged. Circulating calcium, phosphorus, and PTH are homeostatically related. Prior studies linking prostate cancer and circulating calcium and PTH have produced inconclusive results, and no studies have examined the association between serum phosphorus and prostate cancer. The present study examined the relationship between circulating calcium, phosphorus, and PTH and aggressive prostate cancer in a sample of African-American and European American men in the North Carolina-Louisiana Prostate Cancer Project (PCaP). Serum calcium was associated with a modest decreased odds of aggressive prostate cancer though the confidence interval lacked precision. There was suggestion of effect modification by overweight/obesity status, though these results were not statistically significant. High serum phosphorus was associated with an increased odds of aggressive prostate cancer across various categorization approaches. There was a slight inverse association between aggressive prostate cancer and PTH, and this association was modified by number of comorbidities. The joint effects of circulating calcium, phosphorus, PTH, and 25(OH)D were observed. Across categories of low and high analytes, serum phosphorus was associated with increased odds of aggressive prostate cancer. Higher serum phosphorus

may reflect high dietary intake of phosphorus and a less healthy diet overall. A previous study found a positive association between higher dietary phosphorus intake and aggressive prostate cancer risk, corroborating our results. Future studies are warranted to more thoroughly examine the role of serum phosphorus in prostate cancer progression.

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LIST OF ABBREVIATIONS

AA.....African American

Ca.....Calcium

CaSR.....Calcium Sensing Receptor

CCI.....Charlson Comorbidity Index

DRE.....Digital Rectal Exam

EA..... European American

FGF23.....Fibroblastic Growth Factor 23

P.....Phosphorus

PSA.....Prostate Specific Antigen

PTH.....Parathyroid Hormone

PTH-rp.....Parathyroid Hormone-Related Protein

RCT..... Randomized Controlled Trial

25(OH)D.....25-hydroxyvitamin D

CHAPTER 1

INTRODUCTION

1. Statement of the problem

General

Prostate cancer is the second most common cancer diagnosed in men globally, following lung cancer (Bray et al., 2018). In 2018, there were 1,276,106 new prostate cancer diagnoses and 3.8% of cancer deaths were due to prostate cancer (Bray et al., 2018). In the United States, prostate cancer is a leading cause of malignancy among men (Siegel et al., 2019). An estimated 191,930 new cases of prostate cancer will be diagnosed in 2020 and 1 in every 9 men are expected to develop invasive disease in their lifetime (Siegel et al., 2020). The mortality rate for prostate cancer has declined by 53% within the past two decades (Siegel et al., 2018), which could be attributed to advances in early detection and treatment of prostate cancer (Kelly et al., 2017). Recent trends show that prostate cancer was the leading cause of death among men 80 years and older and the third leading cause of death among men 60 to 79 years (Siegel et al., 2019). Between 2010 and 2014, the incidence rates of prostate cancer were 107.0 (per 100,000) among European-American (EA) men, 186.8 (per 100,000) among African-American (AA) men, 58.4 (per 100,000) among Asians/Pacific Islanders, 78.3 (per 100,000) among American Indian/Alaskan Natives, and 97.0 (per 100,000) among Hispanic men (Siegel et al., 2018).

Risk Factors

Age and family history are established risk factors for prostate cancer (Grossman et al., 2018). In addition, certain racial/ethnic groups are at increased risk of prostate cancer incidence and mortality. For decades, AA men have had substantially higher incidence rates of prostate cancer compared to EA men (Odedina et al., 2009). After 55, the risk of prostate cancer substantially increases and peaks between the ages of 70-74 (Gann, 2002). Men with a family history of prostate cancer are 2-3 times as likely to develop prostate cancer as compared to men with no family history of prostate cancer and the risk tends to be stronger with increasing number of family members with prostate cancer (Kicinski et al., 2011). Emerging evidence from epidemiologic studies suggests that modifiable risk factors like obesity and smoking appear to increase risk of prostate cancer incidence, progression, and mortality, but results are only suggestive (Kenfield et al., 2011; Huncharek et al., 2011; Vidal et al., 2014; Lavalette et al., 2018).

Screening

There are two common screening methods for prostate cancer: digital rectal exam (DRE) and prostate specific antigen (PSA). The DRE is a method by which a physician inserts a finger into the rectum to examine the prostate for abnormalities. Prior to the introduction of PSA, which measures the level of PSA in the blood, the DRE was the only screening tool for prostate cancer. Between the late 1980s and early 1990s, the incidence of prostate cancer surged by 82% and a contribution to this trend is the introduction of the PSA screening test (Potosky et al., 1995). Approximately 60% of the prostate cancers detected by PSA screening are overdiagnoses, which means the disease

would not become clinically significant (Sandhu & Andriole, 2012; Welch & Black, 2009).

Due to issues with overdiagnosis and overtreatment in prostate cancer cases that would otherwise remain asymptomatic, the United States Preventive Services Task Force (USPSTF) recommended against routine prostate cancer screening among men of all ages in 2012 (Moyer et al., 2012). Following these recommendations, the frequency of PSA screening and prostate cancer incidence declined (Fleshner et al., Howard et al., 2013; Patel et al.,2018). Concerns about a decline in PSA testing and prostate cancer incidence being misinterpreted and masking increased rates of aggressive prostate cancer arose, specifically among high risk men (Negoita et al., 2018; Ahlering et al., 2019). In an update in 2018, the USPSTF suggested that the decision to undergo PSA screening in men ages 55-69 be at the discretion of the patient and physician, but the recommendation against screening for men over the age of 70 remained unchanged (Grossman et al., 2018).

Treatment and Survival

The primary benefits of prostate cancer screening are early detection and treatment, resulting in more favorable outcomes. Presently, three commonly utilized treatments for localized prostate cancer exist: radical prostatectomy, radiation therapy, and active surveillance (Grosseman et al., 2018). The 10-year survival rate for localized prostate cancer, regardless of treatment type, is estimated to be 99% (Hamdy et al., 2016). For men with advanced prostate cancer, treatment includes hormonal therapy, radiation, chemotherapy, immunotherapy, and bone targeted therapy, and new treatments

are evolving. Despite the treatments available, the 5-year survival rate for advanced/metastatic prostate cancer is approximately 30% (Siegel et al., 2019).

Prostate Cancer in North Carolina and Louisiana

In 2020, there will be an estimated 2,970 and 7,200 incident cases of prostate cancer in Louisiana and North Carolina, respectively (Siegel et al., 2020). Moreover, approximately 450 and 1,010 men in Louisiana and North Carolina, respectively, are expected to die from the disease (Siegel et al., 2020). The prostate cancer mortality rates in North Carolina (19.9 per 100,000) and Louisiana (20.8 per 100,000) are higher than the national rate (19.1 per 100,000) (Siegel et al., 2020). The incidence rate of prostate cancer among AA men in Louisiana (192 cases per 100,000) is higher than the incidence rate among AAs nationally (179 per 100,000) and in North Carolina (183 cases per 100,000) (DeSantis et al., 2019). Furthermore, the prostate cancer specific mortality rates in the US (39.8 per 100,000) and North Carolina (39.6 per 100,000) among AA are slightly higher than that of Louisiana (37.0 per 100,000) (Desantis et al., 2019).

Racial Disparities

On average, AA men are diagnosed with prostate cancer at a younger age (66 years) than EA (69 years) (Howlader et al., 2018). Between 2011 and 2015, the incidence rate of prostate cancer for AA men was approximately 76% higher than that of EA (Desantis et al., 2019). AA men tend to present with more aggressive and advanced stage disease at diagnosis (Powell et al., 2010, Tsodikov et al., 2017; Pietro et al., 2016). Although the prostate cancer mortality rate among men of all races has declined in the last 20 years (Desantis et al., 2019), racial disparities in mortality rates among AA persist. Between 2011 and 2016, the age adjusted mortality rate for AA (179.2 per 100,000) was

significantly higher than that of EA (101.7 per 100,000) (Siegel et al., 2019). The lifetime probability of AA men dying from invasive prostate cancer has recently been reported to be 4%, which is nearly double that of EA men (2.2%) (Desantis et al., 2019). Moreover, studies have found that AA had significantly higher serum PSA levels than EA men, irrespective of demographic and cancer- specific characteristics (Mavropoulos et al., 2007; Leapman et al., 2016). The associations between risk factors that appear to increase risk of prostate cancer and poorer outcomes tend to be more pronounced among AA men (Murphy et al., 2013; Barrington et al., 2015).

Socioeconomic status (SES) influences frequency of screening, access to adequate care, preventive health services, and health behaviors (Pampel et al., 2010; Singh & Jemal., 2017). Socioeconomic factors that differentially impact AA contribute, at least partially, to the racial disparities observed in prostate cancer outcomes (O'keefe et al., 2015; Taksler et al., 2012). Evidence shows that there is a link between SES and prostate cancer mortality, but among AA men the association is stronger than that of EA men (Siegel et al., 2019; Albano et al., 2007; Jemal et al., 2005). Moreover, in a study of low risk prostate cancer patients, after adjusting for socioeconomic parameters and clinicopathological features, AA race was the only predictor of treatment (Albern et al., 2013).

Alongside social factors, genetic and biological characteristics among tumors of AA men with prostate cancer are substantially different than those of EA and could have implications for the disparities in prostate cancer outcomes observed (Aizer et al., 2014). Genetic mutations and biomarkers associated with aggressive prostate cancer tend to be overexpressed or more prevalent in AA men as compared to EA men (Khani et al., 2014;

Kim et al., 2011; Kwabi-Addo et al., 2010). Several studies suggest that AA men are more likely to present with higher Gleason grade tumors at diagnosis as compared to EA men (Powell et al., 2010; Iremashvili et al., 2012).

Calcium, Phosphorus, Parathyroid Hormone (PTH) and Prostate Cancer

Various studies have examined the role of dietary calcium and prostate cancer outcomes but have produced inconsistent results. Several studies have reported that high intakes of dietary calcium are associated with an increased risk of prostate cancer (Mitrou et al., 2007; Kesse et al., 2005) and aggressive disease (Batai et al., 2017; Wilson et al., 2015), but other studies report no association with prostate cancer and/or progression (Tavani et al., 2005; Chan et al., 2000). Research examining the association between prostate cancer and phosphorus intake is not well-studied, and existing studies have produced inconclusive findings. Results from a prospective cohort study on the association between calcium and phosphorus intake and prostate cancer suggest that high phosphorus intake is independently associated with an increase in total, lethal, and aggressive prostate cancer risk (Wilson et al., 2015), whereas other studies produced null results (Chan et al., 2000; Tavani et al., 2005).

Both phosphorus and calcium are found in similar food sources, with phosphorus being more widely distributed in the food supply than calcium. Serum calcium is under tight homeostatic control and therefore, not a biomarker of dietary calcium. Because of the wide distribution of phosphorus in food sources, it is hypothesized that dietary assessments underestimate phosphorus intake. This could contribute to the inconclusive findings on the accuracy of serum phosphorus as a marker of dietary phosphorus (Moore et al., 2015; Uribarri & Calvo, 2014; Calvo et al., 2014).

Under normal homeostatic conditions, serum parathyroid (PTH) is primarily responsible for maintaining calcium and phosphorus homeostasis. As serum calcium decreases, PTH is stimulated and produces the active form of vitamin D (1,25(OH)2D), which works to increase serum calcium concentration (Goltzman et al., 2018). When serum calcium is elevated, PTH secretion is reduced (Goltzman et al., 2018). Conversely, increases in serum phosphorus stimulate PTH secretion (Vorland et al., 2017). In addition to effects on 1,25(OH)2D levels, PTH indirectly stimulates production of fibroblast growth factor 23 (FGF-23), a hormone that functions primarily to reduce serum phosphate levels (Vorland et al., 2017).

Results from in vitro studies suggest that PTH related protein (PTHrP) is a mediator in the development of prostate cancer and metastases in bone (Laio et al., 2008). In a study conducted by Aydin et al. (2014), PTH and PSA were positively correlated among men with and without prostate cancer. Furthermore, calcium sensing receptors (CaSRs) are present on both normal and cancerous prostate cancer cells, and these receptors are expressed at higher levels in metastatic prostate cancer tissues derived from bone (Feng et al., 2014). The homeostatic mechanisms behind serum calcium, serum phosphorus, and PTH are quite complex and evidence on the associations between these variables and prostate cancer outcomes is either limited or has produced mixed findings as described in more detail in Chapter 2.

Specific Aims

The purpose of this study was to examine the association between circulating calcium, phosphorus, and PTH and aggressive prostate cancer. The Specific Aims were as follows:

1. To evaluate the associations between circulating calcium, phosphorus, PTH and prostate cancer aggressiveness independently. We hypothesized that circulating calcium, phosphorus, and PTH would be positively associated with aggressive prostate cancer.

2. To examine the joint associations of circulating calcium, phosphorus and PTH. To compare our results with previous studies, we included plasma vitamin D in the joint analyses; the main effects of plasma vitamin D metabolites have already been published in our dataset (Steck et al., 2015; Ramakrishnan et al., 2019).

3. To examine whether associations between circulating calcium, phosphorus, and PTH and aggressive prostate cancer are modified by body mass index (BMI), comorbidities, or smoking status.

