INTERLEUKIN-1A (IL-1A), IL-1B, IL-1RN, IL-6 AND IL-6R AND PROSTATE CANCER RISK IN AFRICAN AMERICAN AND CAUCASIAN MEN

by

Kwame Afari Appenteng

B.S., City College of the City University of New York, 1998

M.P.H, New York Medical College, 2004

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2009

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This dissertation was presented

by

Kwame Afari Appenteng

It was defended on

April, 17 2009

and approved by

Dissertation Advisor:
Joel Weissfeld, MD, MPH
Associate Professor, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Clareann Bunker, PhD
Associate Professor, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Robert Ferrell, PhD
Professor, Department of Human Genetics
Graduate School of Public Health
University of Pittsburgh

John W. Wilson, PhD
Associate Professor, Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

INTERLEUKIN-1A (IL-1A), IL-1B, IL-1RN, IL-6 AND IL-6R AND PROSTATE CANCER RISK IN AFRICAN AMERICAN AND CAUCASIAN MEN

Kwame Afari Appenteng, PhD

University of Pittsburgh, 2009

Prostate cancer incidence and mortality rates continue to be higher among African Americans than Caucasians. While psychosocial factors may explain some of the disparities, the role played by genetic differences in the two racial groups is not so clear. Emerging evidence suggests an important role of chronic or recurrent inflammation in prostate carcinogenesis. *Interleukin-1 (IL-1)* and *IL-6* are inflammatory genes reported to be associated with prostate cancer risk. *Interleukin-1* and *IL-6* cytokines also decrease bone mineral density (BMD) by inducing osteoclasts to resorb bone matrix. We sought to determine if genotypes of *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* were associated with prostate cancer risk, as well as with selected risk factors, in the two racial groups.

We examined allele frequency distributions of polymorphisms in *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* genes in a cross-sectional study of African American and Caucasian men ages 40 to 80 years old. We also assessed the associations of genotypes of these inflammatory genes and the risk of prostate cancer in a case-control study of the two racial groups. Additionally, we evaluated the associations of bone mineral density and prostate cancer in our sample. We found racial differences in minor allele frequencies, as well as in the associations of single nucleotide polymorphisms of inflammatory genes *IL-1* and *IL-6* and prostate cancer. We also found associations of *IL-1* and *IL-6* genotypes and prostate cancer. Additionally, we found an inverse association of BMD and prostate cancer in both racial groups. Our findings support a growing

body of evidence that chronic or recurrent inflammation play an important role in prostate carcinogenesis, and the possibility of ethnic based differences in susceptibility. Understanding the role of *IL-1* and *IL-6* genes in the development of prostate cancer is of great public health significance because it will enable their possible use as biomarkers for early detection and prompt intervention, increase our understanding of the molecular biology of the disease, open up new avenues for prevention and treatment, as well as explain some of the observed disparities in the disease.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to acknowledge the contributions of many individuals who made this project possible. Dr. Joel Weissfeld was instrumental in funding my training as a cancer epidemiologist by offering me an opportunity on the R25 fellowship training grant. He is an extremely gifted scientist and prudent researcher whose outstanding qualities as a teacher and mentor have helped shaped my thought process and career path. I cannot possibly imagine completing this project successfully without his endless sacrifice, help and guidance.

I am indebted to Mary Yagjian of the University of Pittsburgh Cancer Institute (UPCI), whose oversight and tireless work ensured that all my educational, health, travel and ancillary needs were met in a timely manner. Along with Mary, many of the staff at our UPCI office and research centers helped me every step along the way, for which I am most grateful. My training was made very pleasant by the kind support of friends and fellow trainees: Alicia, Ashley, Cher, Jessica, Ji, Katherine, and Scott, have all become members of my family.

My immense gratitude to Dr. Francesmary Modugno for all the work she did initiating and executing the CAPS project, and for providing me with all the materials I needed for my work. I also thank Claudia Leiras for her work coordinating the CAPS project, and for guiding me through the mountain of paperwork. Dr. James Shikany and Dr. Joel Nelson worked

tirelessly to recruit participants into CAPS, and promptly provided me with all the information I needed for my work, for which I am most grateful.

I would be most ungrateful if I do not say thank you to Dr. Clareann Bunker, Dr. Robert Ferrell and Dr. John Wilson: together with Dr. Joel Weissfeld, they formed the best dissertation committee anyone could possibly ask for. They gave me open accesses to their offices and phones, and guided me through the dissertation process from conception to completion. I am also very thankful to Dr. Brenda Diergaarde and Dr. Susan Moffett for the time they spent reviewing my manuscripts, and for the invaluable contributions they made to this project through their thoughtful and insightful commentary.

I would like to thank Clarence Pearson and Laurie Norris for bringing me to Pittsburgh, and for doing all the hard work for me behind the scenes. Finally, my biggest thank you goes to my family for their patience, dedication and support through all these years; and to God Almighty for His mercies and abundant grace.

1.0 INTRODUCTION

Prostate cancer incidence and mortality rates continue to be higher among African Americans than Caucasians. While psychosocial factors may explain some of the disparities, the role played by genetic differences in the two racial groups is not so clear. Emerging evidence suggests an important role of chronic or recurrent inflammation in prostate tumorigenesis. *Interleukin 1 (IL-1)* and *IL-6* are inflammatory cytokines reported to be associated with prostate cancer risk. Additionally, these cytokines decrease bone mineral density by causing resorption of bone matrix via osteoclast activity. Several studies have reported an association between BMD and cancer of the breast, endometrium and prostate. These studies have primarily assessed BMD as a proxy measurement of a lifetime exposure of specific organs to sex-steroid hormones, among others. The molecular factors that contribute to racial disparities in prostate cancer risk are still unclear. The disease is initially androgen dependent but rapidly becomes androgen independent, and refractory to therapy. Inflammatory cytokines such as *IL-6* have been reported to influence clinical outcome by mediating the transition from androgen dependence to androgen independence. There is a compelling role of chronic or recurrent inflammation in prostate cancer development based on genetic, histopathology, and epidemiologic studies. However, the role of *IL-1* and *IL-6* in prostate cancer risk and in explaining observed racial disparities in the disease are not clearly understood.

The main objective of the current study is to assess whether there are differences in allele frequencies of inflammatory cytokines *IL-1* and *IL-6* gene polymorphisms among African American and Caucasian men, which may partly explain the observed disparities in prostate cancer incidence and mortality rates between the two racial groups. It is also aimed at understanding whether BMD is associates with prostate cancer in the two racial groups. The aims of the current study therefore are to: 1) investigate allele frequencies of polymorphisms of *IL-1* and *IL-6* genes among African Americans and Caucasians, 2) determine whether genotypes of *IL-1* and *IL-6* are associated with prostate cancer in the two racial groups, and 3) determine if bone mineral density is associated with prostate cancer in African Americans and Caucasians.

2.0 REVIEW OF THE LITERATURE

2.1 EPIDEMIOLOGY OF PROSTATE CANCER

Prostate cancer is a major public health problem in the United States. It is the most common nonskin cancer, and the third leading cause of cancer related death among men in the United States[1]. According to the American Cancer Society, there will be approximately 186,320 new cases of prostate cancer in the United States in 2008; in the same year 28,660 men will die from the disease[1]. African Americans are more likely to be diagnosed with and die from the disease as are White Americans [2]. Figure 2.1 shows age-adjusted prostate cancer incidence and mortality rates among AA and Caucasians men ages 50 years and older (1974 to 2004; constructed using SEER 9-registry data). While socioeconomic and hormonal differences are thought to be contributory factors [2-4], the role played by differences in sequence variants of cytokines in the inflammation pathway of these two populations have not been comprehensively examined as part explanation for these disparities.

Recently research efforts have focused on the important role of inflammation in the pathogenesis of prostate cancer. Chronic or recurrent inflammation is known to increase the incidence of malignancies of the bladder, colon, endometrium, esophagus, liver, lung and pancreas [5-9]. Similarly, evidence from epidemiologic, genetic, molecular biology and histopathology studies have suggested a compelling role of inflammation in the development of

prostate cancer [10-13]. The precise mechanism by which inflammation causes cancer is not clearly understood, but it is thought that chronic or recurrent inflammation, which may be a result of immunological conditions, recurrent microbial infections, or chemical irritation, trigger the production of inflammatory cytokine mediators and genotoxic reactive oxygen radicals that increase cell proliferation and promote tumorigenesis [14]. The likelihood of developing cancer may then be dependent upon host response to this inflammatory cascade [15].

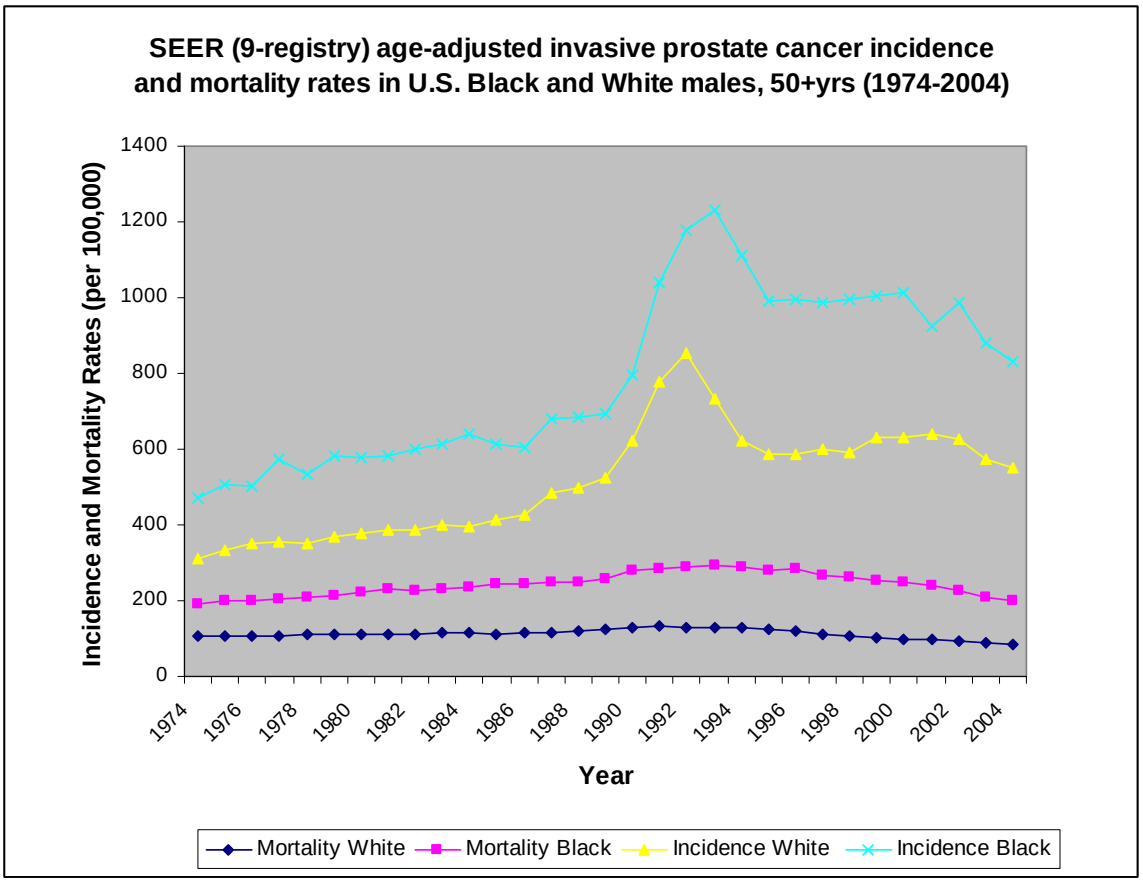


Figure 2.1: Prostate cancer incidence and mortality rates

Efforts by numerous investigators to identify genes in the inflammation pathway that may be involved in prostatic carcinogenesis have partly elucidated the role of a variety of important susceptibility genes and cytokines in prostate cancer pathogenesis [16-20]. The *interleukin-1* (*IL-1*) and *IL-6* family of genes have been reported to be associated with prostatic tumorigenesis [21, 22]. Sequence variants in the *interleukin-1* receptor antagonist *(IL-1RN)* were reported to be associated with prostate cancer risk in a population-based study conducted in Sweden by Lindmark et al [21]. Additionally, endogenous *IL-1* has been reported to promote the invasiveness of malignant cells of the prostate by initiating and completing the process of angiogenesis [23]. *Interleukin-6* regulates the growth and differentiation of prostate carcinomas [24]. Sivashanmugam et al have shown that *IL-6* is involved in the initiation and progression of prostate cancer by mediating the lysophosphatidic acid-regulated cross-talk between stromal and epithelial cells of the prostate gland [25]. The important role of *IL-6* in this regard is evidenced by the fact that neutralization of *IL-6* activity abrogates the conditioned medium (CM) induced mitogenic extracellular signal regulated kinase 1 and 2 (ERK) and signal transducer and activator of transcription 3 (STAT3) in LNCaP cells [25]. Additionally, clinical studies have shown that elevated circulating plasma levels of IL-6 and its soluble receptor are associated with prostate cancer progression and metastasis [26-28].

In addition to playing an active role in the initiation and progression of prostate cancer, *IL-1* and *IL-6* have been shown to influence bone mineral density [29, 30]. Both of these cytokines are known to decrease bone mineral density by resorption of bone matrix through osteoclast activity [31, 32]. Furthermore, serum levels of *IL*-1 and *IL-6* have been found to be associated with bone loss [33, 34]. There are estrogen-dependent changes in the production of these two genes which potentially modify their bioactivity. As a result of decreasing estrogen levels with age, the bone

resorption activity of *IL-1* and *IL-6* increases thereby accelerating the rate of bone loss; however administration of hormone replacement therapy decreases the rate [29]. In addition to estrogen, other hormones such as testosterone, parathyroid hormone, and insulin-like growth factor 1 (IGF-1) are known to influence bone mineral density by regulating *IL-1* and *IL-6* related osteoclast activities [35-38]. Moreover, these same factors, as well as others, such as high calcium intake, and low vitamin D levels, are considered risk factors for prostate cancer [35, 39-44].

To ascertain the long term effect of serum levels of *IL-1* and *IL-6* on the pathogenesis of prostate cancer, there is the need to obtain serial measurements over many years, but this has been difficult, resulting in discrepant findings by various epidemiologic studies [26-28, 45]. Since bone mass reflects a lifetime exposure to *IL-1* and *IL-6*-related osteoclast activity, bone mineral density should serve as a possible surrogate marker for cumulative exposure to these pro-inflammatory cytokines, as well as prostate cancer risk.

2.2 PROSTATE CANCER BIOLOGY

The prostate gland is part of the genitourinary system in men, and surrounds the neck of the male bladder and urethra[46]. It is partly glandular and partly muscular, and has ducts which open into the prostatic portion of the urethra. It measures approximately 3cm x 4cm x 2cm (the size of a walnut), and weighs about 20gm in adults[46]. The exact function of the prostate gland has not been fully defined; however, epithelial cells lining the prostatic glandular acini secrete fluid that becomes a component of seminal fluid[47]. Additionally, these luminal epithelial cells secrete

prostate specific antigen (PSA), a protease that cleaves seminal proteins likely to maintain the fluidity of seminal fluid[47].

2.2.1 Anatomy of the Prostate

There are no true lobar structures in the adult prostate; the generally accepted zones are as described by McNeal, and consist of a peripheral zone representing 70% of the glandular bulk, a central zone which forms 20% of the glandular weight, a transitional zone of 5% of the gland, and a non-glandular anterior fibromuscular zone of stroma[48, 49]. The peripheral zone comprises all the apical and most of the subcapsular area, representing the region of cancer susceptibility. The central zone is thought to be of Wolffian ductal origin and less than 1% of all prostate cancers arise from this zone[46]. Most benign prostate hyperplasia (BPH) forms in the transitional zone[11], which is located in a para-urethral position in the mid-prostate. Carcinoma in this region is uncommon (<20% of all cancers), although it represents isolated tumor formation noted histologically following transurethral prostate resection[46]. The boundary between the transitional zone and the peripheral zone forms the basis of the 'capsule' morphologically noted between benign and malignant-bearing tissues[46]. The prostate rests on the pelvic diaphragm, and communicates with a complex network of blood supply and lymphatic drainage in the hypogastric region. This communication network explains the metastatic spread of prostatic carcinoma to the sacrum, ileum, and lumbar spine.

2.2.2 Histological Features

Greater than 70% of prostate cancers are adenocarcinomas that arise from the peripheral zone[50]. Microscopic foci of latent prostate cancer are found commonly on autopsy, but may appear early in life[51]. Approximately 30% of men over 50 years of age have evidence of latent disease, but because of the very slow growth rate of these microscopic tumors, many never develop to clinical disease[50]. In the normal prostate, each acinus consists of a layer of luminal columnar epithelial cells and a layer of basal cells, surrounded by stromal tissue, including smooth muscle[47, 52]. These acini are connected to each other, and form a ductal system that empties into the prostatic urethra. It is uncertain which cells are at risk of becoming cancerous, but it has been suggested that a cell with a phenotype that is intermediate between a stem cell and an epithelial cell is most likely to undergo neoplastic transformation[53]. Foci of prostate cancer typically consist of a lining of tumor cells surrounded by a lumen. These acini are often small and have lost the characteristic papillary infoldings of an acinus, and the component tumor cells have large nuclei[47]. Once these cells become cancerous, they undergo architectural changes, the extent of which can be described by a histologic grading system referred to as the Gleason scoring system.

2.2.3 Grading

Grading is used to evaluate the aggressiveness of malignant neoplasm, and is based on the idea of cell differentiation. Differentiation refers to the extent to which cells resemble comparable normal cells. Well –differentiated tumors are composed of cells resembling the mature normal cells, poorly differentiated tumors have unspecialized cells[46]. The Gleason system is the most

widely used classification, and it evaluates the architecture of the neoplasia and its relation to the stroma. It defines five patterns and considers a primary (or predominant) and a secondary (or least –abundant) pattern, thereby defining a total score or sum ranging from 2 to 10. Typically a Gleason score of seven or greater is considered to be histologically poorly differentiated.

2.3 RISK FACTORS

There are many risk factors for prostate cancer; these include age, race, androgens, family history, diet, obesity, sexually transmitted infections, vitamin D deficiency, and benign prostatic hyperplasia[47]. The strength of association of each of these risk factors with prostate cancer varies from one risk factor to another, as shown in Table 2. Brief discussions of a few of the strong risk factors are discussed below, other risk factors are incorporated in subsequent text.

2.3.1 Age

Age is the strongest factor influencing the development of prostate cancer; clinical disease rarely occurs before age 40 years[46], and the incidence increases markedly after age 60 years[50].

2.3.2 Race

There are wide disparities in prostate cancer incidence and mortality rates in the United States. African-Americans have approximately twice the incidence and mortality rates as Caucasians[54]. Additionally, African-Americans appear to develop the disease at an earlier age[50]. Prostate

cancer incidence and mortality rates for Asian/Pacific Islander, American Indian/Alaskan Native, or Hispanic are substantially lower than those for Caucasians[54].

2.3.3 Family history

Prostate cancer risk increases between two- and threefold in men with a first degree relative (brother or father) in whom the disease was diagnosed at an early age[55]. The risk is even higher with increasing number of first degree relatives with the disease[56]. The role of inheritance in the development of prostate cancer is further buttressed by findings of twin studies which have shown higher concordance for prostate cancer diagnosis among monozygotic than dizygotic twins [57].

2.3.4 Androgens

Men who have diminished androgen production due to castration, hypogonadism, or enzyme defects of androgen metabolism, such as 5-alpha reductase, have minimal risk for prostate cancer[58]. In a prospective cohort study, Gann et al reported that high pre-diagnosis levels of plasma testosterone was associated with prostate cancer, and an inverse trend was seen with increased levels of sex hormone-binding globuline[59].

2.3.5 Etiologic model

Figure 2.2 shows a proposed etiologic model which integrates host and environmental factors relevant to the current research that may be involved in the development of prostate cancer. In

this model polymorphisms of inflammatory cytokines such as *IL-1* and *IL-6*, which may be determined by race, influences cytokine concentration as well as an individual's susceptibility to infection, which may include sexually transmitted diseases (STDs). Infection of the prostate by a pathogen, whether viral or bacterial, causes cell damage and results in prostate inflammation, as well as elevation of cytokine levels. This process may be mediated by cytokine gene polymorphisms. Chronic or recurrent prostate injury and inflammation may result in the formation of proliferative inflammatory atrophy (PIA) lesions, and subsequently prostatic intraepithelial neoplasia (PIN), and prostate cancer. Race and age are established risk factors of prostate cancer. Levels of hormones such as androgens and estrogens are influenced by race and age. These sex steroid hormones are known to be associated with prostate cancer risk, but also, they are known to influence bone health. High androgen levels, for example, are associated with high BMD. On the other hand the inflammatory cytokines *IL-1* and *IL-6* decrease BMD by inducing osteoclasts to resorb bone. Therefore, BMD reflects long term interplay of opposing factors: those such as sex steroid hormones which cause bone formation, and others such as cytokines which cause bone resorption. Since the prostate gland is chronically under the influence of these sex steroid hormone and cytokines, BMD may therefore serve as a surrogate marker of long term exposure of the prostate gland to these hormones and cytokines.

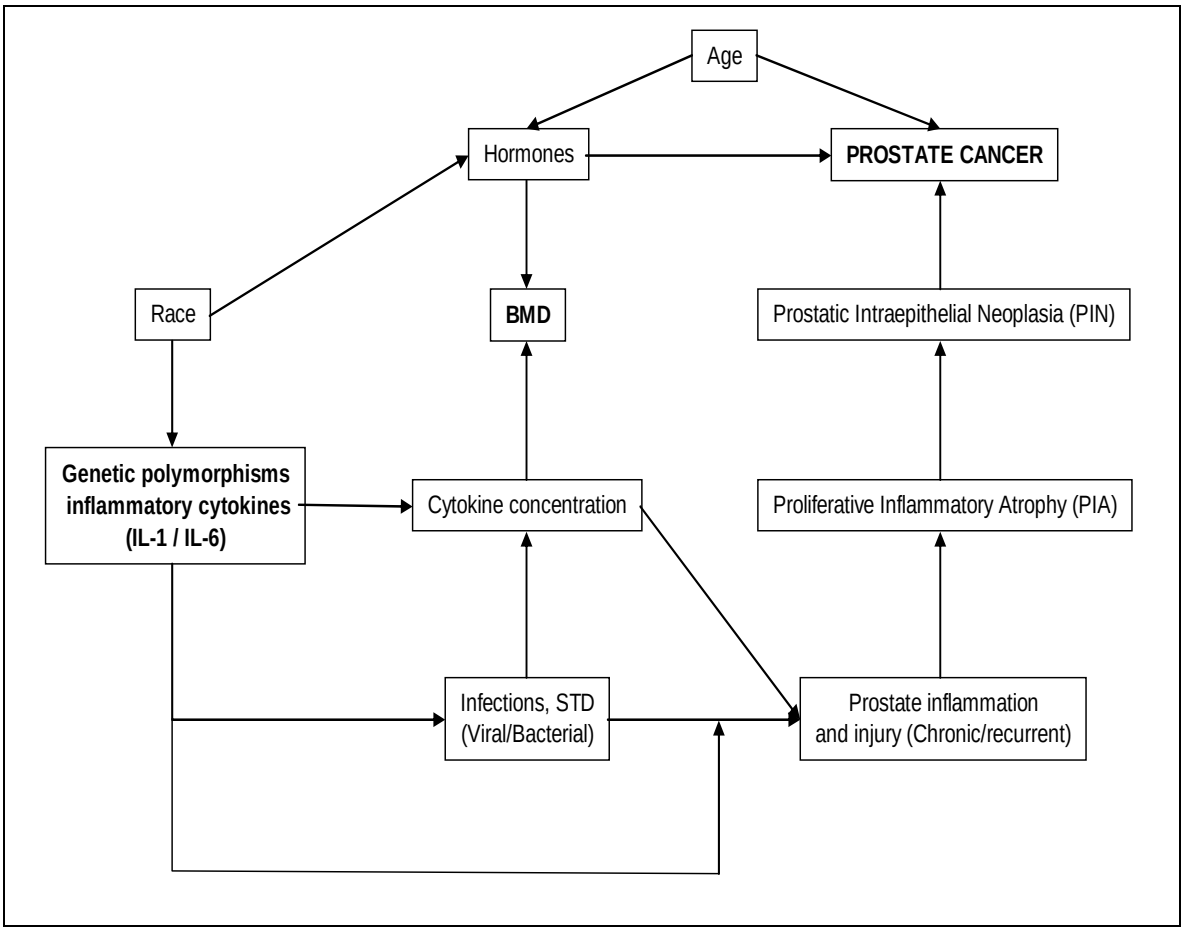


Figure 2.2 : Proposed etiologic model

2.4 PROSTATE CANCER DEVELOPMENT AND PROGRESSION

Androgens are believed to contribute to the development and progression of prostate cancer[47]. Androgen-dependent tumors can be successfully treated with androgen ablation therapy; however, the cancer eventually recurs as an androgen independent tumor and is no longer responsive to treatment[60]. Therefore, the progression from androgen-dependence to androgen-independence is a very important component of prostate cancer development. Recent studies

have shown that androgen-independent activation of the androgen receptor mediates the progression of prostate cancer in the absence of androgen[60, 61]. *Interleukin-6* is a pro-inflammatory cytokine that has been shown to regulate prostate cancer growth and to activate androgen receptor-dependent gene expression in the absence of androgen[60, 61]. Indeed, the important role of inflammatory cytokines in the development of cancer is not limited to the prostate gland, but to several other cancers[5, 8, 62].

2.4.1 Inflammation and Cancer

Chronic or recurrent inflammation is known to increase the incidence of malignancies of the bladder, colon, endometrium, esophagus, liver, lung and pancreas[5-9]. Even though infectious agents and environmental conditions are involved in several of these cancers, inflammation often increases cancer development even further by collaborating with environmental conditions such as dietary derived toxins[63]. It has been suggested that inflammatory cells and cytokines found in tumors are more likely to contribute to tumor growth, progression, and immunosuppression than they are to mount an anti-tumor effect [6].

The exact mechanism by which inflammation causes cancer is unclear, but it is thought to comprise a complex series of events involving the innate and adaptive immune systems [14, 62, 64-66]. Activated phagocytic inflammatory cells of the innate immune system are known to release highly reactive chemical compounds, which includes superoxides, hydrogen peroxide, singlet oxygen and nitric oxide, which causes oxidative or nitrosative damage to DNA in the epithelial cells, or react with other cellular components such as phospholipids, thereby initiating a free-radical chain reaction[67]. These events result in host epithelia cell damage or death, and in order

for the epithelium to maintain its barrier function, resident progenitor and/or stem cells undergo cell division to replace the damaged or dead cells. Epithelial cells that undergo DNA synthesis in the setting of these DNA damaging agents are at an increased risk of mutation[67].

One mechanism by which inflammatory cells aid disease progression is by migration through the extracellular matrix resulting from the release of proteolytic enzymes. Facilitation of epithelial cell invasion into the stromal and vascular compartments ultimately results in the metastasis of tumor cells [64, 65]. In another mechanism, the disruption of cytokine production and regulation, including cytokine deficiencies, leads to increased inflammation and cancer, whether in response to an infectious agent or to chemical carcinogens [68]. Additionally, cell-mediated anti-tumor immune surveillance mechanisms can be dampened by certain immune responses, thereby averting an immune reaction against the tumor that could potentially eliminate the cancer [69].

The important role of oxidant and nitrosative stress in prostate carcinogenesis is evidenced by epidemiologic data which has shown that consumption of certain dietary antioxidants is associated with reduced prostate cancer risk. For example, several epidemiological studies have correlated low selenium with an increased risk of prostate cancer [70-73]. Additionally, a randomized, placebo controlled clinical trial of selenium supplementation for the prevention of recurrent nonmelanoma skin cancer (the Nutritional Prevention of Cancer Study) showed a decrease in incident prostate cancer (overall RR 0.51, 95% CI 0.29-0.87), especially in men with low selenium levels at study entry[74, 75]. Inflammatory cells are also known to secret cytokines that promote epithelial cell proliferation and stimulate angiogenesis[23, 76]. In human multiple myeloma the malignant cells are home to the bone marrow where they stimulate stromal cells to secrete the inflammatory cytokines IL-1, IL-6 and TNF. The cytokines stimulate myeloma cell growth and promote resistance to therapy [77]. In mouse models of metastasis,

treatment with IL-1RN significantly decreases tumor development. This suggests that local production of IL-1 aids in the development of metastasis, because IL-1RN is known to inhibit the actions of IL-1[78]. Furthermore, mice deficient in IL-1B were found to be resistant to the development of experimental metastasis [79].

2.4.2 Inflammation and Prostate Cancer

The role of inflammation in the development of prostate cancer has been examined by several epidemiologic studies through the assessment of the association between prostatitis, and sexually transmitted infections with the disease [80-85]. Additionally, genetics and molecular pathology studies have increased current understanding of the role of inflammation in prostate carcinogenesis [57, 86-90]. Much of the evidence has arisen from studies which assessed the use of nonsteroidal anti-inflammatory drugs (NSAIDS) in prostate cancer, but results have been inconsistent[91-96].

2.4.3 Prostatitis and prostate cancer

Prostatitis manifests clinically with symptoms of dysurea, perineum pain, painful ejaculation, and urinary frequency [97]. It may be classified as acute or chronic based upon National Institute of Health (NIH) consensus classification [98]. In this classification, there are four distinct categories of prostatitis namely: acute bacterial, chronic bacterial, chronic prostatitis/chronic pelvic pain syndrome and asymptomatic inflammatory[98]. This classification was instituted in 1999 by the NIH in an effort to better formalize the diagnostic criteria of prostatitis [98]. Acute bacterial

prostatitis is usually caused by Escherichia coli (E. coli)[99], whereas chronic prostatitis may be caused by several other organisms including E. coli [100, 101].

Even though several epidemiologic studies have reported an association between prostatitis and prostate cancer [80-85], there are a number of factors which makes the exact role of inflammation difficult to study: first, the incidence of prostatitis is uncertain. While the incidence of prostatitis in men aged 40 years and over is between 5-10% there are many men with the condition who are asymptomatic[102]. Moreover, men with symptomatic prostatitis are diagnosed with prostate cancer more frequently due to increased frequency of biopsy[103, 104]. Finally, the offending pathogen is often unknown in many cases of symptomatic prostatitis[105, 106]. These findings appear to imply that host inflammatory responses rather than the cause of the inflammation (infectious agent or chemical compounds) leads to cancer development.

2.4.4 Sexually transmitted infections (STI) and prostate cancer

An association between sexually transmitted infections and prostate cancer has been reported by several epidemiologic studies; while some of these studies relied on self-report[107, 108], others were based on serologic markers[107, 109-111] and prostate tissue[112, 113].The direction and strength of association noted by these studies may be confounded by various factors: the case control studies, and also those which assessed STI status based on interviewing and self-report were subject to interviewer and recall bias. Also, the use of antibiotics to treat common STI such as gonorrhea, Chlamydia, and syphilis is likely to markedly reduce the incidence of prostatitis caused by these agents. Moreover, infectious agents with oncogenic properties, such as human papillomavirus (HPV) may influence prostate carcinogenesis independent of inflammation [13].

Nevertheless the odds of developing prostate cancer in men with a history of gonorrhea or syphilis increases from 1.6 to 3.3 with three or more episodes of gonorrhea[107], indicating that recurrent inflammation may be mediating development of the disease.

2.4.5 Molecular pathology

There are certain characteristic features of the molecular pathogenesis of prostate cancer which highlight the role inflammation in the development of the disease: these include the somatic inactivation of gluthathione S-transferase pi gene (GSTP1) and the strong possibility that (proliferative inflammatory atrophy) PIA lesions are prostate cancer precursors[114-116]. Gluthathione is a tripeptide with antioxidant properties, whose conjugation to various reactive chemical species, including oxidants is catalyzed by the enzyme gluthathione S-transferase (GST)[116]. Silencing of *GSTP1* transcription accompanies somatic CpG island hypermethylation; this results in the loss of GSTP1 expression, and is almost always accompanied by the development of prostate cancer[116]. Expression of GSTP1 is typically induced to high levels at sites of prostatic inflammation and loss of GSTP1 expression is characteristic of prostatic intraepithelial neoplasia (PIN) lesions and prostatic carcinoma[115, 116].

2.4.5.1 Proliferative inflammatory atrophy

Proliferative inflammatory atrophy (PIA) is a term used to describe focal prostate lesions comprised of prostate epithelial cells which are atrophic but have a high proliferative index[116]. These cells show many signs of stress, which include the induction of *GSTP1* and cyclooxygenase-2 (COX-2) expression, as well as features of cells thought to be intermediate in

the differentiation between basal epithelial cells and columnar cells[53, 116-118]. PIA lesions are often found near early adenocarcinoma lesions[119] and high grade prostatic intraepithelial neoplasia (PIN), which are precursor cancer lesions[120]. Furthermore, *GSTP1* CpG island hypermethelation, which is present in 90% of prostate cancer cases, has been found in 6.3% of PIA lesions[121]. Loss of *GSTP1* function has the tendency to mark the transition between PIA lesions and PIN lesions; which is consistent with the possibility that compromised defenses against inflammatory oxidants may initiate carcinogenesis of the prostate[114].

2.4.6 Genetics

Of all human cancers, prostate cancer has been reported to show the greatest hereditability risk [57, 86-88]. The role played by genetics in the development of prostate cancer has been suggested by segregation analyses and linkage studies of familial prostate cancer, which have hinted at specific prostate cancer susceptibility genes; as well as by twin studies which have compared prostate cancer incidence between monozygotic and dizygotic twins[57, 86, 122, 123]. However, the molecular pathogenesis of prostate cancer displays a great deal of heterogeneity between individuals as well as within the affected organ[124]. Currently, the identified somatic gene abnormalities associated with prostate cancer are very diverse, implying that there is not a single dominant molecular pathway required for prostate cancer development[114, 124]. Besides somatic genes, numerous germline prostate cancer susceptibility genes have been identified[125-127]. Support for the role of inflammation in the pathogenesis of prostate cancer is further buttressed by linkage and association studies of genes encoding factors involved in infection response,

oxidant defense enzymes, and inflammatory cytokines. These include *RNASEL*, *MSR1, MnSOD, hOGG1, IL-1, and IL-6*.

2.4.6.1 *RNASEL*

Ribonuclease L (*RNASEL*) is a gene in the innate immune system which encodes a widely expressed latent endoribonuclease that is involved in interferon-inducible RNA degradation pathway[128]. It is activated upon viral infection and has been reported by some studies as a candidate prostate cancer susceptibility gene[17, 18, 128], but not by others[129]. A study by Carpten et al found that variant *RNASEL* alleles Glu256X and Met1Ile encoded defective enzymes and were linked to prostate cancer in certain families[18]. The Arg462Gln allele also encodes a defective *RNASEL* enzyme which has three times less enzymatic activity than the wildtype[130]. Casey et al reported that the fraction of prostate cancer in the population attributable to the Arg462Gln *RNASEL* allele was approximately 13%[130]. Rokman et al noted a higher risk of familial but not sporadic prostate cancer for Gln homozygotes[131]. Furthermore, a recent study by Urisman et al identified a novel gammaretrovirus named Xenotropic MuLV-related virus (XMRV) in stromal cells of 40% of prostate cancer patients homozygous for the R462Q variant of *RNASEL*[132]. The exact mechanism by which defects in an interferon inducible RNA degradation pathway causes prostate cancer is not certain, but is thought to result in decreased interferon-alpha antiviral activity and deficiencies in induction of apoptosis [133]. Shea et al reported no significant association between *RNASEL* or *RNASEL*-inhibitor polymorphisms and prostate cancer risk in the Afro-Caribbean population[129].

2.4.6.2 *MRSI*

The macrophage scavenger receptor-1 (*MSR1*) is a macrophage plasma membrane spanning protein that is capable of binding a variety of ligands, including bacterial lipoteichoic acid, as well as oxidized high-density lipoprotein and low density lipoprotein in the serum[134]. The MSR1 gene is located on 8p22, an area of frequent allelic loss in prostate cancer [19, 134]. Rare germline MSR1 mutations have been linked to prostate cancer susceptibility in some families at high risk for prostate cancer [19]. In addition, the nonsense mutation Arg293X has been detected in approximately 3% of men with sporadic prostate cancer compared to 0.4% of unaffected men (p=0.047)[19]. A population case-control study of African American men found the Asp174Tyr *MSR1* allele in 6.8% of prostate cancer cases and 3.6% in non-cases (p=0.14)[20]. The exact mechanism by which defects in macrophage function might lead to prostate cancer is not clearly understood, however, mice models show vulnerability to Listeria monocytogenes, Staphylococcus aureus, E. coli, and Herpes simplex virus type 1[134-136].

2.4.6.3 *MnSOD*

Manganese superoxide dismutase (*MnSOD*) encodes a mitochondrial enzyme that protects cells against oxidative damage[137]. Reactive oxygen species produced during chronic inflammation and other mechanisms involving oxidative stress are thought to play an important role in prostate carcinogenesis[137]. Superoxide dismutases are located in several human organs, including the prostate gland [138, 139]. In the Alpha-Trocopherol, Beta-Carotene Prevention Study, the Ala16Val allele of *MNSOD* was associated with an increased risk of prostate cancer (OR 1.72, CI 0.96-3.08 for AlaVal homozygotes)[140]. Variants of hoGG1, another gene involved in repair of oxidative genome damage, were found to be associated with increased prostate cancer risk [141].

2.4.7 NSAIDS and Aspirin use and Prostate Cancer

Several recent studies have evaluated the association of aspirin and NSAIDs use and prostate cancer risk[91, 93, 94, 142-144]. Overall, the results of these studies show that aspirin and NSAIDS have a protective effect against prostate cancer; however, there are apparent inconsistencies. The retrospective studies conducted in Canada and France found no significant association between NSAID use and the risk of prostate cancer whereas the prospective studies conducted in the U.S. found significant associations. A possible explanation for the lack of association in the retrospective studies may be detection bias. Participants who use NSAIDS on a regular basis for many years are likely to be health conscious people who also receive frequent medical care, and are therefore more likely to be screened for prostate cancer. Detection bias may also account for the observed reduction in advanced cases of prostate cancer among aspirin users as these patients are more likely to be diagnosed at the early stage of the disease. This is because regular and long term aspirin use is likely to be a result of physician prescription, and patients under routine physician care are more likely to be screened for prostate cancer, leading to early detection. The prospective studies conducted in the U.S all showed a statistically significant protective effect of aspirin and NSAID against prostate cancer, except for the study by Leitzmann et al. They reported a protective effect only for those with metastatic disease who took aspirin for a minimum of 22 days each month, but this was not statistically significant. Besides the studies conducted by Perron et al and Jacobs et al, none of the other studies appears to have directly addressed the effect of dosage and duration of NSAIDS/aspirin use in relation to prostate cancer. Perron's study utilized information from the Quebec health insurance system database, Regie de l'assurance maladie du Quebec (RAMQ), which keeps detailed prescription medication

reimbursement records of residents of Quebec aged 65 years and older. It was a well powered study, and had access to detailed exposure data, and reported a stronger inverse association for larger doses of aspirin, but only among those who used it for 4 years or more. However, because the study relied on database information it could not account for non-prescription NSAIDS or aspirin use, a factor which could have influenced the results. The study by Jacobs et al was a well powered prospective study which obtained detailed dosing and duration information, and found an inverse association among participants who used at least 30 pills of aspirin or NSAIDS each month for a minimum of 5 years. Roberts et al also found an inverse association, which increased with age, but they did not address the effect of dosing. Also, like other prospective studies, information on the use of aspirin and other NSAIDS were obtained at study beginning only, however, reported aspirin/NSAIDS use may change over the course of a study, and this can potentially result in misclassification. In spite of these pitfalls, there is overwhelming evidence from these studies that the use of aspirin and other NSAIDS reduces the risk of prostate cancer, thus buttressing the role of inflammation in prostate carcinogenesis.