Significance of Research

Prostate cancer is the leading cause of malignancy among men in the United States (Siegel et al., 2019). Only three factors have been consistently associated with risk of prostate cancer: age, race/ethnicity, and family history. In recent years, a growing body of evidence linking biomarkers, lifestyle, and sociodemographic factors to prostate cancer development and progression has emerged. To date, several studies have examined the association between serum calcium and prostate cancer (Salem et al., 2013; Schwartz & Skinner, 2012; Van Hemelrijck et al., 2012; Jackson et al., 2015; Skinner & Schwartz, 2008; Halthur et al., 2009). The findings from these studies are inconsistent, as some report no significant increase in risk and others report inverse and positive associations. Furthermore, one study evaluated the impact of PTH and serum calcium on prostate

cancer risk (Brandstedt et al., 2016). In the main effects model, no statistically significant associations were observed between circulating calcium or PTH and risk of prostate cancer. However, positive and negative associations were observed within strata of calcium and PTH.

Current literature is limited by small sample size, lack of information on tumor stage/grade, insufficient covariates, and/or racial homogeneity. No studies have assessed the association between serum phosphorus and prostate cancer outcomes. Calcium, phosphorus, and PTH are homeostatically related, but to our knowledge, no studies have evaluated their independent and/or joint effects in relation to prostate cancer aggressiveness. This study examined the independent effects of circulating calcium, phosphorus, and PTH, along with their joint effects, on prostate cancer aggressiveness in a representative sample of EA and AA men.

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

1. Primary Hypothesis – Circulating Calcium, Phosphorus, PTH and Prostate Cancer

There is biologic plausibility for a role of calcium, phosphorus and PTH in prostate cancer development and progression. In this section, we describe the potential biologic mechanisms and summarize the epidemiologic studies that have examined associations between calcium, phosphorus, and PTH and prostate cancer in humans (Table 2.1).

Calcium

Calcium is the most abundant mineral in the body and 99% of it is stored in the bone (Beto, 2015). It plays a role in vascular functions, muscle contraction, hormone secretion, and nerve stimulation. Dairy products like milk, yogurt, and cheese, and grains are major sources of dietary calcium. In the United Sates, 72% of calcium intake is derived from dairy products (Institute of Medicine, 2011). It is estimated that average intake of calcium ranges from 918 to 1,286 mg/day (Bailey et al., 2010). The recommended daily allowance (RDA) of calcium in adults is 1,000-1,200 mg (Ross et al., 2011). Because serum calcium is tightly regulated, it is not an adequate marker of dietary calcium. Approximately 1% of calcium is found in serum (Beto, 2015) and consists of free ions, protein bound complexes, and ionic complexes (Robertson et al., 1979). The

normal range for total serum calcium is 8.5 to 10.2 mg/dL (Ross et al., 2011). Ionized calcium is the biologically active fraction of serum calcium (Cooper & Gittoes, 2008). Serum calcium homeostasis is tightly regulated for optimal cellular functions (Ladenson, 1973) primarily by PTH and vitamin D acting on the bone, kidney, and GI tract via a negative feedback loop (Cooper & Gittoes, 2008). When calcium concentrations are lower than normal, PTH produces the active form of vitamin D (1,25(OH)2D) in the kidney, which stimulates osteoclasts to break down bone and enhances calcium absorption in the GI tract (Bushinsky & Monk, 1998). When calcium concentrations are elevated, PTH secretion is inhibited and production of vitamin D is reduced (Goltzman et al., 2018). Calcitonin, which acts as an antagonist to PTH, inhibits osteoclasts and decreases the reabsorption of calcium in the kidney, thus restoring serum calcium concentrations back into normal range (Goltzman et al., 2018).

Phosphorus

Phosphorus is the second most abundant mineral in the body, with most of it present in the bone and teeth. It plays a vital role in generation of energy, cell structure, and bone calcification (Takeda, 2004). Since phosphorus is naturally occurring in a variety of foods commonly consumed like meats, beans, nuts and seeds, dairy foods, grains, and food additives, phosphorus deficiencies are not very common (Vorland et al., 2017). The RDA for phosphorus in adult men is 700 mg/day, but intake of phosphorus in the United States tends to exceed the recommended amount (Calvo et al, 2014). Using NHANES data from 2001-2014, McClure et al. (2017) found the average total dietary phosphorus intake for men was 1615 mg/day. Natural sources of phosphorus absorb at a slower rate than phosphorus added to foods during preparation or processing (Calvo,

2014). Increased use of inorganic phosphate in food additives, along with increased consumption of processed foods in the United States, make it difficult to adequately capture phosphorous intake with food and nutrient databases (Calvo, 2014). Gutierrez et al. (2011) suggest that conventional diet assessment methods underestimate phosphorus intake.

Serum phosphate levels vary based on age, but the normal range for adults is 2.5 to 4.5 mg/dL (Penido, 2012). In comparison with calcium and PTH, the homeostatic mechanisms of serum phosphorus are not as controlled (Lederer, 2014). Phosphorus absorption primarily occurs in the intestines and is excreted via urine in the renal system (Chang & Anderson, 2017). Moreover, regulators of phosphate homeostasis include PTH, 1,25(OH)2D, FGF-23, and dietary phosphate intake and absorption (Lederer, 2014; Penido, 2012). Although PTH increases serum calcium levels, it inhibits phosphate reabsorption in the kidney, thus decreasing phosphorus levels in serum (Vorland et al., 2017). 1,25(OH)2D, on the other hand, increases phosphorus absorption in the intestine. FGF23, secreted though osteoclastic activity, inhibits 1,25(OH)2D production and works to decrease serum phosphorus (Vorland et al., 2017).

PTH

The parathyroid gland is comprised of four small glands that produce PTH. PTH's primary function is to maintain calcium homeostasis, but it has secondary effects on phosphorus homeostasis. Attached to the cells of the parathyroid gland are CaSRs, which respond to changes in blood calcium levels and stimulate release when calcium levels are lower than normal or inhibit PTH when calcium levels are elevated (Goltzman et al., 2018). PTH regulates calcium and phosphorus homeostasis in three organ systems: bone,

gastrointestinal tract, and kidney (Goltzman et al., 2018). When serum calcium levels are below normal, PTH stimulates osteoclasts to breakdown bone, which releases calcium and phosphorus into the bloodstream (Quarles, 2008). In the kidney, PTH activates $1,25(OH)_2D$, which increases absorption of calcium and phosphorus in the intestine (Goltzman et al., 2018; Vorland et al., 2017). PTH increases excretion of phosphorus in the kidney, reducing serum phosphorus concentrations (Quarles, 2008). Under healthy homeostatic control, elevated calcium levels will inhibit the release of more PTH (Goltzman et al, 2018; Quarles et al., 2008).

Biological Mechanisms and Implications for Prostate Cancer

The association between dietary calcium intake and risk of prostate cancer has been well-studied, and studies generally suggest high calcium intake increases prostate cancer risk (Aune et al., 2015; Rahmati et al., 2018; Gao et al., 2005). High calcium intake has been associated with lower concentrations of circulating vitamin D (Giovannucci et al., 2006), and vitamin D is hypothesized to decrease risk of prostate cancer, though not all studies are supportive (Song et al., 2018). Evidence on the association between phosphorus intake and prostate cancer is inconsistent and limited. Studies have found that high phosphorus intake is associated with increased risk of advanced disease (Giovannucci et al., 2006; Wilson et al., 2015), while others found a non-significant increase in risk of prostate cancer (Mitrou et al., 2007) or no association (Tseng et al., 2005).

It is hypothesized that high phosphorus intake stimulates PTH, which is thought to aid in the development of prostate cancer cells (Ritchie et al., 1997). PTH related proteins (PTH-rp) are substantially increased in prostate cancer and expressed in over

90% of specimens of localized prostate cancer (Bryden et al., 2002). The preferential target organ for prostate cancer metastases is the bone (Davila et al., 2015). It is hypothesized that PTH-rp, which is involved in bone formation, promotes prostate cancer tumor development and may be a mediator in turning cells to bone metastases (Liao et al., 2008). In stratified analyses, Brandstedt et al. (2016) observed that in the presence of higher serum calcium concentrations, the risk for prostate cancer aggressiveness significantly increased among those in the highest quartile of serum PTH.

Both normal and cancerous prostate cells have calcium receptors (Feng et al., 2014). Several studies have reported no association between the risk of prostate cancer and high serum calcium concentration (Halthur et al., 2009; Skinner & Schwartz, 2008). Though no association between high calcium concentration and prostate cancer risk was found, men with high levels of serum calcium were at an increased risk of fatal prostate cancer (Skinner & Schwartz, 2008; Schwartz & Skinner, 2012). Results from a study in Caribbean men of African ancestry showed a positive association between serum calcium and prostate cancer risk (Jackson et al., 2015). In contrast, results from studies conducted by Halthur et al. (2009) and Salem et al. (2013) suggest that high serum calcium concentrations in specific groups of men may be a protective factor in prostate cancer risk. Interestingly, Bradstedt et al. (2016) observed an inverse association between serum calcium (in the highest quartile) and aggressive prostate cancer in the presence of low concentrations of PTH. On the contrary, some studies report low serum calcium tends to be more prevalent among patients with advanced and metastatic prostate cancer (Sarwar et al., 2017; Raskin et al., 1973). Potential hypothesis for this specific trend is excess secretion of calcium deposition into the bone during osteoblastic

metastases, which decreases serum calcium concentrations (Sarwar et al., 2017; Skinner & Schwartz, 2008). Thus, it is possible that low serum calcium is an effect of metastases or advanced disease rather than a cause.

Potential Confounders

Age

The risk of prostate cancer increases as men age, and recent evidence suggests that older men have an increased risk of aggressive prostate cancer specifically (Brassell et al., 2011, Muralidhar et al., 2015). The average age at prostate cancer diagnosis is 66 years (Rawla, 2019). Using data from nationally representative cancer registries, Siegel et al. (2019) found the probability of being diagnosed with prostate cancer is highest among men 70 and older and lowest among men 49 and younger. In relation to race, AA men are more likely to develop prostate cancer at a younger age as compared to EA men (Nettey et al., 2018). The increased risk could be attributed to chronic inflammatory responses induced by changes in genetic and physiological features that accompany advancing age (Nguyen et al., 2014; Vaidyanathan et al., 2016).

Race

The prostate cancer incidence and mortality rates for AA men are more than twice that of EA men (O'Keefe et al., 2015). Racial differences in prostate cancer incidence, aggressiveness, and survival are influenced by socioeconomic and environmental factors impacting AAs, but these factors do not completely explain the disparities observed. Nonetheless, research suggests that there are genetic ancestry related factors that influence clinicopathological features of prostate cancer (Yamoah et al., 2015). Several studies have found that those with increasing West African ancestry tend to have an

increased risk of prostate cancer and/or aggressive disease (Grizzle et al., 2019; Giri et al., 2009; Conti et al., 2017).

SES

Socioeconomic status (SES) is a leading indicator of health status. In the United States, it is estimated that nearly 34% of cancer deaths between the ages of 25 to 74 would be prevented if socioeconomic disparities did not exist (Siegel et al., 2018). Several studies have found that lower SES is associated with increased risk of late stage and advanced prostate cancer diagnosis (Byers et al., 2008; Clegg et al., 2009). Men with a high SES are more likely to be diagnosed with but have lower mortality rates of prostate cancer (Cheng et al., 2009; Singh et al., 2017). A hypothesis between these observed trends is that men with a higher SES tend to have more access to medical facilities and screening to be diagnosed, and access to treatment they can afford (Rundle et al., 2013). In contrast, men with lower SES are more likely to be uninsured and face inequities regarding access to adequate medical care, screening, and treatment (Singh et al., 2011).

BMI

In the United States, 71% of men are overweight or obese (Odgen et al., 2014). In 2012, it was estimated that approximately 4% of incident cases of cancer in men in the United States were attributed to overweight or obesity (Arnold et al., 2015). Results from a meta-analysis of prospective studies suggest a direct association between risk of advanced prostate cancer and BMI (Discacciati et al., 2012). Moreover, Vidal et al. (2014) observed that although obesity was not associated with risk of overall prostate cancer, it was significantly associated with an increased risk of aggressive disease.

Excess body fat in men tends to be accompanied by higher insulin levels, leptin concentrations and insulin-like-growth-factor-1, which have all been associated with progression of prostate cancer (Mistry et al., 2007). Additionally, obesity promotes systemic inflammation, which is hypothesized to play a role in prostate cancer aggressiveness (Gurel et al., 2014; Stark et al., 2015).

Family history

Family history is another well-established risk factor for prostate cancer. Men with a first degree relative with prostate cancer have double the risk of developing prostate cancer, and this trend is consistent across racial and ethnic groups (Whittemore et al., 1995; Barber et al., 2018). Furthermore, the risk of prostate cancer increases with the number of relatives affected (Chen et al., 2008). Family history of prostate cancer also contributes to risk of aggressive and non-aggressive prostate cancer by nearly two-fold (Chen et al., 2008) and lethal prostate cancer by 72% (Barber et al., 2018). Family history encompasses both genetic predispositions to prostate cancer and shared lifestyle factors. Results from a twin study suggest that the heritability of prostate cancer is approximately 58% (Hjelmborg et al., 2014).

Screening History

There are two commonly used screening tools for prostate cancer - digital rectal exam (DRE) and prostate specific antigen (PSA). The DRE allows a physician to examine the prostate for abnormalities and is often used in combination with the PSA. The DRE has been reported to have low sensitivity in primary care settings (Naji et al., 2018) and only fair reproducibility among urologists, (Smith & Catalona, 1995). Furthermore, results from a study conducted by Okotie et al. (2017) found that prostate

cancers detected by abnormal DRE and PSA tests were more likely to be clinically aggressive as compared to prostate cancers detected by either test alone (Okotie et al., 2007).

High PSA levels are associated with prostate cancer risk, but could be caused by other benign diseases of the prostate (Eastham, 2017). When the PSA screening test was approved for prostate cancer detection in the 1990's, the incidence rate of prostate cancer surged (Potasky, 1995). Consequently, in 2012, the USPSTF recommended against routine PSA screenings for diagnosis due to a substantial increase in diagnosis of prostate cancer cases that would either not progress or remain asymptomatic, (Moyer, 2012). Following these recommendations, a dramatic decrease in PSA testing was observed, raising concerns that this trend could be accompanied by an increase in late stage diagnosis and poorer survival (Patel, 2018; Ahlering, 2019). As an update to the 2012 recommendations, in 2018 the USPSTF suggested that the decision to get PSA screening among men 55 to 69 years old should be influenced by factors like race/ethnicity, family history, and individualized values (Grossman et al., 2018). However, it remained a recommendation that men older than 70 not be screened.

Vitamin D

Vitamin D has been found to mediate biological responses that aid in cancer prevention, cell death, and inhibit the growth of tumors (Chakraborti, 2011). Though vitamin D has been studied for its potential beneficial effects for various cancers (McCullough et al., 2019; Guo et al., 2018), evidence on the influence of vitamin D on prostate cancer is inconsistent. In a study conducted by Albanes et a (2011), men with higher serum concentrations of vitamin D were at an elevated risk of prostate cancer. In

the North Carolina-Louisiana Prostate Cancer Project (PCaP), higher serum 25 hydroxy-vitamin D [25(OH)D] concentrations were associated with increased odds of aggressive prostate cancer in AA men, but had no association in EA men (Steck et al., 2015). In a different study, however, Ahn et al. (2008) reported no association between prostate cancer risk and vitamin D concentration. In contrast, several studies report that vitamin D deficiency increases risk of aggressive prostate cancer (Gilbert et al., 2012; Shui et al., 2012; Nelson et al., 2017). After observing a strong inverse association between tumor vitamin D receptors (VDR) expression and risk of lethal prostate cancer, Hendrickson et al. (2011) suggest that expression of VDR is a predictive marker of prostate cancer development via a biological pathway.

NSAIDs

Chronic inflammation is thought to play a role in prostate cancer development (De Marzo, 2007) and has been associated with high grade, aggressive disease (Gurel et al., 2014; Klink et al., 2013). Nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin inhibit cyclooxygenase-2, which are enzymes that promote inflammation and are independently associated with prostate cancer (Partin, 2001; Cheng, 2007). The lines of evidence on NSAID use and prostate cancer are inconsistent. While some studies suggest an inverse association between NSAID use and prostate cancer incidence and aggressiveness (Vidal et al., 2015; Doat et al., 2017), evidence from other studies suggest no association (Brasky et al., 2010).

Charlson Comorbidity Index

The Charlson Comorbidity Index (CCI) (Charlson et al., 1987) was developed as a weighted index that measures an individual's burden of morbidities and uses this

information to predict mortality. Comorbidities are common among people diagnosed with cancer, with approximately 31% of prostate cancer cases reporting comorbidity (Edwards et al., 2014). Moreover, the CCI is commonly used among clinicians and patients to help guide prostate cancer prognosis and treatment decisions (Berglund et al., 2011; Albertsen et al., 2011). Higher comorbidity scores tend to be associated with poorer disease prognosis and survival, with some suggesting this is due to prostate cancer (Edwards et al., 2014) and others competing risks (Rajan et al., 2017).

Alcohol Intake

The association between alcohol consumption and risk of prostate cancer is inconclusive. A meta-analysis reports increased risk of prostate cancer among all levels of alcohol consumption with a dose-response relationship (Zhao et al., 2016). However, results from a Mendelian randomization study suggest that alcohol consumption does not influence prostate cancer risk, but it may influence disease progression (Brunner et al., 2017). Comparably, Michael et al. (2018) found that although current alcohol use was not associated with prostate cancer risk, high lifetime intake of alcohol was associated with increased risk of aggressive disease at diagnosis. Inconsistencies in associations could be explained by issues with recall bias in case-control studies, measurement error due to self-report of alcohol intake, and overlapping categories of current drinkers versus former drinkers and lifetime intake versus current intake (Michael et al., 2018, Zhao et al., 2016).

Smoking Status

Smoking is a source of established carcinogens (Hecht, 2006) and has been associated with an increased risk of various types of cancer (Bosetti et al., 2012; Freedman et al., 2011; Okeeffe et al., 2018). However, the association between prostate

cancer and smoking is inconclusive. Results from a pooled analysis of 24 cohort studies found that when stratified by amount smoked, current smokers had an increased risk of incident prostate cancer (Huncharek, 2010). In contrast, a prospective cohort study reported that current smokers were less likely to be diagnosed with prostate cancer (Watters et al., 2009). Ho et al (2014) found that although current smokers had an increased risk of aggressive prostate cancer (OR:1.44, 95% CI:1.04, 2.00), current smoking was not associated with risk of low-grade or total prostate cancer. Regarding race, Murphy et al. (2014) found an association between heavy smoking in AA men and odds of aggressive prostate cancer and prostate cancer risk, but no association was observed among EA men.

Table 2.1: Summary table of studies that examined the associations between calcium, phosphorus, PTH, and prostate cancer outcomes								
Lead Author, Yr	**Study Design, Number of Subjects, Location**	**Outcome(s)**	**Exposures**			**Confounders**	**Effect Modifiers**	**Results**
			Ca	**P**	**PTH**			
Jackson et al., 2015	Case control, 224 cases, 248 controls, Jamaica	Prostate cancer	Yes (dietary and serum)	No	No	BMI, education, family history, physical activity, smoking, supplement use; 25(OH)D, phosphorus, and serum calcium as appropriate	Age, BMI, family history	[OR, (95% CI)] T3 vs T1 Dietary calcium: 0.74 (0.36-1.53) Serum calcium: 1.35 (0.80-2.29)
Schwartz et al., 2012	Cohort, 6707, USA	Fatal prostate cancer	Yes (serum ionized and total)	No	No	Age, BMI, serum albumin, serum 25(OH)D	None	[RH, (95% CI)] Ionized calcium: Deaths 0-8 years T3 vs T1

								4.46 (0.89-22.36) Continuous 1.73 (1.13-2.63) Deaths 8+ years T3 vs T1 1.01 (0.23-4.43) Continuous 0.82 (0.35-1.95) Total calcium: Deaths 0-8 years T3 vs T1 1.76 (0.46-6.78) Continuous 1.49 (1.04-2.14) Deaths 8+ years T3 vs T1 1.08 (0.26-4.41) Continuous

								0.75 (0.57-0.99)
Skinner et al., 2008	Cohort 2814, USA	Incident and fatal prostate cancer	Yes (serum)	No	No	Age, BMI, race, family history	None	[RH, (95% CI)] T3 vs T1 Incidence: 1.31 (0.77-2.20) Mortality: 2.68 (0.94-7.64)
Salem et al., 2013	Case control, 194 cases, 317 controls, Iran,	Risk of prostate cancer	Yes (serum)	No	No	Age, BMI, occupation, education level, smoking alcohol, marital status, family history, sex hormones	None	[OR, (95% CI)] T3 vs T1 Total calcium: 0.27 (0.12-0.59) Corrected calcium: 0.25 (0.10-0.58)
Halthur et al., 2009	Cohort, 22391, Sweden	Risk of prostate cancer	Yes (serum)	No	No	Age, smoking status, BMI, marital status, socioeconomic index,	BMI and age	[HR, (95% CI)] Q4 vs Q1 0.94 (0.81-1.08)

						alcohol consumption		
Van Hemelrijck et al., 2012	Cohort, 196022, Sweden	Incident and fatal prostate cancer	Yes (serum)	No	No	SES, albumin, Charlson comorbidity index	Age and overweight	[HR, (95% CI)] Incident: Q4 vs Q1 Total: 0.92 (0.85-0.99) Albumin corrected: 0.91 (0.85-0.98) Aggressive: Q4 vs Q1 Total: 1.10 (0.85-1.41) Albumin corrected: 1.14 (0.89-1.45) Mortality Q4 vs Q1 Total: 0.95 (0.82-1.28) Albumin corrected:

								1.02 (0.82-1.26)
Brandstedt et al., 2016	Nested case control, 943, Sweden	Tumor aggressiveness	Yes (serum)	No	Yes	Age, screening month and year, BMI, education level, alcohol consumption, smoking status	Vitamin D, PTH, calcium	[OR, (95% CI)] Q4 vs Q1 Non-Aggressive PTH: 0.75 (0.56-1.01) Calcium: 1.11 (0.82-1.50) Aggressive PTH: 0.87 (0.59-1.30) Calcium: 1.00 (0.68-1.47)
Wilson et al., 2015	Cohort, 47885, USA	Prostate cancer risk and aggressiveness	Yes (dietary)	Yes (dietary)	No	Age, calendar time, race, height, BMI, at age 21, current BMI, vigorous physical activity, smoking,	None	[RR, (95% CI)] Calcium: High vs ref All cancer 1.16 (0.95-1.43) High grade cancer:

						diabetes, family history, intakes of tomato sauce, α-linolenic acid, supplemental vitamin E, alcohol, energy intake, multivitamin use, history of PSA, dairy, animal protein, phosphorus, calcium		1.51 (.088-2.59) Low grade cancer: 0.76 (0.52-1.11) Phosphorus: Q5 vs Q1 All cancer: 1.13 (1.00-1.27) High grade cancer: 1.51 (1.06-2.17) Low grade cancer: 1.03 (0.85-1.24)
Chan et al., 2000	RCT, 27111, Finland	Prostate cancer risk	Yes (dietary)	Yes (dietary)	No	Intervention group, education, age, BMI, energy, number of years as a smoker	Calcium and phosphorus	[RR, (95% CI)] Q5 vs Q1 Calcium 1.6 (0.8-3.0) Phosphorus 0.8 (0.4-1.5)
Tavani et al., 2005	Case control,	Prostate cancer risk	Yes (dietary)	Yes (dietary)	No	Age, center, education BMI, tobacco	Age, education, BMI, total	[OR, (95% CI)] Q5 vs Q1

	1294 cases, 1451 controls, Italy					smoking, physical activity, total energy, family history	energy intake, family history	Calcium: 1.18 (0.88-1.59) Phosphorus: 1.20 (0.79-1.84)
Kesse et al., 2005	RCT, 2805, France	Prostate cancer risk	Yes (dietary)	Yes (dietary)	No	Occupation, treatment group, smoking status, overall physical activity, energy from fat, energy from other sources, ethanol intake, BMI, family history	Calcium and Phosphorus	[RR, (95% CI)] Total calcium: 2.43 (1.05-5.62) Phosphorus: 1.83 (0.89-3.73)

CHAPTER 3
RESEARCH METHODS

1. Background

The data from this study are derived from the North Carolina-Louisiana Prostate Cancer Project (PCaP), a population-based study consisting only of prostate cancer cases, designed to examine racial differences in prostate cancer aggressiveness. PCaP consists of 1,130 AA cases and 1,128 EA cases from North Carolina and Louisiana. This research is a component of an ancillary PCaP study titled "Vitamin D and Related Genes, Race and Prostate Cancer Aggressiveness" funded by the Department of Defense (Grant # DAMD-11-1-0568; PI: S. Steck).

2. PCaP Methods

Study Population

In the paper, "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", Schroeder et al. (2006) stated: "residents of the North Carolina and Louisiana study areas with a first diagnosis of histologically confirmed adenocarcinoma of the prostate [were] eligible to participate if they [were] 40-79 years old at diagnosis, [could] complete the study interview in English, [did] not live in an institution (nursing home), [were] not cognitively impaired or in a severe debilitated physical state, and [were] not under the influence of alcohol, severely medicated, or

apparently psychotic at the time of interview. Eligible men also must self-identify as at least part African American/Black or Caucasian American/White in response to the open-ended interview question, 'what is your race?' Participants who indicated more than one group [were] asked if one best described them; if not, multiple groups [were] recorded. This classification may be used as a proxy measure of race as a biologic construct, as deemed appropriate for the individual PCaP Consortium projects. Participants [were] asked if they consider themselves to be Cajun, Creole, or Hispanic/Latino prior to the question about race, so that these ethnic groups [were] defined independent of African American or Caucasian American race/ethnicity.