2.5 INFLAMMATORY CYTOKINES AND PROSTATE CANCER

2.5.1 *INTERLEUKIN-6 (IL-6)*

The *IL-6* gene is located on chromosome 7p21[145]. It contains four introns and five exons, and encodes a precursor protein consisting of 212 amino acids with two intrachain disulphide bridges and a 28-residue hydrophobic signal sequence, which has a molecular mass of 21-28 kDa[145, 146].

The gene is expressed in diverse cell types including fibroblasts, endothelial cells, keratinocytes, monocytes, macrophages, T-cells and B-cells, and a variety of tumor cells including the prostate gland[147]. *IL-6* is not constitutively produced under normal circumstances, but its expression is readily induced in response to several stimuli, such as, viral infections, exposure to lipopolysaccharides, and to other cytokines including *IL-1*, TNF-alpha, platelet-derived growth factor, *IL-3* and granulocyte macrophage-colony stimulating factor[148-152]. Both protein kinase C- and cAMP-dependent signal transduction pathways are involved in *IL-6* gene induction[151, 153].

Interleukin-6 is involved in regulating immune and inflammatory responses[154]. In addition to inducing terminal differentiation of B-cells it synergizes with *IL-1* in activating T-cells by inducing *IL-2* responsiveness, and enhances the differentiation of cytotoxic T lymphocytes from thymic precursors[146, 155, 156]. It has a central role in the acute-phase response, acting on hepatocytes to increase the synthesis of acute-phase proteins (haptoglobin, fibrinogen, C-reactive protein,etc) and reducing the secretion of albumin and transferein[157]. It also contributes to the body's defenses by increasing the body temperature and stimulating the release of adrenocoticotropin hormone[158-160]. Other functions include: impairs natural killer cell function; induces bone resorption; stimulates osteoclast formation; induces experimental cancer cachexia; induces platelet-derived growth factor in blood vessels; enhances proliferation of vascular smooth muscle; negative inotropic effect on cardiac myocites; enhances secretion of chorionic gonadotrophin from trophoblasts[154].

2.5.1.1 *Interleukin-6* receptors

Interleukin-6 receptors are found in a wide variety of cell types including epithelial cells, neural cells, fibroblasts, hematopoietic cells, neural cells, B and T cells, macrophages and prostate[146].

The *IL-6* receptor consists of two molecules, an 80-kDa *IL-6* binding protein (alpha chain), and a 130-kDa signal transducer, gp130 (beta-chain)[161, 162]. Cytokine receptors lack intrinsic kinase activity, therefore the IL-6R must interact with another molecule that is capable of recruiting secondary intracellular messengers. Binding of *IL-6* with *IL-6R* triggers the association of *IL-6R* with 2 copies of a 918-amino acid transmembrane protein known as gp130[163]. Binding of *IL-6R* and gp130 stabilizes the interaction between receptor and ligand, resulting in an apparent increase in binding activity[163].

2.5.1.2 Intracellular Signal Transduction

The first step in the intracellular signal transduction cascade is the homodimerization of gp130, which is triggered by *IL-6/IL-6R* binding leading to recruitment and activation of non-receptor protein tyrosine kinases. Activation of 2 distinct pathways then occur:

1) The Janus Kinase (JAK) – signal transducers and activators of transcription (STAT) pathway.
2) The mitogen-actvated protein kinase (MAPK) pathway.

2.5.1.3 The JAK-STAT transduction pathway

The Janus kinase (JAK) family of kinases contain both a kinase and a pseudokinase domain in series[164]. Once activated, this family of kinases phosphorylates and activates STAT transcription factors, particularly STAT3, which then moves into the nucleus to activate transcription of genes containing STAT3 response element. A series of intracellular events may lead to activation of the MAPK pathway. MAPK in turn activates other downstream factors, including additional transcription factors such as serum response factors (SRF). These factors respond to many other

signaling pathways, but together they regulate a variety of complex promoters and enhancers that respond to *IL-6* and other signaling factors. Besides the JAK-STAT and MAPK transduction pathways *IL-6* is thought to mediate cellular activities through other pathways such as the phosphatidylinositol 3-kinase pathway[165].

2.5.1.4 *Interleukin-6* and disease

In 1987 Hirano et al demonstrated the possible involvement of the deregulated expression of the *IL-6* gene in polyclonal B-cell abnormalities in patients with cardiac myxoma[166]. Since then, much evidence has shown that deregulation of *IL-6* production could be involved in a variety of diseases, including autoimmune, inflammatory and malignacies[146]. Polymorphisms of *IL-6* have been shown to be associated with various cancers, such as cervical, oral, colorectal, ovarian cancer, prostate cancer and plasmacytoma[22, 167-171].

The attention of investigators were drawn to the role of *IL-6* in prostate cancer development based on observations by that the disease transitioned from an androgen-dependent tumor, initially responsive to androgen ablation therapy, to an untreatable androgen independent tumor[60]. In-vitro studies by Siegall et al showed that the androgen-independent cell lines DU145 and PC3 and the androgen-dependent cell line LNCaP expressed *IL-6R* on their surfaces, and all three cell lines were susceptible to a chimeric Pseudomonas exotoxin-*IL-6* toxin. Susceptibility was mediated by *IL-6R* as cytotoxic activity was blocked in the presence excess human recombinant *IL-6*[172]. Since then, the expression of mRNA for IL-6R and the gp130 signal transducer has been confirmed in human prostate cancer by other investigators[172-174]. Furthermore, recent in-vitro studies have shown that *IL-6* initiates and promotes prostate tumorigenesis by mediating cross-talk between stromal and epithelial cell of the prostate[25].

Sivashanmugam et al used a co-culture cell assay to identify messangers involved in the cross-talk between human prostate stromal PS30 and epithelial LNCaP cells. After stimulation with lisophosphatidic acid (LPA), the mitogenic extracellular signaling regulated kinase (ERK) signaling pathway in PS30 were activated, but not LNCaP[25]. Co-culture of PS30 and LNCaP cells resulted in the activation ERK in LNCaP that was further increased in response to LPA. When animals were implanted with a mixture of both PS30 and LNCaP tumor cells, they developed larger tumors with higher frequencies compared to LNCaP cells alone. Protein analysis demonstrated that treatment of the PS30 cells with LPA induced synthesis of *IL-6*. By antibody neutralization experiments, it was determined that *IL-6* is responsible for the LPA-induced mitogenic signaling and growth of the LNCaP cells. A major finding of this study was that activation of G protein-coupled receptors (GPCRs) expressed in prostate stromal cells induced the secretion of factors that promoted mitogenic signaling of epithelial cells. Therefore, GCPRs regulate the cross-talk between stromal and epithelial prostate cells, which plays an important role in the initiation and progression of prostate cancer. Interleukin-6 is a major mitogenic growth factor secreted from PS30 cells in response to stimulation with the GPCR ligand LPA[25]. Other in-vitro studies have indicated that *IL-6* induces the progression of prostate tumor epithelial cells[175, 176]. Additionally, several investigators have reported elevated serum levels of *IL-6* upon progression of prostate cancer to androgen independence[26].

2.5.1.5 Serum *interleukin-6* and prostate cancer

The correlation between serum *IL-6* levels and clinical features of prostate cancer has been evaluated by several studies. Twillie et al reported elevated serum *IL-6* levels in 47% of 73 patients with advanced hormone refractory prostate cancer[177]. They measured *IL-6*

concentrations in the ejaculate plasma of healthy men, in primary culture of prostate epithelial cells, in human prostate cancer cell line cultures and in severe combined immunodeficiency mouse xenografts and in the plasma of 73 men with metastatic adenocarcinoma of the prostate, and reported that elevated *IL-6* levels are strongly correlated with objective measures of morbidity[177]. Drachenberg et al assessed the potential of serum *IL-6* levels as a marker of disease in 407 men including 15 controls. They found significantly higher *IL-6* levels in patients with clinically evident hormone-refractory prostate cancer compared to normal controls, as well as those with prostatitis, BPH, localized, and recurrent disease ($p<0.01$)[178]. Wise et al showed that serum *IL-6* levels, and that of other cytokines *IL-4* and *IL-10* were significantly higher among patients with hormone refractory prostate cancer compared to those with hormone-controlled disease ($p=0.02$, 0.01, an 0.0001 respectively)[179]. Nakashima et al evaluated the prognostic significance of serum *IL-6* levels in 74 prostate cancer patients: 23 stage A, 14 stage B, and 37 stage C. They found that serum *IL-6* was significantly correlated with clinical stage of prostate cancer; moreover, a serum *IL-6* concentration of >7 pg/ml was associated with a clinically poorer survival in stages C and D ($p=0.024$)[180].

Another study by Shariat et al assessed plasma *IL-6* and soluble *IL-6R* levels in 44 healthy patients without cancer, 19 men with prostate cancer metastatic to regional lymph nodes, and 10 men with bone-scan proved metastatic prostate cancer. They reported that *IL-6* and soluble *IL-6R* concentrations were significantly higher in patients with bony metastases compared to those with only node-positive disease ($p<0.001$), and in those with nodal metastases compared to organ confined disease and healthy controls ($p=0.042$ and 0.034 respectively). In another cohort of 120 consecutive patients presenting for radical prostatectomy, they found that *IL-6* and soluble *IL-6R* concentrations were associated with a Gleason sum on final histology of

>7(p=0.042 and 0.034) and with prostatic tumor volume (p=0.048 and 0.043). On multivariate analysis the preoperative concentration of soluble *IL-6R* but not *IL-6* predicted postoperative biochemical progression (p<0.040), and also preoperative levels were highest in those with aggressive disease[181]. In a recent study by Michalaki et al, serum levels of *IL-6* and *TNF-alpha* were found to correlate with clinicopathological features in 80 prostate cancer patients and 30 controls. Serum *IL-6* levels in patients with metastatic disease (9.3+/- 7.8 pg/ml) were higher than those in patients with localized disease ((1.3+/-0.8 pg/ml, p<0.001). The levels of both cytokines correlated with the extent of disease[26].

The findings of the aforementioned studies have been consistent in their report of a correlation between serum *IL-6* levels and the clinical features of prostate cancer; however, elevated serum *IL-6* levels may be reflective of the body's inflammatory response to prostate cancer, rather that a cause of the disease. Therefore, the results of these studies may not be interpreted as necessarily confirming a pathogenic role for *IL-6* in prostate carcinogenesis.

Besides serum *IL-6* levels, a few recent studies have examined the relationship between *IL-6* gene polymorphisms and prostate cancer risk, but with mixed result [12, 182-184]. Sun et al tested for an association between sequence variants of *IL-6* in 1,383 cases and 780 controls who participated in the Cancer prostate in Sweden study[185]. They reported no significant association between 6 SNPs and prostate cancer risk. This study was well powered due to its large sample size, but was limited by the number of tagging SNPs (total of 6) examined. In addition, the study population was homogeneous (all Swedish males), therefore the results may not apply to other races, such as African Americans who are known to have the highest rates of prostate cancer. Contrary to the findings of Sun et al, Tan et al. reported that the -174G to C polymorphism of the *IL-6* gene was associated with an overall increased risk of advanced prostate cancer[184]. In a

retrospective analysis of 95 prostate cancer patients, they reported that the distribution of the GC/CC genotype was significantly different between patients with stage T3-T4 tumors compared to those with stage T1-T2 ($p < 0.001$)[184]. They also reported that *IL-6*-174G to C polymorphism was strongly associated Gleason score ($p<0.001$). Additionally, this genotype was reported to be significantly associated with vascular invasion, seminal vesicle involvement, capsular invasion, recurrent disease, and serum PSA elevation. This study was comprised of 89% Whites, 6% Blacks, and 5% other; however there was no stratification of the analyzed data by race, therefore the extent to which race/ethnicity influences *IL-6* gene polymorphism, and subsequently prostate cancer morbidity in the study population could not be ascertained from the results. Furthermore, there was low power due to the small sample size. Nonetheless, this study successfully demonstrated a strong association of the -174G>C polymorphism of the *IL-6* gene with prostate cancer aggressiveness and recurrence, suggesting that genetic differences in the *IL-6* gene could be linked to prostate cancer morbidity.

In another study, Michaud et al examined SNPs in the genes encoding *IL-6, IL-1B, IL-8* and IL-10 in 503 prostate cancer cases and 652 controls enrolled in the Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) screening trial, to evaluate their possible role of genetic susceptibility within the inflammatory pathway in relation to prostate cancer[182]. A total of seven SNPs were evaluated in the four cytokines (*IL-6, IL-1B, IL-8* and *IL-10*), which included one SNP each in *IL-6* and *IL-8*; two SNPs in *IL-1B* and three SNPs in *IL-10*. They reported no association between these seven SNPs and prostate cancer risk. Findings were similar even after stratifying prostate cancer cases by stage and grade, as well as adjusting for NSAID use[182]. This study had a large number of cases, and the nested case-control design reduced potential bias, however, the number of SNPs examined was too few to draw a conclusion. For example, only

one SNP in the *IL-6* gene was examined; it is possible that other unmeasured SNPs in this and the other genes may be associated with prostate cancer. Furthermore, the PLCO is a screening trial, and prostate cancer is often detected early with PSA screening, therefore the results of this study may not be generalizable to men who are less likely to be screened and are diagnosed at a later stage in the disease.

Zheng et al evaluated 9,275 SNPs in 1,086 genes of the inflammation pathway to assess their association with prostate cancer among enrollees in the Cancer Prostate in Sweden study. They first conducted an exploratory stage analysis in 200 familial cases and 200 unaffected controls, followed by a confirmatory stage analysis of 1,223 cases and 676 controls selected randomly from the Cancer Prostate in Sweden study. After the exploratory stage analysis 26 SNPs including *IL-6R* were identified for confirmatory analysis in a larger group. Selection was based on the most significant p-value, allele frequency in controls similar to the reference group (CEPH- Utah residents with ancestry from northern and western Europe), when multiple SNPs within a gene were found to be significant only the most significant one was chosen. The confirmatory analysis resulted in three SNPs that were significantly associated with prostate cancer risk. One of these SNPs, cytokine receptor like factor-1 (CRLF1) was suggested to be a subunit of a cytokine receptor complex, and its mRNA was upregulated by several genes including *IL-6*[186].

This study was comprehensive in the number of SNPs analyzed, and had a large sample size to conduct both exploratory analysis in familial prostate cancer cases, as well as a confirmatory analysis in non-familial cases. It also provided an objective support for an association between prostate cancer and multiple modest-effect genes in the inflammatory pathway. However, the findings of the study are limited to Swedish men, and may not be

generalizable to other populations, such as African Americans, who are reported to have the highest prostate cancer incidence and mortality rates in the world.

A few population-based studies have assessed the relationship between *IL-6* polymorphisms and prostate cancer risk. Two of these studies were based on a homogenous Swedish population, and the other two were conducted in the U.S. Overall these studies reported inconsistent findings. Furthermore, none of the studies was specifically conducted to comprehensively evaluate racial differences in cytokine gene *(IL-1 and IL-6*) polymorphisms as part explanation for the disparities in prostate cancer risk in African Americans compared to Caucasians.

2.5.2 *Interleukin-1 (IL-1)*

The *IL-1* family of cytokines are comprised of two signaling agonists *IL-1 alpha (IL-1A)* and *IL-1 beta (IL-1B)*, and the *IL-1* receptor antagonist *(IL-1RN)* [187]. In spite of low sequence homology but high structural similarity, *IL-18* is now widely accepted as the fourth member of the *IL-1* family[187]. Besides these primary members, there are several other genes which have currently been accepted as members of the interleukin-1 superfamily based on structural similarity, but whose exact properties have not yet been ascertained; these have been assigned a new nomenclature using the expression IL-1F reflecting their being part of a family of related ligands [188]. Members of the IL-1 family are produced by a wide variety of cells, including blood monocytes, tissue macrophages, dendritic cell, B-lymphocytes and NK cell[188].

With the exception of *IL-18*, which resides on chromosome 11, all three known primary members of the IL-1 family are located within a 350 kb span on the q arm of chromosome 2[189].

IL-1A, IL1-B and *IL-1RN* share less than 25% identity with each other but bind to a common signaling receptor; however, *IL-18* shares approximately 18% identity with the other members of the *IL-1* family and binds to a distinct receptor[190]. Each of these primary members of the *IL-1* family is initially synthesized as a precursor molecule without a signal peptide. The N-terminal amino acids are then removed by special proteases, resulting in 'mature' peptides[188]. For example, the 31 kDa precursor form of *IL-1B* is biologically inactive and requires cleavage by a specific intracellular cysteine protease called *IL-1B* converting enzyme (ICE). ICE, which is also termed capsace-1, cleaves the *IL-1B* and *IL-18* precursors resulting in the mature form of *IL-1B* molecule (17.5 kDa) and of *IL-18* molecule (18 kDa) [191].

IL-1 and its related family members are primarily proinflammatory cytokines, and are known to initiate cyclooxygenate type 2 (COX-2), type 2 phospholipase A and inducible nitric oxide synthase (iNOS) in inflammation[187]. This accounts for the large amount of prostaglandin-E2 (PGE-2), platelet activating factor and nitric oxide (NO) produced by cells exposed to IL-1 or in animals or humans injected with *IL-1*[188]. Additionally, *IL-1* promotes the infiltration of inflammatory and immunocompetent cells into extravascular space by increasing the expression of adhesion molecule-1 (ICAM-1) on mesenchymal cells and vascular-cell adhesion molecule-1 (VCAM) on endothelial cells[188]. *Interleukin-1* facilitates angiogenesis by increasing the expression of vascular endothelial growth factor; thereby promoting tumor metastases and blood vessel supply[23].

2.5.2.1 *Interleukin-1* receptors

There are two primary *IL-1* receptors, *IL-1* receptor type 1 (*IL-1RI*) and *IL-1* receptor type 2 (*IL-1RII*); as well as one accessory receptor protein (IL-1R-AcP)[188]. The *IL-1RI* is an 80 kDa

glycoprotein which has three Ig-like domains on its extracellular segment, and a Toll-homology domain in the cytoplasm segment[192, 193]. The Toll- homology domain of the *IL-1R* is necessary for signal transduction[193]. Interleukin-1 signal transduction is initiated by binding of *IL-1* to one chain on the *IL-1RI*, and the formation of a heterodimer with a second, different receptor chain, termed *IL-1* receptor accessory protein (IL-1R-AcP)[194]. The formation of the heterodimer of the IL-1RI with the IL-1R-AcP results in the physical approximation of the Toll homology domains of each chain in the cytoplasmic segments and initiates signal transduction[188]. Of the three primary IL-1 family members (*IL-1A, IL-1B* and *IL-1RN*), *IL-1B* has the lowest affinity for the cell bound form of *IL-1RI*. It has an even lower affinity for the soluble form. *IL-1RN* has the highest binding affinity, and a slow, nearly irreversible off-rate to the cell bound *IL-1RI*[188].

The *IL-1RII* functions as a negative or 'decoy' receptor[195] . Its extracellular segment has three typical Ig-like domains, which includes a transmembrane segment and a short cytoplasmic domain which contains 29 amino acids in humans [196, 197]. The short cytoplasmic domain is unable to initiate signal transduction since there is no Toll-homology domain, therefore, when *IL-1* binds to the cell membrane, *IL-1RII* does not signal[197]. The binding affinity of *IL-1* to the type II receptor is greatest for *IL-1B*, followed by IL-1A and IL-1RN respectively[198] . The function of the type II receptor as a decoy receptor is based on the binding affinity of *IL-1B* to the cell surface form of this receptor, which prevents the ligand to form a complex with the type I receptor and accessory protein[195]. Additionally, the decoy receptor forms a trimeric complex of the *IL-1B* ligand with the type II receptor and the accessory protein, which serves to deprive the functional receptor type I of the accessory chain[199].

2.5.2.2 Signal transduction

Interleukin-1 binding causes activation of two kinases *IRAK-1* (IL-1 Receptor Associated Kinase-1) and *IRAK-2*. *IRAK-1* activates and recruits TNF associated factor-6 (*TRAF-6*) to the IL-1 receptor complex. *TRAF-6* in turn activates two pathways, one leading to activation of nuclear factor kappa beta (NF-kB) and another leading to c-jun activation[200]. A series of intracellular events results in the induction of cyclooxygenase-2 (COX-2) expression by *IL-1*, which occurs through the p38 and p42/44 MAP kinase pathways[201].

2.5.2.3 *Interleukin-1* and prostate cancer

The role of IL-1 in prostate carcinogenesis has been evaluated by assessing the effect of p38 activation on cell proliferation[202]. Ricote et al conducted immunohistochemical and Western blot analysis on 94 prostate samples comprised of normal, BPH and prostate cancer tissues. They reported that overexpression of p38 in BPH, and more intensely, in prostate cancer, enhances cell proliferation[202]. Furthermore, changes in the expression patterns of members of the IL-1 family have been reported as relating to prostate cancer progression[202-205]. Evaluation of 82 prostatic tissues to determine the relationship between these changes and prostate cancer progression showed that high expression levels of *IL-1A* and *IL-1RI* in epithelial cells of BPH and prostate cancer samples were involved in cell proliferation; and that the loss of immune-expression of *IL-1B* and *IL-1RN* was a characteristic feature of prostate cancer compared with normal prostate sample and BPH[203]. Interactions of sequence variants of genes in the inflammation pathway, including IL-1, have been found to be associated with prostate cancer risk [12, 183, 206]. In a recent case-control study, Sun et al analyzed 11 single nucleotide polymorphisms (four in IRAK1 and seven in IRAK4) among 1,383 newly diagnosed prostate cancer patients and 780 population

controls in Sweden. They reported that synergistic effects between variants IRAK4-7987 CG/CC and Toll-like receptor 6-1-10(TLR6-1-10) conferred an excess prostate cancer risk (OR=9.68, p=0.03)[183] . Lindmark et al evaluated the association between sequence variants of the IL-1RN gene and prostate cancer risk in a large population-based case-control study in Sweden[21]. They reported that the most common haplotype (ATGC) was significantly associated with prostate cancer (haplotype – specific p-value = 0.009). Furthermore, the association was strengthened in cases with advanced disease. The findings of this study provided additional support for a role of chronic inflammation in the development of prostate cancer. It had a large sample size, with DNA samples being available from over 1380 histologically characterized cases and 779 controls. Furthermore, the full clinical spectrum of prostate cancer was well represented, with over 40% of the cases having advanced disease. However, test for Hardy-Weinberg equilibrium (HWE) revealed that the genotype frequencies of one of the haplotype tagging SNPs (rs315951) deviated significantly from expected proportions among prostate cancer cases; which may be a result of genotyping errors, selection bias, or population stratification. A third study by Michaud et al[182] which examined genetic polymorphisms of IL-B and prostate cancer risk (discussed above) reported no significant association, but the findings were based on the analysis of only two SNPs in the IL-1B gene.

To date, only three population-based studies (all of which are case-control) have examined the association of polymorphisms in the IL-1 gene and prostate cancer risk[21, 182, 185] , and the findings have been inconsistent. There is some suggestion from two of these studies[21, 183] that the inflammatory cytokine IL-1 plays an important role in prostate cancer risk among Swedish males. It is uncertain whether polymorphisms in this cytokine have the same effect in African Americans and/or White Americans. A comprehensive analysis of polymorphisms in

this cytokine among these two latter populations may help to elucidate the role of this gene in prostate cancer risk in these two racial groups.

The most salient and relevant properties of IL-1 in inflammation are the initiation of COX-2, type 2 phospholipase A and inducible nitric oxide (iNOS)[188]. This accounts for the large amounts of prostaglandin-E2 (PGE2), platelet activating factor and nitric oxide (NO) produced by cells exposed to IL-1[188]. Use of aspirin and other NSAIDs has been shown to be associated with reduced risk of colon cancer; and aspirin use has been shown to reduce the risk of colorectal polyp recurrence[207, 208]. There are indications from laboratory studies that NSAIDS might also influence prostate carcinogenesis, including inhibition of prostate cancer growth and metastases in rodent models[209].

2.6 HORMONES AND PROSTATE CANCER

2.6.1 Androgens

The important effect of sex hormones on the prostate is underscored by the fact that the two peaks of prostatic growth are marked by periods of sex hormone increases: the first being puberty when there is rise in androgen level, while the second peak begins around age 50 years, when there is an increase in the estrogen to androgen ratio[210, 211]. Testosterone is the principal circulating androgen secreted by the Leydig cells of the testis[212]. Approximately 1-2% of the total testosterone in the serum circulates as free testosterone; most of the circulating testosterone, however, are bound to two proteins (sex hormone-binding globulin and albumin)[213]. Free

testosterone enters the prostate gland though passive diffusion, where it is converted to dihydrotestosterone (DHT); this reaction is catalyzed by the membrane bound enzyme, steroid 5 alpha-reductase (SRD5A)[214]. Two isoenzymes have been described, SRD5A1 and SRD5A2, the latter being the predominant enzyme in the prostate[215]. After DHT is formed it binds to the intracytoplasmic androgen-receptor, forming a receptor-androgen complex, which is translocated to the nucleus[216]. The binding of this complex to DNA results in increased protein synthesis, which eventually leads to cell proliferation[216].

2.6.2 Androgen levels and prostate cancer risk

Testosterone and its potent metabolite dihydrotestosterone (DHT) are essential for the normal growth of the prostate, and may play a role in the development of prostate cancer. This is due to the observation that conditions which results in diminished androgen production such as castration, pre-puberty, hypogonadism, or enzyme defects of androgen metabolism, such as 5-alpha reductase, almost never results in prostate cancer [58, 217, 218]. Additionally, there are indications from recent epidemiologic studies to suggest that elevated circulating levels of testosterone are associated with prostate cancer risk[59, 219, 220]. In a prospective cohort study, Gann et al found that high plasma levels of testosterone before diagnosis were associated with increased risk of PC; and an inverse trend with levels of sex hormone globuline[59]. Another study by Ross et al showed that young African American men have higher circulating testosterone levels than their White counterparts and suggest that these higher levels could promote cancer growth, leading to observed higher rates of cancer in African American men [221]. However, other investigators have found no such association[40, 222, 223]. Besides androgen levels, it has also been

suggested that genetic defects in the androgen receptor gene, which results in shorter glutamine (CAG) repeat lengths is associated with prostate cancer risk [224, 225].

2.6.3 Androgen receptor (AR)

The human AR gene locus resides on the long arm of the X chromosome and belongs to the super-family of ligand binding transactivation factors[226]. This receptor mediates the actions of testosterone and DHT in androgen-responsive tissues[227]. Alterations of the AR gene in prostate cancer can result in a wider array of activating steroids and non-steroid ligands[228, 229]. A unique, polymorphic polyglutamine stretch encoded by (CAG)nCAA and polymorphic polyglycine sequence sequence encoded by (GGN)n, are present in the human AR terminus, in addition to polyanaline and polyproline amino acid repeats[230]. Within the normal population, the number of glutamine repeats varies from 9-33 residues and the glycine stretch ranges between 16 and 27 residues[231]. Acidic polyproline and polyglutamine sequence motifs are thought to result in a transcriptional activation function when present in various proteins. Fewer numbers of glutamine residues in this region of the AR are associated with higher levels of gene transactivation than are longer repeat lengths[232]. Additionally, genetic variations in the length of the polyglutamine stretch have been implicated in the progressive nature of prostate cancer [224, 225], and in the neuromuscular degenerative disease known as spinal bulbar muscular atrophy or Kennedy's disease [233].

In prostate cancer, the more transcriptionally active AR with fewer polyglutamine repeat residues is thought to be associated with higher incidence, higher grade, and faster progression of the disease, whereas in Kennedy's disease, abnormally long repeats exceeding 40 glutamine

residues are associated with neuronal degeneration[227]. The mean number of CAG repeats in African-Americans is approximately 20, compared with 22 in White Americans[234, 235]. A series of epidemiologic studies have suggested that the increased risk of developing prostate cancer in African-Americans is related to a reduced frequency of CAG repeats in this polpulation[224, 225, 236, 237].

2.6.4 Steroid 5 alpha-reductase type II (*SRD5A2*)

There are two isoforms of the steroid 5 aplha-reductase enzyme (*SRD5A1* and *SRD5A2*), each of which contains 5 exons, have 50% identity of their nucleotide sequences, and are encoded by different genes[238]. The human type 1 isoform is present at low levels in the prostate but is the predominant isozyme in the skin and liver. It is encoded by a gene on the short arm of chromosome 5[238]. The SRD5A2 is located on the short arm of chromosome 2 and catalyzes the conversion of testosterone to the more bioactive compound, dihydrotestosterone. Dihydrotestosterone, has a greater affinity for the androgen receptor, which results in greater transactivation of androgen responsive genes[239]. Molecular defects in SRD5A2 are responsible for reduced serum and tissue DHT and inadequate virilization of the urogenital sinus and external genitalia observed in some infants with male pseudohermaphroditism due to deficiency of steroid 5 alpha- reductase enzyme[240].

A study by Reichardt et al which evaluated the distribution of a dinucleotide repeat in African Americans, non-Hispanic Caucasians, and Asians found more polymorphisms in this marker than previously reported; with some alleles being specific to African Americans[241]. A subsequent study by the same group which analyzed mutations in SRD5A2 reported one amino

acid substitution, V89L, which replaced valine at codon 89 with leucine[242]. This substitution was a germline DNA polymorphism, and was noted to reduce SDR5A2 activity *in vitro*. The substitution is common among Asians and is thought to partly explain the low risk for prostate cancer in this group[242].

Inhibition of SRD5A2 has been shown to reduce the risk of prostate cancer[243]. The drug finasteride inhibits SRD5A2 activity, thereby blocking the conversion of testosterone to DHT[244]. Finasteride markedly suppresses serum DHT levels, and causes a major decrease in prostate epithelium, most pronounced in the fibromuscular and glandular components of the prostate gland[245]. In the Prostate Cancer Prevention Trial (PCPT) finasteride was found to be chemopreventive against prostatic carcinogenesis: prostate cancer was detected in 18.4% of men randomized to the finasteride group as opposed to 24.4% in the placebo group. This was a 24.8% relative risk reduction in the prevalence of prostate cancer during the trial ($p<0.001$)[243]. These results prompted the early termination of the trial 15 months before the anticipated completion date. However, the trial also reported higher-grade disease among men in the finasteride arm of the study compared to controls, but it is uncertain if this finding was related to histologic or sampling artifact in the study[243].

2.6.5 Growth factors

Several factors such as insulin-like growth factor-1 (IGF1), epidermal growth factor (EGF), keratinocyte growth factor (KGF), and transforming growth factors (TGF)-alpha and -beta, and their respective receptors have been reported as being expressed by prostate cancer cells, even though they are not produced by normal prostate epithelium[246, 247]. These factors are important in

the stromal epithelial cross-talk. Insulin-like growth factor-1 is thought to promote prostate tumorigenesis by stimulating cell proliferation and decreasing apoptosis[246].

In a nested case-control study of men in the Northern Sweden Health and Disease cohort study, Stattin et al reported an increased risk of prostate cancer in men with elevated plasma levels of IGF-1. They found this relation to be strongest among young men, which is suggestive of an early involvement of IGF-1 in the disease process[246]. In another nested case-control study of men in the Physicians' Health Study, Chan et al found a strong positive association between IGF-1 levels and prostate cancer risk[248]. This association was noted to be independent of baseline prostate specific antigen (PSA) levels. Keratinocyte growth factor has also been reported as being involved in the development and progression of prostate cancer, in addition to other human malignancies[249]. It has been shown that in the early stages of the disease, prostate cancer cells produce their own KGF, which serves as a growth advantage. But in later stages of the disease, the KGF receptor is not expressed anymore on these cancer cells.

2.7 BONE MINERAL DENSITY AND PROSTATE CANCER

2.7.1 Bone Formation

Bone formation begins as a cartilage framework which is converted to bone by endochondrial ossification in the long bones and vertebra; and membranous bone formation adjacent to cartilage in flat bones[250]. The primary constituents of bone are organic materials (35%) and inorganic materials (65%) by weight. The organic material is mostly collagen, and gives bone its

flexibility, whereas the inorganic materials are comprised of the minerals calcium and phosphate which forms a calcium phosphate crystal known as hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ [250]. During childhood there is periosteal apposition and endosteal resorption, which results in enlargement of the marrow cavities (modeling)[251]. However, at puberty there is both periosteal and endosteal apposition, resulting in rapid increase in bone mass. Towards the end of puberty, there is epiphyseal closure, due to influence of estrogen, after which modeling activity decreases[251]. The exact age at which bone mass peaks differs between the axial and appendicular skeleton, and between males and females, but is generally thought to be around age 30 years, after which begins to decline[252]. However, removal and replacement of portions of bone continues throughout life (remodeling)[253]. Remodeling compensates for endosteal bone loss and weakening of skeletal structures: it is a complex process initiated by activation, a process which involves hormones, inflammatory factors, and stromal cells of osteoblast lineage. Bone remodeling is under the control of several factors, including parathyroid hormone (PTH), calcitonin, thyroxine, estrogen, androgens, growth hormones, vitamin-D, glucoccorticoids, insulin, prostaglandins, and cytokines[253]. An imbalance in remodeling whereby bone resorption exceeds bone formation results in low bone mineral density[254]. Bone mineral density (BMD) refers to the average concentration of mineral per unit area of bone[255]. Measurement of BMD primarily assesses bone calcium content, and is often used to diagnose osteoporosis[255]. Very low BMD results in osteoporosis and may be largely due to excessive osteoclastogenesis, mediated by IL-1, IL-6 and TNF-alpha[256-258].

2.7.2 Techniques for measuring bone mineral density

There are a variety of techniques for measuring BMD; these include single-energy x-ray absorptiometry (SXA); dual energy x-ray absorptiometry (DXA); peripheral densitometry; quantitative computed tomography (QCT); radiographic absorptiometry (RA); and quantitative ultrasound (QUS). Single x-ray absorptiometry uses a single x-ray energy beam for measurement, and a water bath to simulate soft tissue thickness. DXA scans utilize two x-ray beams: one at a higher energy level and the other at a lower energy level, soft tissue absorption is subtracted, and then the absorption of each beam by bone is used in calculating BMD[255, 259]. Peripheral DXA refers to dual x-ray absorptiometry measured at peripheral sites[255]. Radiographic absorptiometry compares the density of proximal phalanges to a wedge of aluminum of known densities placed on the film alongside the hand. It is used to measure bone density from cortical thickness[260]. The quantitative computed tomography scan provides three dimensional volumetric measurements of bones reported in mg/cc. It uses a conventional CT scanner with a calibration phantom, and measures both trabecular and cortical bone density, and is able to isolate trabecular bone for mineral content evaluation[255]. QUS assesses bone density and structure quantitatively by transmitting ultrasonic waves through bone. It is commonly used to assess bone density of the heel and shin. Of these techniques, the DXA is the most commonly used to measure BMD, and is often employed in obtaining BMD of the spine, hip, and total body[255].

A key measurement issue that needs to be considered when assessing BMD in epidemiologic studies is determining the site to be measured. Deciding whether to measure peripheral or central BMD depends on purpose of the study, cost considerations, age of

participants, expected precision and available technology. Medication use also needs to be taken into consideration because treatment produces smaller changes in peripheral measurements compared to spine or hip bone mineral density.

2.7.3 Bone mineral density and prostate cancer

Hormones such as estrogen, testosterone, parathyroid hormone, and insulin-like growth factor 1 (IGF-1) are known to influence bone mineral density[29, 35-38]. Furthermore, these same factors, as well as others, such as high calcium intake, and low vitamin D levels, are considered risk factors for prostate cancer [35, 39, 41-44]. A few epidemiologic studies have assessed BMD as a surrogate marker of a lifetime prostatic exposure to these various factors, but results have been mixed[261-263]. Bunker et al found an increased risk of prostate cancer with increasing BMD among men aged 60-79 years in a cross-sectional study of 1,725 Afro-Caribbean men who were being screened for cancer in the Tobago Prostate Survey[261]. In this study, prostate cancer risk among participants in the highest quartile of BMD was double that of those in the lowest quartile, independent of age and body mass index (OR 2.12, 95% CI 1.21-3.71, P for trend = 0.004). These results were consistent with the findings of the cohort analyses by Zhang et al[262], which assessed the relationship of bone mass and subsequent prostate cancer risk in 1,012 Caucasian men in the Framingham study. There were 100 incident cases in the Framingham study, most of which were diagnosed at an older age (median age 75.2 years). Cortical bone mineral densities of the metacarpal bones were obtained at a mean participant age of 61 years. There was an increased incidence of prostate cancer in the two higher age-specific quartiles compared to the

lowest quartile. The risk ratio for men in the highest quartile compared to the lowest was 1.6 (95% CI 0.9-3.0, P for trend =0.06)[262].

Contrary to the findings of these two studies, Farhat et al. found a statistically significant inverse association between BMD and prostate cancer in a cohort study of 4,597 men aged 65 years and older, with no prior history of prostate cancer that were followed for an average of 5.2 years[264]. Similarly, Nelson et al found an inverse association of BMD and prostate cancer risk in 94 men followed prospectively in the First National Health and Nutrition Examination Survey (NHANES I) Epidemiologic follow-up Study (NHEFS), but the results were not statistically significant[263]. BMD of the hand was measured at a mean age of 49 years, after which participants were followed for approximately 19 years for diagnosis of prostate cancer. The rate ratio for men in the highest quartile compared to the lowest was 0.72 (95% CI 0.38-1.38, P trend = 0.37)[263]. A key methodological difference that might explain the discrepant results of the NHANES study compared to the Tobago and Framingham studies was BMD data analysis. Whereas the Framingham study employed age-specific BMD quartiles, the Tobago study based the quartiles upon two broad age categories (45-59 years and 60-79 years). In the NHANES study BMD quartiles were calculated across all age groups. Additionally, the NHANES cohort were enrolled at a much younger age (mean age 49 years), and may not have been followed long enough for prostate cancer to develop (total follow-up time was 19 years).