North Carolina enrollment of patients from 42 counties diagnosed on or after July 1, 2004, began in September 2004. The North Carolina study area consists of 42 counties. Louisiana enrollment began in 13 parishes surrounding New Orleans in September 2004, but was discontinued because of Hurricane Katrina (August 29, 2005). This period of data collection (referred to as Louisiana Phase I) included study visits with 122 African American and 95 Caucasian American participants". A second phase of Louisiana enrollment was attained in an expanded study area (including at least eight additional parishes in southern Louisiana)."

Rapid Case Ascertainment

"Eligible North Carolina patients [were] identified by the Rapid Case Ascertainment Core Facility, a collaborative effort of the UNC-Lineberger Comprehensive Cancer Center and the North Carolina Central Cancer Registry (NCCCR). North Carolina state law mandates regular reporting of all newly diagnosed cancers (excluding non-melanoma skin cancers), and the NCCCR is authorized to release

contact and eligibility information to PCaP by the North Carolina Advisory Committee on Cancer Coordination and Control. In Louisiana, eligible patients [were] identified by the Louisiana Tumor Registry (LTR) in the School of Public Health at LSUHSC. LTR operations are mandated by Louisiana law, which directs all hospitals, pathology laboratories, health care facilities, and medical care providers to report cancer cases or provide LTR staff with access to this information. Case ascertainment field representatives abstract[ed] pathology reports, review[ed] information used to screen eligibility and ensure[d] that ascertainment in hospitals and local urology clinics [was] as complete and rapid as possible. These data [were] entered into a relational database that [was] regularly downloaded into the PCaP Subject Tracking Database" (Schroeder et al., 2006).

Randomized Recruitment

"Caucasian Americans account[ed] for a greater proportion of North Carolina patients than African Americans; therefore, a randomized recruitment procedure [was] used to generate comparable ascertainment and enrollment rates by race and state over the entire enrollment period. This sampling method improve[d] efficiency without compromising estimation of main effects and risk difference modification (additive scale 37 interactions) by race, and appropriate analysis requires only that the sampling probabilities are included as stratum-specific offset terms in some analytic models. To apply randomized recruitment, each ascertained case [was] assigned a random number and recruited only if that number [was] less than or equal to its race specific sampling probability, which is 100% for African Americans and 44% for Caucasian Americans" (Schroeder et al., 2006).

Physician Notification

"Recruitment [began] with a mailed request to the diagnosing physician for permission to contact their patient, as mandated by the North Carolina and Louisiana cancer registries. Written physician permission [was] not required; instead, physicians [were] given 3 weeks to notify PCaP if a patient should not be contacted for any reason, including ineligibility due to mental illness or impairment, nursing home residence, or severe physical debilitation. Passive physician permission, and access to patient information under a limited waiver of consent to identify and contact potential PCaP participants, was approved by the UNC and LSUHSC IRBs and DoD HSRRB" (Schroeder et al., 2006).

Enrollment

"Patients with active or passive physician consent [were] sent an introductory letter and brochure describing PCaP. One week later an experienced enrollment specialist [called] to confirm eligibility, explain the study, answer questions, solicit participation, and schedule an in home visit. Demographic and pathology report data (without personally identifiable information) [were] retained for cases who could not be contacted or who decline[d] participation, so that characteristics of non-participants could be 38 compared with those of participants to assess potential selection bias. Reasons for declining participation [were] recorded when known. Enrollment specialists [were] required to make multiple attempts to contact each potential participant. If a valid phone number could not be identified, the patient's urologist [was] asked to provide the patient with the PCaP introductory letter at his next appointment. Patients who could not be contacted within 90 days [were] sent a letter asking them to contact the study directly. If

no contact [was] made within the next 30 days, the patient [was] classified as 'unable to contact'." (Schroeder et al., 2006).

Study Visit

"Participants [were] visited in their home (or other location of their choosing) by a trained Registered Nurse. Participants [were] asked to fast for 6 hr prior to the study visit, which [was] scheduled in the morning whenever possible, and to gather all medications and supplements used in the 2 weeks prior to the visit. Study nurses [began] each visit by explaining the study and obtaining HIPAA authorization and formal written informed consent to: (1) conduct the questionnaire interview, (2) make anthropometric measurements, (3) obtain samples of adipose tissue, blood, urine, and toenails, (4) allow temporary release of paraffin embedded prostate tissue blocks, and (5) allow retrieval and abstraction of medical records. Study consent forms [were] read aloud to illiterate participants in the presence of a witness not associated with PCaP. After consent forms [were] signed, the study nurse collect[ed] biologic samples, [made] anthropometric measurements and administer[ed] the questionnaire. Study visits [took] approximately 4 hr to complete, including two 15-min breaks. Participants [were] partially compensated for their time with a payment of up to $75 for completing the entire PCaP study visit. All study visit protocols [were] documented in a manual of procedures. To ensure consistency, patient safety, and confidentiality, study nurses must be certified and periodically re-certified to conduct all aspects of the visit. Interview and biologic sample collection data [were] reviewed on an ongoing basis to identify variation among study nurses or between study sites that cannot be explained by acceptable or expected trends. In addition, project managers at each site call randomly selected study participants after

study visits [were] completed to assess nurse performance and solicit feedback" (Schroeder et al., 2006).

Anthropometric Measures

"Weight (to the nearest 0.1 kg), height, and waist and hip circumferences (in cm) [were] measured after biologic sample collection using standardized instruments. Participants [were] asked their usual weight and height at age 25 and their weight 1 year prior to the visit" (Schroeder et al., 2006).

Study Questionnaires

"Study nurses administer[ed] a series of structured questionnaires that solicit[ed] information regarding: Background characteristics: self-described race and ethnicity, marital status, religion, education, income, tobacco use, physical activity.

Occupation: current employment, occupation and industry, longest and second occupation and industry, military service, occupations associated with pesticide use.

Family history: prostate cancer in first- and second-degree relatives.

Health status: general health and comorbid conditions.

Health care: usual sources of care, health insurance, traditional health beliefs, perceived access, and quality of care.

Prostate cancer diagnosis and screening history: PSA tests, digital rectal exams, urinary and sexual symptoms, previous prostate biopsies.

Medication survey: all prescription and over-the-counter medications and supplements used in the prior 2 weeks (transcribed by study nurses).

Non-steroidal anti-inflammatory drugs (NSAIDs): frequency and duration of use for prescription and over-the-counter NSAIDs taken during the past 5 years at least once a month for 1 week or longer, with product name show cards to aid recall.

Vitamins and supplements (including herbal products).

Diet History Questionnaire (DHQ): The DHQ was developed by the National Cancer Institute and modified by PCaP Project 3 investigators to include Southern foods. The DHQ asks about intake frequency and usual portion size for 124 food items, as well as food preparation methods. Participants are asked to recall their usual diet for the year prior to diagnosis. Questionnaire responses are linked to the updated DHQ Nutrient Database through the NCI-developed Diet*Calc software to estimate intake of macro- and micronutrients" (Schroeder et al., 2006).

Medical Record Retrieval and Abstraction

"Medical records [were] requested from the diagnosing physician of consenting participants. Trained staff use[d] a relational database designed specifically for PCaP to abstract information concerning comorbid conditions, family history of prostate cancer, urologic symptoms, indications for diagnostic examinations and biopsies, prostate cancer 41 screening examinations, and laboratory assays at or near diagnosis, imaging examinations used in staging, clinical stage and grade (as recorded), and initial treatment information. In addition, abstractors independently derive[d] clinical stage according to a standardized protocol. Pathologic stage, grade, and other prostatectomy data [were] recorded separately, when available" (Schroeder et al., 2006). To ensure consistency between abstractors, approximately 10% of medical records were abstracted by a second abstractor and concordance was ascertained.

Blood Samples and Laboratory Assays

During the study visit, nurses collected approximately 42 ml of blood from consenting participants: serum was removed from red top tubes and aliquoted into 10 cryovials. Plasma was removed from yellow-top tubes and lavender top (EDTA) tubes, and DNA was purified from white blood cells. Louisiana samples were shipped overnight to the Tissue Procurement Facility at UNC-CH for processing. DNA, plasma and serum are stored at -80°C at UNC-CH. Appropriate volumes of biologic samples were aliquoted and shipped overnight on dry ice to the labs at UCLA and University of South Carolina for assays.

Serum calcium and phosphorous were measured at the Clinical Laboratories of Pathology and Laboratory Medicine, UCLA Medical Center using standard colorimetric methods. Intact PTH (84 amino acid chain) was measured in plasma samples using an Enzyme-Linked ImmunoSorbent Assay (ELISA) from ALPCO (Salem, NH) at the University of South Carolina. Briefly, calibrators, controls and patient samples were simultaneously incubated with an enzyme labeled antibody and a biotin coupled antibody in a streptavidin-coated microplate well. Following the incubation period the wells were washed to remove unbound components and the enzyme bound to the solid phase was incubated with a substrate, tetramethylbenzidine (TMB). An acidic stopping solution was added to stop the reaction. The intensity of color measured on a microplate reader at 450nm was directly proportional to the concentration of intact PTH in the sample. Sensitivity of this kit is calculated at 1.72pg/mL. Plasma concentrations of 25(OH)D3 were determined using LC-MS/MS at Heartland Assays, Inc.

3. Variables

Primary Outcome

Prostate cancer aggressiveness was classified based on clinical grade (Gleason grade), clinical stage, and PSA at diagnosis. "PCaP participants were categorized into three categories of aggressiveness: High aggressive cases: Gleason sum ≥ 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 present study, all PCaP research subjects diagnosed with high aggressive cancer and intermediate aggressive cancer subjects who had Gleason sum = 7 with primary Gleason pattern 4 were combined into the high aggressive group. The high aggressive group was compared to a "control" group, which consisted of a random sample of low aggressive cases with Gleason sum <7, stage T1-T2, and PSA<9 ng/ml" (Steck et al., 2015).

Main Exposures: Calcium, Phosphorus, and PTH

Serum Calcium: Expressed in quartiles based on the distribution among low aggressive cases

Serum Phosphorus: Expressed in quartiles based on the distribution among low aggressive cases

Plasma Parathyroid Hormone (PTH): Expressed in quartiles based on the distribution among low aggressive cases

Based on sample size, we categorized the exposure variables into quartiles. However, we added a fifth category to determine if increased risk is apparent in higher categories of the exposure variables. In addition, there are clinical guidelines about normal ranges for each

of circulating calcium, phosphorus, and PTH which we explored using as cutpoints when categorizing these variables. Finally, we examined distributions of the three variables by race to determine if race-specific cutpoints were necessary (as was done in the PCaP vitamin D metabolites papers previously) (Steck et al., 2015; Ramakrishnan et al., 2019).

Potential Confounders and/or Effect Modifiers

Age: Included as a continuous covariate (reported in years)

Alcohol Intake: Included as a continuous variable (reported in servings per day)

BMI: Included as a continuous variable in main effects analysis and categorical variable in stratified analysis (normal [18.5–24.9 kg/m_2], overweight/obese [≥ 25.0 kg/m_2])

Comorbidity: Using the Charlson Comorbidity Index, scores were categorized as 0, 1-2, and ≥ 3 in main effects analysis and dichotomized as 0 comorbidities and ≥ 1 comorbidities in stratified analysis.

Education: Categorized into four levels: less than high school, high school graduate or vocational/technical school; some college or college graduate; some graduate training or graduate/professional degree

Family History: Dichotomized as "yes" if men reported a first degree relative with a history of prostate cancer and "no" for all others

NSAIDs use: Dichotomized into "yes" for those taking NSAIDs regularly and "no" for all others

Race: Classified as European American or African American

Screening History: Defined on four levels: no previous screening history, digital rectal exam only, PSA only, or PSA and digital rectal exam

Smoking Status: Divided into non-smoker, former smoker, current smoker

25-hydroxyvitamin D: Included as a continuous variable in main effects analysis and divided into quartiles based on low aggressive case distribution in joint effects analysis

Missing Data

Any participants missing outcome, covariate, or exposure data were not included in the final analyses.

Statistical Analyses

All procedures were performed using SAS, version 9.4 statistical software to assess:

1. Univariate distributions of exposures, outcomes, and potential covariates. Means and standard deviations for continuous variables and frequencies and proportions for categorical variables were reported by high/low aggressive prostate cancer status and by race.

2. Odds ratios and 95% CIs for high aggressive prostate cancer were calculated using logistic regression for each exposure variable (circulating calcium, phosphorus, and PTH). A simple model included adjustment for age and race, and separately stratified by race (Specific Aim 1).

3. Odds ratios and 95%CIs for high aggressive prostate cancer were calculated using multivariable logistic regression models adjusted for age, race, plasma 25(OH)D, alcohol intake, family history, smoking status, NSAIDs use, education, screening history, BMI, and CCI. The decision to include these covariates in the model was based on previous relevant literature. Models for circulating calcium, phosphorus, and PTH were mutually adjusted for each other (Specific Aim 1).

4. P-values for trend test of quartile categories of calcium, phosphorus, and PTH were calculated by using the continuous variable in the logistic regression model.

5. Joint associations of circulating calcium, phosphorus, PTH, and 25(OH)D were examined using a common referent group and comparing all other combinations to the common referent group. Interaction p-values were determined by including an interaction term in the adjusted models (Specific Aim 2).

6. Effect modification by smoking, comorbidity, and BMI were examined for each exposure variable (circulating calcium, phosphorus, and PTH) using stratified analyses. Interaction p-values were determined by including an interaction term in the adjusted models (Specific Aim 3).

7. Pearson correlations for circulating calcium, phosphorus, and PTH were calculated for all men.

CHAPTER 4
RESULTS

Tables 4.1-4.2: Descriptive Statistics

Subjects missing data on prostate cancer aggressiveness, serum calcium, serum phosphorus, plasma PTH, and/or any of the covariates were excluded. The final sample size consisted of 783 low aggressive prostate cancer cases and 405 high aggressive prostate cancer cases, of which 529 were AA (41.4% high aggressive) and 659 were EA (28.2% high aggressive). High aggressive cases were slightly older at time of diagnosis compared to low aggressive cases (Table 4.1). Most high aggressive and low aggressive cases reported no family history of prostate cancer (71.1% high aggressive vs 66.4% low aggressive). The mean (±SD) serum calcium level did not substantially differ by race (9.3 ± 0.5 mg/dL in AAs vs 9.2 ± 0.4 mg/dL in EAs) (Table 4.2). The mean serum phosphorus level (±SD) in AA and EA men was similar at 4.3 ± 2.4 mg/dl and 4.3 ± 2.3 mg/dl, respectively. The mean (±SD) concentration of plasma PTH was higher among AA men (49.2 ± 35.7 pg/mL) than EA men (43.3 ± 23.9 pg/mL).

Tables 4.3-4.4: Main Effects in the Total Population

As shown in Table 4.3, circulating calcium, phosphorus, and PTH were first categorized into quartiles based on the distribution among low aggressive cases. Though not statistically significant, an inverse association was observed between serum calcium and aggressive prostate cancer (OR_{Q4vsQ1}: 0.75, 95% CI: 0.51-1.09). For serum phosphorus, a higher odds of aggressive prostate cancer was observed in the simple

model which strengthened and remained statistically significant in the adjusted model (OR: Q_3vsQ_1: 2.36, 95% CI:1.60-3.48; OR: Q_4vsQ_1: 2.17, 95% CI:1.43-3.29). In Table 4.4, clinically high 5th categories of serum calcium and phosphorus were added. For calcium, the odds of aggressive prostate cancer reversed in the upper 5th level indicating higher odds of aggressive prostate cancer, though the confidence interval included the null value (OR: 1.44, 95% CI: 0.58-3.53). After adding the 5th category for serum phosphorus, the increased odds of aggressive prostate cancer remained, and the association maintained statistical significance (OR: 2.20, 95% CI: 1.43-3.39). An inverse association between the odds of aggressive prostate cancer and PTH was observed, though this was not statistically significant (OR:Q_4vsQ_1: 0.79, 95% CI:0.55-1.14) (Table 4.3).

Tables 4.5-4.8: Main Effects by Race

The observed inverse association between serum calcium and aggressive prostate cancer persisted in strata of EA and AA men (Table 4.5). Among EA men in the highest quartile of serum calcium, the odds of aggressive prostate cancer decreased by 45% (OR:Q_4vsQ_1: 0.55, 95% CI: 0.31-0.96). The association was not as strong among AA men (ORQ_4vsQ_1: 0.82, 95% CI: 0.47-1.45). For both AA and EA men, the odds of aggressive prostate cancer were significantly increased in the highest two quartiles of serum phosphorus (Table 4.6), with more pronounced results observed among EA men (ORQ_3vsQ_1:3.07, 95% CI: 1.76-5.35; OR:Q_4vsQ_1: 2.63, 95% CI:1.44-4.82) than AA men (OR: Q_3vsQ_1:1.82, 95% CI: 1.02-3.26; OR: Q_4vsQ_1: 1.88, 95% CI:1.02-3.46). There was no consistent association between plasma PTH and prostate cancer aggressiveness for either EA or AA men (Table 4.7). Among AA men in the 3rd PTH quartile, the odds

of aggressive prostate cancer decreased by 44% (OR: 0.56, 95% CI: 0.32-0.97). The mean PTH concentrations of AA men were noticeably higher than that of EA men, so race-specific PTH quartiles were created (Table 4.8). In the simple model, among AA men there was an inverse association between aggressive prostate cancer and plasma PTH for all three upper quartiles. Adjustment for confounders attenuated the associations and the results were no longer statistically significant. There was no clear association between aggressive prostate cancer and plasma PTH among EA within race specific quartiles.

Table 4.9-4.10: Reference Ranges and Median Cutpoints

Clinical cutpoints for circulating calcium, phosphorus, and PTH were created based on reference ranges established by the NIH U.S. National Library of Medicine (Table 4.9). Clinically low and high serum calcium levels were associated with increased odds of aggressive prostate cancer, though the results were not statistically significant (low calcium: OR: 1.35, 95% CI: 0.58-3.18; high calcium: OR: 1.84, 95% CI:0.77-4.38 compared to normal calcium). Among those with serum phosphorus levels below the normal range, the odds of aggressive prostate cancer decreased by 56% in the adjusted model (OR: 0.44, 95% CI: 0.27-0.71). There was indication of a moderate, non-statistically significant increased odds of aggressive prostate cancer among men with serum phosphorus above the normal range (OR: 1.24, 95% CI: 0.90-1.72). The clinically low PTH category consisted of only 2 cases (1 low aggressive case and 1 high aggressive case). Due to insufficient sample size, these participants were excluded from the PTH analysis. There was a slight, non-statistically significant decreased odds of aggressive

prostate cancer among men in the clinically high PTH category (OR: 0.83, 95% CI: 0.61-1.13).

Median cutpoints were established based on the 50th percentile of the low aggressive case distribution for circulating calcium, phosphorus, and PTH (Table 4.10). Among men with serum calcium levels above the median cutpoint 9.2 mg/dL, there no association with aggressive prostate cancer (OR: 0.90, 95% CI: 0.70-1.18). Serum phosphorus levels above the median cutpoint 3.3 mg/dL were associated with increased odds of aggressive prostate cancer (OR: 2.00, 95% CI: 1.53-2.63). The odds of aggressive prostate cancer among those with plasma PTH levels above the median cutpoint of 40.63pg/ml were not significantly different than those with a plasma PTH level at or below the median (OR: 0.85, 95% CI: 0.65-1.10).

Table 4.11: Joint Associations

The joint associations of circulating calcium, phosphorus, PTH, and 25(OH)D were examined using the quartile and median cutpoint variables for each analyte with the low-low joint category as the referent group (Table 4.11). Among those with low serum phosphorus (at or below 3.3. mg/dL) who were in the 2nd quartile of serum calcium the odds of aggressive prostate cancer decreased by 42% (OR: 0.58, 95% CI: 0.34-0.97). Among those with high serum phosphorus (above the median 3.3. mg/dL) and in the lowest quartile of serum calcium, the odds of aggressive prostate cancer increased by nearly two-fold (OR: 1.99, 95% CI: 1.18-3.34). As serum calcium increased, the increased odds of aggressive prostate cancer was attenuated among men with high serum phosphorus; however, the test for interaction between serum calcium and serum

phosphorus was not statistically significant (P=0.49). No joint effects were observed for serum calcium and either high or low PTH or 25(OH)D.

Among those in the 2nd quartile of serum phosphorus with serum calcium 9.2 mg/dL or lower, the odds of aggressive prostate cancer increased by 74% (OR: 1.74; 95% CI: 1.04-2.92). Increasing serum phosphorus substantially increased the risk of aggressive prostate cancer in the presence of serum calcium levels above and below the median. Similar effects were observed in phosphorus quartiles in the presence of low and high PTH and 25(OH)D. Among participants in the 4th quartile of serum phosphorus with PTH above 40.63 pg/mL, the odds of aggressive prostate cancer increased by 185% (OR: 2.85, 95% CI: 1.43-5.66). The odds of aggressive prostate cancer increased by 141% among those in the 4th quartile of serum phosphorus with 25(OH)D concentrations at or above the median 21.14 ng/mL (OR: 2.41, 95% CI: 1.28-4.53).

There were no observed effects of PTH in the presence of low or high serum calcium and 25(OH)D. The odds of aggressive prostate cancer was higher among all quartiles of PTH in the presence of high serum phosphorus concentrations (above the median) with the most pronounced effect being observed in the 1st quartile of PTH in the presence of high serum phosphorus (OR: 2.97, 95% CI: 1.70-5.18). Among men with low serum phosphorus, there were no substantial associations between higher PTH and aggressive prostate cancer compared to men with both low PTH and low phosphorus. There was evidence of statistical interaction between plasma PTH and serum phosphorus (P=0.049).

In the 2nd quartile of 25(OH)D, the odds of aggressive prostate cancer increased among those with low serum calcium (OR: 2.02, 95% CI: 1.22-3.34). Similar results were

observed among men in the 4th quartile of 25(OH)D with high serum calcium (OR: 2.00, 95% CI: 1.15-3.49). Increasing 25(OH)D with serum phosphorus levels below and above the median increased the odds of aggressive prostate cancer, with the strongest association being observed for both high 25(OH)D and high phosphorus (OR: 4.25, 95% CI: 2.27-7.93) though the test for interaction was not statistically significant (P=0.67). The odds of aggressive prostate cancer among men in the 2nd and 4th 25(OH)D quartiles with low PTH were increased. The most pronounced effect was observed in the 2nd quartile of 25(OH)D (OR: 2.45, 95% CI: 1.40-4.29).

Table 4.12: Serum Calcium and Effect Modification by BMI, Comorbidities, or Smoking

BMI, comorbidities, and smoking status did not modify the association between median cutpoints of serum calcium and odds of aggressive prostate cancer. Additionally, there was no evidence of statistical interaction between median cutpoints of serum calcium and BMI, Charlson comorbidity index, or smoking status.

Table 4.13-4.14: Serum Phosphorus and Effect Modification by BMI, Comorbidities, or Smoking

Among those with BMI greater than 24.9 kg/m2, clinically low serum phosphorus (<2.8 mg/dL) was associated with significantly decreased odds of aggressive prostate cancer (OR: 0.43, 95% CI: 0.25-0.74). In participants with no comorbidities, the odds of aggressive prostate cancer were 68% lower among those with low serum phosphorus as compared to those with serum phosphorus within the reference range (2.8-4.5 mg/dL) (OR: 0.32, 95%: 0.15-0.68). Among participants with one or more comorbidities, the odds of aggressive prostate cancer were 51% lower in those with low phosphorus as compared to those with phosphorus within the normal range (OR: 0.49, 95% CI: 0.25-

0.95). The test for interaction between comorbidities and reference ranges of serum phosphorus was statistically significant (P=0.004).

In men with a BMI above 24.9 kg/m2, the odds of aggressive prostate cancer among those with phosphorus levels above the median (>3.3 mg/dL) were significantly higher compared to those with phosphorus levels at or below the median (≤3.3. mg/dL). The odds of aggressive prostate cancer among those with serum phosphorus concentrations above 3.3 mg/dL were significantly increased among those with no comorbidities (OR: 1.83, 95% CI: 1.23-2.71) and those with more than one comorbidity (OR: 2.26, 95% CI: 1.54-3.34). Among men with serum phosphorus above 3.3 mg/dL who never smoked or were former smokers, the odds of aggressive prostate cancer were at least two-fold. Serum phosphorus above 3.3 mg/dL was not associated with increased odds aggressive prostate cancer among current smokers.

Table 4.15-4.16: Plasma PTH and Effect Modification by BMI, Comorbidities or Smoking

Within strata of BMI, comorbidities, and smoking status, the odds of aggressive prostate cancer among men with clinically high PTH (> 55 pg/mL) were not significantly different than that of those with PTH within the reference range (10-55 pg/mL). Similarly, within strata of BMI and smoking status, PTH levels above the median (> 40.63 pg/mL) were not significantly associated with odds of aggressive prostate cancer. Among men with PTH levels above 40.63 pg/mL and one or more comorbidities, the odds of aggressive prostate cancer decreased by 36% (OR: 0.64; 95% CI: 0.44-0.93).

Table 4.17: Pearson Correlations

A weak positive linear association between serum calcium and serum phosphorus was observed (Pearson correlation: 0.20, p value: <.0001). Weak negative linear associations were found between serum calcium and plasma PTH (Pearson correlation: -0.11, p value: 0.0002) and serum phosphorus and plasma PTH (Pearson correlation: -0.04, p value: 0.0002).