2.7.4 *Interleukin-1* and *IL-6* and bone mineral density

The major factor driving low BMD is an imbalance in bone resorption which exceeds bone formation; this occurs due to excessive bone resorption ability of osteoclasts. Interluekin-1 and

IL-6 play a central role in bone turnover by stimulating osteoclastogenesis, a key factor responsible for increasing bone resorption[30]. Functional polymorphisms of *IL-1* and *IL-6* have been reported to be associated with bone mineral density[38, 257, 265-268]. Kim et al examine the relation between *IL-1* and *IL-1RN* polymorphisms and BMD in 202 postmenopausal Korean women. They reported that women carrying the A2 allele of the *IL-1 RN* gene had a significantly lower BMD than those without the allele, and the A2 allele was more common in osteoporotic women than in those without osteoporosis[265] Nemetz et al reported that the presence of the *IL1B*-511 allele was associated with significantly lower Z scores and a higher risk of osteopenia and osteoporosis in patients with irritable bowel disease[268].

Moffett et al evaluated the relationship between the *IL-6* G-174C polymorphism and BMD, the rate of decline in BMD, and the risk of fracture in 3376 Caucasian women aged 65 years and older among participants in the Study of Osteoporotic fractures (SOF) [257]. They reported the lowest BMD of the proximal and distal radius among women with the G/G genotype, intermediate in heterozygotes, and highest in women with the C/C genotype ($p < 0.05$). In addition, women with the C/C genotype experienced a slower rate of decline in total hip and femoral neck BMD compared with the G/G genotype ($p < 0.05$)[257]. In another study Lorentzon et al investigated the *IL-6* G-174C polymorphism in relation to BMD during and after puberty in 90 teenage males, and reported that participants with the CC genotype had a higher BMD of the femoral neck and lumbar spine, compared to those with the GG genotype ($p < 0.5$)[38]. These findings of an association between *IL-6* gene polymorphisms and low BMD have been corroborated by several other studies[30, 267, 269-271]. A study by Ota et al in which 192 sibling pairs of Japanese women from 136 families were genotyped for microsatellite polymorphisms in or near *IL-6, IL-6R,* calcium-sensing receptor (CASR), and matrix Gla protein (MGP) genes,

found that the *IL-6* locus was linked to a decrease in BMD (T value for all women = -2.0405; postmenopausal women = -2.406; p values <.05).

To ascertain the long term effect of serum levels of *IL-1* and *IL-6* on the pathogenesis of prostate cancer, there is the need to obtain serial measurements over many years, but this has been difficult, resulting in discrepant findings by various investigators [26-28, 45]. Since bone mass reflects a lifetime exposure of bone to *IL-1* and *IL-6*, bone mineral density should serve as a possible surrogate marker for cumulative exposure of the prostate to these inflammatory cytokines.

2.8 SIGNIFICANCE

The molecular factors that contribute to prostate cancer risk as well as racial disparities in morbidity and mortality are not clearly understood. Evidence from epidemiologic, genetic, molecular biology and histopathology studies suggest a compelling role of chronic or recurrent inflammation in the development of prostate cancer. The disease is initially androgen dependent but rapidly becomes androgen independent, and refractory to therapy. Inflammatory cytokines such as *IL-6* have been reported to influence clinical outcome by mediating the transition from androgen dependence to androgen independence. However, the role of *IL-1* and *IL-6* in prostate cancer risk and in explaining observed racial disparities in the disease are not clearly understood. Understanding how these various factors influence prostate carcinogenesis is of great public health significance because it will enable their use for early identification of those at increased

risk of the disease. This may result in early detection of the disease, and prompt intervention. Additionally, it will increase our understanding of the molecular biology of the disease, which may open up new avenues for prostate cancer prevention and treatment. It may also help to explain some of the observed racial disparities in prostate cancer risk.

3.0 PAPER 1: ALLELE FREQUENCIES OF INFLAMMATORY CYTOKINES INTERLEUKIN-1A (IL-1A), IL-1B, IL-1RN, IL-6 AND IL-6R IN AFRICAN AMERICANS AND CAUCASIANS: THE CANCER AND PROSTATE STUDY

Kwame Appenteng, MPH, [1] Clearann Bunker, PhD,[1] Joel Nelson, MD,[2] John Wilson, PhD,[3] Robert Ferrell, PhD,[4] Brenda Diergaarde, PhD,[1] Susan Moffett,[4] PhD, Francesmary Modugno, PhD,[1] Claudia Leiras, MPH,[1] James Shikany, DrPH, [5] Joel Weissfeld, MD, MPH,[1]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[2]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

[3]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[4]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[5] Department of Preventative Medicine, University of Alabama at Birmingham, Birmingham, Alabama

3.1 ABSTRACT

Objective: To assess allele frequencies of inflammatory cytokines IL-1A, IL-1B, IL-1RN, IL-6 and IL-6R gene polymorphisms among African American and Caucasian men.

Methods: We conducted a cross-sectional study of 286 African-American (AA) and Caucasian men ages 40-80y who were controls in the Cancer and Prostate Study (CAPS) and enrolled between 2001 and 2006. Tagging single nucleotide polymorphisms (tagSNPs) and putative functional SNPs in IL-1A, IL-1B, IL-1RN, IL-6 and IL-6R were selected using HapMap, Haploview and FastSNP. Genomic DNA was extracted from whole blood, and genotyping was conducted using on the Sequenom iPLEX gold. We conducted descriptive analysis on all subjects. We used the allele chi-square test to compare minor allele frequencies between AA and Caucasians in SAS/Genetics 9.2.

Results: Genotyping information was successfully obtained on 53 SNPs in 5 genes in the inflammation pathway for 59 AA and 227 Caucasians controls. There were significant MAF differences ($p < 0.05$) in at least 50% of the SNPs in each of the 5 genes between the two racial groups.

Conclusion: Minor allele frequencies were significantly different in 50% or more SNPs in each of the five inflammatory genes, between AA and Caucasians.

3.2 INTRODUCTION

Inflammatory cytokines are important mediators of the immune system, and play a major role in the development of various diseases[8, 9]. Single nucleotide polymorphisms (SNPs) at the regulatory regions of cytokine genes are associated with modification of protein expression, and have been reported to influence morbidity[170, 184, 272, 273]. It has been suggested that as a result of genetic heterogeneity, individual response to immune system insults, which is a result of differences in patterns of cytokine expression, may differ from one person to another[274]. Thus host response is a major component of chronic or recurrent inflammatory state[15]. The interleukin-1 *(IL-1)* and *interleukin-6 (IL-6)* family of genes promote inflammation and have been associated with prostatic carcinogenesis [21, 22, 26]. Sequence variants in the interleukin-1 receptor antagonist *(IL-1RN)* and *IL-6* have been reported to be associated with prostate cancer[21, 184]. It has been suggested that ethnicity is strongly associated with cytokine gene polymorphisms, and is subsequently an important determinant of differences in disease susceptibility and morbidity in various racial groups[274].

The purpose of this study is to determine whether allele frequencies of pro-inflammatory cytokines IL-1 and IL6 are the same or different in African American and Caucasian men. Our hypothesis is that allele frequencies of *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* are different in African American compared to Caucasian men.

3.3 MATERIALS AND METHODS

3.3.1 Study Sample

Subjects in the current study were controls from an existing prostate cancer case-control study known as the Cancer and Prostate Study (CAPS). Briefly, the Cancer and Prostate Study (CAPS) was a case-control study designed to assess the individual and joint associations of bone mineral density (BMD) and sex hormone gene polymorphisms in prostate cancer risk. Enrollment into CAPS started in December of 2001 and was completed in January of 2006, and included Caucasian and African American (AA) men aged 40 to 80 years. Cases were men with recently diagnosed prostate cancer (within 3 months of enrollment into the study; confirmed diagnosis based on pathology report). Controls were men without a history of prostate cancer. All participants in CAPS gave informed consent. The study was approved by the institutional review boards (IRB) of the University of Pittsburgh and the University of Alabama at Birmingham.

Potential enrollees were excluded if they used glucocorticoids (>6 months); used testosterone (>3 months); had a history of hyper- or hypothyroidism, hyperparathyroidism, renal disease or bone disease. Other exclusion criteria included bilateral hip replacement; kidney transplant; previous diagnosis of prostate cancer or any other cancer besides basal and squamous cell skin cancer; evidence of bone metastases among prostate cancer cases; and PSA levels above 3.0 ng/ml among controls. Controls were frequency matched to cases by age and race.

Participants in CAPS were recruited from Pittsburgh and Alabama. In Pittsburgh, recruitment was conducted at two sites: The University of Pittsburgh Medical Center (UPMC), and the Veterans Administration Medical Center (VA). Recruitment in Alabama was conducted

at the University of Alabama in Birmingham (UAB) Medical Center. A total of 593 Caucasian and African American men were enrolled in CAPS. Controls from Pittsburgh numbered 253, and were recruited from the local community, as well as from University of Pittsburgh employees, by sending out flyers. Controls were frequency age-matched to cases. Majority of the controls were from the ongoing Prostate, Lung, Colorectal, and Ovarian study (PLCO). A total of 10 participants were recruited from the VA Medical Center in Pittsburgh, comprising of 3 cases and 7 controls.

In Alabama, information about the CAPS study was advertised in the *UAB Reporter*. Additionally, flyers were sent out to local residents and University of Alabama employees, and brochures were placed in waiting rooms of Birmingham area urologist offices. Forty-four community-based controls were enrolled at the UAB Medical center, majority of who were from the ongoing PLCO trial. Other control enrollees included UAB employees, as well as local Birmingham residents who responded to advertisements and flyers. The total number of subjects (controls) in the current study is therefore 286, comprising 59 AA and 227 Caucasians.

An interviewer administered standardized questionnaire was used to collect demographic and prostate cancer risk factor information. Whole blood, height and weight measurements, bone mineral density (BMD, Hologic DEXA) were obtained at a single clinic visit. Samples were stored in a -70^{o}C freezer in the Department of Epidemiology in the Graduate School of Public Health at the University of Pittsburgh.

3.3.2 Selection of Single Nucleotide Polymorphisms

Single Nucleotide Polymorphisms (SNPs) of *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* were selected using two web-based programs – HapMap Phase 1 and 2 (HapMap Data Rel 22/phase II April 07, on NCBI B36 assembly, dbSNP b126) and Functional Analysis and Selection Tool for Single Nucleotide Polymorphisms (FastSNP). Information on HapMap and FastSNP is available to the public online, and the programs can be downloaded free of charge at the respective websites: http://www.hapmap.org and http://www.fastsnp.ibms.sinica.edu.tw.

HapMap was used to obtain SNP information per gene for Caucasians (CEU) and Blacks (Yoruba-YRI) separately, and the information was downloaded into Haploview. The Tagger approach was used in Haploview to select tagSNPs for CEU and YRI separately, at a minor allele frequency (MAF) of at least 10%, and a pair-wise correlation (r^2 of 0.80 or greater). This procedure identified a total of 51 tagSNPs in both racial groups. To complement the list of SNPs obtained from HapMap, we ran the five candidate genes through FastSNP, resulting in the identification of 2 additional potentially functional SNPs, bringing the total number of SNPs for genotyping analysis to 53.

3.3.3 Laboratory assay

Genomic DNA was isolated from EDTA anticoagulated whole blood by standard methods using the Puregene kit (Gentra Systems). Genotyping was carried out on the Sequenom platform.

3.3.3.1 Sequenom i-PLEX Gold SNP Assay

Primer Design: Three primers were designed for each locus of interest using Mass Array Assay Design version 3.1 (Sequenom Inc., San Diego, CA). The two amplification primers flanked the polymorphic site to provide for sample amplification, while the single MassExtend primer lay immediately adjacent to allow for allelic discrimination via single base extension. Assay Design software determined pooling of primer sets to optimize multiplex reactions. Mass modifications are incorporated in the design of the MassExtend primers to maximize the mass differential between primers of different loci within a given multiplex pool.

Sample Amplification: Target loci were amplified within the samples by multiplex polymerase chain reaction (PCR) in 1X PCR buffer (Qiagen) containing 3.5 mM $MgCl_2$, 25 mM dNTPs, 500 nM each forward and reverse amplification primer within the multiplex pool and 2.5 U HotStar Taq (Qiagen). PCR conditions were: 95^{o}C for 15 minutes for taq activation followed by 45 cycles of 94^{o}C for 20 seconds, 56^{o}C for 20 seconds and 72^{o}C for 1 minute. A single extension for 1 minute at 72^{o}C completed the PCR reaction. Deoxyribonucleotide triphosphates (dNTPs) and primers were removed by incubation with 0.5 U shrimp alkaline phosphotase (SAP) at 37^{o}C for 40 minutes. SAP was inactivated by incubation at 87^{o}C for 5 minutes.

MassExtend: Excess MassExtend primers corresponding to the loci represented by the amplification primers used were pooled. Higher mass primers were added at a higher concentration to adjust for signal drop off during spectra acquisition. Single base extension was carried out in 0.2X iPLEX buffer plus, 1X termination mix (containing mass modified termination nucleotides), 1X iPLEX enzyme and primers at 0.84 μM, 1.04 μM and 1.25 μM as appropriate to the relative mass of the primer. A double cycle amplification program performed

40 cycles of denaturation at 94 ^{o}C for 5 seconds followed by 5 cycles of 52^{o}C for 5 seconds, 80 ^{o}C for 5 seconds, back to 94 ^{o}C for a total of 200 cycles. A final extension at 72 ^{o}C for 3 minutes completed the amplification. Clean resin and water was added to the MassExtend reaction products. Samples were incubated in clean resin at room temperature with mixing for 5 minutes and centrifuged at 3200 x g for 5 minutes.

NanoDispense, Spectra acquisition and analysis: Samples were dispensed to a SpectraChip using the MassArray Nanodispenser according to manufacturer's instructions. Spectra chips were loaded into the MassArray analyzer and spectra acquired for each sample. MassArray Typer software used the known mass of the MassExtend primers to identify each locus, and the increase caused by each distinct nucleotide to identify the alleles present in the sample.

Whenever appropriate, alleles that were not automatically identified by the computer software were directly read and assigned by the operator. We observed 100% concordance rates in two randomly replicated samples. Centre d'Etude du Polymorphisme Humain (CEPH) positive controls and water negative controls were included in two 96 well plates as part of quality control measures. For 52 of 53 SNP assays we were able to obtain genotyping results for over 97% of subjects analyzed. One SNP assay (rs11265613) produced a genotype result in 94% of subjects tested.

3.3.4 Data Analysis

We analyzed our data in SAS/Genetics version 9.2. There were 304 controls in CAPS. From this number 18 subjects were excluded due to lack of sufficient samples for genotyping, or low call

rates. The total number of controls included in our analysis is 286. Genotype frequencies observed among AA controls were consistent with HWE, as per the allele test in SAS/Genetics 9.2. Two *IL-1RN* loci (rs3181052 and rs4252019) and two *IL-6R* loci (rs4393147 and rs7518199) departed from HWE among Caucasian controls ($0.024 < P < 0.050$, exact test).

We conducted race-specific descriptive analyses on all 286 subjects included in our analyses for the following risk factors: age (years), height (cm), weight (kg), body mass index (kg/m^2), personal history of benign prostatic hypertrophy (BPH) or prostatitis ("yes" or " no"), family history of prostate cancer ("yes" or "no"), and history of regular aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) use ("yes" or "no"). Regular aspirin or NSAIDs use was defined as taking these medications at least five to seven days per week for the twelve months preceding enrollment into the study.

Our reference minor allele frequencies for Blacks and Whites were based on genotyping information provided by HapMap on YRI and CEU populations respectively. In the HapMap project 30 sets of samples from two parents and an adult child (trio) were obtained from the Yoruba people of Ibadan, Nigeria for genotyping analyses. The project also collected samples from 30 trios in the United States, who were residents of Utah (CEU population), with northern and western European ancestry by the Centre d'Etude du Polymorphisme Humain (CEPH). Genotyping information is available to the public for free download at: http://www.hapmap.org .

We downloaded the YRI and CEU genotyping data provided by HapMap. We excluded offspring genotyping information, bringing the total HapMap sample included in our analyses to Yoruba (n=60), and CEU (n = 60). This sample served as the HapMap references for each of our racial groups. We assessed allele frequencies in our reference groups using SAS/Genetics version 9.2.

In our controls we characterized race-specific distributions of the variant allele at each locus based on the rare allele observed in our White control group using the allele Chi-square test. We compared race-specific allele frequencies at each locus between our control groups (observed) and the HapMap sample (reference). Our assessment of a significant minor allele frequency difference between the observed and reference group at each locus, was based on the allele test ($p<0.05$). Additionally, we compared minor allele frequencies at each locus between our Black and White control groups.

3.4 RESULTS

Selected characteristics of our control subjects are shown in Table 3.1. A total of 286 men who enrolled in CAPS and whose blood samples were satisfactorily genotyped were included in our analyses. There were 59 AA and 227 Caucasian controls, mean age in the total study sample were 61.1 ± 7.1 years. Caucasians were slightly taller than AA, but weighed less and also had a lower body mass index (BMI); however, these differences were not statistically significant. A significantly higher proportion of Caucasians had a history of benign prostatic hypertrophy (BPH) than AA, but there was no significant difference in the proportion of controls with a family history of prostate cancer between the two racial groups

A list *of IL-1A, IL-1B, IL-1RN, IL-6 and IL-6R* SNPs that were genotyped is shown in Table 3.2. Genes are ordered according to chromosome and SNPs by position across the respective genes. Genotyping information on 53 SNPs in 5 genes in the inflammation pathway are shown for 286 AA and Caucasian control subjects (Table 3.3). Race-specific minor allele frequency (MAF) comparisons between observed and reference Black and White groups are shown in Table 3.3. There was 1 SNPs in our AA control group in which data was unavailable in the reference group (*IL-1A*-rs20540), therefore, the total number of SNPs available for comparison between the Black observed vs. reference group was 52. Similarly, among Whites, there were 2 SNPs in our control group in which data was unavailable in the reference group (*IL-1A*-rs20540, *IL-6R*-rs-7549338); therefore, the total number of SNPs available for comparison in the White observed vs. reference group was 51. We noted that there were 6 significant ($P<0.05$) MAF differences between the observed vs. reference groups in Blacks, compared to no significant differences in Whites. Graphs comparing MAF in observed vs. reference groups in

Blacks and Whites are shown in Figures 3.1 and 3.2. As illustrated in these figures there were greater numbers of significant differences in MAF between the reference vs. observed group in Blacks, compared to Whites. Race-specific control group allele frequency and HWE test results are shown in Table 3.3.

Table 3.4 shows comparisons of MAF in AA and Caucasian control subjects. Significant differences in MAF between AA and Caucasian were observed in 64% of all SNPs genotyped. Of SNPs showing significant differences, 79% were highly significantly different ($p<0.01$) between the two racial groups. In each of the 5 genes we observed 50% or more markers with significant MAF differences between the two racial groups (*IL-1A*, 50%; *IL-1B*, 60%; *IL-1RN*, 63%; *IL-6*, 71%; and *IL-6R,* 68%). A lesser proportion of AA controls (0.229) carried the variant allele (A) at *IL-1A*-rs17561 than Caucasians (0.278), but the difference was not statistically significant (Table 3.4). The GG genotype of this particular marker up-regulates the protein encoded by the *IL-1A* gene, and was found more commonly among AA controls (61%) than Caucasian controls (53%). Similarly, a lesser proportion of AA controls (0.169) than Caucasians (0.223) carried the variant allele at *IL-1-B*-rs1143634, but the difference was not statistically significant (Table 4). The (C) allele at this locus up-regulates *IL-1B* secretion, and was carried more commonly by AA controls (83%) compared to Caucasians control (80%).

3.5 DISCUSSION

We compared MAFs of inflammatory gene markers between AA and Caucasian controls. We found significant differences in the distribution of variant alleles at several *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* loci in the two racial groups. We made race-specific comparisons of MAF of *IL-1* and *IL-6* gene markers between observed and HapMap reference groups and found that approximately 12% of MAFs were significantly different in our Black comparison group, but none in our White comparison group. These differences may reflect greater admixture in the African American population compared to Caucasians, and is an important consideration in disease susceptibility[275, 276]. Cytokine gene polymorphisms have been reported to influence disease susceptibility, severity and clinical outcome[184, 277]. Racial differences in the distribution of inflammatory marker allele frequencies have been reported to influence allograft rejection, and in rheumatoid arthritis development and response to treatment [274, 278-280].

In this study, there were significant MAF differences between the two racial groups in 50% of *IL-1A*, 60% of *IL-1B* and 63% of *IL-1RN* SNPs. These differences may underlie some of the disparities in clinical outcome noted between the two racial groups. *Interleukin-1A and IL-1B* up-regulate the division of immune cells, as well as promote cell growth, differentiation and migration[188]. They also inhibit apoptosis and induce angiogenesis, thereby promoting tumor growth[23, 188]. Their action, however, is inhibited by the binding of the *IL-1RN* to the *IL-1* receptor. *Interleukin-1* and its related family members are primarily inflammatory cytokines, and are known to initiate cyclooxygenate type 2 (COX-2), type 2 phospholipase A and inducible nitric oxide synthase (iNOS) in inflammation[187].

The GG polymorphism at *IL-1A*-rs17561 produces an alanine-to-serine amino acid substitution at codon 114 of the *IL-1* cytokine protein and has been associated with an increased risk of atopy[281]. In a recent study which compared differences in cytokine gene polymorphisms among healthy primiparous African American (N = 179) and Caucasian (N = 396) women seeking prenatal care prior to 20 weeks' gestation in Pittsburgh, Pennsylvania, Ness et al reported that the up-regulating GG genotype at *IL-1A*-4845 (*IL-1A*-rs17561) was found in 59.7% of AA women compared to Caucasian women (47.3%)[282]. In the current study the GG genotype was found in 61% of AA men compared to 53% Caucasians. The (C) allele at *IL-1B*-rs1143634 has been associated with an increased secretion of IL-1B in activated macrophages in vitro[283]. Ness et al reported that the (C) allele at *IL-1B*-3597 (rs1143634) was found in 86% of AA women compared to 77% in Caucasians[282]. In the current study the (C) allele was found in approximately 83% of AA men compared to 80% of Caucasians. We extend the findings of Ness et al by studying a greater number of inflammatory markers, and by focusing on AA and Caucasian men. *IL-1A*-rs20540 is a putatively functional SNP, involved in splicing regulation (http://www.fastsnp.ibms.sinica.edu.tw). The exact functions of the other *IL-1* SNPs are not clearly known, but they are candidates with biological plausibility, being that they are clustered in highly conserved genomic regions in different vertebrate species (http://genome.ucsc.edu).

Of the *IL-6* gene SNPs evaluated in this study 71% of *IL-6* and 68% of *IL-6R* MAFs were significantly different between AA and Caucasians. Due to the pleiotropic nature of *IL-6* such differences in MAFs could result in disparities in disease development, progression and treatment response between the two racial groups. *Interleukin-6* is involved in regulating immune and inflammatory responses[154]. In addition to inducing terminal differentiation of B-cells it synergizes with *IL-1* in activating T-cells by inducing *IL-2* responsiveness, and enhances

the differentiation of cytotoxic T lymphocytes from thymic precursors[146, 155, 156]. It has a central role in the acute-phase response, acting on hepatocytes to increase the synthesis of acute-phase proteins (haptoglobin, fibrinogen, C-reactive protein) and reducing the secretion of albumin and transferein[157]. It also contributes to the body's defenses by increasing the body temperature and stimulating the release of adrenocoticotropin hormone[158-160]. Other functions include impairment of natural killer cell function, induction of bone resorption via stimulation of osteoclast formation, and induction of experimental cancer cachexia. *IL-6* also induces platelet-derived growth factor in blood vessels, enhances proliferation of vascular smooth muscle, and has a negative inotropic effect on cardiac myocites.[154].

Attention to the role of *IL-6* in the pathogenesis of prostate cancer was drawn by the observation that the disease transitioned from an androgen-dependent tumor, initially responsive to androgen ablation therapy, to an untreatable androgen independent tumor[60]. In-vitro studies by Siegall at al showed that the androgen-independent cell lines DU145 and PC3 and the androgen-dependent cell line LNCaP expressed *IL-6R* on their surfaces, and all three cell lines were susceptible to a chimeric Pseudomonas exotoxin-IL-6 toxin. Susceptibility was mediated by *IL-6R* as cytotoxic activity was blocked by excess human recombinant IL-6[172]. Since then, the expression of mRNA for *IL-6R* and the gp130 signal transducer has been confirmed in human prostate cancer by several investigators[172-174]. Furthermore, recent in-vitro studies have shown that *IL-6* initiates and promotes prostate tumorigenesis by mediating cross-talk between stromal and epithelial cell of the prostate[25].

IL-6R -rs28730736 is a putatively functional SNP (missense), and is involved in splicing regulation (http://www.fastsnp.ibms.sinica.edu.tw). The variant allele of this SNP was carried by 15% of AA controls in our study, but was absent in our Caucasian controls, who were 100%

homozygous for the common allele. Two *IL-6* loci (rs1554606 and rs2069845) were in strong pair-wise LD (r^2=0.99) in Caucasians. Greater than a third of the IL6R SNPs evaluated in this study were in strong linkage disequilibrium with each other ($r^2 \geq 0.90$). While we are not certain of the exact functions of these SNPs, several of them appear to be candidates with biological plausibility, because they are clustered in highly conserved genomic regions in different vertebrate species (http://genome.ucsc.edu).

Our study had several limitations. Our tag SNP panel was based on Phase II of the International HapMap project, and may not have captured a more comprehensive set of tag SNPs as provided in Phase III of the HapMap project. We studied a limited number of inflammatory markers, even though the true assessment of differences in MAFs may involve a larger number of inflammatory markers, and include others in areas of the genome outside our tag region. We also relied on self reporting of race, even though there is admixture among African Americans.

In spite of these limitations, our study has several strengths. It is one of the first to directly perform a three-way comparison of MAFs in AA vs. YRI of West Africa, AA vs. Caucasians, and Caucasians vs. CEU of Utah within the context of a comprehensive set of inflammatory cytokines. Such a comparison adds breadth and depth to our understanding of the heterogeneity of allele frequency distributions in the population, and the basis for predisposition or susceptibility to certain diseases. It identified two putatively functional inflammatory markers (*IL-1A*-rs20540 and *IL-6R*- rs28730736) with variant alleles found in African Americans, but not Caucasians, that may or may not be associated with prostate cancer, but have not as yet been reported in the literature in relation to the disease. Beyond reporting similar findings as previous studies, the current study demonstrated that there are differences in allele frequency distributions

of markers of inflammation in the two racial groups that may contribute to disparities in inflammation mediated diseases.

Even though we do not know the exact functions of all the SNPs in this study there is the possibility AA carry a greater proportion of alleles that up-regulate pro-inflammatory cytokines compared to Caucasians thereby leading to differentially poor clinical outcomes in AA. Future studies may want to consider examining a wide variety of markers which up-regulate pro-inflammatory cytokines in these two groups, as well as those with marginal effect on prostate cancer and other diseases in order to determine susceptibility. Understanding the differences in allele frequencies of *IL-1* and *IL-6* gene is of great public health significance because it is the first step in understanding the basis for some of the observed disparities in the disease between the two racial groups. It will also enable their possible use as biomarkers for early detection and prompt intervention, as well as increase our understanding of the molecular biology of the disease.

3.6 TABLES AND FIGURES

Table 3.1 : General characteristics of control subjects

	African Americans			Caucasians				All subjects		
	Mean	SD	N	Mean	SD	N	p-value	Mean	SD	N
Age in years	61.1	9.8	59	61.1	6.2	227	0.9514	61.1	7.1	286
Height (cm)	174.7	5.3	59	176	6.3	227	0.0784	175.9	6.1	286
Weight (kg)	92	17.1	59	90.6	15.5	227	0.5775	90.9	15.8	286
BMI (kg/m^2)	30	5	59	29.1	4.4	227	0.2058	29.3	4.5	286
Father or brother with history of prostate cancer	N	(%)		N	(%)		p-value	N	(%)	
Yes	3	5.1		10	4.4		0.8234	13	4.6	
No	56	94.9		217	95.6			273	95.4	
Personal medical history of BPH or prostatitis										
Yes	10	16.9		128	56.4		<.0001	138	48.2	
No	49	83.1		99	43.6			148	51.8	

BMI - Body mass index

BPH - Benign prostatic hyperplasia

Table 3.2 :SNPs included in genotyping analysis

Chromosome	Gene	SNP identification number							
Chr1	*IL-6R*	rs4845617	rs6427641	rs11265610	rs12083537	rs1386821	rs4075015	rs4601580	rs4845618
		rs7549338	rs7518199	rs4553185	rs4393147	rs4537545	rs4845626	rs28730736	rs11265618
		rs10159236	rs4329505	rs4509570	rs2229238	rs4072391	rs4379670		
Chr2	*IL-1A*	rs3783590	rs2856836	rs17561	rs20540	rs2856838	rs1609682	rs3783526	rs2856837
Chr2	*IL-1B*	rs1143643	rs1143634	rs1143633	rs3136558	rs1143630			
Chr2	*IL-1RN*	rs3181052	rs1794066	rs1794067	rs2071459	rs432014	rs380092	rs452204	rs4252019
		rs315955	rs315951	rs9005					
Chr7	*IL-6*	rs2069837	rs2069840	rs1554606	rs2069842	rs1548216	rs2069843	rs2069845	

Genes are odered by chromosome. SNPs are presented by position across the gene. SNPs (N=51) were selected from HapMap/Haploview using the Tagger approach, MAF > 0.8, r2 > 0.1 . Two additional SNPs were selected from FastSNP

Chr - Chromosome; SNP - Single nucleotide polymorphism; IL-1A - Interleukin-1A; IL-1B - Interleukin-1B; IL1RN - Interleukin-1 receptor antagonist; IL-6 - Interleukin-6; IL-6R - IL-6R

Table 3.3 : Control group allele frequency and Hardy-Weinberg Equilibrium (HWE) test result, by race

		Black				White			
		Allele frequency [2]				Allele frequency [2]			
Gene variant	Allele [1]	Observed	Reference	Allele [3]	HWE [4]	Observed	Reference	Allele [3]	HWE [4]
IL1A									
rs3783590	A	0.093	0.229	0.005 *	1.0000	0.002	0.000	0.613	1.0000
rs2856836	C	0.229	0.133	0.056	0.4574	0.278	0.308	0.506	0.6204
rs17561	T	0.229	0.133	0.056	0.4778	0.278	0.308	0.506	0.6204
rs20540	T	0.051			1.0000	0.000			
rs2856838	T	0.364	0.358	0.922	0.7784	0.388	0.400	0.805	0.7846
rs1609682	C	0.203	0.183	0.695	1.0000	0.336	0.292	0.362	1.0000
rs3783526	A	0.042	0.008	0.094	0.0862	0.333	0.292	0.394	1.0000
rs2856837	T	0.254	0.183	0.186	1.0000	0.278	0.308	0.506	0.6192
IL1B									
rs1143643	A	0.195	0.150	0.359	0.4314	0.339	0.392	0.284	0.0724
rs1143634	T	0.169	0.100	0.116	0.6616	0.202	0.223	0.623	0.8440
rs1143633	A	0.237	0.178	0.261	0.1582	0.338	0.397	0.242	0.0566
rs3136558	C	0.136	0.153		0.5760	0.187	0.323		0.8322
rs1143630	A	0.288	0.254	0.563	0.5388	0.064	0.040	0.361	1.0000
IL1RN									
rs3181052	A	0.153	0.217	0.203	0.6122	0.082	0.119	0.213	0.0446 *
rs1794066	G	0.390	0.475	0.185	0.2744	0.398	0.390	0.868	0.8834
rs1794067	A	0.288	0.183	0.057	0.7470	0.308	0.271	0.433	0.5346
rs2071459	T	0.161	0.283	0.023 *	0.6304	0.082	0.119	0.218	0.0506
rs432014	C	0.203	0.175	0.576	0.6744	0.308	0.271	0.433	0.5252
rs380092	T	0.703	0.833	0.017 *	0.7504	0.275	0.314	0.403	0.8770
rs452204	A	0.491	0.600	0.094	0.3052	0.396	0.390	0.895	0.8928
rs4252019	T	0.364	0.592	0.000 *	0.7878	0.090	0.127	0.231	0.0240 *
rs315955	C	0.138	0.108	0.489	1.0000	0.000	0.000		
rs315951	C	0.407	0.533	0.051	1.0000	0.240	0.263	0.610	1.0000
rs9005	A	0.254	0.175	0.136	0.1738	0.335	0.305	0.540	0.5582

Table 3.3 (continued)

		Black				White			
		Allele frequency [2]				Allele frequency [2]			
Gene variant	Allele [1]	Observed	Reference	Allele [3]	HWE [4]	Observed	Reference	Allele [3]	HWE [4]
IL6									
rs2069837	G	0.144	0.158	0.759	0.3240	0.073	0.067	0.820	0.3314
rs2069840	G	0.127	0.169	0.360	0.2108	0.347	0.317	0.529	0.4596
rs1554606	T	0.356	0.267	0.143	0.3988	0.445	0.542	0.059	0.8898
rs2069842	A	0.042	0.102	0.078	0.0840	0.000	0.000		
rs1548216	C	0.169	0.217	0.357	0.3512	0.018	0.000	0.157	1.0000
rs2069843	A	0.093	0.175	0.064	0.3968	0.018	0.017	0.943	1.0000
rs2069845	G	0.347	0.300	0.434	0.2658	0.444	0.542	0.057	1.0000
IL6R									
rs4845617	A	0.379	0.373	0.919	0.5776	0.383	0.397	0.793	0.5618
rs6427641	G	0.703	0.730	0.664	1.0000	0.423	0.396		0.5868
rs11265610	C	0.272	0.356	0.168	0.7324	0.000	0.000		
rs12083537	G	0.280	0.233	0.413	0.3502	0.203	0.136	0.112	0.3052
rs1386821	C	0.110	0.092	0.636	0.1208	0.185	0.167	0.642	0.3800
rs4075015	A	0.110	0.042	0.046 *	1.0000	0.423	0.458	0.486	1.0000
rs4601580	T	0.570	0.608	0.553	0.4246	0.415	0.508	0.068	0.2150
rs4845618	G	0.542	0.575	0.612	0.7930	0.434	0.508	0.145	0.1380
rs7549338	C	0.297	0.417	0.053	0.5490	0.421			0.2764
rs7518199	C	0.186	0.100	0.057	0.3988	0.410	0.347	0.218	0.0392 *
rs4553185	C	0.578	0.508	0.286	0.2848	0.430	0.492	0.227	0.1304
rs4393147	T	0.103	0.042	0.066	0.4820	0.410	0.345	0.202	0.0398 *
rs4537545	T	0.644	0.692	0.436	0.7700	0.421	0.339	0.107	0.1704
rs4845626	T	0.475	0.442	0.610	0.5968	0.167	0.158	0.812	1.0000
rs28730736	A	0.153	0.167		1.0000	0.000	0.000		
rs11265618	T	0.407	0.383	0.711	0.7880	0.170	0.158	0.769	1.0000
rs10159236	A	0.220	0.115	0.038 *	0.7230	0.161	0.109	0.174	1.0000
rs4329505	C	0.415	0.467	0.424	1.0000	0.159	0.158	0.995	0.8040
rs4509570	G	0.500	0.614	0.081	1.0000	0.242	0.328	0.061	0.7250
rs2229238	T	0.161	0.208	0.347	1.0000	0.196	0.258	0.136	0.8350
rs4072391	T	0.263	0.336	0.220	0.1832	0.196	0.254	0.165	0.8324
rs4379670	T	0.161	0.192	0.535	1.0000	0.196	0.242	0.271	0.8386

1. Rare allele observed in white control group
2. Reference allele frequency obtained from HapMap or EntrezSNP database
3. Observed vs. reference allele frequency (allele test), with asterisk (*) to indicate $p<0.05$
4. Hardy-Weinberg-Equilibrium p-value, exact method (5000 permutations), with asterisk (*) to indicate $p < 0.05$

Table 3.4 : Allele frequencies in Black and White control subjects

		Black		White		Allele
Gene variant	Allele	Freq	N	Freq	N	test [1]
IL1A						
rs3783590	A	0.093	59	0.002	227	***
rs2856836	C	0.229	59	0.278	227	
rs17561	T	0.229	59	0.278	227	
rs20540	T	0.051	59	0.000	227	***
rs2856838	T	0.364	59	0.388	227	
rs1609682	C	0.203	59	0.336	225	**
rs3783526	A	0.042	59	0.333	227	***
rs2856837	T	0.254	59	0.278	227	
IL1B						
rs1143643	A	0.195	59	0.339	227	**
rs1143634	T	0.169	59	0.202	225	
rs1143633	A	0.237	59	0.338	226	*
rs3136558	C	0.136	59	0.187	227	
rs1143630	A	0.288	59	0.064	227	***
IL1RN						
rs3181052	A	0.153	59	0.082	226	*
rs1794066	G	0.390	59	0.398	226	
rs1794067	A	0.288	59	0.308	227	
rs2071459	T	0.161	59	0.082	225	*
rs432014	C	0.203	59	0.308	227	*
rs380092	T	0.703	59	0.275	224	***
rs452204	A	0.491	58	0.396	227	
rs4252019	T	0.364	59	0.090	227	***
rs315955	C	0.138	58	0.000	227	***
rs315951	C	0.407	59	0.240	225	***
rs9005	A	0.254	59	0.335	227	

Table 3.4 (continued)

		Black		White		Allele
Gene variant	Allele	Freq	N	Freq	N	test [1]
IL6						
rs2069837	G	0.144	59	0.073	227	*
rs2069840	G	0.127	59	0.347	226	***
rs1554606	T	0.356	59	0.445	227	
rs2069842	A	0.042	59	0.000	225	***
rs1548216	C	0.169	59	0.018	227	***
rs2069843	A	0.093	59	0.018	227	***
rs2069845	G	0.347	59	0.444	223	
IL6R						
rs4845617	A	0.379	58	0.383	227	
rs6427641	G	0.703	59	0.423	227	***
rs11265610	C	0.272	57	0.000	214	***
rs12083537	G	0.280	59	0.203	227	
rs1386821	C	0.110	59	0.185	227	
rs4075015	A	0.110	59	0.423	227	***
rs4601580	T	0.570	57	0.415	224	**
rs4845618	G	0.542	59	0.434	227	*
rs7549338	C	0.297	59	0.421	227	*
rs7518199	C	0.186	59	0.410	227	***
rs4553185	C	0.578	58	0.430	227	**
rs4393147	T	0.103	58	0.410	227	***
rs4537545	T	0.644	59	0.421	227	***
rs4845626	T	0.475	59	0.167	227	***
rs28730736	A	0.153	59	0.000	225	***
rs11265618	T	0.407	59	0.170	227	***
rs10159236	A	0.220	59	0.161	227	
rs4329505	C	0.415	59	0.159	227	***
rs4509570	G	0.500	59	0.242	227	***
rs2229238	T	0.161	59	0.196	227	
rs4072391	T	0.263	59	0.196	227	
rs4379670	T	0.161	59	0.196	227	

1. Allele test p-value, p<0.001***, p<0.01**, p<0.05*

2. Hardy-Weinberg-Equilibrium p-value, exact method (5000 permutations), with asterisk (*) to indicate $p < 0.05$

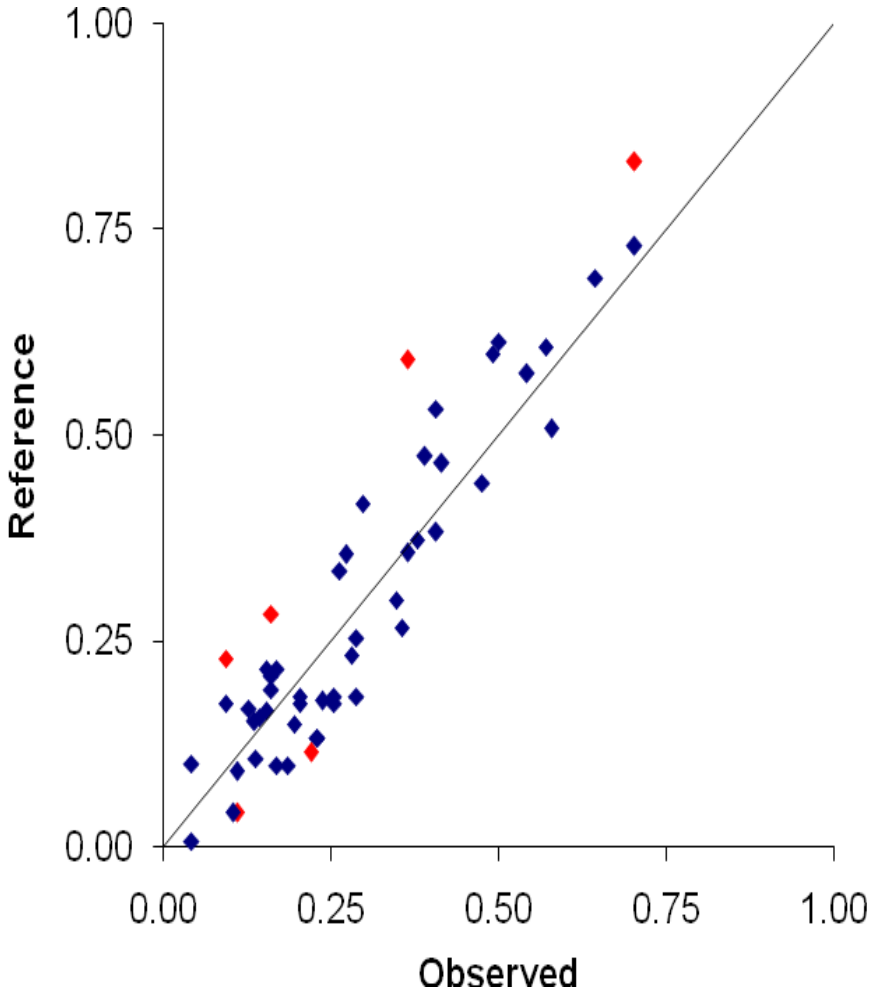


Figure 3.1: SNP by SNP comparison of allele frequencies observed in Black control group (N=59) and calculated for the HapMap YRI reference population (N=60 mother-father pairs). Reference and observed allele frequencies for SNPs shown red differ significantly (allele test, p-value <0.05).