Table 4.1 Characteristics of PCaP participants by high and low aggressiveness				
Characteristics	**Low Aggressive (N =783)**		**High Aggressive (N =405)**	
	Mean (SD)		**Mean (SD)**	
Age, years	62 (8)		64 (8)	
Body mass index, kg/m2	29.0 (5.0)		29.6 (5.9)	
Alcoholic drinks, grams/day	1.2 (3.5)		1.7 (5.2)	
Serum calcium, mg/dL	9.3 (0.4)		9.3 (0.5)	
Serum phosphorus, mg/dL	4.2 (2.4)		4.4 (2.3)	
Plasma parathyroid hormone, pg/mL	45.4 (29.0)		46.9 (31.6)	
Plasma 25-hydroxyvitamin D, ng/mL	21.6 (8.9)		21.5 (10.4)	
	N	**%**	**N**	**%**
Race				
African American	310	39.6	219	54.1
European American	473	60.4	186	45.9
Family History of Prostate Cancer				
No affected 1st-degree relative	520	66.4	288	71.1
At least 1 affected 1st-degree relative	206	26.3	87	21.5
Don't know	57	7.3	30	7.4
Education				
Graduate/professional degree	126	16.1	49	12.1
Some college or college graduate	291	37.2	139	34.3
High school grad or voc/tech school	250	31.9	111	27.4

Less than high school education	116	14.8	106	26.2
Screening History (Digital and PSA)				
No previous screening history	64	8.2	67	16.5
Previous DRE only	103	13.2	84	20.7
Previous PSA only	28	3.6	20	4.9
Previous history of digital rectal exam and PSA	588	75.1	234	57.8
Smoking Status				
Non-smoker	300	38.3	115	28.4
Former smoker	383	48.9	218	53.8
Current smoker	100	12.8	72	17.8
NSAIDs Use				
No	298	38.1	149	36.8
Yes	485	61.9	256	63.2
Charlson Comorbidity Index				
No comorbidities	413	52.8	189	46.7
1, 2, or 3 comorbidities	323	41.2	186	45.9
More than 3 comorbidities	47	6.0	30	7.4

[a]Prostate cancer aggressiveness is defined as the severity of the cancer at diagnosis based on combinations of the Gleason score, morphologic stage, and PSA as follows: high aggressive, Gleason sum ≥ 8 OR PSA > 20 ng/mL OR Gleason sum = 7 (4 +3) OR Gleason sum = 7 and stage T3- T4; low aggressive, Gleason sum < 7 and stage T1-T2 and PSA < 9 ng/mL

Table 4.2 Characteristics of PCaP participants by race				
Characteristics	**African Americans (N =529)**		**European Americans (N =659)**	
	Mean (SD)		**Mean (SD)**	
Age, years	61 (8)		64 (8)	
Body mass index, kg/m2	29.1 (5.9)		29.2 (4.9)	
Alcoholic drinks, grams/day	1.5 (4.7)		1.2 (3.6)	
Serum calcium, mg/dL	9.3 (0.5)		9.2 (0.4)	
Serum phosphorus, mg/dL	4.3 (2.4)		4.3 (2.3)	
Plasma parathyroid hormone, pg/mL	49.2 (35.7)		43.3 (23.9)	
Plasma 25-hydroxyvitamin D, ng/mL	17.7 (7.6)		24.6 (9.6)	
	N	**%**	**N**	**%**
Prostate cancer classification				
High aggressive	219	41.4	186	28.2
Low aggressive	310	58.6	473	71.8
Family History of Prostate Cancer				
No affected 1st-degree relative	343	64.8	465	70.6
At least 1 affected 1st-degree relative	140	26.5	153	23.2
Don't know	46	8.7	41	6.2
Education				
Graduate/professional degree	32	6.1	143	21.7
Some college or college graduate	158	29.9	272	41.3

High school grad or voc/tech school	177	33.5	184	27.9
Less than high school education	162	30.6	60	9.1
Screening History (Digital and PSA)				
No previous screening history	88	16.6	43	6.5
Previous DRE only	125	23.6	62	9.4
Previous PSA only	18	3.4	30	4.6
Previous history of digital rectal exam and PSA	298	56.3	524	79.5
Smoking Status				
Non-smoker	158	29.9	257	39.0
Former smoker	258	48.8	343	52.1
Current smoker	113	21.4	59	9.0
NSAIDs Use				
No	232	43.9	215	32.6
Yes	297	56.1	444	67.4
Charlson Comorbidity Index				
No comorbidities	257	48.6	345	52.4
1, 2, or 3 comorbidities	240	45.4	269	40.8
More than 3 comorbidities	32	6.1	45	6.8

[a]Prostate cancer aggressiveness is defined as the severity of the cancer at diagnosis based on combinations of the Gleason score, morphologic stage, and PSA as follows: high aggressive,

Gleason sum ≥ 8 OR PSA > 20 ng/mL OR Gleason sum = 7 (4 +3) OR Gleason sum = 7 and stage T3- T4; low aggressive, Gleason sum < 7 and stage T1-T2 and PSA < 9 ng/mL

Table 4.3 Association between concentrations of circulating calcium, phosphorus, and PTH (quartiles) and aggressive prostate cancer							
		Simple Model[a]			**Adjusted Model[b]**		
	N, High/ Low aggressive cases	**OR**	**95% CI**	**P_{trend}**	**OR**	**95% CI**	**P_{trend}**
Serum calcium (mg/dL) quartiles				0.95			0.65
≤ 8.9	107/177	1	Ref		1	Ref	
9.0-9.2	101/216	0.75	0.53-1.06		0.74	0.51-1.06	
9.3-9.5	94/195	0.79	0.56-1.12		0.78	0.54-1.13	
> 9.5	103/195	0.82	0.58-1.17		0.75	0.51-1.09	
Serum phosphorus (mg/dL) quartiles				0.07			0.06
≤ 2.9	58/176	1	Ref		1	Ref	
3.0-3.3	81/210	1.18	0.79-1.76		1.26	0.83-1.90	
3.4-4.1	151/206	2.27	1.57-3.29		2.36	1.60-3.48	
> 4.1	115/191	1.92	1.30-2.82		2.17	1.43-3.29	
Plasma parathyroid hormone (mg/dL) quartiles				0.87			0.67
≤ 30.584	114/196	1	Ref		1	Ref	
30.585-40.63	95/195	0.85	0.61-1.21		0.97	0.68-1.39	
40.64-54.2145	98/196	0.82	0.59-1.16		0.88	0.62-1.26	
> 54.2145	98/196	0.75	0.53-1.06		0.79	0.55-1.14	

[a]Adjusted for age and race

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, plasma 25-hydroxyvitamin D, and mutually adjusted for circulating calcium, phosphorus, and PTH

Table 4.4 Association between concentrations of serum calcium and phosphorus[a] (including a fifth category of values considered abnormally high clinically) and aggressive prostate cancer

		Simple Model[b]		Adjusted Model[c]	
	N, High/ Low aggressive cases	**OR**	**95% CI**	**OR**	**95% CI**
Serum calcium (mg/dL), 5 levels					
≤ 8.9	107/177	1	Ref	1	Ref
9.0-9.2	101/216	0.75	0.53-1.06	0.74	0.51-1.05
9.3-9.5	94/195	0.79	0.56-1.13	0.78	0.54-1.13
9.6-10.2	90/184	0.77	0.53-1.10	0.71	0.48-1.04
> 10.2	13/11	1.74	0.74-4.10	1.44	0.58-3.53
Serum phosphorus (mg/dL), 5 levels					
≤ 2.9	58/176	1	Ref	1	Ref
3.0-3.3	81/210	1.18	0.79-1.76	1.26	0.83-1.90
3.4-4.1	151/206	2.27	1.57-3.29	2.36	1.60-3.49
4.1-4.5	18/33	1.64	0.85-3.18	2.04	1.02-4.05
> 4.5	97/158	1.98	1.33-2.95	2.20	1.43-3.39

[a] PTH not included in this table because the clinically high cutpoint was similar to the fourth quartile cutpoint in Table 4.3

[b]Adjusted for age and race

[c]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, plasma 25-hydroxyvitamin D and PTH, and mutually adjusted for serum calcium and serum phosphorus

Table 4.5 Association between concentrations of serum calcium (quartiles) and aggressive prostate cancer by race using uniform cutpoints					
		Simple Model[a]		**Adjusted Model[b]**	
Serum calcium (mg/dL) quartiles	**N, High/low aggressive**	**OR**	**95% CI**	**OR**	**95% CI**
African Americans					
≤ 8.9	45/54	1	Ref	1	Ref
9.0-9.2	55/81	0.84	0.50-1.42	0.77	0.43-1.37
9.3-9.5	48/80	0.76	0.44-1.30	0.69	0.38-1.24
> 9.5	71/95	0.96	0.58-1.60	0.82	0.47-1.45
European Americans					
≤ 8.9	62/123	1	Ref	1	Ref
9.0-9.2	46/135	0.68	0.43-1.08	0.69	0.42-1.11
9.3-9.5	46/115	0.84	0.52-1.33	0.85	0.51-1.41
> 9.5	32/100	0.68	0.41-1.13	0.55	0.31-0.96

[a]Adjusted for age

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, and circulating 25-hydroxyvitamin D, phosphorus, and PTH

Table 4.6 Association between concentrations of serum phosphorus (quartiles) and aggressive prostate cancer by race using uniform cutpoints					
		Simple Model[a]		**Adjusted Model[b]**	
Serum phosphorus (mg/dL) quartiles	**N, High/low aggressive**	**OR**	**95% CI**	**OR**	**95% CI**
African Americans					
≤ 2.9	31/61	1	Ref	1	Ref
3.0-3.3	45/81	1.11	0.63-1.96	1.15	0.61-2.14
3.4-4.1	79/91	1.77	1.04-3.00	1.82	1.02-3.26
> 4.1	64/77	1.72	0.99-2.99	1.88	1.02-3.46
European Americans					
≤ 2.9	27/115	1	Ref	1	Ref
3.0-3.3	36/129	1.25	0.71-2.21	1.37	0.76-2.49
3.4-4.1	72/115	2.90	1.72-4.89	3.07	1.76-5.35
> 4.1	51/114	2.12	1.23-3.66	2.63	1.44-4.82

[a]Adjusted for age

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, and circulating 25-hydroxyvitamin D, calcium, and PTH

Table 4.7 Association between concentrations of plasma PTH (quartiles) and aggressive prostate cancer by race using uniform cutpoints					
		Simple Model[a]		**Adjusted Model[b]**	
PTH (pg/mL) quartiles	**N, High/low aggressive**	**OR**	**95% CI**	**OR**	**95% CI**
African American					
≤ 30.584	65/67	1	Ref	1	Ref
30.585-40.63	46/65	0.70	0.42-1.17	0.92	0.52-1.61
40.64-54.2145	45/85	0.51	0.31-0.85	0.56	0.32-0.97
> 54.2145	63/93	0.65	0.40-1.04	0.81	0.48-1.38
European American					
≤ 30.584	49/129	1	Ref	1	Ref
30.585-40.63	49/130	1.05	0.66-1.69	1.15	0.70-1.91
40.64-54.2145	53/111	1.30	0.81-2.08	1.42	0.86-2.34
> 54.2145	35/103	0.85	0.51-1.43	0.90	0.52-1.56

[a]Adjusted for age

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, and circulating 25-hydroxyvitamin D, calcium, and phosphorus

Table 4.8 Association between concentrations of plasma PTH and aggressive prostate cancer by race using race-specific cutpoints							
		Simple Model[a]			**Adjusted Model[b]**		
PTH (pg/mL) race-specific quartiles	**High/ Low aggressive cases**	**OR**	**95% CI**	**P_{trend}**	**OR**	**95% CI**	**P_{trend}**
African American				0.43			0.13
≤ 32.49	76/77	1	Ref		1	Ref	
32.50-43.75	49/78	0.61	0.38-0.99		0.72	0.43-1.22	
43.76-56.94	44/77	0.55	0.34-0.91		0.59	0.34-1.01	
> 56.94	50/78	0.61	0.37-0.98		0.70	0.41-1.20	
European American				0.37			0.80
≤ 29.20	61/151	1	ref		1	Ref	
29.21-38.54	56/137	0.97	0.59-1.60		1.06	0.62-1.80	
38.55-51.59	35/98	1.30	0.81-2.09		1.46	0.88-2.41	
> 51.59	34/87	0.88	0.53-1.46		0.95	0.55-1.62	

[a]Adjusted for age

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, and circulating 25-hydroxyvitamin D, calcium, and phosphorus

Table 4.9 Association between circulating calcium, phosphorus, and PTH (clinical ranges, using normal as the referent group) and aggressive prostate cancer					
		Simple Model[a]		**Adjusted Model[b]**	
Clinical cutpoints of serum analytes[c]	**High/ Low aggressive cases**	**OR**	**95% CI**	**OR**	**95% CI**
Serum calcium (mg/dL)					
Low (< 8.5)	11/14	1.54	0.68-3.50	1.35	0.58-3.18
Normal (8.5-10.2)	381/758	1.00	Ref	1.00	Ref
High (> 10.2)	13/11	2.16	0.95-4.93	1.84	0.77-4.38
Serum phosphorus (mg/dL)					
Low (< 2.8)	25/92	0.48	0.30-0.78	0.44	0.27-0.71
Normal (2.8-4.5)	283/533	1.00	Ref	1.00	Ref
High (> 4.5)	97/158	1.21	0.90-1.63	1.24	0.90-1.72
Plasma parathyroid hormone (pg/mL)					
Normal (10-55)	311/596	1.00	Ref	1.00	Ref
High (> 55)	93/186	0.85	0.63-1.14	0.83	0.61-1.13