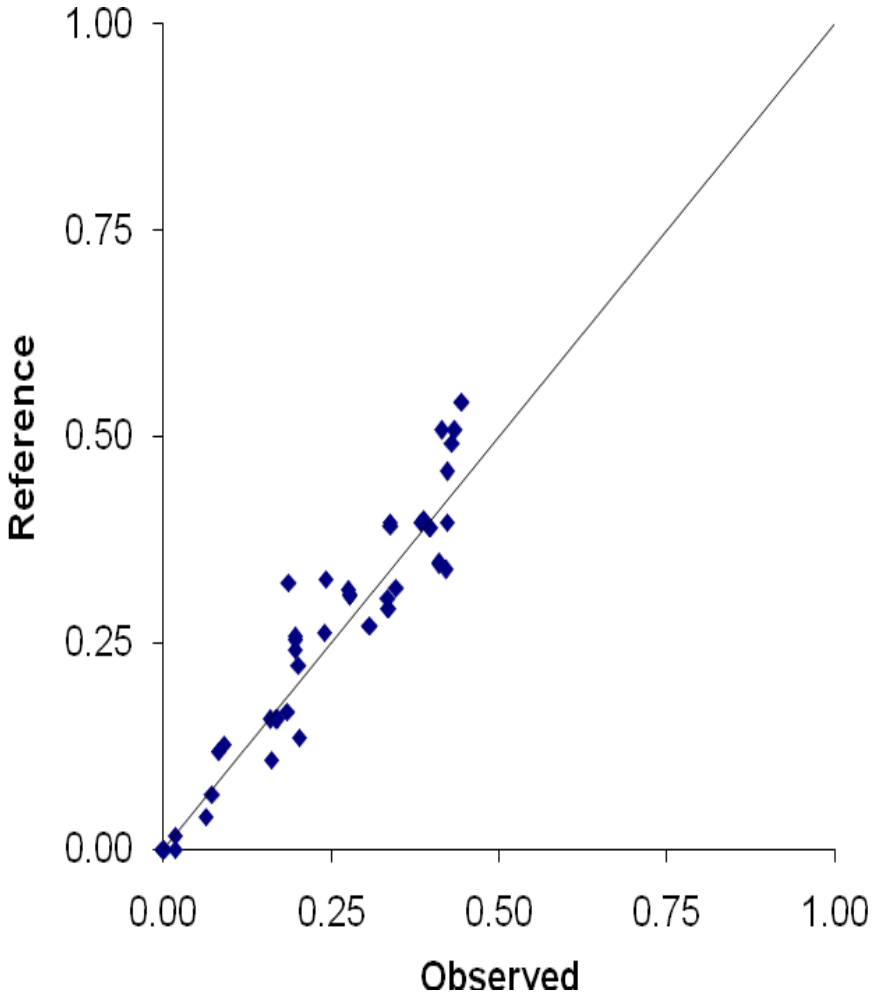


Figure 3.2: SNP by SNP comparison of allele frequencies observed in White control group (N=227) and calculated for the HapMap CEU reference population (N=60 mother-father pairs). Reference and observed allele frequencies for SNPs shown red differ significantly (allele test, p-value <0.05).

4.0 PAPER 2: INFLAMMATORY CYTOKINES INTERLEUKIN-1A (IL-1A), IL-1B, IL-1RN, IL-6 AND IL-6R AND PROSTATE CANCER RISK IN AFRICAN AMERICAN~ AND CAUCASIAN MEN: THE CANCER AND PROSTATE STUDY

Kwame Appenteng, MPH, [1] Clearann Bunker, PhD,[1] Joel Nelson, MD,[2] John Wilson, PhD,[3] Robert Ferrell, PhD,[4] Brenda Diergaarde, PhD,[1] Susan Moffett,[4] PhD, Francesmary Modugno, PhD,[1] Claudia Leiras, MPH,[1] James Shikany, DrPH, [5] Joel Weissfeld, MD, MPH,[1]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[2]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

[3]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[4]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[5] Department of Preventative Medicine, University of Alabama at Birmingham, Birmingham, Alabama

4.1 ABSTRACT

Objective: To assess association of common polymorphisms in inflammatory genes *interleukin-1A (IL-1A), IL-1B, IL-1RN, IL-6* and *IL-6R* and risk of prostate cancer in African American (AA) and Caucasian men.

Methods: We conducted a matched case-control study of 558 African-American (AA) and Caucasian men ages 40-80y who enrolled in the Cancer and Prostate Study (CAPS) between 2001 and 2006. A total of 53 single nucleotide polymorphisms (SNPs) were selected using HapMap, Haploview and FastSNP. Genomic DNA was extracted from whole blood, and genotyping was done using Sequenom iPlex Gold.

Results: Genotyping information was successfully obtained on 53 SNPs for 113 AA (54 cases and 59 controls) and 445 Caucasians (218 cases and 227 controls). Three *IL-1RN* SNPs (rs452204, rs425019, and rs9005) were associated with a personal history of BPH or prostatitis in AA, but not in Caucasian controls. Three *IL-1B* SNPs (rs1143643, rs1143633 and rs1143630) were significantly associated with a family history of prostate cancer in Caucasian, but not in AA controls. *IL-1RN*-rs432014, *IL-1RN*-rs9005, and *IL-6R*-rs4845626 were significantly associated with prostate cancer in AA in the dominant model. Three *IL-1RN* SNPs (rs3181052, rs2071459, and rs4252019) were associated with prostate cancer in Caucasians in the dominant model. We identified two putative functional SNPs (*1L-1A*-rs20540 and *IL-6R*-rs28730736) whose variant alleles are carried by AA, but not by Caucasians.

Conclusion: We found statistically significant prostate cancer associations of two *IL-1RN* SNPs in AA, three *IL-1RN* SNPs in Caucasians, and one *IL-6R* in AA.

4.2 INTRODUCTION

Prostate cancer is a major public health problem in the United States. It is the most common nonskin cancer, and the second leading cause of cancer related death among men in the United States[1]. According to the American Cancer Society, there will be approximately 186,320 new cases of prostate cancer in the United States in 2008; in the same year approximately 28,660 men will die from the disease.[1] African Americans (AA) are twice as likely to be diagnosed with and die from the disease as are White Americans [2]. Socioeconomic and hormonal differences are thought to be contributory factors [2-4], however, the role played by differences in sequence variants of genes in the inflammation pathway of these two racial groups have not been comprehensively examined as part explanation for these disparities.

Chronic or recurrent inflammation is known to increase the incidence of malignancies of the bladder, colon, endometrium, esophagus, liver, lung and pancreas [5-9]. Similarly, evidence from epidemiologic, genetic, molecular biology and histopathology studies have suggested a compelling role of inflammation in the development of prostate cancer [10-13]. The precise mechanism by which inflammation causes cancer is currently not clearly understood, but it is thought that chronic or recurrent inflammation, which may be a result of immunological conditions, recurrent microbial infections, or chemical irritation, trigger the production of inflammatory cytokine mediators and genotoxic reactive oxygen radicals that increase cell proliferation and promote tumorigenesis [14]. The likelihood of developing cancer may then be dependent upon precise host response to this inflammatory cascade [15].

The interleukin-1 *(IL-1)* and interleukin-6 (*IL-6*) family of genes promote inflammation, and have been reported to be associated with prostatic tumorigenesis [21, 22]. Sequence variants in

the interleukin-1 receptor antagonist *(IL-1RN*) have been reported to be associated with prostate cancer risk in a population-based study conducted in Sweden [21]. Additionally, endogenous *IL-1* has been reported to promote the invasiveness of malignant cells of the prostate by initiating and completing the process of angiogenesis [23]. Interleukin-6 regulates the growth and differentiation of prostate carcinomas [24], and has been shown in laboratory studies to be involved in the initiation and progression of prostate cancer by mediating the lysophosphatidic acid-regulated cross-talk between stromal and epithelial cells of the prostate gland [25]. Additionally, clinical studies have shown that elevated circulating plasma levels of *IL-6* and its soluble receptor are associated with prostate cancer progression and metastasis [26-28].

Even though prostate cancer incidence and mortality rates have been reportedly higher in African Americans than Caucasians for several decades[1], the molecular factors that contribute to these racial disparities are still unclear. The disease is initially androgen dependent but rapidly becomes androgen independent, and refractory to therapy. Inflammatory cytokines have been reported to influence clinical outcome of prostate cancer by mediating the transition from androgen dependence to androgen independence[60]. Evidence from epidemiologic, genetic, molecular biology and histopathology studies have suggested a compelling role of chronic or recurrent inflammation in the development of prostate cancer[10-13, 126]. The role of inflammatory genes *IL-1* and *IL-6* in prostate cancer risk and in explaining observed racial disparities in the disease are not clearly understood. However, cytokine gene polymorphisms have been reported to be strongly associated with ethnicity[274], and these differences have been suggested to partly explain the apparent influence of ethnicity on disease outcome, such as allograft rejection[274] . The purpose of this study is to determine if genotypes of polymorphisms in inflammatory genes

IL-1A, IL-1B, IL-1RN, IL-6 and IL-6R are related to prostate cancer risk in African American and Caucasian men.

4.3 MATERIALS AND METHODS

4.3.1 Study sample

Participants in the current study were selected from an existing prostate cancer case-control study known as the Cancer and Prostate Study (CAPS). Briefly, CAPS was a case-control study designed to assess the individual and joint associations of bone mineral density (BMD) and sex hormone gene polymorphism with prostate cancer. Enrollment into CAPS started in December of 2001 and was completed in January of 2006, and included Caucasian and African American (AA) men aged 40 to 80 years. Cases were men with recently diagnosed prostate cancer (within 3 months of enrollment into the study; confirmed diagnosis based on pathology report). Controls were men without a history of prostate cancer. All participants in CAPS gave informed consent. The study was approved by the institutional review boards (IRB) of the University of Pittsburgh and the University of Alabama at Birmingham.

Potential enrollees were excluded if they used glucocorticoids (>6 months); used testosterone (>3 months); had a history of hyper- or hypothyroidism, hyperparathyroidism, renal disease or bone disease. Other exclusion criteria included bilateral hip replacement; kidney transplant; previous diagnosis of prostate cancer or any other cancer besides basal and squamous cell skin cancer; evidence of bone metastases among prostate cancer cases; and PSA levels above 3.0 ng/ml among controls. Controls were frequency matched to cases by age and race.

Participants in CAPS were recruited from Pittsburgh and Alabama. In Pittsburgh, recruitment was conducted at two sites: The University of Pittsburgh Medical Center (UPMC), and the Veterans Administration Medical Center (VA). Recruitment in Alabama was conducted

at the University of Alabama in Birmingham (UAB) Medical Center. A total of 593 Caucasian and African American men were enrolled in CAPS. There were a total of 244 cases recruited at UPMC, all of who were referrals from one institution-based urology practice. Patients at this practice were mostly community-based residents from Pittsburgh, many of who were referred for specialty care by their primary care physicians. All recruited cases from this urology practice underwent radical prostatectomy within 3 months of diagnosis. The urologist personally informed potential subjects about the study for the first time during their second post-operative follow-up visit. Interested men were then referred to the CAPS research team at UPMC for further study details and enrollment information. Controls from Pittsburgh numbered 253, and were recruited from the local community, as well as from University of Pittsburgh employees, by sending out flyers. Controls were frequency matched by age and race to cases. Majority of the controls were from the ongoing Prostate, Lung, Colorectal, and Ovarian study (PLCO). A total of 10 participants were recruited from the VA Medical Center in Pittsburgh, comprising of 3 cases and 7 controls.

In Alabama, information about the CAPS study was advertised in the *UAB Reporter*. Additionally, flyers were sent out to local residents and University of Alabama employees, and brochures were placed in waiting rooms of Birmingham area urologist offices. Urologists at three community-based urology practices informed potential case subjects about the study, and then referred interested parties to an on-site study recruiter from UAB Medical Center, who provided detailed information about the study. A total of 42 cases were enrolled at UAB Medical Center, which included 41 referrals from the three community-based urology practices and one subject who was referred by word of mouth from another study participant. Forty-four community-based controls were enrolled at the UAB Medical center, majority of who were from the ongoing

PLCO trial. Other control enrollees included UAB employees, as well as local Birmingham residents who responded to advertisements and flyers. The total number of CAPS subjects eligible for the current study was therefore 593, comprising 122 AA and 471 Caucasians.

An interviewer administered standardized questionnaire was used to collect demographic and prostate cancer risk factor information. Whole blood, height and weight measurements, bone mineral density (BMD, Hologic DEXA) were obtained at a single clinic visit. Samples were stored in a -70^{o}C freezer in the Department of Epidemiology in the Graduate School of Public Health at the University of Pittsburgh.

4.3.2 Selection of Single Nucleotide Polymorphisms

Single Nucleotide Polymorphisms (SNPs) in *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* were selected using two web-based programs – HapMap Phase 1 and 2 (HapMap Data Rel 22/phase II April 07, on NCBI B36 assembly, dbSNP b126) and Functional Analysis and Selection Tool for Single Nucleotide Polymorphisms (FastSNP). HapMap and FastSNP information is available to the public online, and the programs can be downloaded free of charge at the respective websites: http://www.hapmap.org and http://www.fastsnp.ibms.sinica.edu.tw.

HapMap was used to obtain SNP information per gene for Caucasians (CEU) and Blacks (Yoruba-YRI) separately, and the information was downloaded into Haploview. The Tagger approach was used in Haploview to select TagSNPs for CEU and YRI separately, at a minor allele frequency (MAF) of at least 10%, and a pair-wise correlation (r^2 of 0.80 or greater). This procedure identified a total of 51 TagSNPs in both racial groups. Haploview information is available to the public online, and the program can be downloaded free of charge at:

www.broad.mit.edu/haploview/haploview-downloads . To compliment the list of SNPs obtained from Haploview, we ran the five candidate genes through FastSNP, resulting in the identification of 2 additional potentially functional SNPs, bringing the total number of SNPs for genotyping analysis to 53.

4.3.3 Laboratory Assay

Genomic DNA was isolated from EDTA anticoagulated whole blood by standard methods using the Puregene kit (Gentra Systems). Genotyping was carried out on the Sequenom platform.

4.3.3.1 Sequenom i-PLEX Gold SNP Assay

Primer Design: Three primers were designed for each locus of interest using Mass Array Assay Design. The two amplification primers flanked the polymorphic site to provide for sample amplification, while the single MassExtend primer lay immediately adjacent to allow for allelic discrimination via single base extension. Assay Design software determined pooling of primer sets to optimize multiplex reactions. Mass modifications are incorporated in the design of the MassExtend primers to maximize the mass differential between primers of different loci within a given multiplex pool.

Sample Amplification: Target loci were amplified within the samples by multiplex polymerase chain reaction (PCR) in 1X PCR buffer (Qiagen) containing 3.5 mM $MgCl_2$, 25 mM dNTPs, 500 nM each forward and reverse amplification primer within the multiplex pool and 2.5 U HotStar Taq (Qiagen). PCR conditions were: 95^{o}C for 15 minutes for taq activation followed by 45 cycles of 94^{o}C for 20 seconds, 56^{o}C for 20 seconds and 72^{o}C for 1 minute. A single

extension for 1 minute at 72°C completed the PCR reaction. Deoxyribonucleotide triphosphates (dNTPs) and primers were removed by incubation with 0.5 U shrimp alkaline phosphotase (SAP) at 37 °C for 40 minutes. SAP was inactivated by incubation at 87 °C for 5 minutes.

MassExtend: Excess MassExtend primers corresponding to the loci represented by the amplification primers used were pooled. Higher mass primers were added at a higher concentration to adjust for signal drop off during spectra acquisition. Single base extension was carried out in 0.2X iPLEX buffer plus, 1X termination mix (containing mass modified termination nucleotides), 1X iPLEX enzyme and primers at 0.84 μM, 1.04 μM and 1.25 μM as appropriate to the relative mass of the primer. A double cycle amplification program performed 40 cycles of denaturation at 94 °C for 5 seconds followed by 5 cycles of 52°C for 5 seconds, 80 °C for 5 seconds, back to 94 °C for a total of 200 cycles. A final extension at 72 °C for 3 minutes completed the amplification. Clean resin and water were added to the MassExtend reaction products. Samples were incubated in clean resin at room temperature with mixing for 5 minutes and centrifuged at 3200 x g for 5 minutes.

NanoDispense, Spectra acquisition and analysis: Samples were dispensed to a SpectraChip using the MassArray Nanodispenser according to manufacturer's instructions. Spectra chips were loaded into the MassArray analyzer and spectra acquired for each sample. MassArray Typer software used the known mass of the MassExtend primers to identify each locus, and the increase caused by each distinct nucleotide to identify the alleles present in the sample.

Whenever appropriate, alleles that were not automatically identified by the computer software were directly read and assigned by the operator. We observed 100% concordance rates

in two randomly replicated samples. Centre d'Etude du Polymorphisme Humain (CEPH) positive controls and water negative controls were included in two 96 well plates as part of quality control measures. For 52 of 53 SNP assays we were able to obtain genotyping results for over 97% of subjects analyzed. One SNP assay (rs11265613) produced a genotype result in 94% of subjects tested.

4.3.4 Data Analysis

We analyzed our data in SAS/Genetics version 9.2. We excluded 31 study subjects due to lack of sufficient sample for genotyping. Four additional subjects were excluded due to low call rates (<85%), bringing the total number of subjects excluded from our analysis to 35. Therefore the total number of subjects included for analysis in the current study is 558, comprising 113 AA and 445 Caucasians. Genotype frequencies observed among AA controls were consistent with HWE. Two *IL-1RN* loci (rs3181052 and rs4252019) and two *IL-6R* loci (rs4393147 and rs7518199) departed from HWE among Caucasian controls ($0.024 < P < 0.050$, exact test).

We conducted race-specific descriptive analyses, by case-control status, on all 558 subjects included in our analyses for the following risk factors: age (years), height (cm), weight (kg), body mass index (kg/m^2), personal history of benign prostatic hypertrophy (BPH) or prostatitis ("yes" or " no"), family history of prostate cancer ("yes" or "no"), and history of regular aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) use ("yes" or "no"). Family history of prostate cancer was defined as a father, brother or half brother with a history of prostate cancer. Regular aspirin or NSAIDs use was defined as taking any of these medications at least five to seven days per week for the twelve months preceding enrollment into the study.

To determine whether differences existed between subjects included (n=558), and those excluded (n=35) from our analyses, we performed descriptive analyses comparing these two groups using the chi-square test for categorical variables, and the t-test for continuous variables.

In our control groups, we characterized race-specific distributions of individuals homozygous or heterozygous for the variant allele at each locus, for the following risk factors: age (years), height (cm), weight (kg), body mass index (kg/m^2), hip bone mineral density (BMD, gm/cm^2), personal history of benign prostatic hypertrophy (BPH) or prostatitis ("yes" or " no"), and family history of prostate cancer ("yes" or "no"), using the chi-square test for categorical variables, and the t-test for continuous variables.

We compared genotype frequencies at each locus by case-control status, according to race, using the chi-square test. Our analyses of the association of each inflammatory gene marker and prostate cancer were based on genotype and trend chi-square tests. We assumed a dominant model of inheritance to evaluate the magnitude of association (OR, and 95% CI) between genotype and prostate cancer. Race- and age- stratified conditional logistic regression models (unadjusted and adjusted) were fitted to assess the association of each marker and prostate cancer, by race, and for all subjects. Age stratification was based on the following age categories (40-54y, 55-59y, 60-64y, and 65-80y). Each conditional logistic regression model was adjusted for the following risk factors individually: personal history of BPH or prostatitis, family (father, brother, or half brother) history of prostate cancer, BMI (based on race-specific tertile cutpoints in the control groups: Blacks – 27.8 and 32.0 kg/m^2; Whites – 26.83 and 30.59 kg/m^2), and BMD (based on race-specific tertile cutpoints in the control groups: Blacks – 1.005 and 1.124 gm/cm^2; Whites – 0.972 and 1.070 gm/cm^2). Race-specific haplotype blocks were constructed for *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* genes in Haploview 4.1[284]. Definition of haplotype

blocks were based on pairwise measures of linkage disequilibrium (LD) as calculated by Haploview[285]. Loci in strong LD were combined into haplotypes. Race-specific distributions of haplotype frequencies and their corresponding p-values were generated by Haploview, for each gene, by case-control status.

4.4 RESULTS

Selected subject characteristics are shown in Table 4.1. A total of 558 men who enrolled in CAPS and whose blood samples were satisfactorily genotyped were included in our analyses. There were 113 AA (54 cases and 59 controls), and 445 Caucasians (218 cases and 227 controls). Controls had a slightly higher body mass index (BMI) than cases, and were also slightly older, but these differences were not statistically significant. A first degree relative with a history of prostate cancer was significantly associated with prostate cancer in both races. A personal medical history of benign prostatic hyperplasia (BPH) or prostatitis was statistically significantly higher among AA cases than controls, however, among Caucasians, it was statistically significantly lower in cases than controls.

A list *of IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* SNPs that were genotyped is shown in Table 4.2. Genes are ordered according to chromosome and SNPs by position across the respective genes. Genotyping results for the 53 SNPs are shown for 558 AA and Caucasian subjects (Tables 4.3 to 4.5, and in the Appendix Tables A1.1 to A1.5). Race-specific distributions of control subjects with one or two copies of the variant allele at each locus are categorized by selected risk factors: personal history of prostatitis or BPH, and family history of prostate cancer distributions are shown in Tables 4.3 and 4.4. Figures 4.1 to 4.5 show race-specific haplotype block organization and corresponding haplotype frequencies for *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R*. Appendix Tables A1.1 to A1.5 show race-specific distributions of control subjects homozygous or heterozygous for the variant allele at each of the 53 loci in relation to the following risk factors: age, height, weight, BMI, and BMD.

4.4.1 *IL-1* genes and selected risk factors in controls

A personal history of BPH or prostatitis was significantly less common among AA controls heterozygous or homozygous for the variant alleles at three *IL-1RN* SNPs (rs452204, rs425019, and rs9005) compared to AA controls homozygous for the common allele (Table 3). African American controls heterozygous or homozygous for the variant (T) allele at *IL-1A*-rs20540 weighed significantly less, and had a significantly lower BMI than non-carriers (Tables A1.3 and A1.4). No other significant associations were observed between *IL-1* genes and selected risk factors in AA controls.

A family history of prostate cancer was significantly more common among Caucasian controls heterozygous or homozygous for the variant alleles at three *IL-1B* SNPs (rs1143643, rs1143633 and rs1143630) compared to Caucasian controls homozygous for the common allele (Table 4.4). Two of these SNPs (rs1143643 and rs1143633) were in linkage disequilibrium (LD) with each other (r^2=0.97) (Fig. 4.2). Caucasian controls heterozygous or homozygous for the variant allele (T) at *IL-1A*-rs2856838 had a significantly lower mean hip BMD than non-carriers (Table A1.5). No other significant associations were observed between *IL-1* genes and selected risk factors in Caucasian controls.

4.4.2 Associations of *IL-1* markers and prostate cancer

A greater proportion of AA cases (0.72) than controls (0.61) were homozygous for the common allele (G) at *IL-1A*-rs17561, but the difference was not statistically significant (Table 4.5). In Caucasians a slightly lesser proportion of cases (0.5) than controls (0.53) were homozygous for

the common allele at *IL-1A*-rs17561, but the difference was not statistically significant (Table 4.5). The GG genotype of this particular marker up-regulates the protein encoded by the *IL-1A* gene. The common allele (C) at *IL-1-B*-rs1143634 was not significantly associated with prostate cancer in AA or Caucasians (Table 4.5). However, the (C) allele at this locus up-regulates *IL-1B* secretion, and was carried more commonly by AA controls (83%) compared to Caucasians control (80%). *IL-1-B*-rs1143634 was in strong pair-wise linkage disequilibrium with *IL-1-B*-rs1143633 in Caucasians, Figure 4.2.

Among AA increasing doses of the variant alleles were significantly associated with prostate cancer at two *IL-1RN* loci (rs432014 and rs9005, Table 4.5). In the dominant inheritance model the variant alleles of both of these SNPs were associated with a significantly decreased risk of prostate cancer in AA (Table 4.5). In Caucasians, increasing doses of the variant alleles at three *IL-1RN* loci (IL-1RN-rs3181052, IL-1RN-rs2071459 and *IL-1RN*-rs4252019 were significantly associated with prostate cancer (Table 4.5). The variant alleles at each of these loci were significantly associated with an increased risk of prostate cancer in Caucasians (Table 4.5). *IL-1RN*-rs2071459 was in linkage disequilibrium with *IL-1RN*-rs3181052 (r^2=0.92), Figure 4.3. Alleles from both of these SNPs were part of an *IL-1RN* haplotype (AGGTT). This haplotype was found more commonly in Caucasian prostate cancer cases (11.7%) than controls (8.1%), but the difference was not statistically significant (p = 0.765), Figure 4.3.

4.4.3 *IL-6* genes and selected risk factors in controls

A family history of prostate cancer was significantly less common among AA controls heterozygous or homozygous for the variant alleles at *IL-6R*-rs1554606 (Table 4.4) compared to

non-carriers. However, among AA controls, a significantly greater proportion of individuals heterozygous or homozygous for the variant allele at *IL-6R*-rs4393147 had a family history of prostate cancer compared to non-carriers (Table 4.4). Five *IL-6R* SNPs were significantly associated with anthropometric measures in AA controls heterozygous or homozygous for the variant alleles compared to non-carriers: weight (rs4845617 and rs11265610, Table A1.3), BMI (rs11265610 and rs4537545, Table A1.4), and BMD (rs4537545, Table A1.5). No other associations of *IL-6* or *IL-6R* SNPs and selected risk factors were observed in AA controls.

Caucasian controls heterozygous or homozygous for the variant allele at two *IL-6* loci (rs1554606 and rs2069845) had a significantly higher mean BMD than non-carriers (Table A1.5). These two SNPs were in strong pair-wise LD (r^2=0.99), Figure 4.5. Seven *IL-6R* SNPs (rs4845618, rs7549338, rs4553185, rs4845626, rs11265618, rs10159236 and rs4329505) were all significantly associated with BMD in Caucasian controls heterozygous or homozygous for the variant alleles compared to non-carriers, (Table A1.5). Four of these SNPs (rs4845626, rs11265618, rs10159236 and rs4329505) were in pair-wise LD with each other ($r^2 \geq 0.90$). No other *IL-6* or *IL-6R* loci were associated with selected risk factors.

4.4.4 Associations of *IL-6* markers and prostate cancer

Increasing doses of the variant allele (T) at *IL-6R*-rs4845626 was associated with decreased prostate cancer risk in AA (Table 4.5). Individuals heterozygous or homozygous for the variant allele had a protective effect against prostate cancer in the dominant inheritance model, compared to non-carriers (Table 4.5). No other statistically significant associations of *IL-6* markers and prostate cancer were noted in our dominant inheritance model in either race.

4.5 DISCUSSION

We examined the associations of *IL-1 and IL-6* gene polymorphisms and the risk of prostate cancer in AA and Caucasians. The current study was undertaken because recent studies have reported mounting evidence for potential associations of sequence variants of these pro-inflammatory genes and prostate cancer[21, 24] . Moreover, prostate cancer incidence and mortality rates have been consistently higher among AA than Caucasians for several decades[1, 54]. Furthermore, racial differences in immune modulating genes are well documented [277], and it has been suggested that such differences may influence disparities in clinical outcome between AA and Caucasians[274]. We sought to determine if genotypes of *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* were associated with prostate cancer risk, as well as with selected risk factors, in the two racial groups. We found racial differences in the associations of SNPs of pro-inflammatory genes *IL-1* and *IL-6* and prostate cancer, as well as with selected risk factors. Our findings support a growing body of evidence that chronic or recurrent inflammation play an important role in prostate carcinogenesis[10-13], and the possibility of ethnic based differences in susceptibility.

In the current study, several *IL-1* SNPs were observed to be associated with prostate cancer, and also with selected risk factors. *Interleukin-1A* and *IL-1B* up-regulate the division of immune cells, as well as promote cell growth, differentiation and migration[188]. They also inhibit apoptosis and induce angiogenesis, thereby promoting tumor growth. Their action, however, is inhibited by the binding of the *IL-1RN* to the *IL-1* receptor. Interleukin-1 and its related family members are primarily inflammatory cytokines, and are known to induce the release of cyclooxygenase-2 (COX-2), type 2 phospholipase A and inducible nitric oxide synthase in inflammation[187]. This accounts for the large amount of prostaglandin-E2 (PGE-2), platelet

activating factor and nitric oxide produced by cells exposed to *IL-1* or in animals or humans injected with *IL-1*[188]. Additionally, *IL-1* promotes the infiltration of inflammatory and immunocompetent cells into extravascular space by increasing the expression of adhesion molecule-1 on mesenchymal cells and vascular-cell adhesion molecule-1 on endothelial cells[188]. Since host response to inflammation (chronic or recurrent) is known to influence cancer development[15], race or ethnicity based differential immune responses to inflammation may translate to differential cancer development in the given groups.

The GG polymorphism at *IL-1A*-rs17561 produces an alanine-to-serine amino acid substitution at codon 114 of the IL-1 cytokine protein and has been associated with an increased risk of atopy[281]. In a recent study which compared differences in cytokine gene polymorphisms among healthy primiparous African American (N = 179) and Caucasian (N = 396) women seeking prenatal care prior to 20 weeks' gestation in Pittsburgh, Pennsylvania, Ness et al reported that the up-regulating GG genotype at *IL-1A*-4845 (*IL-1A*-rs17561) was found in 59.7% of AA women compared to Caucasian women (47.3%)[282]. In the current study the GG genotype was found in 61% of AA men compared to 53% Caucasians. The (C) allele at *IL-1B*-rs1143634 has been associated with an increased secretion of IL-1B in activated macrophages in vitro[283]. Ness et al reported that the (C) allele at *IL-1B*-3597 (rs1143634) was found in 86% of AA women compared to 77% in Caucasians[282]. In the current study the (C) allele was found in approximately 83% of AA men compared to 80% of Caucasians.

We observed significant racial differences in the distribution of variant alleles of *IL-1* and *IL-6* SNPs (*IL-1A* 50%, *IL-1B* 60%, *IL-1RN* 64%, *IL-6* 71% and *IL-6R* 68%) in the current study. The extent to which these differences influenced prostate cancer risk in our cases is unclear, but we observed, for instance, that while a personal medical history of benign prostatic hypertrophy

(BPH) or prostatitis was significantly higher in AA cases than controls, it was significantly lower in Caucasian cases than controls (Table 4.1). A personal medical history of prostatitis has been reported by several studies to be associated with an increased risk of prostate cancer[83, 84]. We also observed noteworthy differences in the markers of association between the AA and Caucasians. The three *IL-1RN* SNPs (rs452204, rs4252019 and rs9005) that were associated with a personal history of BPH or prostatitis in controls showed significance exclusively in AA, whereas *IL-1B* SNPs (rs1143643, rs1143633 and rs1143630) showed significant associations with a family history of prostate cancer that were observed exclusively in Caucasian controls. *IL-1B*-rs1143643 is involved in splicing regulation, and was in strong LD with rs11436633 (r^2=97) in Caucasains. While we are not certain of the functional significance of all of the other markers, we find their polarizing racial predilection very striking. The variant allele of *IL-1A*-rs20540 was observed only in AA (5% of controls). Caucasians were 100% homozygous for the common allele. *IL-1A*-rs20540 is a putatively functional SNP, involved in splicing regulation. No significant association of this marker and prostate cancer was observed in the dominant inheritance model. To our knowledge, this marker has not been reported in the literature as being associated with prostate cancer to date.

Two *IL-1RN* SNPS (rs432014 and rs9005) were significantly associated with prostate cancer in AA. Three SNPs at *IL-1RN* (rs3181052, rs2071459 and rs425019) were significantly associated with prostate cancer in Caucasians in our dominant model. Two of these SNPs (rs3181052 and rs2071459) were in strong linkage disequilibrium (r^2=0.97). The exact functions of these SNPs are not clearly known, but they are candidates with biological plausibility, being that they are clustered in highly conserved genomic regions in different vertebrate species (http://genome.ucsc.edu). Two *IL-1RN* haplotypes that were significantly associated with

prostate cancer in AA subjects (GG and AC) provided a protective effect against the disease; however, the single *IL-1RN* haplotype (TCG) that was significantly associated with prostate cancer in Caucasians increased the risk of disease. We were unable to document an association of *IL-1RN*-rs315951 and prostate cancer, as reported by Lindmark et al[21]. In their study, rs315951 was one of four SNPs that formed a common haplotype which was strongly associated with prostate cancer[21].

Interleukin-1 is one of the most potent inducers of COX-2, which plays a key role in the inflammatory process[188]. Aspirin and NSAIDs are known to exert their anti-inflammatory properties by selectively inhibiting the release of COX-2. We observed a high prevalence of ASA or NSAIDs use in our Caucasian subjects compared to AA. Among our controls 51% of Caucasians and 27% of AA used ASA or NSAIDs regularly. Similarly, in our cases 46% of Caucasians and 30% of AA used ASA or NSAIDs on a regular basis. Several population studies have reported a reduction in prostate cancer risk by regular use of ASA or NSAIDs[91, 94, 144]. If the pattern of ASA or NSAIDs use among men in our study in any way reflects the patterns of use among men in the general population, then this may imply that there is greater attenuation of the disease in Caucasians than AA from regular ASA or NSAIDs use, and may partly explain the differences in prostate cancer rates between the two racial groups.

IL-6R-rs4845626 showed the most significant association with prostate cancer (P_{trend}=0.0022). The variant allele (T) at this marker was 3 times more likely to be carried by AA in our study than Caucasians. Individuals heterozygous or homozygous for the variant allele at this marker had a protective effect against prostate cancer compared to non-carriers, even after adjusting for a personal history of BPH or prostatitis in our conditional logistic regression model. The variant allele of another *IL-6R* SNP rs28730736 was carried by 15% of AA controls in our

study, but was absent in our Caucasian controls, who were 100% homozygous for the common allele. This is a functionally relevant SNP (missense), and is involved in splicing regulation. To our knowledge, this marker has not been reported in the literature as being associated with prostate cancer, to date. Among AA, one of the most commonly occurring haplotypes was *IL-6R*-CC (Figure 4.5, block 4), and was associated with an increased risk of prostate cancer. The haplotype with the most significant association with prostate cancer was *IL-6R*-GG (Figure 4.5, block 2). This haplotype was found in AA, but it provided a protective effect against the disease. In the current study, we did not directly genotype *IL6*-174 (rs1800795), however one of the SNPs we genotyped, *IL-6*-rs1554606 was in pair-wise linkage disequilibrium with *IL-6*-174 (r^2=0.90). The *G allele of *IL-6*-174 up-regulates the production of *IL-6* and has been reported by some studies to be carried more commonly by AA than Caucasians[274, 282].

We were unable to document an association of *IL-6R*-rs4329505 and prostate cancer, as reported by Zheng et al[12]. In their study of 9,275 SNPs in 1,086 genes in the inflammation pathway, *IL-6*-rs432905 was identified as one of 26 SNPs which were strongly associated with prostate cancer in the first stage of the study, but not in the confirmatory stage, where only three other SNPs showed association[12].

Interleukin-6 is involved in regulating immune and inflammatory responses[154]. In addition to inducing terminal differentiation of B-cells it synergizes with *IL-1* in activating T-cells by inducing *IL-2* responsiveness, and enhances the differentiation of cytotoxic T lymphocytes from thymic precursors[146, 155, 156]. It has a central role in the acute-phase response, acting on hepatocytes to increase the synthesis of acute-phase proteins (haptoglobin, fibrinogen, C-reactive protein,etc) and reducing the secretion of albumin and transferein[157]. It also contributes to the body's defenses by increasing the body temperature and stimulating the release

of adrenocoticotropin hormone[158-160]. Other functions include: impairment of natural killer cell function; induction of bone resorption; stimulation of osteoclast formation; induction experimental cancer cachexia; induction of platelet-derived growth factor in blood vessels; enhancement of proliferation of vascular smooth muscle; negative inotropic effect on cardiac myocites; enhancement of secretion of chorionic gonadotrophin from trophoblasts[154].

Attention to the role of *IL-6* in the pathogenesis of prostate cancer was drawn by the observation that the disease transitioned from an androgen-dependent tumor, initially responsive to androgen ablation therapy, to an untreatable androgen independent tumor[60]. In-vitro studies by Siegall at al showed that the androgen-independent prostate cancer cell lines DU145 and PC3 and the androgen-dependent cell line LNCaP expressed *IL-6R* on their surfaces, and all three cell lines were susceptible to a chimeric Pseudomonas exotoxin-*IL-6* toxin. Susceptibility was mediated by *IL-6R* as cytotoxic activity was blocked by in the presence excess human recombinant *IL-6*[172]. Since then, the expression of mRNA for *IL-6R* and the gp130 signal transducer has been confirmed in human prostate cancer by several other investigators[172-174]. Furthermore, recent in-vitro studies have shown that *IL-6* initiates and promotes prostate tumorigenesis by mediating cross-talk between stromal and epithelial cell of the prostate[25].

In the current study, we observed associations of *IL-6* and *IL-6R* and several prostate cancer risk factors. *Interleukin-6* and *IL-6R* were significantly associated height and weight in AA, but not Caucasians (Appendix, Tables A1.2 and A1.3). While tall stature has been reported by some studies as being associated with an increased risk of prostate cancer[286-288], the association of weight and prostate cancer is not so clear. Additionally, of the 11 SNPs that showed associations with BMD two were *IL-6* markers and the other eight were *IL-6R* markers (Appendix, Table A1.5). Several of these SNPs were in strong linkage disequilibrium with each

other in Caucasians. BMD has been reported by several studies to be associated with prostate cancer[261, 263, 264]. With the exception of *IL-6R*-rs4845618, all statistically significant associations of *IL-6* SNPs with BMD in our study were observed exclusively in Caucasians.

Our study had several limitations. We relied on self reporting of race, even though there is an approximately 7-20% admixture among African Americans, which may tend to decrease our observed race-specific associations. Our tag SNP panel was based on Phase II of the International HapMap project, and may not have captured a more comprehensive set of tag SNPs as provided in Phase III of the HapMap project. In spite of our best efforts to include a comprehensive set of markers in our study, there is always the possibility of excluding markers of true association.

In spite of these limitations, our study has several strengths. It is one of the first to directly assess prostate cancer risk in AA and Caucasian men within the context of a comprehensive set of inflammatory cytokines. It evaluated the associations of the inflammatory cytokines and commonly reported risk factors of prostate cancer in the two racial groups. It identified two putative functional SNPs (*IL-1A*-rs20540 and *IL-6R*- rs28730736) in which variant alleles were observed in AA, but not Caucasians. These two SNPs have not been previously reported in the literature as being associated with prostate cancer. Prostate cancer is a very complex disease, whose development may involve a combination of numerous risk factors under a wide variety of conditions. Therefore no given risk factor (s) may explain all the variability in the disease, and its susceptibility. We believe however, that the racial predilection of some of the makers identified in our study should prompt further investigation into the roles of some of these inflammatory cytokines in the development of the disease in both racial groups.

In conclusion, we found racial differences in the associations of inflammatory genes *IL-1* and *IL-6* and prostate cancer, as well as with selected risk factors. These findings suggest differences in response to inflammation which may be ethnic or race based. Our findings also support a growing body of evidence that chronic or recurrent inflammation plays an important role in the development of prostate cancer[10-13]. The differences in genotype and haplotype frequencies between the two racial groups in themselves may not mean much if the variants involved are nonfunctional. Even though we are not certain of the exact functions of several of the SNPs that showed significant associations with prostate cancer in the current study, we are of the opinion that a number of them might be potentially functional, because they cluster in highly conserved regions of the genome in several vertebrate species. There is the possibility that the higher rates of prostate cancer in AA compared to Caucasians may be due to differential up-regulation of cytokines that promote or sustain inflammation in AA compared to Caucasians. Future studies may want to consider examining the function of some of the SNPs identified in this study to be significantly associated with prostate cancer. Additionally, future studies may include a wide variety of markers which up-regulate pro-inflammatory cytokines in these two groups, as well as those with marginal effect on prostate cancer in order to determine susceptibility. Results from this study also suggest the need to closely examine the *IL-1RN,* as well as the *IL-6R* including its alpha- (gp80) and beta- (gp130) subunits in order to further understand some of the disparities noted between AA and Caucasians. Understanding the role of *IL-1* and *IL-6* genes in the development of prostate cancer is of great public health significance because it will enable their possible use as biomarkers for early detection and prompt intervention, increase our understanding of the molecular biology of the disease, open up new

avenues for prevention and treatment, as well as explain some of the observed disparities in the disease.

4.6 TABLES AND FIGURES

Table 4.1 : General characteristics of CAPS subjects

	Black					White					All				
Attribute	Cases N=54		Controls N=59			Cases N=218		Controls N=227			Cases N=272		Controls N=286		
	Mean	(SD)	Mean	(SD)	p-value	Mean	(SD)	Mean	(SD)	p-value	Mean	(SD)	Mean	(SD)	p-value
Age (yrs)	59.7	-7.7	61.1	-9.8	0.42	61	-6.5	61.1	-6.2	0.78	60.7	-6.8	61.1	-7.1	0.49
Height (cm)	177.3	-7.3	174.7	-5.3	0.04	176.1	(6.5)[1]	176.2	-6.3	0.84	176.3	(6.6)[1]	175.9	-6.1	0.44
Weight (kg)	93.8	-18.3	92	-17.1	0.58	87.8	(12.6)[1]	90.6	-15.5	0.04	89	(14.1)[1]	90.9	-15.8	0.14
BMI (kg/m^2)	29.8	-5.2	30	-5	0.79	28.3	(3.5)[1]	29.1	-4.4	0.03	28.6	(4.0)[1]	29.3	-4.5	0.04
Hip BMD (gm/cm^2) 1.	05	-0.16	1.08	-0.16	0.3276	1.01	-0.14	1.02	-0.14	0.1614	1.02	-0.14	1.04	-0.14	0.08
	N	(%)	N	(%)	p-value	N	(%)	N	(%)	p-value	N	(%)	N	(%)	p-value
Father or brother with prostate cancer	9	-17	3	-5	0.05	41	-19	10	-4	<0.0001	50	-18	13	-5	<0.0001
Personal history of BPH or prostatitis	19	-35	10	-17	0.03	93	-43	128	-56	<0.01	112	-41	138	-48	0.09
Regular use of ASA or NSAIDS	16	-30	16	-27	0.73	101	-46	115	-51	0.36	117	-43	131	-46	0.51

1. Attribute information missing for one subject

Table 4.2 : SNPs included in genotyping analysis

Chromosome	Gene	SNP identification number							
Chr1	*IL-6R*	rs4845617	rs6427641	rs11265610	rs12083537	rs1386821	rs4075015	rs4601580	rs4845618
		rs7549338	rs7518199	rs4553185	rs4393147	rs4537545	rs4845626	rs28730736	rs11265618
		rs10159236	rs4329505	rs4509570	rs2229238	rs4072391	rs4379670		
Chr2	*IL-1A*	rs3783590	rs2856836	rs17561	rs20540	rs2856838	rs1609682	rs3783526	rs2856837
Chr2	*IL-1B*	rs1143643	rs1143634	rs1143633	rs3136558	rs1143630			
Chr2	*IL-1RN*	rs3181052	rs1794066	rs1794067	rs2071459	rs432014	rs380092	rs452204	rs4252019
		rs315955	rs315951	rs9005					
Chr7	*IL-6*	rs2069837	rs2069840	rs1554606	rs2069842	rs1548216	rs2069843	rs2069845	

Genes are odered by chromosome. SNPs are presented by position across the gene. SNPs (N=51) were selected from HapMap/Haploview using the Tagger approach, MAF > 0.8, r2 > 0.1 . Two additional SNPs were selected from FastSNP

Chr - Chromosome; SNP - Single nucleotide polymorphism; IL-1A - Interleukin-1A; IL-1B - Interleukin-1B; IL1RN - Interleukin-1 receptor antagonist; IL-6 - Interleukin-6; IL-6R - IL-6R

Table 4.3 : Control subjects, number (N) with a personal history of BPH or prostatitis

	Black				White			
	History of BPH or Prostatitis				History of BPH or Prostatitis			
	No	Yes			No	Yes		
Locus	% (N)	% (N)	Exact p-value		% (N)	% (N)	Exact p-value	
IL1A								
rs3783590	22.4 (49)	0.0 (10)	0.1827		0.0 (99)	0.8 (128)	1	
rs2856836	40.8 (49)	30.0 (10)	0.7255		45.5 (99)	48.4 (128)	0.6899	
rs17561	40.8 (49)	30.0 (10)	0.7225		45.5 (99)	48.4 (128)	0.6889	
rs20540	12.2 (49)	0.0 (10)	0.5768		0.0 (99)	0.0 (128)		
rs2856838	61.2 (49)	60.0 (10)	1		63.6 (99)	60.9 (128)	0.7827	
rs1609682	34.7 (49)	50.0 (10)	0.477		55.1 (98)	56.7 (127)	0.8924	
rs3783526	6.1 (49)	10.0 (10)	0.5345		54.5 (99)	56.3 (128)	0.8929	
rs2856837	46.9 (49)	30.0 (10)	0.488		45.5 (99)	48.4 (128)	0.6889	
IL1B								
rs1143643	36.7 (49)	40.0 (10)	1		61.6 (99)	57.0 (128)	0.4996	
rs1143634	32.7 (49)	20.0 (10)	0.708		32.7 (98)	40.2 (127)	0.2673	
rs1143633	42.9 (49)	60.0 (10)	0.4881		61.6 (99)	57.5 (127)	0.5859	
rs3136558	26.5 (49)	30.0 (10)	1		30.3 (99)	37.5 (128)	0.2642	
rs1143630	46.9 (49)	50.0 (10)	1		17.2 (99)	8.6 (128)	0.0665	
IL1RN								
rs3181052	28.6 (49)	20.0 (10)	0.7128		12.2 (98)	16.4 (128)	0.4492	
rs1794066	63.3 (49)	40.0 (10)	0.2892		61.6 (99)	66.1 (127)	0.8935	
rs1794067	53.1 (49)	40.0 (10)	0.5062		52.5 (99)	53.9 (128)		
rs2071459	30.6 (49)	20.0 (10)	0.708		12.1 (99)	16.7 (126)	0.4481	
rs432014	38.8 (49)	20.0 (10)	0.4699		52.5 (99)	53.9 (128)	0.8935	
rs380092	87.8 (49)	100.0 (10)	0.5768		44.4 (99)	50.4 (125)	0.4199	
rs452204	77.1 (48)	40.0 (10)	0.0499	*	61.6 (99)	65.6 (128)	0.5784	
rs4252019	67.3 (49)	30.0 (10)	0.0373	*	15.2 (99)	16.4 (128)	0.856	
rs315955	25.0 (48)	30.0 (10)	0.7082		0.0 (99)	0.0 (128)		
rs315951	63.3 (49)	70.0 (10)	1		39.8 (98)	44.1 (127)	0.5865	
rs9005	46.9 (49)	10.0 (10)	0.0376	*	54.5 (99)	58.6 (128)	0.5897	

Table 4.3 (continued)

	Black			White		
	History of BPH or Prostatitis			History of BPH or Prostatitis		
	No	Yes		No	Yes	
Locus	% (N)	% (N)	Exact p-value	% (N)	% (N)	Exact p-value
IL6						
rs2069837	20.4 (49)	50.0 (10)	0.1037	13.1 (99)	14.1 (128)	1
rs2069840	24.5 (49)	10.0 (10)	0.4324	52.5 (99)	59.1 (127)	0.3466
rs1554606	57.1 (49)	50.0 (10)	0.7365	73.7 (99)	66.4 (128)	0.2479
rs2069842	8.2 (49)	0.0 (10)	1	0.0 (99)	0.0 (126)	
rs1548216	28.6 (49)	30.0 (10)	1	6.1 (99)	1.6 (128)	0.0817
rs2069843	18.4 (49)	10.0 (10)	1	6.1 (99)	1.6 (128)	0.0817
rs2069845	55.1 (49)	50.0 (10)	1	73.7 (99)	65.3 (124)	0.192
IL6R						
rs4845617	62.5 (48)	70.0 (10)	0.7333	61.6 (99)	64.1 (128)	0.7818
rs6427641	89.8 (49)	100.0 (10)	0.5768	64.6 (99)	70.3 (128)	0.392
rs11265610	46.8 (47)	40.0 (10)	0.7412	0.0 (93)	0.0 (121)	
rs12083537	44.9 (49)	50.0 (10)	1	35.4 (99)	35.2 (128)	1
rs1386821	20.4 (49)	10.0 (10)	0.6697	33.3 (99)	32.0 (128)	0.8868
rs4075015	22.4 (49)	20.0 (10)	1	64.6 (99)	68.8 (128)	0.57
rs4601580	74.5 (47)	100.0 (10)	0.0997	59.6 (99)	67.2 (125)	0.2642
rs4845618	75.5 (49)	90.0 (10)	0.4324	71.7 (99)	69.5 (128)	0.7702
rs7549338	49.0 (49)	70.0 (10)	0.3056	69.7 (99)	67.2 (128)	0.7739
rs7518199	36.7 (49)	10.0 (10)	0.1446	70.7 (99)	67.2 (128)	0.6652
rs4553185	85.4 (48)	90.0 (10)	1	71.7 (99)	68.8 (128)	0.6632
rs4393147	22.9 (48)	0.0 (10)	0.1824	70.7 (99)	67.2 (128)	0.6652
rs4537545	87.8 (49)	80.0 (10)	0.6126	67.7 (99)	69.5 (128)	0.7747
rs4845626	77.6 (49)	60.0 (10)	0.257	28.3 (99)	32.8 (128)	0.4734
rs28730736	28.6 (49)	30.0 (10)	1	0.0 (99)	0.0 (126)	
rs11265618	67.3 (49)	60.0 (10)	0.7809	28.3 (99)	33.6 (128)	0.4704
rs10159236	40.8 (49)	40.0 (10)	1	26.3 (99)	32.0 (128)	0.3806
rs4329505	67.3 (49)	60.0 (10)	0.7209	26.3 (99)	31.3 (128)	0.4625
rs4509570	73.5 (49)	80.0 (10)	1	44.4 (99)	42.2 (128)	0.7875
rs2229238	30.6 (49)	30.0 (10)	1	38.4 (99)	32.8 (128)	0.4033
rs4072391	42.9 (49)	40.0 (10)	1	38.4 (99)	32.8 (128)	0.4033
rs4379670	30.6 (49)	30.0 (10)	1	38.4 (99)	32.8 (128)	0.4033

1. Fisher's Exact test with asterisk (*) to indicate $p<0.05$

Table 4.4 : Control subjects, number (N) with and without a family history of prostate cancer

	Black			White			
	Family history of prostate cancer			Family history of prostate cancer			
	No	Yes		No	Yes		
Locus	% (N)	% (N)	p-value	% (N)	% (N)	p-value [1]	
IL1A							
rs3783590	17.9 (56)	33.3 (3)	0.468	0.5 (217)	0.0 (10)	1	
rs2856836	39.3 (56)	33.3 (3)	1	47.9 (217)	30.0 (10)	0.341	
rs17561	39.3 (56)	33.3 (3)	1	47.9 (217)	30.0 (10)	0.341	
rs20540	10.7 (56)	0.0 (3)	1	0.0 (217)	0.0 (10)		
rs2856838	58.9 (56)	100.0 (3)	0.2741	61.3 (217)	80.0 (10)	0.3258	
rs1609682	37.5 (56)	33.3 (3)	1	56.3 (215)	50.0 (10)	0.7522	
rs3783526	5.4 (56)	33.3 (3)	0.193	55.8 (217)	50.0 (10)	0.7544	
rs2856837	44.6 (56)	33.3 (3)	1	47.9 (217)	30.0 (10)	0.341	
IL1B							
rs1143643	35.7 (56)	66.7 (3)	0.5493	57.6 (217)	90.0 (10)	0.0503	*
rs1143634	30.4 (56)	33.3 (3)	1	37.7 (215)	20.0 (10)	0.3306	
rs1143633	44.6 (56)	66.7 (3)	0.5881	57.9 (216)	90.0 (10)	0.0511	*
rs3136558	26.8 (56)	33.3 (3)	1	35.0 (217)	20.0 (10)	0.5004	
rs1143630	48.2 (56)	33.3 (3)	1	11.1 (217)	40.0 (10)	0.0233	*
IL1RN							
rs3181052	26.8 (56)	33.3 (3)	1	15.3 (216)	0.0 (10)	0.3647	
rs1794066	57.1 (56)	100.0 (3)	0.2636	64.4 (216)	60.0 (10)	0.7484	
rs1794067	51.8 (56)	33.3 (3)	0.612	53.0 (217)	60.0 (10)	0.754	
rs2071459	28.6 (56)	33.3 (3)	1	15.3 (215)	0.0 (10)	0.3648	
rs432014	33.9 (56)	66.7 (3)	0.2864	53.0 (217)	60.0 (10)	0.754	
rs380092	89.3 (56)	100.0 (3)	1	48.6 (214)	30.0 (10)	0.3376	
rs452204	69.1 (55)	100.0 (3)	1	64.1 (217)	60.0 (10)	0.7503	
rs4252019	60.7 (56)	66.7 (3)	1	16.1 (217)	10.0 (10)	1	
rs315955	27.3 (55)	0.0 (3)	0.561	0.0 (217)	0.0 (10)		
rs315951	62.5 (56)	100.0 (3)	0.5459	42.3 (215)	40.0 (10)	1	
rs9005	41.1 (56)	33.3 (3)	1	57.1 (217)	50.0 (10)	0.7491	

Table 4.4 (continued)

	Black			White		
	Family history of prostate cancer			Family history of prostate cancer		
	No	Yes		No	Yes	
Locus	% (N)	% (N)	p-value	% (N)	% (N)	p-value [1]
IL6						
rs2069837	25.0 (56)	33.3 (3)	1	13.4 (217)	20.0 (10)	0.6304
rs2069840	21.4 (56)	33.3 (3)	0.5331	57.4 (216)	30.0 (10)	0.109
rs1554606	58.9 (56)	0.0 (3)	0.08	68.7 (217)	90.0 (10)	0.2895
rs2069842	7.1 (56)	0.0 (3)	1	0.0 (215)	0.0 (10)	
rs1548216	30.4 (56)	0.0 (3)	0.5498	3.2 (217)	10.0 (10)	0.3067
rs2069843	17.9 (56)	0.0 (3)	1	3.2 (217)	10.0 (10)	0.3067
rs2069845	57.1 (56)	0.0 (3)	0.09	68.1 (213)	90.0 (10)	0.1799
IL6R						
rs4845617	61.8 (55)	100.0 (3)	0.5467	63.6 (217)	50.0 (10)	0.5051
rs6427641	91.1 (56)	100.0 (3)	1	67.7 (217)	70.0 (10)	1
rs11265610	47.3 (55)	0.0 (2)	0.495	0.0 (204)	0.0 (10)	
rs12083537	44.6 (56)	66.7 (3)	0.5881	35.0 (217)	40.0 (10)	0.7446
rs1386821	17.9 (56)	33.3 (3)	0.468	32.7 (217)	30.0 (10)	1
rs4075015	21.4 (56)	33.3 (3)	0.5331	66.8 (217)	70.0 (10)	1
rs4601580	79.6 (54)	66.7 (3)	0.515	63.1 (214)	80.0 (10)	0.3356
rs4845618	78.6 (56)	66.7 (3)	0.5331	70.0 (217)	80.0 (10)	0.7272
rs7549338	51.8 (56)	66.7 (3)	1	67.7 (217)	80.0 (10)	0.5098
rs7518199	30.4 (56)	66.7 (3)	0.2402	69.1 (217)	60.0 (10)	0.5084
rs4553185	87.3 (55)	66.7 (3)	0.3648	69.6 (217)	80.0 (10)	0.7271
rs4393147	16.4 (55)	66.7 (3)	0.0891	69.1 (217)	60.0 (10)	0.5084
rs4537545	87.5 (56)	66.7 (3)	0.3594	69.1 (217)	60.0 (10)	0.5084
rs4845626	76.8 (56)	33.3 (3)	0.1561	30.9 (217)	30.0 (10)	1
rs28730736	30.4 (56)	0.0 (3)	0.5498	0.0 (215)	0.0 (10)	
rs11265618	67.9 (56)	33.3 (3)	0.263	31.3 (217)	30.0 (10)	1
rs10159236	41.1 (56)	33.3 (3)	1	29.5 (217)	30.0 (10)	1
rs4329505	67.9 (56)	33.3 (3)	0.263	29.0 (217)	30.0 (10)	1
rs4509570	76.8 (56)	33.3 (3)	0.1561	43.3 (217)	40.0 (10)	1
rs2229238	30.4 (56)	33.3 (3)	1	35.0 (217)	40.0 (10)	0.7446
rs4072391	42.9 (56)	33.3 (3)	1	35.0 (217)	40.0 (10)	0.7446
rs4379670	30.4 (56)	33.3 (3)	1	35.0 (217)	40.0 (10)	0.7446

1. Fisher's Exact test with asterisk (*) to indicate $p<0.05$

Table 4.5 : Single SNPs (ordered by gene and marker position) and prostate cancer in cases and controls

			Black							White					
	Alleles		Frequencies [1]		Statistical tests [2]			Dominant model [3]		Frequencies [1]		Statistical tests [2]			Dominant model [3]	
Gene variant	Rare	Common	Case	Control	Global	Trend		OR	CI	Case	Control	Global	Trend		OR	CI
IL1A																
rs3783590	A	T	1/11/42	0/11/48	0.5540	0.5026		1.25	0.50-3.12	0/1/217	0/1/226	0.9771	0.9771		1.04	0.06-16.8
rs2856836	C	T	3/12/39	4/19/36	0.4436	0.2737		0.60	0.27-1.33	23/86/109	19/88/120	0.6871	0.4186		1.12	0.77-1.63
rs17561	T	G	3/12/39	4/19/36	0.4436	0.2737		0.60	0.27-1.33	22/87/109	19/88/120	0.7513	0.4600		1.12	0.77-1.63
rs20540	T	C	0/7/47	0/6/53	0.6420	0.6420		1.32	0.41-4.19	0/0/218	0/0/227					
rs2856838	T	C	8/22/24	7/29/23	0.6606	0.8456		0.80	0.38-1.69	43/96/79	35/106/86	0.4889	0.3784		1.07	0.73-1.58
rs1609682	C	A	3/13/38	2/20/37	0.4772	0.6085		0.71	0.32-1.56	14/91/110	25/101/99	0.1369	0.0554		0.75	0.52-1.09
rs3783526	A	G	0/6/48	1/3/55	0.3232	0.6666		1.72	0.46-6.45	15/92/111	25/101/101	0.2008	0.0847		0.77	0.53-1.12
rs2856837	T	C	4/15/35	4/22/33	0.5587	0.4831		0.69	0.32-1.47	22/87/109	19/88/120	0.7513	0.4600		1.12	0.77-1.63
IL1B																
rs1143643	A	G	1/19/34	1/21/37	0.9972	0.9924		0.99	0.46-2.12	29/102/87	20/114/93	0.3106	0.3707		1.05	0.72-1.53
rs1143634	T	C	1/17/36	2/16/41	0.7914	0.8971		1.14	0.52-2.51	12/78/127	8/75/142	0.4605	0.2346		1.21	0.83-1.78
rs1143633	A	G	2/21/31	1/26/32	0.7185	0.9107		0.88	0.42-1.85	27/101/87	19/115/92	0.3387	0.4763		1.01	0.69-1.48
rs3136558	C	T	2/16/36	0/16/43	0.3006	0.2946		1.34	0.60-3.01	9/78/131	7/71/149	0.4596	0.2142		1.27	0.86-1.87
rs1143630	A	C	2/22/30	6/22/31	0.4068	0.4206		0.89	0.42-1.86	3/24/191	1/27/199	0.5602	0.7755		1.00	0.57-1.77
IL1RN																
rs3181052	A	G	2/20/32	2/14/43	0.2929	0.1798		1.85	0.84-4.07	2/49/165	4/29/193	0.0206	0.0486	*	1.81	1.11-2.94
rs1794066	G	A	6/26/22	11/24/24	0.4919	0.5652		1.00	0.47-2.11	34/104/79	35/110/81	0.9876	0.9533		0.98	0.66-1.44
rs1794067	A	G	3/13/38	4/26/29	0.0647	0.0510		0.41	0.19-0.88	16/86/114	19/102/106	0.4410	0.2420		0.78	0.54-1.14
rs2071459	T	C	3/20/30	2/15/42	0.2725	0.1209		1.89	0.87-4.14	1/48/165	4/29/192	0.0161	0.0891	*	1.73	1.06-2.81
rs432014	C	T	0/8/46	3/18/38	0.0247	0.0067	*	0.31	0.13-0.79	16/86/114	19/102/106	0.4410	0.2420		0.78	0.54-1.14
rs380092	T	A	22/21/10	30/23/6	0.3668	0.1794		0.49	0.16-1.45	21/88/105	16/91/117	0.5636	0.3420		1.14	0.78-1.65
rs452204	A	G	10/25/19	16/25/17	0.5080	0.2847		0.76	0.35-1.69	32/105/81	35/110/82	0.9633	0.7852		0.96	0.65-1.41
rs4252019	T	C	9/22/23	7/29/23	0.6091	0.9270		0.86	0.41-1.83	4/52/162	5/31/191	0.0221	0.0318	*	1.83	1.15-2.93
rs315955	C	G	1/6/45	1/14/43	0.2314	0.1602		0.45	0.17-1.20	0/0/218	0/0/227					
rs315951	C	G	12/22/20	10/28/21	0.7025	0.7799		0.94	0.44-2.02	20/79/119	13/82/130	0.3836	0.2724		1.14	0.78-1.66
rs9005	A	G	1/10/43	6/18/35	0.0394	0.0110	*	0.37	0.16-0.87	24/86/107	23/106/98	0.3205	0.4086		0.78	0.54-1.14

Table 4.5 (continued)

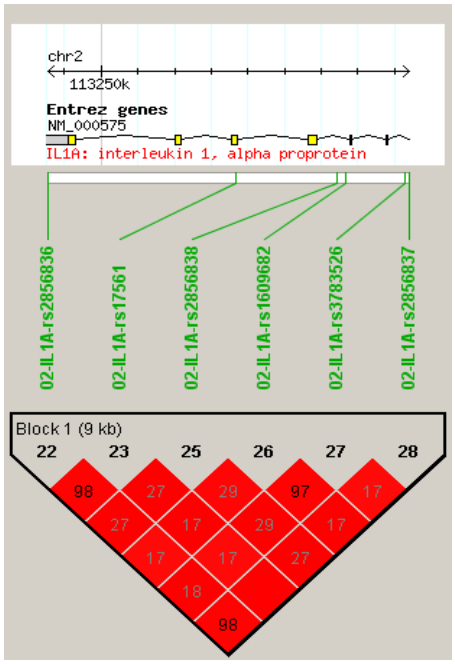
			Black						White					
	Alleles		Frequencies [1]		Statistical tests [2]		Dominant model [3]		Frequencies [1]		Statistical tests [2]		Dominant model [3]	
Gene variant	Rare	Common	Case	Control	Global	Trend	OR	CI	Case	Control	Global	Trend	OR	CI
IL6														
rs2069837	G	A	0/10/44	2/13/44	0.3372	0.2409	0.67	0.27-1.64	0/25/193	2/29/196	0.3433	0.3547	0.82	0.47-1.44
rs2069840	G	C	1/12/41	2/11/46	0.8010	0.9569	1.12	0.47-2.70	33/98/86	30/97/99	0.6444	0.3557	1.19	0.81-1.73
rs1554606	T	G	5/29/19	9/24/26	0.3029	0.8502	1.41	0.66-3.02	35/110/73	44/114/69	0.5982	0.3269	0.87	0.58-1.29
rs2069842	A	G	0/7/46	1/3/55	0.2134	0.4579	2.09	0.58-7.60	0/2/212	0/0/225	0.1461	0.1461	5.31	0.25-111.
rs1548216	C	G	3/15/36	3/14/42	0.8714	0.6482	1.24	0.56-2.74	0/9/209	0/8/219	0.7396	0.7396	1.18	0.45-3.11
rs2069843	A	G	1/11/42	1/9/49	0.7717	0.5219	1.40	0.55-3.57	0/13/205	0/8/219	0.2251	0.2251	1.74	0.71-4.27
rs2069845	G	A	5/29/19	9/23/27	0.2330	0.7482	1.51	0.71-3.23	35/106/72	44/110/69	0.6268	0.3556	0.88	0.59-1.31
IL6R														
rs4845617	A	G	3/26/25	7/30/21	0.3511	0.1633	0.66	0.31-1.40	34/107/77	31/112/84	0.8290	0.5737	1.08	0.73-1.58
rs6427641	G	A	19/29/6	29/25/5	0.3241	0.1711	0.74	0.21-2.58	39/117/62	38/116/73	0.6936	0.4488	1.19	0.80-1.79
rs11265610	C	T	5/21/26	5/21/31	0.9005	0.6771	1.19	0.56-2.53	0/3/203	0/0/214	0.0764	0.0764	7.38	0.38-144.
rs12083537	G	A	6/18/30	6/21/32	0.9636	0.9765	0.95	0.45-1.99	7/81/128	12/68/147	0.1745	0.5270	1.26	0.86-1.86
rs1386821	C	A	1/14/39	2/9/48	0.3440	0.4141	1.68	0.69-4.07	6/79/133	10/64/153	0.1502	0.3698	1.32	0.90-1.95
rs4075015	A	T	0/16/38	0/13/46	0.3558	0.3558	1.49	0.64-3.48	35/110/71	40/112/75	0.9103	0.8486	1.01	0.68-1.50
rs4601580	T	A	19/22/12	20/25/12	0.9648	0.9535	0.91	0.37-2.25	39/103/71	43/100/81	0.7331	0.7764	1.13	0.76-1.68
rs4845618	G	T	13/26/15	18/28/13	0.6691	0.3705	0.73	0.31-1.73	38/112/68	37/123/67	0.8379	0.9319	0.92	0.62-1.38
rs7549338	C	G	8/25/21	4/27/28	0.3340	0.1801	1.42	0.67-3.00	36/108/73	36/119/72	0.8543	0.8534	0.92	0.62-1.36
rs7518199	C	A	5/22/27	3/16/40	0.1529	0.0621	2.11	0.98-4.52	28/113/77	30/126/71	0.6578	0.4735	0.83	0.56-1.24
rs4553185	C	T	16/28/9	17/33/8	0.8718	0.8529	0.78	0.28-2.20	38/110/70	36/123/68	0.7310	0.9276	0.90	0.60-1.35
rs4393147	T	C	3/15/36	1/10/47	0.1902	0.0686	2.14	0.90-5.08	28/113/77	30/126/71	0.6578	0.4735	0.83	0.56-1.24
rs4537545	T	C	16/31/7	25/26/8	0.3224	0.3323	1.05	0.35-3.13	30/115/71	35/121/71	0.8763	0.6193	0.93	0.62-1.39
rs4845626	T	G	2/27/25	12/32/15	0.0072	0.0022 *	0.40	0.18-0.87	10/65/143	6/64/157	0.4772	0.2910	1.18	0.79-1.75
rs28730736	A	G	1/14/38	1/16/42	0.9940	0.9729	0.98	0.43-2.22	0/0/214	0/0/225				
rs11265618	T	C	2/26/26	9/30/20	0.0702	0.0321	0.55	0.26-1.18	10/66/142	6/65/156	0.4762	0.2903	1.18	0.79-1.75
rs10159236	A	C	0/15/39	2/22/35	0.1896	0.0939	0.56	0.25-1.24	8/62/146	6/61/160	0.7183	0.4383	1.14	0.77-1.71
rs4329505	C	T	6/22/26	10/29/20	0.2826	0.1230	0.55	0.26-1.18	8/62/148	6/60/161	0.7105	0.4235	1.15	0.77-1.73
rs4509570	G	C	10/34/10	15/29/15	0.3362	1.0000	1.50	0.61-3.70	9/84/123	12/86/129	0.8513	0.8246	1.00	0.68-1.45
rs2229238	T	C	3/22/29	1/17/41	0.1752	0.0625	1.96	0.91-4.24	4/72/142	9/71/147	0.3995	0.6254	0.98	0.67-1.45
rs4072391	T	C	8/22/24	6/19/34	0.3657	0.1743	1.70	0.81-3.58	4/73/141	9/71/147	0.3878	0.6902	1.00	0.68-1.48
rs4379670	T	A	3/22/29	1/17/41	0.1752	0.0625	1.96	0.91-4.24	5/71/142	9/71/147	0.5922	0.6924	0.98	0.67-1.45

Legend: OR odds ratio; CI 95% confidence interval

1. Number with two copies of the rare allele/Number with one copy of the rare allele/Number with no copies of the rare allele
2. P-values for global test and trend test with an asterisk (*) to indicate genotype test p-value < 0.05 and trend test p-value < 0.10
3. Risk group has one or two copies and the reference group no copies of the rare allele

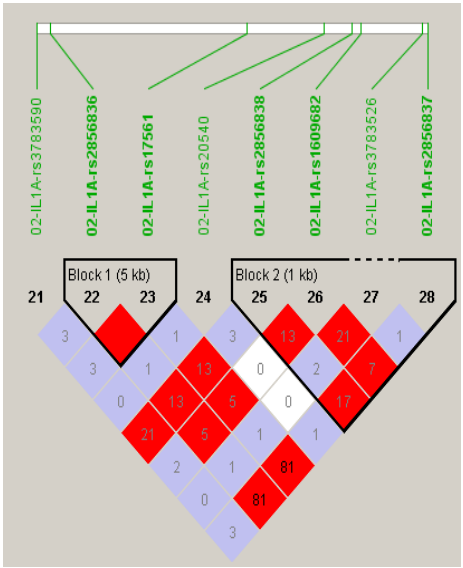
IL-1A

White



Gene	Block	Haplotype	Frequency Case	Frequency Control	P value
IL-1A	1	TGTAGC	0.417	0.385	0.33
		TGCCAC	0.278	0.333	0.07
		CTCAGT	0.298	0.278	0.5

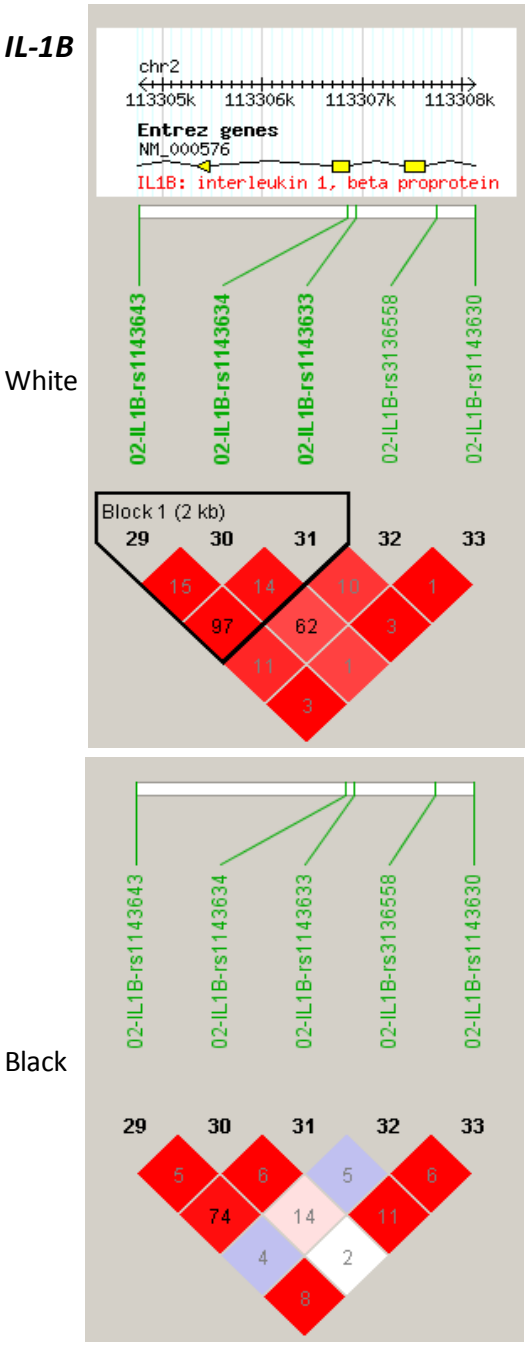
Black



Gene	Block	Haplotype	Frequency Case	Frequency Control	P value
IL-1A	1	TG	0.833	0.771	0.24
		CT	0.167	0.229	0.24
	2	TAC	0.352	0.364	0.84
		CAT	0.213	0.254	0.46
		CAC	0.259	0.178	0.14
		CCC	0.176	0.203	0.6

Figure 4.1: Haplotype block organization of *IL-1A* in Blacks and Whites (left), and the corresponding case-control haplotype frequencies (right).

Figures 4-1 to 4-5 show haplotypes observed in five genes and case-control haplotype frequencies, by race. Analyses were completed in Haploview 4.1 (Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and Haplotype maps. Bioinformatics 2005;21:263-265), with default confidence interval method used to define haplotype blocks (Gabriel SB, Schaffner SF, Nguyen H et al. The structure of Haplotype blocks in the human genome. Science 2002; 296:2225-2229). Haplotype maps show R-squared values. Haplotype maps use shades between pink and red to indicate magnitude of D-prime (LOD≥2) and pale blue to indicate D-prime =1, (LOD<2).



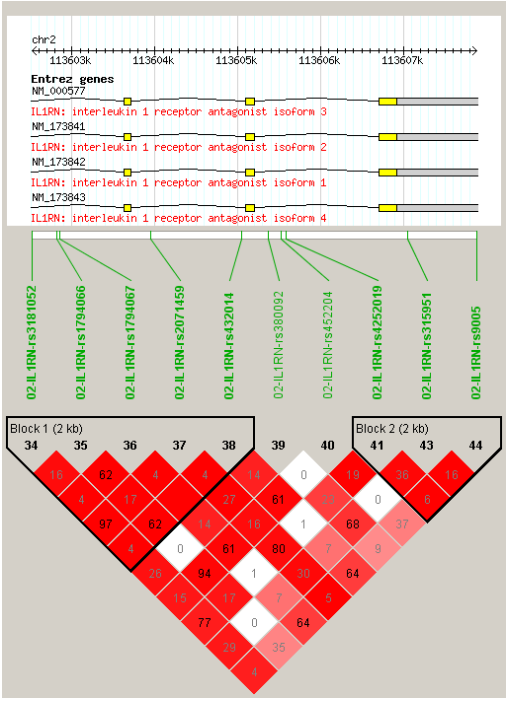
Frequency

Gene	Block	Haplotype	Case	Control	P value
IL-1B	1	GCG	0.398	0.455	0.09
		ACA	0.365	0.337	0.39
		GTG	0.233	0.202	0.26

Figure 4.2 : Haplotype block organization of *IL-1B* in Blacks and Whites (left), and the corresponding case-control haplotype frequencies in Whites (right).