[a]Adjusted for age and race

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, plasma 25-hydroxyvitamin D, and mutually adjusted for circulating calcium, phosphorus, and PTH

[c]Clinical cutpoints based on reference ranges established by the NIH U.S. National Library of Medicine

Table 4.10 Association between circulating calcium, phosphorus, and PTH (median cutpoints) and aggressive prostate cancer					
		Simple Model[a]		**Adjusted Model[b]**	
Median cutpoints of serum analytes[c]	**High/ Low aggressive cases**	**OR**	**95% CI**	**OR**	**95% CI**
Serum calcium (mg/dL)					
≤ 9.2	208/393	1.00	Ref	1 .00	Ref
> 9.2	197/390	0.94	0.73-1.20	0.90	0.70-1.18
Serum phosphorus (mg/dL)					
≤ 3.3	139/386	1.00	Ref	1	Ref
> 3.3	266/397	1.91	1.48-2.47	2.00	1.53-2.63
Plasma parathyroid hormone (pg/mL)					
≤ 40.63	209/391	1.00	Ref	1.00	Ref
> 40.63	196/392	0.85	0.66-1.09	0.85	0.65-1.10

[a]Adjusted for age and race

[b]Additionally adjusted for BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, plasma 25-hydroxyvitamin D, and mutually adjusted for circulating calcium, serum phosphorus, and PTH

[c]Median cutpoints established based on the 50th percentile of low aggressive case distribution

Table 4.11 Joint effects of circulating calcium, phosphorus, PTH, and vitamin D[a]								
	Median Cutpoints[b]							
	Serum calcium		**Serum phosphorus**		**Plasma parathyroid hormone**		**Plasma vitamin D**	
Quartiles of analytes	≤ 9.2	> 9.2	≤ 3.3	>3.3	≤40.63	> 40.63	≤ 21.14	> 21.14
Serum calcium (mg/dL)								
≤ 8.9			1.00	1.99 (1.18-3.34)	1.00	1.22 (0.71-2.09)	1.00	1.14 (0.67-1.92)
9.0-9.2			0.58 (0.34-0.97)	1.54 (0.95-2.48)	0.94 (0.55-1.63)	0.76 (0.43-1.35)	0.82 (0.51-1.33)	0.75 (0.44-1.26)
9.3-9.5			0.62 (0.34-1.12)	1.41 (0.88-2.27)	1.09 (0.63-1.88)	0.71 (0.39-1.29)	0.84 (0.50-1.40)	0.84 (0.50-1.41)
> 9.5			0.81 (0.44-1.48)	1.18 (0.75-1.86)	1.00 (0.57-1.74)	0.73 (0.40-1.31)	0.67 (0.41-1.11)	1.00 (0.59-1.67)
$P_{interaction}$			0.49		0.39		0.56	
Serum phosphorus (mg/dL)								
≤ 2.9	1.00	1.55 (0.81-2.97)			1.00	1.61 (0.81-3.21)	1.00	1.04 (0.55-1.95)
3.0-3.3	1.74	1.04			1.62	1.84	1.45	1.13

	(1.04-2.92)	(0.56-1.90)			(0.81-3.25)	(0.93-3.67)	(0.82-2.59	(0.61-2.08)
3.4-4.1	2.81 (1.71-4.62)	2.53 (1.51-4.24)			4.05 (2.12-7.74)	2.44 (1.25-4.79)	2.03 (1.17-3.53)	2.74 (1.57-4.81)
> 4.1	3.41 (1.85-6.32)	2.07 (1.27-3.37)			3.13 (1.60-6.12)	2.85 (1.43-5.66)	1.93 (1.11-3.37)	2.41 (1.28-4.53)
$P_{interaction}$	0.10				0.09		0.45	
Plasma parathyroid hormone (pg/mL)								
≤ 30.584	1.00	1.52 (0.92-2.54)	1.00	2.97 (1.70-5.18)			1.00	0.96 (0.58-1.58)
30.585-40.63	1.44 (0.84-2.46)	1.09 (0.63-1.89)	1.16 (0.61-2.21)	2.71 (1.53-4.79)			1.07 (0.63-1.81)	0.85 (0.51-1.44)
40.64-54.2145	1.25 (0.74-2.13)	1.05 (0.60-1.84)	1.70 (0.94-3.08)	1.84 (1.02-3.34)			0.75 (0.45-1.23)	0.97 (0.57-1.65)
> 54.2145	1.27 (0.75-2.14)	0.79 (0.44-1.41)	1.05 (0.57-1.95)	2.30 (1.28-4.15)			0.66 (0.40-1.07)	0.92 (0.52-1.64)
$P_{interaction}$	0.09		0.049				0.40	
Plasma vitamin D (ng/mL)								
≤ 15.15	1.00	0.91	1.00	1.87	1.00	0.96		

		(0.54-1.54)		(1.05-3.34)		(0.55-1.68)	
15.16-21.14	2.02 (1.22-3.34)	1.67 (0.98-2.82)	2.22 (1.22-4.07)	3.77 (2.08-6.81)	2.45 (1.40-4.29)	1.46 (0.83-2.57)	
21.15-26.18	1.51 (0.85-2.68)	1.26 (0.73-2.19)	1.27 (0.65-2.50)	3.20 (1.71-5.99)	1.26 (0.70-2.30)	1.60 (0.86-2.97)	
> 26.18	1.69 (0.98-2.93)	2.00 (1.15-3.49)	1.78 (0.93-3.39)	4.25 (2.27-7.93)	1.96 (1.10-3.49)	1.77 (0.95-3.32)	
$P_{interaction}$	0.74		0.67		0.22		

[a]All models adjusted for age, race, BMI, alcohol intake, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index, smoking status, education level, and mutually adjusted for circulating calcium, phosphorus, PTH, and 25-hydroxyvitamin D

[b]Median cutpoints established based on the 50th percentile of low aggressive case distribution

	Table 4.12 Associations between median cutpoints of serum calcium and prostate cancer aggressiveness in strata of BMI, comorbidities, and smoking status				
	Median Cutpoints of Serum Calcium (mg/dL)[b]				
	N (high/low aggressive cases)	**Low Calcium (≤9.2)**	**N (high/low aggressive cases)**	**High Calcium (>9.2)**	
		OR		**OR (95% CI)**	**$P_{interaction}$**
BMI (kg/m^2)[c]					0.30
Normal (18.5-24.9)	27/64	1.00 (ref)	45/79	1.33 (0.69-2.58)	
Overweight/Obese (≥ 25)	177/325	1.00 (ref)	152/308	0.84 (0.62-1.12)	
Charlson Comorbidity Index					0.06
No comorbidities	104/202	1.00 (ref)	85/211	0.77 (0.53-1.13)	
≥ 1 comorbidities	104/191	1.00 (ref)	112/179	1.04 (0.71-1.52)	
Smoking Status					0.07
Never smoker	67/143	1.00 (ref)	48/157	0.65 (0.40-1.06)	
Former Smoker	109/205	1.00 (ref)	109/178	1.11 (0.76-1.62)	
Current smoker	32/45	1.00 (ref)	40/55	0.97 (0.48-1.97)	

a. Adjusted for age, race, BMI (in BMI unstratified groups), alcohol consumption, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index (in Charlson Comorbidity Index unstratified groups), smoking status (in smoking unstratified groups), education level, and circulating phosphorus, PTH, and 25-hydroxyvitamin D

b. Median cutpoints established based on the 50^{th} percentile of low aggressive case distribution

c. BMI cutpoints established by the World Health Organization (WHO)

	Table 4.13 Associations between clinical ranges of serum phosphorus and prostate cancer aggressiveness in strata of BMI, comorbidities, and smoking status						
	Clinical Ranges of Serum Phosphorus (mg/dL)[b]						
	Low Phosphorus (≤2.8)		**Normal Phosphorus (2.8-4.5)**		**High Phosphorus (>4.5)**		
	N (high/low aggressive cases)	**OR (95% CI)**	**N (high/low aggressive cases)**	**OR**	**N (high/low aggressive cases)**	**OR (95% CI)**	**$P_{interaction}$**
BMI (kg/m^2)[c]							0.97
Normal (18.5-24.9)	4/18	0.36 (0.10-1.25)	51/98	1.00 (ref)	17/27	1.27 (0.57-2.86)	
Overweight/Obese (≥ 25)	21/73	0.43 (0.25-0.74)	229/430	1.00 (ref)	79/130	1.32 (0.91-1.89)	
Charlson Comorbidity Index							0.004
No comorbidities	10/42	0.32 (0.15-0.68)	141/273	1.00 (ref)	38/98	0.66 (0.40-1.07)	
≥ 1 comorbidities	15/50	0.49 (0.25-0.95)	142/260	1.00 (ref)	59/60	2.34 (1.46-3.76)	
Smoking Status							0.14
Never smoker	11/27	0.96 (0.41-2.27)	75/211	1.00 (ref)	29/62	1.61 (0.88-2.94)	
Former Smoker	10/55	0.25 (0.12-0.53)	157/252	1.00 (ref)	51/76	1.15 (0.72-1.84)	

Current smoker	4/10	0.71 (0.17-2.90)	51/70	1.00 (ref)	17/20	1.16 (0.49-2.79)	

a. Adjusted for age, race, BMI (in BMI unstratified groups), alcohol consumption, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index (in Charlson Comorbidity Index unstratified groups), smoking status (in smoking unstratified groups), education level, and circulating calcium, PTH, and 25-hydroxyvitamin D

b. Clinical cutpoints based on reference ranges established by the NIH U.S. National Library of Medicine

c. BMI cutpoints established by the World Health Organization (WHO)

	Table 4.14 Associations between serum phosphorus and prostate cancer aggressiveness in strata of BMI, comorbidities, and smoking status				
	Median Cutpoint of Serum Phosphorus (mg/dL)[b]				
	Low Phosphorus (≤3.3)		**High Phosphorus (>3.3)**		
	N (high/low aggressive cases)	**OR**	**N (high/low aggressive cases)**	**OR (95% CI)**	**$P_{interaction}$**
BMI (kg/m^2)[c]					0.71
Normal (18.5-24.9)	24/67	1.00 (ref)	48/76	1.85 (0.95-3.63)	
Overweight/Obese (≥ 25)	114/316	1.00 (ref)	215/317	2.12 (1.57-2.88)	
Charlson Comorbidity Index					0.22
No comorbidities	68/195	1.00 (ref)	121/218	1.83 (1.23-2.71)	
≥ 1 comorbidities	71/191	1.00 (ref)	145/179	2.26 (1.54-3.34)	
Smoking Status					0.42
Never smoker	36/151	1.00 (ref)	79/149	2.64 (1.57-4.43)	
Former Smoker	78/193	1.00 (ref)	140/190	2.02 (1.38-2.97)	
Current smoker	25/42	1.00 (ref)	47/58	1.10 (0.53-2.27)	

a. Adjusted for age, race, BMI (in BMI unstratified groups), alcohol consumption, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index (in Charlson Comorbidity Index unstratified groups), smoking status (in smoking unstratified groups), education level, and circulating calcium, PTH, and 25-hydroxyvitamin D

b. Median cutpoints established based on the 50th percentile of low aggressive case distribution

c. BMI cutpoints established by the World Health Organization (WHO)

Table 4.15 Associations between clinical ranges of plasma PTH and prostate cancer aggressiveness in strata of BMI, comorbidities, and smoking status					
	Clinical Ranges of Plasma PTH (pg/mL)[b]				
	Normal PTH (10-55)		**High PTH (> 55)**		
	N (high/low aggressive cases)	**OR**	**N (high/low aggressive cases)**	**OR (95% CI)[b]**	**$P_{interaction}$**
BMI (kg/m^2)[c]					0.85
Normal (18.5-24.9)	57/112	1.00 (ref)	14/30	0.79 (0.35-1.79)	
Overweight/Obese (≥ 25)	252/477	1.00 (ref)	77/156	0.81 (0.58-1.14)	
Charlson Comorbidity Index					0.92
No comorbidities	148/320	1.00 (ref)	41/93	0.80 (0.51-1.27)	
≥ 1 comorbidities	163/276	1.00 (ref)	52/93	0.82 (0.54-1.26)	
Smoking Status					0.72
Never smoker	92/230	1.00 (ref)	23/70	0.76 (0.42-1.39)	
Former Smoker	161/283	1.00 (ref)	56/99	0.80 (0.52-1.21)	
Current smoker	58/83	1.00 (ref)	14/17	1.04 (0.43-2.54)	

a. Adjusted for age, race, BMI (in BMI unstratified groups), alcohol consumption, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index (in Charlson Comorbidity Index unstratified groups), smoking status (in smoking unstratified groups), education level, and circulating calcium, PTH, and 25-hydroxyvitamin D

b. Clinical cutpoints based on reference ranges established by the NIH U.S. National Library of Medicine

c. BMI cutpoints established by the World Health Organization (WHO)