IL-1RN

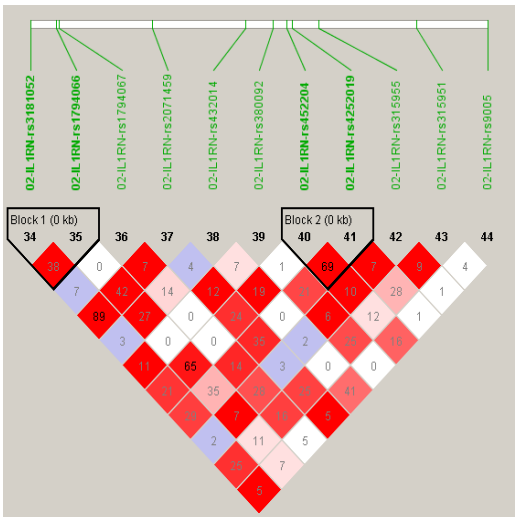
White



Frequency

Gene	Block	Haplotype	Case	Control	P value
IL-1RN	1	GAGCT	0.06	0.603	0.94
		GGACC	0.271	0.308	0.21
		AGGTT	0.117	0.081	0.08
	2	CGG	0.417	0.426	0.78
		CGA	0.309	0.335	0.41
		CCG	0.138	0.149	0.6572
		TCG	0.136	0.09	0.03

Black



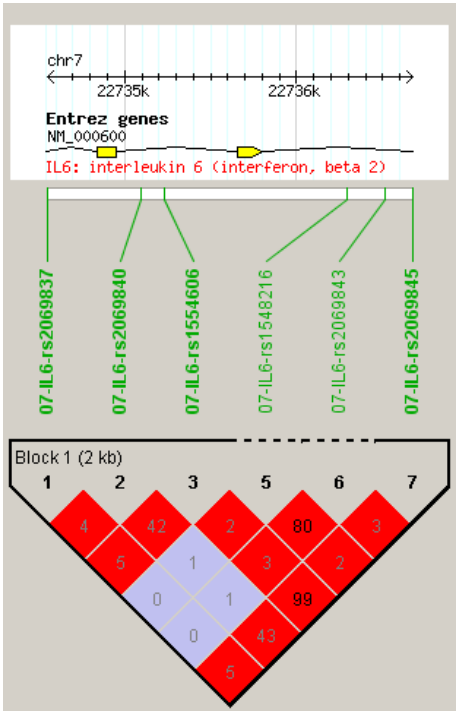
Frequency

Gene	Block	Haplotype	Case	Control	P value
IL-1RN	1	GA	0.648	0.61	0.55
		GG	0.13	0.237	0.04
		AG	0.222	0.153	0.18
	2	GC	0.583	0.507	0.25
		AT	0.37	0.364	0.93
		AC	0.046	0.128	0.03

Figure 4.3 : Haplotype block organization of *IL-1RN* in Blacks and Whites (left), and the corresponding case-control haplotype frequencies (right).

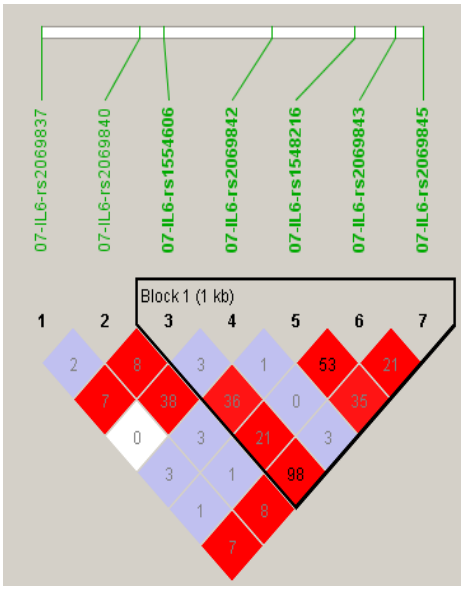
IL-6

White



Gene	Block	Haplotype	Frequency Case	Frequency Control	P value
IL-6	1	ACTG	0.413	0.445	0.33
		AGGA	0.379	0.347	0.32
		ACGA	0.15	0.133	0.45
		GCGA	0.057	0.073	0.35

Black

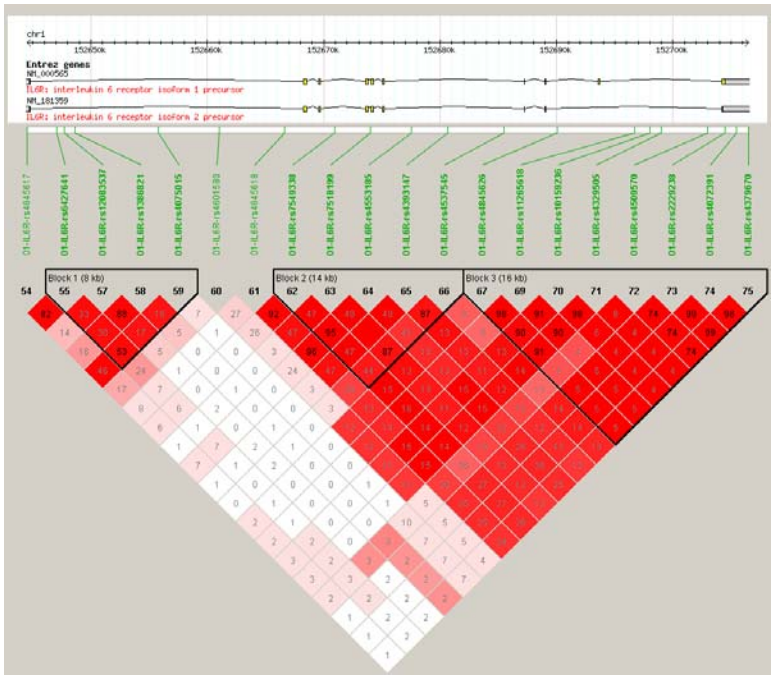


Gene	Block	Haplotype	Frequency Case	Frequency Control	P value
IL-6	1	GGGGA	0.554	0.601	0.47
		TGGGG	0.186	0.179	0.89
		TGCAG	0.12	0.093	0.51
		TGCGG	0.064	0.075	0.73
		GAGGA	0.066	0.042	0.43

Figure 4.4 : Haplotype block organization of *IL-6* in Blacks and Whites (left), and the corresponding case-control haplotype frequencies (right).

IL-6R

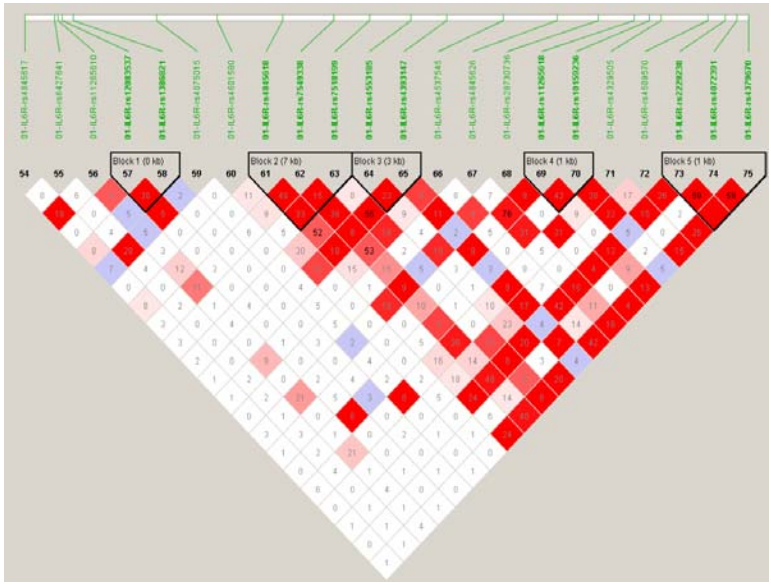
White



Frequency

Gene	Block	Haplotype	Case	Control	P value
IL-6R	1	AAAA	0.413	0.418	0.89
		GAAT	0.225	0.223	0.94
		GGCT	0.203	0.178	0.33
		AAAT	0.134	0.156	0.35
		GGAT	0.014	0.017	0.68
	2	CACCC	0.409	0.408	0.98
		GCTTT	0.381	0.399	0.58
		GATCC	0.18	0.16	0.42
		GACCT	0.011	0.009	0.69
	3	GCCTCCCA	0.58	0.597	0.61
		GCCTGTTT	0.181	0.196	0.57
		TTACCCCA	0.179	0.159	0.42
		GCCTGCCA	0.037	0.037	0.98
		TTCTGCCA	0.014	0.009	0.49

Black



Frequency

Gene	Block	Haplotype	Case	Control	P value
IL-6R	1	AA	0.722	0.72	0.97
		GA	0.13	0.169	0.4
		GC	0.148	0.11	0.39
	2	GCA	0.38	0.297	0.19
		TGA	0.222	0.271	0.39
		TGC	0.296	0.186	0.05
		GGA	0.102	0.246	0
	3	CC	0.568	0.578	0.88
		TC	0.238	0.319	0.18
		TT	0.194	0.103	0.05
	4	CC	0.722	0.593	0.04
		TA	0.139	0.22	0.11
		TC	0.139	0.186	0.33
	5	CCA	0.648	0.737	0.15
		TTT	0.259	0.161	0.07
		CTA	0.093	0.102	0.82

Figure 4.5 : Haplotype block organization of *IL-6R* in Blacks and Whites (left), and the corresponding case-control haplotype frequencies (right).

5.0 PAPER 3: BONE MINERAL DENSITY IS INVERSELY ASSOCIATED WITH PROSTATE CANCER IN AFRICAN AMERICAN AND CAUCASIAN MEN

Kwame Appenteng, MPH, [1] Clearann Bunker, PhD,[1] Joel Nelson, MD,[2] John Wilson, PhD,[3] Robert Ferrell, PhD,[4] Francesmary Modugno, PhD,[1] Claudia Leiras, MPH,[1] James Shikany, DrPH, [5] Joel Weissfeld, MD, MPH,[1]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[2]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

[3]Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[4]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

[5] Department of Preventative Medicine, University of Alabama at Birmingham, Birmingham, Alabama

5.1 ABSTRACT

Objective: To evaluate the association between bone mineral density (BMD) – a possible surrogate of cumulative exposure to inflammatory cytokines – and prostate cancer in African Americans and Caucasians.

Method: We conducted a case-control study of 591 African-American and Caucasian men (287 recently diagnosed cases, and 304 controls with normal prostate specific antigen (PSA) levels, and no history of prostate cancer). In the current study, we included men, aged 40-80y, who enrolled in the Cancer and Prostate Study (CAPS) between 2001 and 2006. Controls were frequency matched by age and race to cases. BMD of the total hip was obtained using dual X-ray absorptiometry (DXA) scan. All prostate cancer cases were confirmed histologically. We evaluated the association between total hip BMD and prostate cancer.

Results: Mean ages of cases and controls were 60.7 years (standard deviation (SD), 6.7) and 61.2 years (SD, 7.1) respectively. Mean body mass index (BMI) of cases was lower than controls, 28.5 kg/m^2 (SD, 3.9) and 29.3 kg/m^2 (SD, 4.5) respectively (p = 0.036). Mean BMD was lower in cases than controls, 1.017 gm/cm^2 (SD 0.130) vs. 1.036 gm/cm^2 (SD 0.140) (p = 0.10). A statistically significant inverse association was noted between hip BMD (continuous) and prostate cancer in a conditional logistic regression model (both races) adjusting for age, race and marital status (OR = 0.28, 95% confidence interval (CI) = 0.08 – 0.97, p = 0.045). A marginally significant inverse association between hip BMD (continuous) and prostate cancer was noted among Caucasians (OR = 0.26, 95% CI = 0.06 – 1.08, p = 0.063), but not AA (OR = 0.41, 95% CI = 0.39 – 4.22, p = 0.450), adjusting for age and marital status.

Conclusion: Hip BMD is inversely associated with prostate cancer in this case-control study of African-American and Caucasian men, ages 40-80 years.

5.2 INTRODUCTION

Prostate cancer is a major public health problem in the United States. It is the most common non-skin cancer, and the second leading cause of cancer-related death among men in the United States[1]. African Americans have higher incidence and mortality rates than Caucasians[1]. Socioeconomic and hormonal differences are thought to be contributory factors[2-4]. However, the roles of inflammatory cytokines and bone mineral density in these two populations have not been comprehensively examined as part explanation for these disparities.

Chronic or recurrent inflammation is known to increase the incidence of malignancies of the bladder, colon, endometrium, esophagus, liver, lung and pancreas[5-9]. Evidence from epidemiologic, genetic, molecular biology and histopathology studies have suggested a compelling role of inflammation in the development of prostate cancer [10-13]. The precise mechanism by which inflammation causes cancer is not clearly understood, but it is thought that chronic or recurrent inflammation, which may be a result of immunological conditions, recurrent microbial infections, or chemical irritation, trigger the production of inflammatory cytokine mediators and genotoxic reactive oxygen radicals that increase cell proliferation and promote tumorigenesis [14]. The likelihood of developing cancer may then be dependent upon precise host response to this inflammatory cascade [15].

The inflammatory *cytokines interleukin-1 (IL-1)* and *IL-6* have been reported to influence the initiation and progression of prostate cancer[23-25]. Additionally, these cytokines have been shown to decrease bone mineral density by resorption of bone matrix via osteoclast activity [29-32]. Furthermore, serum levels of *IL-1* and *IL-6* have been reported to be associated with bone loss [33, 34]. Serum sex steroid hormone levels have been reported by some studies to be associated with

prostate cancer[59, 219], but not by others[40, 289]. Additionally, some[224, 225] but not all[40, 290] epidemiologic studies have reported an association between a shorter length of the androgen receptor gene CAG repeat sequence and an increased risk of prostate cancer. This gene mediates the effect of testosterone and dihydrotestosterone (DHT) in androgen-responsive tissues[291]. Shorter CAG repeats have been reported to result in an increased transactivational activity of the androgen receptor. African Americans have been reported by some studies to have a shorter mean CAG repeat length than Caucasians[292, 293].

Androgens and estrogen play a major role in bone health, including bone development and the attainment of peak bone mass [294]. The sexual dimorphism of the skeleton noted during adolescence is attributable to androgens[295]. Additionally, sex steroid hormones are involved in age-related bone loss[294]. Estrogen deficiency is a major cause of bone loss during menopause[296], however, this process is prevented or reversed by estrogen replacement therapy[29]. Free testosterone levels below the median has been reported to be an independent predictor of osteoporosis-related fractures and x-ray verified vertebral fractures in elderly men [297]. Besides estrogen and testosterone, other hormones such as parathyroid hormone (PTH) and insulin-like growth factor 1 (IGF-1) influence bone mineral density by regulating the bone resorption activities of IL-1 and IL-6[35-39]. Moreover, these same factors, as well as others, such as calcium, and vitamin D, are considered risk factors for prostate cancer [35, 39-44].

Several studies have documented an association between BMD and cancer of the breast[298, 299], endometrium[300], and prostate[261, 262]. These studies have primarily assessed BMD as a proxy measurement of a lifetime exposure of specific organs to sex-steroid hormones, among others. Bone health is influenced by a variety of hormones, including estrogen and testosterone, whose bone-building ability is counteracted by the bone resorption activities of the inflammatory

cytokines IL-1 and IL-6. To ascertain the long term effect of serum levels of IL-1 and IL-6 on the pathogenesis of prostate cancer, there is the need to obtain serial measurements over many years, but this has been difficult, resulting in discrepant findings by various epidemiologic studies [26-28, 45]. The purpose of this study, therefore, is to evaluate the relationship between bone mineral density (BMD), a possible surrogate of cumulative exposure to inflammatory cytokines IL-1 and IL-6, and prostate cancer in African Americans and Caucasians. We hypothesized that high BMD would be associated with prostate cancer in both racial groups.

5.3 MATERIALS AND METHODS

5.3.1 Study sample

Participants in the current study were from an existing prostate cancer study known as the Cancer and Prostate Study (CAPS). Briefly, CAPS was a case-control study designed to assess the individual and joint roles of bone mineral density (BMD) and sex hormone gene polymorphisms in prostate cancer risk. Enrollment into CAPS started in December of 2001 and was completed in January of 2006, and included Caucasian and African American (AA) men aged 40 to 80 years. Cases were men with recently diagnosed prostate cancer (within 3 months of enrollment into the study; confirmed diagnosis based on pathology report). Controls were men without a history of prostate cancer. All participants in CAPS gave informed consent. The study was approved by the institutional review boards (IRB) of the University of Pittsburgh and the University of Alabama at Birmingham.

Potential enrollees were excluded if they used glucocorticoids (>6 months); used testosterone (>3 months); had a history of hyper- or hypothyroidism, hyperparathyroidism, renal disease or bone disease. Other exclusion criteria included bilateral hip replacement; kidney transplant; previous diagnosis of prostate cancer or any other cancer besides basal and squamous cell skin cancer; evidence of bone metastases among prostate cancer cases; and PSA levels above 3.0 ng/ml among controls. Controls were frequency matched to cases by age and race.

Participants in CAPS were recruited from Pittsburgh and Alabama. In Pittsburgh, recruitment was conducted at two sites: The University of Pittsburgh Medical Center (UPMC), and the Veterans Administration Medical Center (VA). Recruitment in Alabama was conducted

at the University of Alabama in Birmingham (UAB) Medical Center. A total of 591 Caucasian and African American men who enrolled in CAPS are included in our analysis. There were a total of 242 cases recruited at UPMC, all of who were referrals from one institution-based urology practice. Patients at this practice were mostly community-based residents from Pittsburgh, many of who were referred for specialty care by their primary care physicians. All recruited cases from this urology practice underwent radical prostatectomy within 3 months of diagnosis. The urologist personally informed potential subjects about the study for the first time during their second post-operative follow-up visit. Interested men were then referred to the CAPS research team at UPMC for further study details and enrollment information. Controls from Pittsburgh numbered 253, and were recruited from the local community, as well as from the University of Pittsburgh employees, by sending out flyers. Participants were signed up in particular age groups and race in the order they called until the group was filled. Majority of the controls were from the ongoing Prostate, Lung, Colorectal, and Ovarian study (PLCO). A total of 10 participants were recruited from the VA Medical Center in Pittsburgh, comprising of 3 cases and 7 controls.

In Alabama, information about the CAPS study was advertised in the *UAB Reporter*. Additionally, flyers were sent out to local residents and University of Alabama employees, and brochures were placed in waiting rooms of Birmingham area urologist offices. Urologists at three community-based urology practices informed potential case subjects about the study, and then referred interested parties to an on-site study recruiter from UAB Medical Center, who provided detailed information about the study. A total of 42 cases were enrolled at UAB Medical Center, which included 41 referrals from the three community-based urology practices and one subject who was referred by word of mouth from another study participant. Forty-four community-based

controls were enrolled at the UAB Medical center, majority of who were from the ongoing PLCO trial. Other control enrollees included UAB employees, as well as local Birmingham residents who responded to advertisements and flyers.

5.3.2 Assessment of bone mineral density

All enrollees underwent measurement of total hip bone mineral density by Dual-energy X-ray Absorptiometry (DXA) using a Hologic QDR-4500A bone densitometer (Hologic Inc., Waltham MA). Quality control was assessed by daily quality control scans with a phantom provided by the manufacturer. All DXA scans were interpreted by one radiologist, and all results were recorded on a standard study form.

5.3.3 Data analysis

We analyzed our data using the following variables: age (40-49y, 50-59y, 60-69y, 70y+), race (African-American and Caucasian), education (less than high school, high school graduate, technical training or college), marital status (currently married, never or previously married), history of benign prostatic hyperplasia or prostatitis (yes or no), father or brother with a history of prostate cancer (yes or no), history of regular nonsteroidal anti-inflammatory drugs (NSAIDS) or aspirin use (yes or no). Regular aspirin or NSAIDS use was defined as taking these drugs at least five to seven days per week for the past twelve months. Variable information was obtained via interviewer-administered standardized questionnaire. Other variables included height, weight and body mass index (BMI, measured in kg/m^2). Height and weight were measured directly by

research staff. Weight was measured in kilograms using a standard beam balance, and without shoes or heavy clothing. Height was measured in centimeters using a wall- mounted Harpenden stadiometer, without shoes, and at the peak of deep inspiration. The height and beam balance weight information on eight participants were either missing or incomplete: these measurements were therefore imputed using DXA estimates.

We calculated the 25^{th}, 50^{th}, and 75^{th} percentile hip BMD cut points in controls by race, and for all subjects. We assessed associations between selected risk factors and hip BMD quartiles in controls by race, and for all controls, using a chi-squared test for categorical variables. These risk factors included age, level of education, marital status, history of benign prostatic hyperplasia (BPH) or prostatitis, father or brother with history of prostate cancer, history of regular aspirin or NSAIDS use, weight, height, and BMI.

Additionally, we evaluated associations between risk factors and prostate cancer, by race, and for all subjects using conditional logistic regression. To examine the relation between hip BMD (continuous) and prostate cancer, we fitted age-adjusted and multiply adjusted conditional logistic regression models. We also used conditional logistic regression to assess the relation between hip BMD (quartiles) and prostate cancer. Our considerations for statistical significance were based on a two-sided p-value of 0.05 or less.

5.4 RESULTS

The mean ages of cases (n = 287) and controls (n = 304) were 60.7 years (SD, 6.7) and 61.2 years (SD, 7.1) respectively. Mean body mass index (BMI) of cases was lower than controls, 28.5 kg/m^2 (SD, 3.9) and 29.3 kg/m^2 (SD, 4.5) respectively ($p = 0.036$). Race-specific associations between risk factors and hip BMD in control subjects are shown in Table 5.1. The 25^{th}, 50^{th} and 75^{th} percentile hip BMD cut points are shown for AA, Caucasians, and all subjects. African Americans had higher cut points (0.975 gm/cm^2, 1.055 gm/cm^2, 1.171 gm/cm^2) than Caucasians (0.932 gm/cm^2, 1.023 gm/cm^2, 1.113 gm/cm^2). Marital status (currently married vs. never / previously married) was significantly associated with hip BMD in the race-combined group ($p < 0.001$), and in Caucasians ($p < 0.001$), but not in AA ($p = 0.148$). Hip BMD was significantly associated with weight ($p=0.0029$, AA, and $p<0.0001$, Caucasians) and BMI ($p=0.0011$, AA, and $p<0.0001$, Caucasians), but height was not ($p= 0.1227$ vs. $p =0.0774$ in AA and Caucasians respectively). Hip BMD appeared unrelated to age, education, history of BPH or prostatitis, father or brother with history of prostate cancer and aspirin or NSAID use in either racial group.

Table 5.2 shows race-specific associations between risk factors and prostate cancer. Significant associations were noted between each of the following risk factors and prostate cancer in the race-combined group: marital status ($p<.0001$), father or brother with a history of prostate cancer ($p<.0001$), weight ($p=0.0079$), and BMI ($p=0.0074$). Similar associations were noted in Caucasians, but not in AA: marital status ($p<.0001$ vs. 0.2781), father or brother with a history of prostate cancer ($p<.0001$ vs. $p= 0.0228$), weight ($p=0.0066$ vs. $p=0.0792$), BMI

(p=0.0357 vs. p=0.1147). Mean BMD was lower in cases than controls, 1.017 gm/cm^2 (SD, 0.130) vs. 1.036 gm/cm^2 (SD, 0.140) (p = 0.10).

The relationship between hip BMD and prostate cancer was assessed using conditional logistic regression. Covariates in our multiply-adjusted model included age, race, educational level, marital status, history of BPH or prostatitis, father or brother with history of prostate cancer, body weight, height, BMI, and aspirin or NSAIDS use (Table 5.3). Separate models were fitted with hip BMD entered as a continuous, and as a categorical (quartile) variable. A statistically significant inverse association was noted between hip BMD and prostate cancer (all subjects), in the model in which hip BMD was entered as a continuous variable, adjusting for age, race, and marital status (OR = 0.28, 95% CI = 0.08 – 0.97, p = 0.045). The strengths of association between hip BMD (continuous) and prostate cancer (both races) were similar among older men (70 years or older, OR = 0.32, 95% CI = 0.01-8.63, p = 0.496) and younger men (<70 years old, OR = 0.28, 95% CI = 0.07-1.06, p = 0.060)**,** adjusting for race and marital status**.** Results in Caucasians were similar to those of both races combined among subjects in the highest hip BMD quartile after adjusting for marital-status (OR = 0.57, 95% CI = 0.33 -0.97, p =0.2254; vs. OR = 0.58, 95% CI = 0.36 – 0.93, p = 0.1061) respectively. Results were also similar in models in which hip BMD was entered as a continuous variable (OR = 0.25, 95% CI = 0.06 – 1.08, p =0.0628; vs. OR = 0.28, 95% CI = 0.08 – 0.97, p = 0.0454) among Caucasians and both races combined respectively. The magnitude of association was different for AA, but the direction of association was the same.

5.5 DISCUSSION

Our analyses suggest an inverse association between hip BMD and prostate cancer among participants in this case-control study of African-American and Caucasian men, ages 40-80 years. However, statistical significance was only noted in the parsimonious model (all participants) in which BMD was entered as a continuous variable, adjusting for race, age, and marital status.

Our results are consistent with the findings of Nelson et al., who reported an inverse association between BMD and prostate cancer risk in 2,769 men followed prospectively in the First National Health and Nutrition Examination Survey (NHANES I) Epidemiologic follow-up Study (NHEFS), but this association was not statistically significant [263]. BMD of the hand was measured at a mean age of 49 years, after which participants were followed for approximately 19 years for diagnosis of prostate cancer. Mean age at diagnosis of prostate cancer was not reported. There were 94 incident cases of prostate cancer, and the rate ratio for men in the highest quartile compared to the lowest was 0.72 (95% CI 0.38-1.38, P trend = 0.37) [263]. Farhat et al. reported a statistically significant inverse association between BMD and prostate cancer in a cohort study of 4,597 men aged 65 years and older, with no prior history of prostate cancer that were followed for an average of 5.2 years[264].

Contrary to these findings, Bunker et al reported an increased risk of prostate cancer with increasing BMD quartiles among men ages 60-79 years in a cross-sectional study of 1,725 Afro-Caribbean men who were being screened for cancer in the Tobago Prostate Survey [261]. In this study, prostate cancer risk among participants in the highest quartile of BMD was double that of those in the lowest quartile, independent of age and body mass index (OR 2.12, 95% CI 1.21-

3.71, P for trend = 0.004)[261]. These results were consistent with the findings of Zhang et al, in a cohort study which assessed the relationship of bone mass and subsequent prostate cancer risk in 1,012 Caucasian men in the Framingham study [262]. There were 100 incident cases in the Framingham study, most of which were diagnosed at an older age (median age 75.2 years). Cortical bone mineral densities of the metacarpal bones were obtained at a mean participant age of 61 years. They reported an increased incidence of prostate cancer in the two higher age-specific quartiles compared to the lowest quartile. The risk ratio for men in the highest quartile compared to the lowest was 1.6 (95% CI 0.9-3.0, P for trend =0.06) [262]. McGlynn et al reported a significantly decreased risk of prostate cancer (standardized incident ratio [SIR] = 0.74 95% CI = 0.54-0.98) in a cohort of 3,055 Danish men hospitalized with a diagnosis of osteoporosis[301]. Notably, subgroup analysis showed that the association was only significant in men 70 years or older (SIR = 0.62, 95% CI = 0.41-0.91), but not in men 69 years or younger (SIR = 0.97, 95% CI = 0.59-1.50)[301].

The cohort in NHANES was enrolled at a much younger age (mean age 49 years), bringing the mean age at the end of the 19 year follow-up period to approximately 68 years. Even though the median age at prostate cancer diagnosis was not reported in NHANES, it appears likely to be considerably less than the median age of 75.2 years reported by the Framingham study. Age is an important risk factor in prostate cancer development, as well as BMD determination; as one ages the risk of developing prostate cancer increases, but BMD decreases. Therefore, there is the possibility that the observed relation between BMD and prostate cancer risk reverses as one transitions from a younger to an older age group, which may represent shifting androgen to cytokine ratio with age.

Previous studies[261, 262, 301] appear to suggest an association between high testosterone levels and prostate cancer risk, because men with higher BMD, as reported by these studies, are also likely to have higher testosterone levels. An association between high levels of testosterone and prostate cancer has been reported by some studies[59, 219, 225], but not others[40, 302]. Testosterone and estrogen maintain bone homeostasis by counteracting the bone resorption activities of inflammatory cytokines such as *IL-1* and *IL-6*. The major factor driving low BMD is an imbalance in bone resorption which exceeds bone formation; this occurs due to excessive bone resorption activity of osteoclasts [30]. The inflammatory cytokines *IL-1* and *IL-6* play a central role in bone turnover by stimulating osteoclastogenesis[30 266]. The bioactivity of *IL-1* and *IL-6* may be potentially modified by sex-steroid hormones[29]. For example, decreasing BMD in aging men due to subnormal testosterone levels can be reversed with testosterone treatment[303]. Similarly, the accelerated rate of bone loss in aging women can be decelerated by administration of estrogen [29]. The reversal of bone loss in these instances are likely a direct result of the counteractive effect of these sex-steroid hormones on the bone resorption activities of inflammatory cytokines such as *IL-1* and *IL-6*[29, 30], among other factors. Furthermore, several studies have reported an association of sequence variants of these inflammatory cytokines and bone mineral density[38, 257, 265, 267]. Besides their roles in bone resorption, *IL-1* and *IL-6* have been reported to influence the initiation and progression of prostate cancer[23-25]. These cytokines mediate the rapid development of prostate cancer, which starts as an androgen-dependent disease initially responsive to androgen ablation therapy, but which invariably progresses to an androgen-independent disease, which is refractory to treatment, and is characterized by recurrent growth and metastasis, predominantly to bone[25, 114].

We report an inverse association between hip BMD (continuous) and prostate cancer in our case control study of African-American and Caucasian men ages 40-80 years. Our results did not change considerably when we examined the relation of hip BMD and prostate cancer in advanced age (70 years and older) versus younger age (69 years and younger), suggesting that sex-hormone levels may not be the dominating etiologic factor of prostate cancer in our sample. After adjusting for race, age and marital-status, we obtained results in the combined racial groups which approximated the results in Caucasians (BMD quartiles and continuous), implying that our overall results were possibly driven by Caucasians. Even though the magnitude of association was different in the two racial groups, the direction of association was the same, suggesting a transcendent commonality in the causative factors of prostate cancer in both Caucasians and AA in this study.

Besides the increasing bone resorption activities of *IL-1* and *IL-6* with age, another possible explanation for the inverse association between BMD and prostate cancer observed in this study may be vitamin D deficiency. Vitamin D is essential for bone health, and has also been associated with prostate cancer[304]. Low levels of vitamin D have been reported to be associated with a decrease in BMD[305], as well as increase the risk of prostate cancer[44]. Levels are reported to decrease with age[306, 307], and are lower in AA compared to Caucasians[305]. We did not measure Vitamin D levels in our subjects, but it might be reasonable to speculate that the vitamin D levels in our AA and Caucasian subjects of mean age approximately 61 years, will be lower that the levels in the general population. The possibility exists that the rate of change of BMD over time (or "BMD velocity/deceleration") may be a better predictor of the risk of disease, rather than an instantaneous measurement. A prospective study which assesses serial measurements of BMD, hormones such as androgen and estrogen, and cytokines will help elucidate this relationship, as

well as serve an important public health purpose to further help in identifying those at increased risk, in order to initiate early intervention.

This study has several limitations. First, we relied on self-reporting of race in spite of the fact that 7-20% of African Americans have Caucasian admixture. Furthermore, a disproportionately higher number of Caucasians participated in the study compared to African Americans (79% versus 21%); therefore our statistical analysis for African Americans in this population may be underpowered. Our analysis was based on hip BMD; it is possible that BMD of other sites may provide a different outcome in regard to the association between BMD and prostate cancer. Among our cases, approximately 85% were married, and nearly 76% had a technical training or college background. A combination of these factors is likely to result in an increase in doctor visits due to availability of health insurance, or pressure from spouses to get medical check-up, which may lead to prostate cancer detection. This group is also apt to self select into ongoing research studies due to the likelihood of being more informed.

There are also several strengths. The mean race-specific hip BMD measurements in our controls were similar to those reported by Leder et al[308], thereby validating our sample externally. Additionally, all our case diagnoses were based on pathology report, reducing the likelihood of misclassification. Furthermore, case recruitment was restricted to those with early diagnosed prostate cancer (within 3 months of diagnosis) thereby limiting the likelihood of bone metastasis. Age and race frequency matching of cases to controls improved the comparability of the two groups.

In conclusion we found an inverse association between hip BMD and prostate cancer in our case control study of African-American and Caucasian men ages 40-80 years. With a few exceptions, this finding was consistent in both races and across all models; however, statistical

significance was observed only in the age-, race- and marital status-adjusted parsimonious model (combined races) in which hip BMD was entered as a continuous variable. The long term effect of inflammatory cytokines on bone during the aging process needs to be carefully studied along with vitamin D status and hormonal factors in order to fully understand the relation between bone mineral density and prostate cancer.

5.6 TABLES AND FIGURES

Table 5.1 : Race-specific associations between risk factors and hip BMD in controls

	Black					White					All races				
Attribute	N	25th p-tile	50th p-tile	75th p-tile	p-value	N	25th p-tile	50th p-tile	75th p-tile	p-value	N	25th p-tile	50th p-tile	75th p-tile	p-value
ALL	66	0.975	1.055	1.171		238	0.932	1.023	1.113		304	0.939	1.031	1.123	
Age (years)					0.5197					0.6933					0.2582
40-49	8	1.031	1.119	1.206		7	1.006	1.048	1.090		15	1.006	1.065	1.171	
50-59	18	0.956	1.044	1.094		89	0.956	1.023	1.094		107	0.956	1.023	1.094	
60-69	31	0.959	1.061	1.199		121	0.921	1.031	1.134		152	0.923	1.037	1.141	
70+	9	0.983	1.005	1.181		21	0.904	1.001	1.101		30	0.923	1.003	1.135	
Education					0.8413					0.4457					0.8349
Less than high school	9	0.990	1.005	1.058		5	0.929	0.932	0.972		14	0.959	0.993	1.058	
High school graduate	14	0.974	1.052	1.129		39	0.893	1.044	1.135		53	0.919	1.044	1.131	
Technical training or college	43	0.972	1.074	1.181		194	0.936	1.023	1.110		237	0.947	1.031	1.122	
Marital status					0.148					<0.001					<0.001
Married	41	0.996	1.074	1.199		179	0.958	1.044	1.131		220	0.969	1.045	1.141	
Other (never/previously married)	25	0.972	1.042	1.096		59	0.890	0.956	1.042		84	0.911	0.985	1.069	

Note: p-value (Kruskall-Wallis)
Height missing for N=1 black control, weight missing for N=1 black control, BMI missing for N=1 black control
The height and weight estimates of 3 controls with missing data points were based on DXA measurements
Race-specific height quartile cut-points, blacks: 171.1, 174.1, 178.5 cm, white: 172.1, 176.2, 180.0 cm
Race-specific weight quartile cut-points, blacks: 78.4, 91.35, 102.5 kg, whites: 77.5, 89.35, 100. 0 kg

Table 5.1 (continued)

	Black					White					All races				
Attribute	N	25th p-tile	50th p-tile	75th p-tile	p-value	N	25th p-tile	50th p-tile	75th p-tile	p-value	N	25th p-tile	50th p-tile	75th p-tile	p-value
ALL	66	0.975	1.055	1.171		238	0.932	1.023	1.113		304	0.939	1.031	1.123	
History of BPH or prostatitis					0.1123					0.5737					0.6849
Yes	12	1.030	1.109	1.264		132	0.936	1.023	1.124		144	0.937	1.033	1.132	
No	54	0.974	1.044	1.156		106	0.931	1.023	1.108		160	0.951	1.029	1.113	
Father or brother with prostate cancer					0.4357					0.8824					0.5866
Yes	4	1.029	1.079	1.228		11	0.929	1.045	1.094		15	0.936	1.045	1.096	
No	62	0.974	1.049	1.171		227	0.932	1.023	1.116		289	0.940	1.028	1.124	
Height					0.1227					0.0774					0.0110
Quartile 1 (low)	14	0.954	0.0.993	1.074		59	0.881	0.987	1.072		73	0.886	0.990	1.072	
Quartile 2	18	1.016	1.093	1.290		61	0.925	1.032	1.131		79	0.951	1.052	1.139	
Quartile 3	17	0.972	1.065	1.156		59	0.953	1.039	1.103		76	0.955	1.039	1.107	
Quartile 4 (high)	17	0.997	1.046	1.171		59	0.975	1.025	1.139		76	0.976	1.039	1.149	
Weight					0.0029					<.0001					<.0001
Quartile 1 (low)	18	0.901	0.978	1.022		59	0.867	0.938	1.041		77	0.876	0.951	1.037	
Quartile 2	14	1.042	1.102	1.186		60	0.920	1.015	1.072		74	0.929	1.031	1.101	
Quartile 3	17	0.974	1.065	1.156		59	0.975	1.032	1.139		76	0.975	1.046	1.141	
Quartile 4 (high)	17	1.020	1.082	1.253		60	1.014	1.106	1.178		77	1.018	1.102	1.193	
Body mass index (kg/m^2)					0.0011					<.0001					<.0001
Normal (18.5-24.9)	12	0.900	0.964	0.999		43	0.875	0.951	1.041		55	0.878	0.956	1.021	
Overweight (25.0-29.9)	21	0.996	1.084	1.199		110	0.922	1.016	1.079		131	0.929	1.025	1.097	
Obese (30.0+)	33	1.016	1.082	1.171		85	0.988	1.068	1.169		118	0.997	1.072	1.171	
Aspirin or NSAIDS					0.8685					0.6612					0.9384
Yes	18	0.974	1.018	1.199		120	0.934	1.032	1.118		138	0.947	1.024	1.125	
No	48	0.987	1.063	1.164		118	0.931	1.016	1.108		166	0.938	1.038	1.121	

Note: p-value (Kruskall-Wallis)
Height missing for N=1 black control, weight missing for N=1 black control, BMI missing for N=1 black control
The height and weight estimates of 3 controls with missing data points were based on DXA measurements
Race-specific height quartile cut-points, blacks: 171.1, 174.1, 178.5 cm, white: 172.1, 176.2, 180.0 cm
Race-specific weight quartile cut-points, blacks: 78.4, 91.35, 102.5 kg, whites: 77.5, 89.35, 100. 0 kg