Table 4.16 Associations between median cutpoints of plasma PTH and prostate cancer aggressiveness in strata of BMI, comorbidities, and smoking status					
	Median Cutpoints of Plasma PTH (pg/mL)[b]				
	Low PTH (≤40.63)		**High PTH (>40.63)**		
	N (high/low aggressive cases)	**OR**	**N (high/low aggressive cases)**	**OR (95% CI)[b]**	**$P_{interaction}$**
BMI (kg/m2)[c]					0.65
Normal (18.5-24.9)	42/77	1.00 (ref)	30/66	0.82 (0.43-1.57)	
Overweight/Obese (≥ 25)	165/308	1.00 (ref)	164/325	0.86 (0.64-1.15)	
Charlson Comorbidity Index					0.03
No comorbidities	89/216	1.00 (ref)	100/197	1.12 (0.77-1.63)	
≥ 1 comorbidities	120/175	1.00 (ref)	96/195	0.64 (0.44-0.93)	
Smoking Status					0.99
Never smoker	60/152	1.00 (ref)	55/148	0.88 (0.54-1.44)	
Former Smoker	108/185	1.00 (ref)	110/198	0.86 (0.60-1.25)	
Current smoker	41/54	1.00 (ref)	31/46	0.77 (0.38-1.55)	

a. Adjusted for age, race, BMI (in BMI unstratified groups), alcohol consumption, family history of prostate cancer, previous screening history, NSAIDs use, Charlson comorbidity index (in Charlson Comorbidity Index unstratified groups), smoking status (in smoking unstratified groups), education level, and circulating calcium, phosphorus, and 25-hydroxyvitamin D

b. Median cutpoints established based on the 50th percentile of low aggressive case distribution

c. BMI cutpoints established by the World Health Organization (WHO)

Table 4.17 Pearson Correlations between circulating calcium, phosphorus, and PTH		
Pearson Correlation		
	Serum phosphorus	**Plasma PTH**
Serum calcium	0.20 (<.0001)	-0.11 (.0002)
Serum phosphorus		-0.04 (0.0002)

CHAPTER 5

DISCUSSION

Summary and Significance of Results

This study examined the association between circulating calcium, phosphorus, and PTH and the odds of aggressive prostate cancer using data from PCaP, a racially diverse case-only study of prostate cancer in North Carolina and Louisiana. Exposure variables were categorized using various cutpoints: 1) quartiles using uniform cutpoints determined by distributions among low aggressive cases, with or without a "clinically high" fifth category for calcium and phosphorus, 2) quartiles using race-specific cutpoints according to distribution among low aggressive cases by race, 3) clinical reference ranges established by the U.S National Library of Medicine, and 4) high and low categories based on median distribution of exposures among low aggressive cases.

In this study, serum calcium was associated with a modestly decreased odds of aggressive prostate cancer. Among the few studies that have examined the association between serum calcium and aggressive prostate cancer, results were inconclusive. Brandstedt et al. (2016) reported no association and Van Hemeljrick et al. (2013) found a weak positive, non-statistically significant association between serum calcium and aggressive prostate cancer. While our study consisted of relatively similar distributions of EA and AA men, previous studies were predominately comprised of European men. Additionally, quartile cutpoints for serum calcium in both previous studies were higher

than quartile cutpoints in the present study. Furthermore, both previous studies used pre-diagnostic serum concentrations, and Van Hemeljrick et al. (2013) reported that serum measurements were taken 8 years prior to prostate cancer diagnosis. In the present study, serum measurements were fasting and taken within four months after diagnosis. Thus, differences in timing of blood collection, populations studied, and other study design differences may explain the differences in results between our study and the afore-mentioned previous studies.

Our study found that the odds of aggressive prostate cancer increased among men in the clinically low and high serum calcium categories as compared to those in the normal calcium range. Sample sizes among these lower and upper categories were relatively small, which could potentially explain why this result differed from the main effects analyses for serum calcium. Results of two previous studies were comparable with ours, though the primary outcome of these studies was total prostate cancer rather than aggressive prostate cancer (Jackson et al., 2015; Skinner and Schwartz, 2008). The study conducted by Skinner and Schwartz (2008) consisted of only 85 incident cases of prostate cancer, and serum samples were, on average, collected 9.9 years prior to prostate cancer diagnosis. The Jackson et al. (2015) study was conducted in Jamaica, and was more similar to ours because post-diagnostic serum concentrations were used, though they were non-fasting.

Evidence generally suggests that high calcium intake increases the risk of prostate cancer (Rahmati et al., 2018; Aune et al., 2015), but it is difficult to disentangle whether these effects are a result of dietary calcium specifically or rather a marker of a diet high in calcium-rich dairy products which may be associated with other risk factors such as

saturated fat intake or obesity (Wilson et al., 2015). Moreover, serum calcium is not an adequate marker of dietary calcium. Serum calcium is tightly regulated, which may explain the conflicting, weak to modest associations observed. However, it is biologically plausible for serum calcium to be implicated in the progression of prostate cancer. Results from previous studies suggest that normal and cancerous prostate cells express the CaSR, though expression is higher among metastatic prostate cancer tissues (Feng et al., 2014). Additionally, CaSR has been associated with increased risk of fatal prostate cancer (Ahearn et al., 2016). Calcium is released into serum during bone remodeling, and it is possible that during this process, elevated serum calcium stimulates expression of the CaSR in tumor cells (Feng et al., 2014; Ahearn et al., 2016).

Results from stratified analyses suggest that overweight/obesity status modified the association between serum calcium and aggressive prostate cancer, though the interaction was not statistically significant. Similar to our findings, Halthur et al. (2009) reported decreased risk of total prostate cancer for high serum calcium among those who had a BMI above 25 kg/m2. It is suggested that obesity hinders the metabolism of vitamin D (Lagunova et al., 2009), a calcium regulating hormone that has been postulated to decrease the risk of prostate cancer progression, though recent studies have produced inconsistent results (Murphy et al., 2013; Gao et al., 2018).

Across various categorization approaches, higher serum phosphorus was significantly associated with increased odds of aggressive prostate cancer, while men with clinically low serum phosphorus had decreased odds compared to men in the clinically normal range. In a study examining the association between risk of prostate cancer and phosphorus intake, Wilson et al. (2015) found that high dietary phosphorus

was independently associated with an increased risk of prostate cancer. High serum phosphorus could be indicative of a diet high in phosphorus, which is commonly found in dairy products and processed foods. However, there is suggestion of only a weak to modest correlation between dietary phosphorus intake and serum phosphorus in the general population without renal disease (de Boer, Rue, & Kestenbaum, 2009; Gutiérrez et al., 2012). Due to increased availability and use of phosphate in food additives, it is difficult to adequately measure dietary phosphorus intake using conventional dietary assessment methods (Calvo et al., 2014). Since it is suggested that these assessment methods underestimate dietary phosphorus intake, it could also be hypothesized that this underestimation attenuates the true associations between serum phosphorus and dietary phosphorus (de Boer, Rue, & Kestenbaum, 2009). Thus, utilizing serum concentrations, as was done in our study, may be a better indicator of phosphorus exposure or status than dietary intake data.

Comorbidities and smoking status modified the association between serum phosphorus and aggressive prostate cancer. The odds of aggressive prostate cancer were significantly increased among men with clinically high phosphorus and one or more comorbidities, while a modest decreased risk for clinically high serum phosphorus was observed among those with no comorbidities ($p_{interaction}=0.004$). Research suggests that men with a higher number of comorbidities have poorer prostate cancer prognosis, which could be attributed to the cancer itself or competing risks (Edwards et al., 2014; Rajan et al., 2017). Using the median cutpoint serum phosphorus variable, the odds of aggressive prostate cancer were significantly increased among men with high serum phosphorus who were never smokers or former smokers, while there was no association with high

serum phosphorus among current smokers. Current smokers tend to have a higher risk of total and aggressive prostate cancer (Huncharek, 2010; Ho et al., 2014). Perhaps high serum phosphorus does not substantially increase the odds of aggressive prostate cancer beyond that of smoking, which could explain why there was no significantly increased odds in this group of men.

Elevated serum phosphorus leads to release of PTH, which has been found to promote progression of prostate tumor cells (Ritchie et al., 1997). Additionally, PTH related proteins (PTH-rp), though fundamentally different from PTH, are expressed in approximately 90% of localized prostate cancer cases (Bryden et al., 2002). PTH and PTH-rp share a common receptor, which is highly expressed in prostate cancer bone metastasis (Bryden et al., 2002). A recent study among Korean men reported no difference in serum PTH levels for men with low (≤7) or high (>8) Gleason grade prostate cancer, though men with prostate cancer had higher PTH levels compared to men with benign prostatic hyperplasia. They also observed substantially lower PTH levels after radical prostectomy (Kim et al., 2020). In the present study, in both the total population and race specific quartiles, PTH was non-significantly associated with a slight decreased odds of aggressive prostate cancer. This finding is consistent with that of the study conducted by Brandstedt and colleagues (2016). The biological pathways behind these findings are inconclusive. There was evidence of interaction and effect modification between PTH and number of comorbidities. The odds of aggressive prostate cancer among men with high PTH and one or more comorbidities were significantly decreased. Since PTH is a clinical marker of other diseases, this result could be the effect of competing risks or poor health.

In the joint effects analysis, across categories of low and high serum calcium, PTH, and 25(OH)D, high serum phosphorus was consistently associated with increased odds of aggressive prostate cancer, with the exception that increasing serum calcium appeared to attenuate the effect of high serum phosphorus on the odds of aggressive prostate cancer though the interaction was not statistically significant. There was evidence of statistical interaction between PTH and serum phosphorus, wherein the odds of aggressive prostate cancer were significantly increased across PTH quartiles with phosphorus above the median. These results are notably different from the main effects analysis for PTH, which further suggests that serum phosphorus is the driver for increased odds of aggressive prostate cancer in this study.

Similar effects were observed for quartiles of 25(OH)D and phosphorus with increasing odds observed in all quartiles of 25(OH)D among those with high phosphorus compared to the joint low 25(OH)D-low phosphorus referent group. However, among men with low calcium, low phosphorus, or low PTH, the 2nd quartile of 25(OH)D was significantly associated with increased odds of aggressive prostate cancer compared to men in the lowest category of each. These may be spurious associations given the number of comparisons in this study. The relationship between 25(OH)D and aggressive prostate cancer is mixed. Several epidemiologic studies have suggested that high 25(OH)D levels may protect against aggressive prostate cancer (Gilbert et al., 2012; Nelson et al., 2016). However, in a previous study published from our dataset, Steck et al. (2015) found that 25(OH)D was positively associated with odds of aggressive prostate cancer among AA men, and calcium intake modified this association.

Strengths and Limitations

Our study benefited from several strengths. Participants were recruited into the study using a randomized recruitment procedure to allow for comparable enrollment rates of EA and AA men. Though AA men have an increased risk of total and aggressive prostate cancer, they are historically underrepresented in research studies. Additionally, because circulating calcium, phosphorus, PTH, and vitamin D are homeostatically related, they were mutually adjusted for one another.

Despite its strengths, the study was not without limitations. This was a case-only study, so comparison to a set of healthy controls was not feasible. Due to the racial composition of PCaP, the generalizability of the results of this study may only be applicable to EA and AA men. The biomarkers obtained from PCaP were taken post-diagnosis, so it is difficult to determine if high serum phosphorus preceded or is an effect of aggressive prostate cancer. Sufficient data on demographic, clinical, and lifestyle factors were available to adjust for potential confounders, but due to the observational design of the study, residual confounding remains a possibility. Though the study consisted of 1,188 men, results from stratified analysis were limited in statistical power given the small sample sizes within strata. Another limitation is that we were not able to adjust for renal disease, which could impact circulating calcium, phosphorus, and PTH concentrations. Finally, blood samples were taken only once, and circulating concentrations could fluctuate throughout the day or from day to day.

Recommendations for Future Research

This study found statistically significant associations between serum phosphorus and the odds of aggressive prostate cancer. To our knowledge, this is the first study that

has examined the role of serum phosphorus in prostate cancer. Due to this, it is difficult to compare our findings to others. Further studies examining the role of serum phosphorus and aggressive prostate cancer should be conducted to confirm the results in the present study. Specifically, a prospective study would be best to determine if high serum phosphorus is a pre-diagnostic marker of aggressive prostate cancer or a result of aggressive prostate cancer. Since the evidence on whether serum phosphorus is an adequate marker of dietary phosphorus is limited, future studies could examine if similar effects are observed among dietary phosphorus and serum phosphorus in the same sample. Additionally, studies thoroughly examining factors that influence serum phosphorus levels in the general population are recommended.

Inconsistencies on the associations between aggressive prostate cancer and serum calcium persist. Though findings from previous studies suggest a positive association between PTH and metastatic prostate cancer, findings from the present study and the study conducted by Brandstedt et al. (2016) suggest a slight, non-significant inverse association between PTH and aggressive prostate cancer. The roles of PTH and serum calcium in aggressive prostate cancer remain unclear, and future studies should thoroughly examine the independent effects of these biomarkers on prostate cancer progression. If it is confirmed that circulating calcium, phosphorus, and PTH influence the progression of prostate cancer, there could be implications for incorporating them into strategies used to help guide clinical decisions.

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