Table 5.2 : Race-specific associations between risk factors and prostate cancer

	Blacks					Whites					All races				
	Cases		Controls			Cases		Controls			Cases		Controls		
Attribute	N	%	N	%	p-value	N	%	N	%	p-value	N	%	N	%	p-value
ALL	56	100.0	66	100.0		231	100.0	238	100.0		287	100.0	304	100.0	
Age (years)					0.3579					0.9998					0.8900
40-49	5	8.9	8	12.1		7	3.0	7	2.9		12	4.2	15	4.9	
50-59	22	39.3	18	27.3		86	37.2	89	37.4		108	37.6	107	35.2	
60-69	19	33.9	31	47.0		118	51.1	121	50.8		137	47.7	152	50.0	
70+	10	17.9	9	13.6		20	8.7	21	8.8		30	10.5	30	9.9	
Education					0.6088					0.9287					0.7952
Less than high school	8	14.3	9	13.6		4	1.7	5	2.1		12	4.2	14	4.6	
High school graduate	16	28.6	14	21.2		40	17.3	39	16.4		56	19.5	53	17.4	
Technical training or college	32	57.1	43	65.2		187	81.0	194	81.5		219	76.3	237	78.0	
Marital status					0.2781					<.0001					<.0001
Married	40	71.4	41	62.1		208	90.0	179	75.2		248	86.4	220	72.4	
Other (never/previously married)	16	28.6	25	37.9		23	10.0	59	24.8		39	13.6	84	27.6	
History of BPH or prostatitis					0.0283					0.0026					0.0889
Yes	20	35.7	12	18.2		96	41.6	132	55.5		116	40.4	144	47.4	
No	36	64.3	54	81.8		135	58.4	106	44.5		171	59.6	160	52.6	
Father or brother with prostate cancer					0.0228					<.0001					<.0001
Yes	11	19.6	4	6.1		44	19.0	11	4.6		55	19.2	15	4.9	
No	45	80.4	62	93.9		187	81.0	227	95.4		232	80.8	289	95.1	
Height					0.0439					0.6240					0.2021
Quartile 1 (low)	13	23.2	14	21.2		63	27.3	59	24.8		76	26.5	73	24.0	
Quartile 2	5	8.9	18	27.3		55	23.8	61	25.6		60	20.9	79	26.0	
Quartile 3	14	25.0	17	25.8		48	20.8	59	24.8		62	21.6	76	25.0	
Quartile 4 (high)	24	42.9	17	25.8		65	28.1	59	24.8		89	31.0	76	25.0	
Weight					0.9390					0.0059					0.0064
Quartile 1 (low)	12	21.4	18	27.3		46	19.9	59	24.8		58	20.2	77	25.3	
Quartile 2	17	30.4	14	21.2		91	39.4	60	25.2		108	37.6	74	24.3	
Quartile 3	6	10.7	17	25.8		55	23.8	59	24.8		61	21.3	76	25.0	
Quartile 4 (high)	21	37.5	17	25.8		39	16.9	60	25.2		60	20.9	77	25.3	
Body mass index (kg/m^2)					0.1230					0.0352					0.0053
Normal (18.5-24.9)	7	12.5	12	18.2		38	16.5	43	18.1		45	15.7	55	18.1	
Overweight (25.0-29.9)	28	50.0	21	31.8		133	57.6	110	46.2		161	56.1	131	43.1	
Obese (30.0+)	21	37.5	33	50.0		60	26.0	85	35.7		81	28.2	118	38.8	
Total hip bone mineral density					0.1680					0.3666					0.2524
Quartile 1 (low)	19	33.9	16	24.2		66	28.6	59	24.8		85	29.6	75	24.7	
Quartile 2	6	10.7	17	25.8		59	25.5	59	24.8		65	22.6	76	25.0	
Quartile 3	17	30.4	16	24.2		63	27.3	60	25.2		80	27.9	76	25.0	
Quartile 4 (high)	14	25.0	17	25.8		43	18.6	60	25.2		57	19.9	77	25.3	
Aspirin or NSAIDS					0.7074					0.427					0.6527
Yes	17	30.4	18	27.3		108	46.8	120	50.4		125	43.6	138	45.4	
No	39	69.6	48	72.7		123	53.2	118	49.6		162	56.4	166	54.6	

Note: p-value (Wald test)
Uses conditional logistic regression

Table 5.3 : Unadjusted and adjusted associations of hip BMD (quartiles and continuous) and prostate cancer, by race, and for all subjects

	Hip BMD quartile										
		Q2		Q3		Q4			Hip BMD (g/cm^2)		
	Q1 Reference	OR	95% CI	OR	95% CI	OR	95% CI	p-value	OR	95% CI	p-value
Blacks	(19/16)	(6/17)		(17/16)		(14/17)					
Age-adjusted		0.26	0.08 - 0.82	1.08	0.41 - 2.86	0.78	0.29 - 2.08	0.0950	0.57	0.06 - 5.57	0.6248
Marital status-adjusted		0.24	0.07 - 0.78	1.13	0.42 - 3.03	0.63	0.22 - 1.76	0.0670	0.41	0.39 - 4.22	0.4504
Multiply adjusted		0.20	0.05 - 0.77	1.12	0.35-3.58	0.54	0.17 - 1.72	0.0611	0.11	0.01 - 2.09	0.1422
White	(66/59)	(59/59)		(63/60)		(43/60)					
Age-adjusted		0.89	0.54 - 1.48	0.94	0.57 - 1.55	0.64	0.38 - 1.09	0.3727	0.38	0.09 - 1.55	0.1748
Marital status-adjusted		0.82	0.49 - 1.38	0.8	0.47 - 1.34	0.57	0.33 - 0.97	0.2254	0.25	0.06 - 1.08	0.0628
Multiply adjusted		0.89	0.51 - 1.56	0.90	0.51 - 1.57	0.73	0.40 - 1.34	0.7930	0.45	0.09 - 2.32	0.3361
Both races	(85/75)	(65/76)		(80/76)		(57/77)					
Age-adjusted		0.74	0.47 -1.16	0.95	0.61 - 1.49	0.67	0.42 - 1.06	0.2435	0.42	0.13 - 1.40	0.1582
Marital status-adjusted		0.68	0.42 - 1.08	0.86	0.55 - 1.37	0.58	0.36 - 0.93	0.1061	0.28	0.08 - 0.97	0.0454
Multiply adjusted		0.66	0.40 - 1.08	0.90	0.55 - 1.44	0.64	0.38 - 1.07	0.2123	0.35	0.09 - 1.38	0.1341

Notes

Hip BMD quartile cut-point are 0.975, 1.055, and 1.171 g/cm^2 for blacks and 0.932, 1.023, and 1.113 g/cm^2 for whites.

p-value -- statistical significance of Hip BMD quartile in logistic regression model (Wald test)

ORs for analyses that model hip BMD as a continuous variable represent the relative odds of prostate cancer per change in hip BMD of 0.1 g/cm^2

Uses conditional logistic regression

Age adjusment considers age in four categories (40-49, 50-59, 60-69, and 70+ year of age)

Multiply adjusted models adjusts for, education (three categories), marital status (two categories), history of BPH (two categories), history of prostatitis (two categories),

family history of prostate cancer (two categories), body weight (continuous), height (continuous),

body mass index (continuous)

6.0 GENERAL DISUSSION

We examined allele frequency distributions of polymorphisms in *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6* genes in African Americans and Caucasians. We also assessed the associations of genotypes of these inflammatory genes and the risk of prostate cancer in the two racial groups. Additionally, we evaluated the associations of bone mineral density and prostate cancer in our case control study of AA and Caucasian men ages 40 to 80 years old. The current study was undertaken because recent studies have reported mounting evidence for potential associations of sequence variants of these inflammatory genes and prostate cancer[21, 24] . Furthermore, there have been recent reports of an association of BMD with prostate cancer; and *IL-1 and IL-6* are known to decrease BMD by inducing osteoclasts to resorb bone. Prostate cancer incidence and mortality rates have been consistently higher among AA than Caucasians for several decades[1, 54]. Furthermore, racial differences in immune modulating genes are well documented [277], and it has been suggested that such differences may influence disparities in clinical outcome between AA and Caucasians[274]. We sought to determine if genotypes of *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* were associated with prostate cancer risk, as well as with selected risk factors, in the two racial groups. We found racial differences in MAFs, as well as in the associations of SNPs of inflammatory genes *IL-1* and *IL-6* and prostate cancer. We also found an inverse association of BMD and prostate cancer in both racial groups. Our findings support a growing body of evidence

that chronic or recurrent inflammation play an important role in prostate carcinogenesis[10-13], and the possibility of ethnic based differences in susceptibility.

6.1 PAPER #1

In our first aim we assessed allele frequencies of inflammatory cytokines IL-1 and IL6 gene polymorphisms to determine if there were differences between African American and Caucasian men without prostate cancer. We compared MAFs of inflammatory gene markers between AA and Caucasian controls. We found 50% or greater significant differences in the distribution of variant alleles in *IL-1A, IL-1B, IL-1RN, IL-6* and *IL-6R* genes between the two racial groups. We also made race-specific comparisons of MAF of *IL-1* and *IL-6* gene markers between observed and HapMap reference groups and found that approximately 12% of MAFs were significantly different in our Black comparison group, but none in our White comparison group. These differences may reflect greater admixture in the African American population compared to Caucasians, and is an important consideration in disease susceptibility[275, 276]. Cytokine gene polymorphisms have been reported to influence disease susceptibility, severity and clinical outcome[184, 277]. Racial differences in the distribution of inflammatory marker allele frequencies have been reported to influence allograft rejection, and in rheumatoid arthritis development and response to treatment [274, 278-280]. Inter-ethnicity differences in the frequencies of cytokine gene variants are well document, and the extent to which these differences influenced disease in our subjects is unclear, but they may, to some degree, have served as a

basis for some of the differences in marker-disease and marker-risk factor associations we observed in our second specific aim.

6.2 PAPER #2

This part of our project examined the associations of *IL-1 and IL-6* gene polymorphisms and the risk of prostate cancer in AA and Caucasians. We found racial differences in the associations of SNPs of pro-inflammatory genes *IL-1* and *IL-6* and prostate cancer, as well as with selected risk factors. Two *IL-1RN* markers (rs432014 and rs9005), and one *IL-6R* marker (rs4845626) were associated with prostate cancer in AA, but to these markers provided a protective effect against prostate cancer in African Americans homozygous or heterozygous for the variant allele. On the other hand, the markers that were significantly associated with prostate cancer in Caucasians (*IL-1RN*-rs3181052, *IL-1RN*-rs2071459 and *IL-1RN*-rs4252019) all increased prostate cancer risk in this racial group. We observed that two of these SNPs (*IL-1RN*-rs3181052 and *IL-1RN*-rs2071459) were associated with a higher risk of prostate cancer among AA than Caucasians; however, there were no statistically significant differences between AA individuals homozygous and heterozygous for the variant allele compared to non-carriers.

The overarching theme of markers of disease association in this study is that *IL-1RN* plays an important in the development of prostate cancer among our subjects. Of the six SNPs that were significantly associated with prostate cancer in our subjects five (83%) were *IL-1RN* SNPs. This underscores the important role of inflammation in prostate cancer development because *IL-1RN* is the natural inhibitor of the pro-inflammatory genes *IL-1A* and *IL-1B*[188], and

therefore certain polymorphisms in the gene which may impair its normal activities may result in disease. Polymorphisms in *IL-1RN* have been reported to be associated with bladder cancer[309], BPH[310] and several other cancers[311]. Additionally, IL-1RN has been used to treat melanoma[311]. In our specific first specific aim (paper one) we reported that MAFs of approximately 63% of *IL-1RN* markers were significantly different between AA and Caucasians. There is a possibility that these differences partly explain the differences in disease associations observed in our second specific aim. We also observed an association of one *IL-6R* SNP with prostate cancer in AA, while several other IL-6R markers were significantly associated with BMD in Caucasians controls.

6.3 PAPER #3

In our third aim, we assessed the association of BMD and prostate cancer in our subjects. Our analyses suggested an inverse association of hip BMD and prostate cancer in our participants. Even though several of our results were not statistically significant, the inverse association was consistent across all our models. An inverse association of BMD and prostate cancer has been reported by some[263, 264], but not all[261, 262] epidemiologic studies. A possible explanation for the inverse association between BMD and prostate cancer observed in this study may be due to increased bone resorption activity of *IL-1* and *IL-6* which exceeds bone formation in our sample. In the current study nine *IL-6* and *IL-6R* markers showed significant associations with BMD in Caucasian controls. One of these SNPs showed the strongest association with prostate cancer in AA individuals homozygous or heterozygous for the variant allele (*IL-6R*-rs4845625, Ptrend

=0.0022). This relationship underscores the important role of these inflammatory markers in BMD as well as prostate cancer. Another possible explanation could be vitamin D deficiency. Vitamin D is essential for bone health, and has also been associated with prostate cancer[304]. Low levels of vitamin D have been reported to be associated with a decrease in BMD[305], as well as increase the risk of prostate cancer[44].

6.4 CONCLUSION

In conclusion, the aggregate findings of these papers suggest that inflammation plays an important role in prostate cancer, and that differences in allele frequencies between AA and Caucasians may be partly influencing the observed disparities in prostate cancer rates between these two racial groups. These findings suggest differences in response to inflammation which may be ethnic or race based. Limitations of this study include relying on self reporting of race, even though there is an approximately 7-20% admixture among African Americans, which may tend to decrease our observed race-specific associations. Our tag SNP panel was based on Phase II of the International HapMap project, and may not have captured a more comprehensive set of tag SNPs as provided in Phase III of the HapMap project. In spite of our best efforts to include a comprehensive set of markers in our study, there is always the possibility of excluding markers of true association. Also, our analysis of BMD was based on hip BMD; it is possible that BMD of other sites may provide a different outcome in regard to the association between BMD and prostate cancer. Among our cases, approximately 85% were married, and nearly 76% had a

technical training or college background. This group is also apt to self select into ongoing research studies due to the likelihood of being more informed.

In spite of these limitations, our study has several strengths. It is one of the first to directly assess prostate cancer risk in AA and Caucasian men within the context of a comprehensive set of inflammatory cytokines. It evaluated the associations of the inflammatory cytokines and commonly reported risk factors of prostate cancer in the two racial groups. It identified two putative functional SNPs (*IL-1A*-rs20540 and *IL-6R*- rs28730736) in which variant alleles were observed in African Americans, but not Caucasians. We report race-specific mean hip BMD in our controls that corroborate those reported by Leder et. al.[308], therefore our sample is validated externally. Additionally, all our case diagnoses were based on pathology report confirmed by one pathologist, there reducing the likelihood of misclassification. Furthermore, case recruitment was restricted to those with early diagnosed prostate cancer (within 3 months of diagnosis) thereby limiting the likelihood of bone metastasis. Age and race frequency matching of cases to controls improved the comparability of the two groups.

6.5 FUTURE DIRECTIONS

Future studies may want to closely examine the *IL-1RN,* as well as the *IL-6R* including its alpha- (gp80) and beta- (gp130) subunits in order to further understand some of the disparities noted between AA and Caucasians. Also, a prospective study which assesses serial measurements of BMD, hormones, and cytokines will help elucidate the role of inflammation in disease disparities. Understanding the role of *IL-1* and *IL-6* genes in the development of prostate cancer

is of great public health significance because it will enable their possible use as biomarkers for early detection and prompt intervention, increase our understanding of the molecular biology of the disease, open up new avenues for prevention and treatment, as well as explain some of the observed disparities in the disease.

APPENDIX

ASSOCIATIONS OF IL-1A, IL-1B, IL-1RN, IL-6 AND IL-6R POLYMORPHISMS AND SELECTED RISK FACTORS

Table A1.1: Characteristics of subjects included and those excluded from genotyping analysis

	Subjects included (n = 558)		Subjects excluded (n=35)		
Attribute	Mean	SD	Mean	SD	p-value
Age	60.9	6.9	61.7	6.8	0.5295
Height (cm)	176.1	6.4	176.4	6.7	0.7631
Weight (kg)	89.9	87.4	87.4	14.8	0.3313
BMI (kg/m^2)	29.0	4.3	28.0	4.0	0.1806
Father or brother with history of prostate cancer, n (%)					
Yes	63	90.0	7	10.0	0.1214
No	495	94.7	28	5.4	
Personal medical history of BPH or prostatitis, n(%)					
Yes	250	95.8	11	4.2	0.1221
No	308	92.8	24	7.2	

Note: Subject exclusion was based on poor sample quality or lack of sufficient samples for genotyping

Table A1.2 : Control subjects with and without a variant allele, number (N) and age (years, mean and standard deviation – SD), by locus and race

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		Age			Age				Age			Age		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL1A														
rs3783590	11	60.1	11.8	48	61.3	9.4	0.7175	1	80.0		226	61.1	6.1	
rs2856836	23	61.4	9.9	36	60.9	9.9	0.8415	107	60.5	5.7	120	61.7	6.6	0.1455
rs17561	23	61.4	9.9	36	60.9	9.9	0.8415	107	60.5	5.7	120	61.7	6.6	0.1455
rs20540	6	54.7	8.1	53	61.8	9.8	0.0917							
rs2856838	36	61.9	9.7	23	59.8	10.1	0.4257	141	61.5	6.2	86	60.6	6.2	0.3130
rs1609682	22	59.4	11.2	37	62.1	8.9	0.3206	126	60.9	6.0	99	61.4	6.4	0.5232
rs3783526	4	61.5	11.8	55	61.0	9.8	0.9282	126	60.9	6.1	101	61.4	6.4	0.5357
rs2856837	26	62.2	9.6	33	60.2	10.0	0.4549	107	60.5	5.7	120	61.7	6.6	0.1455
IL1B														
rs1143643	22	64.1	9.5	37	59.2	9.6	0.0632	134	61.2	6.2	93	61.0	6.3	0.8128
rs1143634	18	62.2	9.2	41	60.6	10.1	0.5536	83	61.0	5.8	142	61.3	6.4	0.6915
rs1143633	27	63.4	9.8	32	59.1	9.5	0.0873	134	61.4	6.4	92	60.8	6.0	0.4863
rs3136558	16	63.5	8.4	43	60.2	10.2	0.2485	78	60.4	5.7	149	61.5	6.4	0.1871
rs1143630	28	61.0	9.9	31	61.2	9.9	0.9394	28	63.4	7.6	199	60.8	5.9	0.0442 *
IL1RN														
rs3181052	16	60.3	9.3	43	61.4	10.1	0.6995	33	61.3	5.8	193	61.1	6.3	0.8926
rs1794066	35	60.5	10.1	24	62.0	9.5	0.5680	145	61.0	6.2	81	61.4	6.2	0.6334
rs1794067	30	62.2	9.4	29	59.9	10.3	0.3859	121	61.0	6.3	106	61.4	6.1	0.6063
rs2071459	17	60.8	9.3	42	61.2	10.1	0.8815	33	61.3	5.8	192	61.2	6.3	0.9212
rs432014	21	60.6	10.9	38	61.3	9.3	0.7964	121	61.0	6.3	106	61.4	6.1	0.6063
rs380092	53	60.9	9.8	6	62.5	10.3	0.7093	107	61.3	5.6	117	61.1	6.8	0.7831
rs452204	41	60.5	10.7	17	62.9	7.1	0.3958	145	61.0	6.2	82	61.4	6.2	0.6779
rs4252019	36	60.0	10.9	23	62.7	7.7	0.3122	36	60.9	6.4	191	61.2	6.2	0.7618
rs315955	15	61.1	9.8	43	61.0	10.0	0.9946							
rs315951	38	60.4	9.5	21	62.2	10.5	0.5003	95	61.1	6.1	130	61.1	6.3	0.9686
rs9005	24	62.1	10.8	35	60.4	9.2	0.5147	129	60.9	6.1	98	61.5	6.3	0.4343

Table A1.2 (continued)

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		Age			Age				Age			Age		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL6														
rs2069837	15	60.8	11.7	44	61.2	9.2	0.9038	31	61.0	6.2	196	61.2	6.2	0.9100
rs2069840	13	60.6	10.8	46	61.2	9.6	0.8524	127	61.4	5.8	99	60.8	6.8	0.4548
rs1554606	33	59.9	9.7	26	62.5	10.0	0.3106	158	61.1	6.4	69	61.3	5.9	0.7516
rs2069842	4	59.5	8.2	55	61.2	10.0	0.7436							
rs1548216	17	62.4	9.6	42	60.5	9.9	0.5077	8	58.9	5.9	219	61.2	6.2	0.2924
rs2069843	10	61.6	10.2	49	61.0	9.8	0.8525	8	58.9	5.9	219	61.2	6.2	0.2924
rs2069845	32	60.2	9.6	27	62.1	10.1	0.4738	154	61.1	6.4	69	61.3	5.9	0.8050
IL6R														
rs4845617	37	60.4	10.1	21	62.9	9.3	0.3529	143	61.4	6.4	84	60.7	5.8	0.3700
rs6427641	54	60.8	10.0	5	64.2	7.9	0.4601	154	61.4	6.3	73	60.6	6.0	0.3864
rs11265610	26	62.5	10.0	31	60.8	9.3	0.5279							
rs12083537	27	61.0	9.6	32	61.1	10.1	0.9826	80	60.2	5.7	147	61.7	6.4	0.0934
rs1386821	11	56.3	11.1	48	62.2	9.3	0.0718	74	60.1	5.7	153	61.7	6.4	0.0787
rs4075015	13	65.2	5.7	46	59.9	10.4	0.0889	152	61.1	6.1	75	61.3	6.5	0.7898
rs4601580	45	61.9	10.0	12	59.6	9.3	0.4741	143	61.4	6.1	81	60.6	6.5	0.3608
rs4845618	46	60.7	10.4	13	62.2	7.8	0.6323	160	61.2	6.2	67	61.1	6.3	0.9064
rs7549338	31	61.6	10.2	28	60.5	9.5	0.6763	155	61.3	6.3	72	60.9	6.1	0.7007
rs7518199	19	58.7	10.1	40	62.2	9.6	0.2007	156	61.3	6.5	71	60.9	5.6	0.6353
rs4553185	50	61.5	10.2	8	59.8	6.9	0.6425	159	61.2	6.2	68	61.1	6.2	0.8856
rs4393147	11	58.0	9.8	47	62.0	9.7	0.2226	156	61.3	6.5	71	60.9	5.6	0.6353
rs4537545	51	61.1	10.0	8	60.9	9.2	0.9529	156	61.3	6.4	71	60.9	5.7	0.7022
rs4845626	44	60.5	9.9	15	62.6	9.7	0.4882	70	60.7	6.1	157	61.4	6.3	0.4259
rs28730736	17	64.3	8.9	42	59.8	9.9	0.1084							
rs11265618	39	60.9	10.2	20	61.5	9.3	0.8324	71	60.6	6.1	156	61.4	6.3	0.4125
rs10159236	24	60.6	10.5	35	61.4	9.4	0.7563	67	60.8	6.2	160	61.3	6.2	0.5585
rs4329505	39	60.4	10.6	20	62.3	8.1	0.4942	66	60.8	6.2	161	61.3	6.2	0.5750
rs4509570	44	61.9	9.4	15	58.6	10.8	0.2626	98	61.4	5.7	129	60.9	6.6	0.5280
rs2229238	18	61.8	10.2	41	60.7	9.7	0.6947	80	61.4	5.8	147	61.0	6.4	0.6076
rs4072391	25	61.4	9.1	34	60.8	10.4	0.8050	80	61.4	5.8	147	61.0	6.4	0.6076
rs4379670	18	61.8	10.2	41	60.7	9.7	0.6947	80	61.4	5.8	147	61.0	6.4	0.6076

1. t-test with asterisk (*) to indicate $p<0.05$

Table A1.3 : Control subjects with and without a variant allele, number (N) and height (cm, mean and standard deviation – SD), by locus and race

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		Height			Height				Height			Height		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL1A														
rs3783590	10	174.8	5.9	46	174.4	4.9	0.8104	1	175.5		226	176.2	6.3	
rs2856836	23	173.8	3.2	33	174.9	6.0	0.4517	107	176.0	5.9	120	176.3	6.6	0.7226
rs17561	23	173.8	3.2	33	174.9	6.0	0.4517	107	176.0	5.9	120	176.3	6.6	0.7226
rs20540	6	172.1	1.9	50	174.7	5.3	0.2294							
rs2856838	34	174.2	5.6	22	174.8	4.2	0.7143	141	175.9	6.6	86	176.6	5.7	0.4006
rs1609682	20	175.6	5.3	36	173.8	4.9	0.2150	126	176.4	6.1	99	175.8	6.6	0.4453
rs3783526	4	175.4	2.6	52	174.4	5.2	0.7074	126	176.5	6.1	101	175.8	6.5	0.3701
rs2856837	26	173.5	3.3	30	175.3	6.1	0.1977	107	176.0	5.9	120	176.3	6.6	0.7226
IL1B														
rs1143643	21	173.5	6.1	35	175.0	4.3	0.2711	134	176.3	6.9	93	176.0	5.3	0.7510
rs1143634	18	173.5	3.4	38	174.9	5.7	0.3230	83	176.1	5.7	142	176.3	6.6	0.8264
rs1143633	26	173.6	5.7	30	175.2	4.4	0.2570	134	176.5	6.9	92	175.8	5.3	0.4298
rs3136558	16	172.7	3.5	40	175.1	5.5	0.1060	78	176.0	6.0	149	176.3	6.4	0.6875
rs1143630	25	174.9	5.0	31	174.1	5.1	0.5352	28	175.4	5.5	199	176.3	6.4	0.4819
IL1RN														
rs3181052	15	174.9	4.9	41	174.3	5.2	0.7051	33	175.3	6.2	193	176.4	6.3	0.3469
rs1794066	34	174.6	4.9	22	174.3	5.5	0.8213	145	176.2	5.8	81	176.1	7.1	0.9092
rs1794067	29	175.3	5.7	27	173.5	4.2	0.1826	121	176.4	5.7	106	176.0	6.9	0.6160
rs2071459	16	174.3	5.3	40	174.5	5.0	0.8939	33	175.3	6.2	192	176.4	6.3	0.3389
rs432014	21	175.0	5.1	35	174.1	5.1	0.5663	121	176.4	5.7	106	176.0	6.9	0.6160
rs380092	50	174.7	5.2	6	172.6	3.0	0.3489	107	176.5	6.8	117	176.0	5.8	0.5019
rs452204	40	174.3	4.8	15	174.9	6.0	0.6961	145	176.2	5.8	82	176.1	7.1	0.8597
rs4252019	35	174.7	5.0	21	174.1	5.3	0.6790	36	175.6	6.4	191	176.3	6.3	0.5732
rs315955	14	173.8	6.2	41	174.4	4.6	0.6996							
rs315951	37	174.6	4.4	19	174.2	6.3	0.8261	95	176.2	6.9	130	176.1	5.8	0.9824
rs9005	23	174.3	5.2	33	174.5	5.1	0.9013	129	176.4	5.7	98	176.0	7.0	0.6481

Table A1.3 (continued)

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		Height			Height				Height			Height		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL6														
rs2069837	13	175.8	4.0	43	174.0	5.3	0.2700	31	177.6	6.4	196	176.0	6.2	0.1893
rs2069840	13	172.9	3.8	43	174.9	5.3	0.2025	127	176.3	6.5	99	176.2	5.9	0.9227
rs1554606	31	174.0	5.2	25	175.0	4.9	0.4779	158	176.4	6.2	69	175.8	6.4	0.5518
rs2069842	4	172.1	3.6	52	174.6	5.1	0.3510							
rs1548216	15	173.9	6.7	41	174.6	4.4	0.6308	8	178.6	5.4	219	176.1	6.3	0.2635
rs2069843	9	173.6	7.2	47	174.6	4.6	0.5953	8	178.6	5.4	219	176.1	6.3	0.2635
rs2069845	30	174.1	5.3	26	174.9	4.8	0.5485	154	176.5	6.2	69	175.8	6.4	0.4749
IL6R														
rs4845617	35	175.0	5.2	20	173.6	4.9	0.3498	143	176.0	6.2	84	176.5	6.3	0.5611
rs6427641	51	174.5	5.2	5	174.2	3.5	0.9174	154	176.0	6.2	73	176.6	6.5	0.5190
rs11265610	24	173.4	5.4	30	175.2	4.8	0.2154							
rs12083537	26	174.1	5.7	30	174.7	4.6	0.6689	80	176.6	6.4	147	176.0	6.2	0.4929
rs1386821	11	174.9	6.3	45	174.3	4.8	0.7321	74	176.6	6.6	153	176.0	6.1	0.4445
rs4075015	13	174.0	4.4	43	174.6	5.3	0.7245	152	176.0	6.4	75	176.7	6.0	0.4326
rs4601580	42	175.0	5.4	12	172.8	3.6	0.1843	143	176.0	6.5	81	176.5	6.0	0.5888
rs4845618	44	174.4	4.6	12	174.6	6.8	0.8923	160	176.2	6.1	67	176.0	6.8	0.8160
rs7549338	29	174.4	3.8	27	174.5	6.2	0.9573	155	176.1	6.1	72	176.3	6.6	0.8522
rs7518199	19	173.7	4.4	37	174.8	5.4	0.4603	156	176.4	6.4	71	175.7	5.9	0.4267
rs4553185	48	174.2	4.7	7	176.6	7.6	0.2503	159	176.2	6.1	68	176.0	6.7	0.8153
rs4393147	11	174.5	5.0	44	174.5	5.2	0.9911	156	176.4	6.4	71	175.7	5.9	0.4267
rs4537545	48	174.7	5.3	8	172.8	3.3	0.3337	156	176.4	6.5	71	175.7	5.8	0.4109
rs4845626	43	174.8	5.1	13	173.3	4.9	0.3737	70	176.2	6.6	157	176.2	6.1	0.9688
rs28730736	15	172.3	6.0	41	175.2	4.5	0.0564							
rs11265618	38	174.6	5.4	18	174.1	4.4	0.7587	71	176.2	6.6	156	176.2	6.1	0.9764
rs10159236	23	173.9	5.3	33	174.8	4.9	0.5217	67	175.9	6.6	160	176.3	6.1	0.6931
rs4329505	36	173.6	4.9	20	176.0	5.1	0.0977	66	175.9	6.7	161	176.3	6.1	0.6409
rs4509570	42	174.1	4.8	14	175.6	5.7	0.3178	98	177.0	6.1	129	175.6	6.4	0.1013
rs2229238	16	173.1	3.5	40	175.0	5.5	0.2161	80	177.2	6.0	147	175.7	6.4	0.0810
rs4072391	22	173.6	4.7	34	175.0	5.3	0.3299	80	177.2	6.0	147	175.7	6.4	0.0810
rs4379670	16	173.1	3.5	40	175.0	5.5	0.2161	80	177.2	6.0	147	175.7	6.4	0.0810

1. t-test with asterisk (*) to indicate $p<0.05$

Table A1.4 : Control subjects with and without a variant allele, number (N) and weight (kg, mean and standard deviation – SD), by locus and race

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		Weight			Weight				Weight			Weight		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL1A														
rs3783590	10	93.0	14.3	46	92.7	18.0	0.9654	1	80.8		226	90.6	15.6	
rs2856836	23	92.8	15.5	33	92.7	18.7	0.9838	107	89.5	15.6	120	91.5	15.5	0.3343
rs17561	23	92.8	15.5	33	92.7	18.7	0.9838	107	89.5	15.6	120	91.5	15.5	0.3343
rs20540	6	77.8	6.8	50	94.6	17.3	0.0239 *							
rs2856838	33	93.3	18.3	23	92.1	16.1	0.8093	141	90.9	15.8	86	90.0	15.2	0.6717
rs1609682	21	94.8	14.7	35	91.6	18.8	0.4981	126	90.6	14.6	99	90.5	16.9	0.9439
rs3783526	4	90.0	7.1	52	93.0	17.9	0.7420	126	90.6	14.6	101	90.5	16.7	0.9696
rs2856837	25	93.0	15.5	31	92.6	18.9	0.9189	107	89.5	15.6	120	91.5	15.5	0.3343
IL1B														
rs1143643	20	91.0	20.8	36	93.8	15.2	0.5640	134	90.8	15.9	93	90.2	15.1	0.7822
rs1143634	18	93.2	14.0	38	92.6	18.8	0.8985	83	89.9	15.0	142	91.0	15.7	0.5938
rs1143633	25	91.5	20.7	31	93.8	14.3	0.6133	134	90.8	15.7	92	90.0	15.3	0.6857
rs3136558	15	94.1	19.5	41	92.3	16.7	0.7286	78	89.7	15.8	149	91.0	15.4	0.5464
rs1143630	26	93.2	14.0	30	92.5	20.0	0.8815	28	90.9	17.4	199	90.5	15.3	0.9044
IL1RN														
rs3181052	14	88.9	15.5	42	94.1	17.9	0.3371	33	90.2	17.0	193	90.7	15.3	0.8562
rs1794066	33	90.8	15.4	23	95.7	19.7	0.3015	145	90.9	15.8	81	90.2	15.2	0.7561
rs1794067	29	96.2	16.8	27	89.1	17.4	0.1263	121	90.6	15.1	106	90.6	16.1	0.9983
rs2071459	15	87.6	15.7	41	94.7	17.7	0.1788	33	90.2	17.0	192	90.6	15.2	0.8656
rs432014	20	93.7	15.4	36	92.3	18.5	0.7643	121	90.6	15.1	106	90.6	16.1	0.9983
rs380092	50	92.7	18.1	6	93.5	8.8	0.9116	107	91.9	16.2	117	89.4	14.7	0.2250
rs452204	39	90.7	15.2	16	99.3	20.5	0.0933	145	90.6	15.7	82	90.5	15.3	0.9375
rs4252019	34	89.9	16.3	22	97.2	18.3	0.1219	36	89.8	17.5	191	90.7	15.2	0.7593
rs315955	15	93.6	18.6	40	92.9	17.0	0.8944							
rs315951	37	92.7	18.9	19	93.0	14.1	0.9469	95	90.8	16.8	130	90.0	14.4	0.6823
rs9005	22	90.8	15.2	34	94.1	18.7	0.4857	129	91.4	14.9	98	89.5	16.4	0.3445

Table A1.4 (continued)

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		Weight			Weight				Weight			Weight		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL6														
rs2069837	13	95.3	15.8	43	92.0	17.8	0.5592	31	93.8	17.4	196	90.1	15.2	0.2107
rs2069840	13	86.5	15.2	43	94.7	17.6	0.1347	127	90.1	14.7	99	91.4	16.6	0.5213
rs1554606	31	90.3	15.7	25	95.8	19.0	0.2379	158	91.7	15.9	69	88.0	14.4	0.0943
rs2069842	4	81.9	19.9	52	93.6	17.0	0.1945							
rs1548216	15	91.2	17.9	41	93.4	17.3	0.6855	8	83.9	16.9	219	90.8	15.5	0.2180
rs2069843	9	92.2	22.5	47	92.9	16.4	0.9069	8	83.9	16.9	219	90.8	15.5	0.2180
rs2069845	30	89.9	15.9	26	96.1	18.6	0.1894	154	91.8	15.9	69	88.0	14.4	0.0856
IL6R														
rs4845617	35	96.4	18.9	20	86.0	12.3	0.0319 *	143	91.4	15.9	84	89.2	14.9	0.2998
rs6427641	51	92.8	18.0	5	92.9	9.1	0.9917	154	91.5	15.6	73	88.7	15.4	0.1995
rs11265610	25	85.7	16.5	29	98.7	15.8	0.0047 *							
rs12083537	26	92.8	19.3	30	92.8	15.7	0.9907	80	93.3	15.5	147	89.1	15.4	0.0540
rs1386821	10	92.6	23.7	46	92.8	15.9	0.9759	74	93.1	16.0	153	89.3	15.2	0.0834
rs4075015	13	96.5	18.2	43	91.7	17.1	0.3876	152	89.9	15.7	75	91.9	15.1	0.3560
rs4601580	42	95.1	17.7	12	86.2	14.0	0.1164	143	89.3	15.0	81	92.7	16.4	0.1215
rs4845618	43	93.5	17.3	13	90.4	18.0	0.5784	160	89.7	15.2	67	92.7	16.3	0.1850
rs7549338	28	94.8	17.5	28	90.8	17.2	0.3845	155	89.4	15.2	72	93.0	16.1	0.1077
rs7518199	19	87.5	13.8	37	95.5	18.4	0.0997	156	91.4	15.8	71	88.8	14.9	0.2537
rs4553185	47	92.8	17.0	8	91.7	21.3	0.8712	159	89.6	15.1	68	92.8	16.4	0.1679
rs4393147	11	89.9	13.8	44	93.3	18.3	0.5606	156	91.4	15.8	71	88.8	14.9	0.2537
rs4537545	49	91.5	16.5	7	102.1	21.5	0.1308	156	91.1	15.6	71	89.4	15.4	0.4567
rs4845626	43	91.9	17.1	13	95.6	18.3	0.5137	70	92.4	14.9	157	89.8	15.8	0.2512
rs28730736	16	87.1	17.7	40	95.1	16.8	0.1179							
rs11265618	38	91.7	17.7	18	95.1	16.7	0.4881	71	92.2	14.9	156	89.8	15.8	0.2841
rs10159236	23	93.1	18.0	33	92.6	17.1	0.9111	67	91.9	15.1	160	90.0	15.7	0.4047
rs4329505	37	90.4	17.2	19	97.5	17.0	0.1440	66	92.0	15.2	161	90.0	15.7	0.3628
rs4509570	41	92.1	16.6	15	94.5	19.5	0.6549	98	90.5	16.1	129	90.6	15.1	0.9786
rs2229238	15	89.7	17.3	41	93.9	17.4	0.4212	80	90.5	16.2	147	90.6	15.2	0.9326
rs4072391	22	91.0	18.6	34	93.9	16.6	0.5382	80	90.5	16.2	147	90.6	15.2	0.9326
rs4379670	15	89.7	17.3	41	93.9	17.4	0.4212	80	90.5	16.2	147	90.6	15.2	0.9326

1. t-test with asterisk (*) to indicate $p<0.05$

Table A1.5 : Control subjects with and without a variant allele, number (N) and body mass index (BMI, kg/m^2, mean and standard deviation – SD), by locus and race

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		BMI			BMI				BMI			BMI		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL1A														
rs3783590	10	30.3	3.6	45	30.1	5.2	0.9065	1	26.2		226	29.1	4.4	
rs2856836	23	30.7	4.9	32	29.8	5.0	0.5030	107	28.8	4.2	120	29.4	4.5	0.3072
rs17561	23	30.7	4.9	32	29.8	5.0	0.5030	107	28.8	4.2	120	29.4	4.5	0.3072
rs20540	6	26.3	2.6	49	30.6	4.9	0.0412 *							
rs2856838	33	30.5	5.3	22	29.6	4.3	0.4932	141	29.3	4.5	86	28.8	4.2	0.3553
rs1609682	20	30.2	3.8	35	30.1	5.5	0.9530	126	29.1	4.2	99	29.2	4.6	0.8368
rs3783526	4	29.2	1.5	51	30.2	5.1	0.6958	126	29.0	4.2	101	29.2	4.6	0.7551
rs2856837	25	30.8	4.9	30	29.6	5.0	0.3936	107	28.8	4.2	120	29.4	4.5	0.3072
IL1B														
rs1143643	20	30.0	6.0	35	30.3	4.2	0.8401	134	29.2	4.4	93	29.1	4.3	0.8652
rs1143634	18	30.9	4.3	37	29.8	5.2	0.4229	83	28.9	4.0	142	29.3	4.6	0.5693
rs1143633	25	30.1	6.0	30	30.2	4.0	0.9457	134	29.1	4.4	92	29.0	4.3	0.9128
rs3136558	15	31.3	5.9	40	29.7	4.5	0.2808	78	28.9	4.2	149	29.2	4.5	0.5407
rs1143630	25	30.0	3.7	30	30.3	5.8	0.8157	28	29.5	5.2	199	29.1	4.3	0.6118
IL1RN														
rs3181052	14	28.9	4.8	41	30.6	4.9	0.2623	33	29.3	4.7	193	29.1	4.3	0.8418
rs1794066	33	29.7	4.8	22	30.9	5.1	0.3701	145	29.2	4.4	81	29.1	4.4	0.8307
rs1794067	28	30.8	4.9	27	29.5	5.0	0.3106	121	29.0	4.2	106	29.2	4.6	0.7889
rs2071459	15	28.6	4.7	40	30.7	4.9	0.1630	33	29.3	4.7	192	29.1	4.3	0.8245
rs432014	20	30.5	4.8	35	30.0	5.0	0.7458	121	29.0	4.2	106	29.2	4.6	0.7889
rs380092	49	30.0	5.1	6	31.4	3.1	0.5128	107	29.4	4.5	117	28.8	4.2	0.3010
rs452204	39	29.8	4.7	15	31.7	5.2	0.1976	145	29.1	4.3	82	29.2	4.5	0.9312
rs4252019	34	29.3	5.0	21	31.5	4.7	0.1144	36	29.0	4.8	191	29.1	4.3	0.8842
rs315955	14	30.1	4.5	40	30.4	5.0	0.8492							
rs315951	36	30.0	5.5	19	30.6	3.8	0.6647	95	29.2	4.6	130	29.0	4.2	0.7191
rs9005	22	29.7	4.7	33	30.5	5.1	0.5832	129	29.3	4.2	98	28.8	4.6	0.3774

Table A1.5 (continued)

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		BMI			BMI				BMI			BMI		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL6														
rs2069837	13	30.8	4.6	42	30.0	5.1	0.6158	31	29.7	4.8	196	29.0	4.3	0.4383
rs2069840	13	28.9	4.5	42	30.6	5.0	0.2832	127	29.0	4.1	99	29.4	4.7	0.4678
rs1554606	30	29.3	4.6	25	31.1	5.2	0.1774	158	29.4	4.5	69	28.4	4.0	0.1001
rs2069842	4	27.6	6.4	51	30.4	4.8	0.2857							
rs1548216	14	29.2	5.0	41	30.5	4.9	0.3920	8	26.2	4.0	219	29.2	4.4	0.0507
rs2069843	8	28.8	6.0	47	30.4	4.8	0.4068	8	26.2	4.0	219	29.2	4.4	0.0507
rs2069845	29	29.2	4.6	26	31.3	5.1	0.1169	154	29.4	4.5	69	28.4	4.0	0.1005
IL6R														
rs4845617	34	31.0	5.3	20	28.5	3.9	0.0725	143	29.4	4.5	84	28.6	4.2	0.1558
rs6427641	50	30.1	5.1	5	30.6	3.0	0.8358	154	29.5	4.3	73	28.4	4.4	0.0831
rs11265610	24	27.9	4.3	29	32.0	4.7	0.0016 *							
rs12083537	25	29.9	5.2	30	30.4	4.8	0.7482	80	29.8	4.2	147	28.7	4.4	0.0674
rs1386821	10	29.8	6.6	45	30.2	4.5	0.7903	74	29.8	4.3	153	28.8	4.4	0.1228
rs4075015	13	31.8	5.3	42	29.7	4.8	0.1754	152	29.0	4.4	75	29.4	4.3	0.4748
rs4601580	41	30.6	4.9	12	28.9	4.5	0.2734	143	28.8	4.2	81	29.7	4.7	0.1318
rs4845618	43	30.6	5.1	12	28.6	4.2	0.2210	160	28.8	4.3	67	29.8	4.5	0.1136
rs7549338	28	31.0	5.1	27	29.3	4.7	0.2092	155	28.8	4.3	72	29.9	4.5	0.0861
rs7518199	19	28.9	4.2	36	30.8	5.2	0.1845	156	29.3	4.4	71	28.7	4.3	0.3723
rs4553185	47	30.4	4.9	7	27.7	4.5	0.1764	159	28.8	4.3	68	29.9	4.6	0.0967
rs4393147	11	29.5	4.2	43	30.2	5.1	0.6640	156	29.3	4.4	71	28.7	4.3	0.3723
rs4537545	48	29.6	4.5	7	33.8	6.4	0.0373 *	156	29.2	4.4	71	28.9	4.4	0.6460
rs4845626	43	30.0	5.0	12	30.7	4.8	0.6568	70	29.7	4.2	157	28.9	4.5	0.1820
rs28730736	15	28.3	3.9	40	30.9	5.1	0.0879							
rs11265618	38	30.0	5.1	17	30.6	4.5	0.6689	71	29.6	4.2	156	28.9	4.5	0.2224
rs10159236	23	30.7	5.5	32	29.8	4.5	0.4830	67	29.6	4.3	160	28.9	4.4	0.2470
rs4329505	36	29.6	5.0	19	31.3	4.8	0.2358	66	29.7	4.3	161	28.9	4.4	0.2025
rs4509570	41	30.3	4.8	14	29.9	5.4	0.7874	98	28.9	4.6	129	29.3	4.2	0.4294
rs2229238	15	29.7	5.1	40	30.3	4.9	0.6799	80	28.8	4.7	147	29.3	4.2	0.3825
rs4072391	21	29.4	4.8	34	30.6	5.0	0.3790	80	28.8	4.7	147	29.3	4.2	0.3825
rs4379670	15	29.7	5.1	40	30.3	4.9	0.6799	80	28.8	4.7	147	29.3	4.2	0.3825

1. t-test with asterisk (*) to indicate $p<0.05$

Table A1.6 : Control subjects with and without a variant allele, number (N) and hip bone mineral density (BMD, gm/cm^2, mean and standard deviation – SD), by locus and race

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		BMD			BMD				BMD			BMD		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL1A														
rs3783590	11	1.08	0.10	48	1.08	0.17	0.9419	1	1.10		226	1.02	0.14	0.5773
rs2856836	23	1.08	0.18	36	1.08	0.15	0.9351	107	1.03	0.14	120	1.02	0.13	0.8505
rs17561	23	1.08	0.18	36	1.08	0.15	0.9351	107	1.03	0.14	120	1.02	0.13	0.8505
rs20540	6	1.03	0.13	53	1.09	0.16	0.4024							
rs2856838	36	1.09	0.16	23	1.06	0.17	0.5113	141	1.01	0.14	86	1.05	0.13	0.0136 *
rs1609682	22	1.10	0.18	37	1.07	0.15	0.4384	126	1.03	0.13	99	1.01	0.15	0.3014
rs3783526	4	1.20	0.11	55	1.07	0.16	0.1321	126	1.03	0.13	101	1.01	0.15	0.2970
rs2856837	26	1.08	0.17	33	1.08	0.15	0.9379	107	1.03	0.14	120	1.02	0.13	0.8505
IL1B														
rs1143643	22	1.10	0.18	37	1.07	0.15	0.5610	134	1.02	0.14	93	1.04	0.14	0.3550
rs1143634	18	1.06	0.16	41	1.09	0.16	0.4693	83	1.02	0.14	142	1.03	0.13	0.4790
rs1143633	27	1.07	0.18	32	1.08	0.15	0.8361	134	1.02	0.13	92	1.04	0.14	0.3290
rs3136558	16	1.05	0.14	43	1.09	0.17	0.4717	78	1.01	0.15	149	1.03	0.13	0.3962
rs1143630	28	1.10	0.15	31	1.06	0.17	0.4098	28	1.02	0.15	199	1.03	0.13	0.7290
IL1RN														
rs3181052	16	1.02	0.15	43	1.10	0.16	0.0837	33	1.04	0.13	193	1.02	0.14	0.4790
rs1794066	35	1.07	0.16	24	1.10	0.16	0.4314	145	1.02	0.14	81	1.02	0.14	0.9872
rs1794067	30	1.08	0.16	29	1.07	0.17	0.7826	121	1.02	0.14	106	1.03	0.14	0.9768
rs2071459	17	1.03	0.15	42	1.10	0.16	0.1395	33	1.04	0.13	192	1.02	0.14	0.4843
rs432014	21	1.07	0.18	38	1.09	0.15	0.6628	121	1.02	0.14	106	1.03	0.14	0.9768
rs380092	53	1.09	0.16	6	1.02	0.14	0.3155	107	1.03	0.14	117	1.02	0.14	0.9135
rs452204	41	1.07	0.15	17	1.11	0.19	0.3524	145	1.02	0.14	82	1.03	0.14	0.9251
rs4252019	36	1.07	0.14	23	1.10	0.19	0.5155	36	1.03	0.13	191	1.02	0.14	0.7817
rs315955	15	1.09	0.10	43	1.08	0.18	0.8121							
rs315951	38	1.09	0.18	21	1.05	0.12	0.3223	95	1.02	0.14	130	1.03	0.13	0.9878
rs9005	24	1.07	0.17	35	1.09	0.16	0.7122	129	1.03	0.13	98	1.02	0.14	0.7328

Table A1.6 (continued)

	Black							White						
	Controls with variant allele			Controls without variant allele				Controls with variant allele			Controls without variant allele			
		BMD			BMD				BMD			BMD		
Locus	N	Mean	SD	N	Mean	SD	p-value [1]	N	Mean	SD	N	Mean	SD	p-value [1]
IL6														
rs2069837	15	1.10	0.20	44	1.07	0.15	0.5069	31	1.04	0.14	196	1.02	0.14	0.5803
rs2069840	13	1.01	0.12	46	1.10	0.16	0.0667	127	1.02	0.13	99	1.03	0.14	0.3511
rs1554606	33	1.07	0.14	26	1.09	0.19	0.6230	158	1.04	0.13	69	1.00	0.14	0.0402 *
rs2069842	4	1.05	0.15	55	1.08	0.16	0.7135							
rs1548216	17	1.07	0.15	42	1.08	0.16	0.8302	8	1.03	0.12	219	1.02	0.14	0.9421
rs2069843	10	1.10	0.17	49	1.07	0.16	0.6049	8	1.03	0.12	219	1.02	0.14	0.9421
rs2069845	32	1.07	0.14	27	1.09	0.18	0.6557	154	1.04	0.14	69	1.00	0.14	0.0425 *
IL6R														
rs4845617	37	1.08	0.17	21	1.07	0.15	0.8154	143	1.03	0.14	84	1.01	0.13	0.2397
rs6427641	54	1.09	0.16	5	0.99	0.15	0.1883	154	1.03	0.14	73	1.01	0.13	0.1991
rs11265610	26	1.06	0.14	31	1.10	0.17	0.3645							
rs12083537	27	1.09	0.15	32	1.07	0.17	0.6697	80	1.04	0.14	147	1.02	0.13	0.3436
rs1386821	11	1.09	0.14	48	1.08	0.17	0.7487	74	1.03	0.14	153	1.02	0.13	0.5086
rs4075015	13	1.14	0.21	46	1.06	0.14	0.1494	152	1.02	0.13	75	1.04	0.14	0.1398
rs4601580	45	1.09	0.17	12	1.06	0.12	0.5690	143	1.02	0.14	81	1.03	0.13	0.8321
rs4845618	46	1.09	0.17	13	1.04	0.12	0.2916	160	1.01	0.13	67	1.07	0.14	0.0022 *
rs7549338	31	1.10	0.18	28	1.06	0.13	0.4050	155	1.01	0.13	72	1.07	0.13	0.0022 *
rs7518199	19	1.03	0.12	40	1.10	0.17	0.0949	156	1.03	0.14	71	1.01	0.14	0.4475
rs4553185	50	1.08	0.17	8	1.10	0.14	0.7580	159	1.01	0.13	68	1.07	0.14	0.0017 *
rs4393147	11	1.03	0.16	47	1.09	0.16	0.2534	156	1.03	0.14	71	1.01	0.14	0.4475
rs4537545	51	1.06	0.14	8	1.21	0.23	0.0114 *	156	1.03	0.14	71	1.02	0.14	0.9153
rs4845626	44	1.08	0.13	15	1.08	0.23	0.9450	70	1.05	0.14	157	1.01	0.14	0.0409 *
rs28730736	17	1.06	0.15	42	1.09	0.16	0.4819							
rs11265618	39	1.08	0.12	20	1.08	0.22	0.9086	71	1.05	0.13	156	1.01	0.14	0.0402 *
rs10159236	24	1.06	0.13	35	1.09	0.18	0.5247	67	1.05	0.14	160	1.01	0.13	0.0351 *
rs4329505	39	1.06	0.14	20	1.12	0.20	0.1697	66	1.05	0.14	161	1.01	0.13	0.0356 *
rs4509570	44	1.09	0.16	15	1.05	0.17	0.3564	98	1.01	0.14	129	1.04	0.13	0.0760
rs2229238	18	1.07	0.20	41	1.09	0.14	0.6752	80	1.00	0.14	147	1.04	0.13	0.1009
rs4072391	25	1.05	0.19	34	1.10	0.13	0.2153	80	1.00	0.14	147	1.04	0.13	0.1009
rs4379670	18	1.07	0.20	41	1.09	0.14	0.6752	80	1.00	0.14	147	1.04	0.13	0.1009

1. t-test with asterisk (*) to indicate $p<0.05$

BIBLIOGRAPHY

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
2. Albano JD WE, Jemal A, Anderson R et al. Cancer mortality in the United States by education level and race. J Natl Cancer Inst 2007;99:1384-94.
3. Du XL FS, Coker AL, et al. . Racial disparity and socioeconomic status in association with survival in older men with local/regional stage prostate carcinoma. Cancer 2006;106:1276-85.
4. Robbins AS YD, Parikh-Patel A. Differences in prognostic factors and survival among White and Black men with prostate cancer, California, 1995-2004. Am J Epidemiol 2007;166:71-8.
5. Balkwill F, Coussens LM. Cancer: an inflammatory link. Nature 2004;431:405-6.
6. Balkwill F MA. Inflammation and cancer: Back to Virchow? The Lancet 2001;357:539-45.
7. Modugno F, Ness RB, Chen C, Weiss NS. Inflammation and endometrial cancer: a hypothesis. Cancer Epidemiol Biomarkers Prev 2005;14:2840-7.
8. Macarthur M HG, and El-Omar EM. Inflammation and Cancer. Role of chronic inflammation and cytokine gene polymorhism in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol 2004;286:G515-20.
9. Michaud DS. Chronic inflammation and bladder cancer. Urol Oncol 2007;25:260-8.
10. Nelson WG, De Marzo AM, DeWeese TL, Isaacs WB. The role of inflammation in the pathogenesis of prostate cancer. J Urol 2004;172:S6-11; discussion S-2.
11. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer 2007;7:256-69.
12. Zheng SL LW, Wiklund F et al. . A Comprehensive Association Study for Genes in Inflammation Pathway Provides Support for Their Roles in Prostate Cancer Risk in the CAPS Study. The Prostate 2006;66:1556-64.
13. Palapattu GS, Sutcliffe S, Bastian PJ, et al. Prostate carcinogenesis and inflammation: emerging insights. Carcinogenesis 2005;26:1170-81.
14. Dennis LK LC, and Torner JC. Epidemiologic association between prostatitis and prostate cancer. Urology 2002;60:78-83.
15. Goldstraw MA, Fitzpatrick JM, Kirby RS. What is the role of inflammation in the pathogenesis of prostate cancer? BJU Int 2007;99:966-8.
16. McCarron SL ES, Evans PR et al. . Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002;62:3369-72.

17. Casey G NP, Plummer SJ, et al. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. Nat Genet 2002;32:581-3.
18. Carpten J NN, Isaacs S, et al. . Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet 2002;30:181-4.
19. Xu J ZS, Komiya A, et al. Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat Genet 2002;32:321-5.
20. Miller DC ZS, Dunn RL, et al. Germ-line mutations of the macrophage scavenger receptor 1 gene: association with prostate cancer risk in African-American men. Cancer Res 2003;63:3486-9.
21. Lindmark F, Zheng SL, Wiklund F, et al. Interleukin-1 receptor antagonist haplotype associated with prostate cancer risk. Br J Cancer 2005;93:493-7.
22. Culig Z. Interleukin-6 polymorphism: expression and pleiotropic regulation in human prostate cancer. J Urol 2005;174:417.
23. Voronov E, Shouval DS, Krelin Y, et al. IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci U S A 2003;100:2645-50.
24. Culig Z SH, Bartsch G, et al. Interleukin-6 regulation of prostate cancer cell growth. J Cell Biochem 2005;95:497-505.
25. Sivashanmugam P, Tang L, Daaka Y. Interleukin 6 mediates the lysophosphatidic acid-regulated cross-talk between stromal and epithelial prostate cancer cells. J Biol Chem 2004;279:21154-9.
26. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer 2004;90:2312-6.
27. Nakashima J TM, Horiguchi Y et al. Serum interleukin-6 as a prognostic factor in patients with prostate cancer
Clin Cancer Res 2000;6:2702-6.
28. Adler HL, McCurdy MA, Kattan MW, Timme TL, Scardino PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor-beta1 in patients with metastatic prostatic carcinoma. J Urol 1999;161:182-7.
29. Abrahamsen B, Bonnevie-Nielsen V, Ebbesen EN, Gram J, Beck-Nielsen H. Cytokines and bone loss in a 5-year longitudinal study--hormone replacement therapy suppresses serum soluble interleukin-6 receptor and increases interleukin-1-receptor antagonist: the Danish Osteoporosis Prevention Study. J Bone Miner Res 2000;15:1545-54.
30. Xiao P, Liu PY, Lu Y, et al. Association tests of interleukin-6 (IL-6) and type II tumor necrosis factor receptor (TNFR2) genes with bone mineral density in Caucasians using a re-sampling approach. Hum Genet 2005;117:340-8.
31. Manolagas S. Role of cytokines in bone resorption. Bone 1995;17:63S-7S.
32. Ishimi Y MC, Jin CH et al. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 1990;145:3297-303.
33. Fornari MC PS, Niveloni S, et al. Pre- and post-treatment serum levels of cytokine IL-1 beta, IL-6, and IL-1 receptor antagonist in celiac disease. Are they related to the associated osteopenia? Am J Gastroenterol 1998;93:413-8.
34. Scheidt-Nave C, Bismar H, Leidig-Bruckner G, et al. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab 2001;86:2032-42.

35. Lu Y, Xiao G, Galson DL, et al. PTHrP-induced MCP-1 production by human bone marrow endothelial cells and osteoblasts promotes osteoclast differentiation and prostate cancer cell proliferation and invasion in vitro. International journal of cancer 2007;121:724-33.
36. Olney R. Regulation of bone mass by growth hormone. Med Pediatr Oncol 2003;41:228-34.
37. Wang J ED, Veys EM, Verbruggen G. Insulin-like growth factor 1-induced interleukin-1 receptor II overides the activity of interleukin I and controls the homeostasis of the extracellular matrix of cartilage. Arthritis Rheum 2003;48:1281-91.
38. Lorentzon M, Lorentzon R, Nordstrom P. Interleukin-6 gene polymorphism is related to bone mineral density during and after puberty in healthy white males: a cross-sectional and longitudinal study. J Bone Miner Res 2000;15:1944-9.
39. Wolk A, Mantzoros CS, Andersson SO, et al. Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study. J Natl Cancer Inst 1998;90:911-5.
40. Platz EA LM, Rifai N et al. Sex steroid hormones and the androgen receptor gene CAG and subsequent risk of prostate cancer in the prostate specific antigen era. Cancer Epidemiol Biomarkers Prev 2005;14:1262-9.
41. Giovannucci E LY, Stampfer MJ, Willet WC. A prospective study of calcium intake and incident and fatal prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:203-10.
42. Rodriguez C MM, Mondul AM, et al. Calcium, dairy products, and risk of prostate cancer in a prospective cohort of United States men. Cancer Epidemiol Biomarkers Prev 2003;12:597-603.
43. Christakos S DP, Shen Q, et al. New insights into the mechanisms involved in the pleitropic actions of 1,25dihydroxyvitamin D3. Ann N Y Acad Sci 2006;1068:194-203.
44. Rukin NJ LC, Moo S, et al. Prostate cancer susceptibility is mediated by interactions between exposure to ultraviolet radiation and polymorphism in the 5' haplotype block of the vitamin D receptor gene. Cancer Lett 2007;247:328-35.
45. Akimoto S, Okumura A, Fuse H. Relationship between serum levels of interleukin-6, tumor necrosis factor-alpha and bone turnover markers in prostate cancer patients. Endocr J 1998;45:183-9.
46. Syrigos K, ed. Prostate Cancer: Biology, Diagnosis, and Management. New York: Oxford University Press; 2001.
47. Schottenfeld D FJ, JF, ed. Cancer Epemiology and Prevention. 3rd ed. New York: Oxford University Press, Inc; 2006.
48. McNeal JE. The zonal anatomy of the prostate. Prostate 1981;2:35-49.
49. McNeal JE. Normal and pathologic anatomy of prostate. Urology 1981;17:11-6.
50. Kirby RS BM. Prostate Cancer. Oxford: Health Press Limited
2004.
51. Montironi R, Mazzucchelli R, Pomante R. Preneoplastic lesions of the prostate. Adv Clin Path 1997;1:35-47.
52. Nemeth JA, Lee C. Prostatic ductal system in rats: regional variation in stromal organization. Prostate 1996;28:124-8.
53. van Leenders GJ, Gage WR, Hicks JL, et al. Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. Am J Pathol 2003;162:1529-37.
54. Jemal A SR, Ward E, Murray T, Xu J, Thun MJ. Cancer Statistics, 2007. CA: A Cancer Journal for Clinicians 2007;57:43-66.

55. Stanford JL, Ostrander EA. Familial prostate cancer. Epidemiol Rev 2001;23:19-23.
56. Whittemore AS, Wu AH, Kolonel LN, et al. Family history and prostate cancer risk in black, white, and Asian men in the United States and Canada. Am J Epidemiol 1995;141:732-40.
57. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78-85.
58. Imperato-McGinley J, Gautier T, Zirinsky K, et al. Prostate visualization studies in males homozygous and heterozygous for 5 alpha-reductase deficiency. J Clin Endocrinol Metab 1992;75:1022-6.
59. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. J Natl Cancer Inst 1996;88:1118-26.
60. Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. Cancer Res 2000;60:2132-5.
61. Hobisch A, Eder IE, Putz T, et al. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. Cancer Res 1998;58:4640-5.
62. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.
63. Groopman JD aKT. Role of metabolism and viruses in aflotoxin-induced liver cancer. Toxicol Appl Pharmacol 2005;206:131-7.
64. Condeelis J aPJ. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis
Cell 2006;124:263-6.
65. Lewis CE aPJ. Distinct role of macrophages in different tumor microenvironments Cancer Res 2006;66:605-12.
66. de Visser KE EA, Coussens LM. Paradoxical roles of the immune system during cancer development. Nature Rev Cancer 2006;6:24-37.
67. Ames BN CL, Willet WC. The cause and prevention of cancer Proc Natl Acad Sci U S A 1995;92:5258-65.
68. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 2004;4:11-22.
69. Langowski JL, Zhang X, Wu L, et al. IL-23 promotes tumour incidence and growth. Nature 2006;442:461-5.
70. Yoshizawa K WW, Morris SJ et al. Study of prediagnostic selenium in toenails and the risk of prostate cancer J Natl Cancer Inst 1998;90:1219.
71. Brooks JD ME, Chan DW et al. Plasma selenium level before diagnosis and the risk of prostate cancer development. J Urol 2001;166:2034.
72. Vogt TM ZR, Graubard BI et al. Serum selenium and risk of prostate cancer in U.S. blacks and whites. International journal of cancer 2003;103:664.
73. van den Brandt PA ZM, Bode P, and Goldbohm RA. Toenail selenium levels and subsequent risk of prostate cancer: a prospective cohort study. . Cancer Epidemiol Biomarkers Prev 2003;12:866.
74. Clark LC CGJ, Turnbull BW et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized control trial. Nutritional Prevention of Cancer Study Group. JAMA 1996;276:1957-63

75. Clark LC, Dalkin B, Krongrad A, et al. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br J Urol 1998;81:730-4.
76. Coussens LM, Tinkle CL, Hanahan D, Werb Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 2000;103:481-90.
77. Tricot GJ. New insights into role of microenvironment in multiple myeloma. Int J Hematol 2002;76 Suppl 1:334-6.
78. Daniels NA, Ewing SK, Zmuda JM, Wilt TJ, Bauer DC. Correlates and prevalence of prostatitis in a large community-based cohort of older men. Urology 2005;66:964-70.
79. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, et al. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci U S A 2000;97:734-9.
80. Hiatt RA AM, Klastyl AL, Sidney S. Alcohol consumption, smoking, and other risk factors and prostate cancer in a large health plan cohort in California (United States). Cance Causes Control 1994 5:66-72.
81. Nakata S IK, and Yamanaka H. Study of risk factors for prostatic cancer. Acta Urologica Japonica 1993;39:1017-24.
82. Wagenlehner FM, Elkahwaji JE, Algaba F, et al. The role of inflammation and infection in the pathogenesis of prostate carcinoma. BJU Int 2007;100:733-7.
83. Sarma AV, McLaughlin JC, Wallner LP, et al. Sexual behavior, sexually transmitted diseases and prostatitis: the risk of prostate cancer in black men. The Journal of urology 2006;176:1108-13.
84. Rosenblatt KA, Wicklund KG, Stanford JL. Sexual factors and the risk of prostate cancer. Am J Epidemiol 2001;153:1152-8.
85. Rosenblatt KA, Carter JJ, Iwasaki LM, Galloway DA, Stanford JL. Serologic evidence of human papillomavirus 16 and 18 infections and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2003;12:763-8.
86. Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC. Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci U S A 1992;89:3367-71.
87. Page WF, Braun MM, Partin AW, Caporaso N, Walsh P. Heredity and prostate cancer: a study of World War II veteran twins. Prostate 1997;33:240-5.
88. Gronberg H, Damber L, Damber JE. Studies of genetic factors in prostate cancer in a twin population. J Urol 1994;152:1484-7; discussion 7-9.
89. Hughes C, Murphy A, Martin C, Sheils O, O'Leary J. Molecular pathology of prostate cancer. J Clin Pathol 2005;58:673-84.
90. Nelson WG, De Marzo AM, Deweese TL, et al. Preneoplastic prostate lesions: an opportunity for prostate cancer prevention. Ann N Y Acad Sci 2001;952:135-44.
91. Habel LA, Zhao W, Stanford JL. Daily aspirin use and prostate cancer risk in a large, multiracial cohort in the US. Cancer Causes Control 2002;13:427-34.
92. Liu X, Plummer SJ, Nock NL, Casey G, Witte JS. Nonsteroidal antiinflammatory drugs and decreased risk of advanced prostate cancer: modification by lymphotoxin alpha. Am J Epidemiol 2006;164:984-9.
93. Leitzmann MF, Stampfer MJ, Ma J, et al. Aspirin use in relation to risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1108-11.

94. Jacobs EJ, Rodriguez C, Mondul AM, et al. A large cohort study of aspirin and other nonsteroidal anti-inflammatory drugs and prostate cancer incidence. J Natl Cancer Inst 2005;97:975-80.
95. Mahmud S, Franco E, Aprikian A. Prostate cancer and use of nonsteroidal anti-inflammatory drugs: systematic review and meta-analysis. Br J Cancer 2004;90:93-9.
96. Nelson JE, Harris RE. Inverse association of prostate cancer and non-steroidal anti-inflammatory drugs (NSAIDs): results of a case-control study. Oncol Rep 2000;7:169-70.
97. Krieger JN, Ross SO, Penson DF, Riley DE. Symptoms and inflammation in chronic prostatitis/chronic pelvic pain syndrome. Urology 2002;60:959-63.
98. Krieger JN, Nyberg L, Jr., Nickel JC. NIH consensus definition and classification of prostatitis. Jama 1999;282:236-7.
99. Mitsumori K, Terai A, Yamamoto S, Ishitoya S, Yoshida O. Virulence characteristics of Escherichia coli in acute bacterial prostatitis. J Infect Dis 1999;180:1378-81.
100. Skerk V, Schonwald S, Krhen I, et al. Aetiology of chronic prostatitis. Int J Antimicrob Agents 2002;19:471-4.
101. Krieger JN, Ross SO, Riley DE. Chronic prostatitis: epidemiology and role of infection. Urology 2002;60:8-12; discussion 3.
102. Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. BJU Int 1999;84:976-81.
103. Roberts RO, Lieber MM, Rhodes T, Girman CJ, Bostwick DG, Jacobsen SJ. Prevalence of a physician-assigned diagnosis of prostatitis: the Olmsted County Study of Urinary Symptoms and Health Status Among Men. Urology 1998;51:578-84.
104. Carver BS, Bozeman CB, Williams BJ, Venable DD. The prevalence of men with National Institutes of Health category IV prostatitis and association with serum prostate specific antigen. J Urol 2003;169:589-91.
105. Schaeffer AJ, Knauss JS, Landis JR, et al. Leukocyte and bacterial counts do not correlate with severity of symptoms in men with chronic prostatitis: the National Institutes of Health Chronic Prostatitis Cohort Study. J Urol 2002;168:1048-53.
106. True LD, Berger RE, Rothman I, Ross SO, Krieger JN. Prostate histopathology and the chronic prostatitis/chronic pelvic pain syndrome: a prospective biopsy study. J Urol 1999;162:2014-8.
107. Hayes RB, Pottern LM, Strickler H, et al. Sexual behaviour, STDs and risks for prostate cancer. Br J Cancer 2000;82:718-25.
108. Mandel JS, Schuman LM. Sexual factors and prostatic cancer: results from a case-control study. J Gerontol 1987;42:259-64.
109. Dillner J, Knekt P, Boman J, et al. Sero-epidemiological association between human-papillomavirus infection and risk of prostate cancer. International journal of cancer 1998;75:564-7.
110. Hisada M, Rabkin CS, Strickler HD, Wright WE, Christianson RE, van den Berg BJ. Human papillomavirus antibody and risk of prostate cancer. Jama 2000;283:340-1.
111. Sutcliffe S, Giovannucci E, Gaydos CA, et al. Plasma antibodies against Chlamydia trachomatis, human papillomavirus, and human herpesvirus type 8 in relation to prostate cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2007;16:1573-80.

112. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol 2003;170:998-1002.
113. Serth J, Panitz F, Paeslack U, Kuczyk MA, Jonas U. Increased levels of human papillomavirus type 16 DNA in a subset of prostate cancers. Cancer Res 1999;59:823-5.
114. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med 2003;349:366-81.
115. Lin X, Tascilar M, Lee WH, et al. GSTP1 CpG island hypermethylation is responsible for the absence of GSTP1 expression in human prostate cancer cells. Am J Pathol 2001;159:1815-26.
116. De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am J Pathol 1999;155:1985-92.
117. Zha S, Gage WR, Sauvageot J, et al. Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. Cancer Res 2001;61:8617-23.
118. Parsons JK, Nelson CP, Gage WR, Nelson WG, Kensler TW, De Marzo AM. GSTA1 expression in normal, preneoplastic, and neoplastic human prostate tissue. Prostate 2001;49:30-7.
119. Putzi MJ, De Marzo AM. Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. Urology 2000;56:828-32.
120. McNeal JE, Bostwick DG. Intraductal dysplasia: a premalignant lesion of the prostate. Hum Pathol 1986;17:64-71.
121. Nakayama M, Bennett CJ, Hicks JL, et al. Hypermethylation of the human glutathione S-transferase-pi gene (GSTP1) CpG island is present in a subset of proliferative inflammatory atrophy lesions but not in normal or hyperplastic epithelium of the prostate: a detailed study using laser-capture microdissection. Am J Pathol 2003;163:923-33.
122. Baffoe-Bonnie AB, Kittles RA, Gillanders E, et al. Genome-wide linkage of 77 families from the African American Hereditary Prostate Cancer Study (AAHPC). Prostate 2007;67:22-31.
123. Smith JR, Freije D, Carpten JD, et al. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 1996;274:1371-4.
124. Abate-Shen C, Shen MM. Molecular genetics of prostate cancer. Genes Dev 2000;14:2410-34.
125. Chin JL, Reiter RE. Genetic alterations in prostate cancer. Curr Urol Rep 2004;5:157-65.
126. De Marzo AM, DeWeese TL, Platz EA, et al. Pathological and molecular mechanisms of prostate carcinogenesis: implications for diagnosis, detection, prevention, and treatment. J Cell Biochem 2004;91:459-77.
127. Deutsch E, Maggiorella L, Eschwege P, Bourhis J, Soria JC, Abdulkarim B. Environmental, genetic, and molecular features of prostate cancer. Lancet Oncol 2004;5:303-13.
128. Silverman RH. Implications for RNase L in prostate cancer biology. Biochemistry 2003;42:1805-12.
129. Shea PR, Ishwad CS, Bunker CH, Patrick AL, Kuller LH, Ferrell RE. RNASEL and RNASEL-inhibitor variation and prostate cancer risk in Afro-Caribbeans. Prostate 2008;68:354-9.
130. Casey G, Neville PJ, Plummer SJ, et al. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. Nat Genet 2002;32:581-3.

131. Rokman A, Ikonen T, Seppala EH, et al. Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. Am J Hum Genet 2002;70:1299-304.
132. Urisman A, Molinaro RJ, Fischer N, et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog 2006;2:e25.
133. Zhou A, Paranjape J, Brown TL, et al. Interferon action and apoptosis are defective in mice devoid of 2',5'-oligoadenylate-dependent RNase L. EMBO J 1997;16:6355-63.
134. Platt N, Gordon S. Is the class A macrophage scavenger receptor (SR-A) multifunctional? - The mouse's tale. J Clin Invest 2001;108:649-54.
135. Suzuki H, Kurihara Y, Takeya M, et al. A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature 1997;386:292-6.
136. Thomas CA, Li Y, Kodama T, Suzuki H, Silverstein SC, El Khoury J. Protection from lethal gram-positive infection by macrophage scavenger receptor-dependent phagocytosis. J Exp Med 2000;191:147-56.
137. Kang D, Lee KM, Park SK, et al. Functional variant of manganese superoxide dismutase (SOD2 V16A) polymorphism is associated with prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer study. Cancer Epidemiol Biomarkers Prev 2007;16:1581-6.
138. Li N, Oberley TD, Oberley LW, Zhong W. Overexpression of manganese superoxide dismutase in DU145 human prostate carcinoma cells has multiple effects on cell phenotype. Prostate 1998;35:221-33.
139. Bostwick DG, Alexander EE, Singh R, et al. Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. Cancer 2000;89:123-34.
140. Woodson K, Tangrea JA, Lehman TA, et al. Manganese superoxide dismutase (MnSOD) polymorphism, alpha-tocopherol supplementation and prostate cancer risk in the alpha-tocopherol, beta-carotene cancer prevention study (Finland). Cancer Causes Control 2003;14:513-8.
141. Xu J, Zheng SL, Turner A, et al. Associations between hOGG1 sequence variants and prostate cancer susceptibility. Cancer Res 2002;62:2253-7.
142. Irani J, Ravery V, Pariente JL, et al. Effect of nonsteroidal anti-inflammatory agents and finasteride on prostate cancer risk. J Urol 2002;168:1985-8.
143. Perron L, Bairati I, Moore L, Meyer F. Dosage, duration and timing of nonsteroidal antiinflammatory drug use and risk of prostate cancer. International journal of cancer 2003;106:409-15.
144. Roberts RO, Jacobson DJ, Girman CJ, Rhodes T, Lieber MM, Jacobsen SJ. A population-based study of daily nonsteroidal anti-inflammatory drug use and prostate cancer. Mayo Clin Proc 2002;77:219-25.
145. Bowcock AM, Kidd JR, Lathrop GM, et al. The human "interferon-beta 2/hepatocyte stimulating factor/interleukin-6" gene: DNA polymorphism studies and localization to chromosome 7p21. Genomics 1988;3:8-16.
146. Kishimoto T. Interleukin-6 (IL-6). 4th ed. London: Elsevier Science Ltd; 2003.
147. Van Snick J. Interleukin-6: an overview. Annu Rev Immunol 1990;8:253-78.
148. Cayphas S, Van Damme J, Vink A, Simpson RJ, Billiau A, Van Snick J. Identification of an interleukin HP1-like plasmacytoma growth factor produced by L cells in response to viral infection. J Immunol 1987;139:2965-9.

149. Nordan RP, Potter M. A macrophage-derived factor required by plasmacytomas for survival and proliferation in vitro. Science 1986;233:566-9.
150. Shalaby MR, Waage A, Espevik T. Cytokine regulation of interleukin 6 production by human endothelial cells. Cell Immunol 1989;121:372-82.
151. Zhang Y, Lin JX, Vilcek J. Synthesis of interleukin 6 (interferon-beta 2/B cell stimulatory factor 2) in human fibroblasts is triggered by an increase in intracellular cyclic AMP. J Biol Chem 1988;263:6177-82.
152. Kohase M, May LT, Tamm I, Vilcek J, Sehgal PB. A cytokine network in human diploid fibroblasts: interactions of beta-interferons, tumor necrosis factor, platelet-derived growth factor, and interleukin-1. Mol Cell Biol 1987;7:273-80.
153. Sehgal PB, Walther Z, Tamm I. Rapid enhancement of beta 2-interferon/B-cell differentiation factor BSF-2 gene expression in human fibroblasts by diacylglycerols and the calcium ionophore A23187. Proc Natl Acad Sci U S A 1987;84:3663-7.
154. Corcoran NM, Costello AJ. Interleukin-6: minor player or starring role in the development of hormone-refractory prostate cancer? BJU Int 2003;91:545-53.
155. Lotz M, Jirik F, Kabouridis P, et al. B cell stimulating factor 2/interleukin 6 is a costimulant for human thymocytes and T lymphocytes. J Exp Med 1988;167:1253-8.
156. Takai Y, Wong GG, Clark SC, Burakoff SJ, Herrmann SH. B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. J Immunol 1988;140:508-12.
157. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990;265:621-36.
158. Wigmore SJ, Fearon KC, Ross JA, McNally SJ, Welch WJ, Garden OJ. Febrile-range temperature but not heat shock augments the acute phase response to interleukin-6 in human hepatoma cells. Am J Physiol Gastrointest Liver Physiol 2006;290:G903-11.
159. Karlsson JO, Yarmush ML, Toner M. Interaction between heat shock and interleukin 6 stimulation in the acute-phase response of human hepatoma (HepG2) cells. Hepatology 1998;28:994-1004.
160. Marinkovic S, Jahreis GP, Wong GG, Baumann H. IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo. J Immunol 1989;142:808-12.
161. Yamasaki K, Taga T, Hirata Y, et al. Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. Science 1988;241:825-8.
162. Taga T, Hibi M, Hirata Y, et al. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 1989;58:573-81.
163. Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. Blood 1995;86:1243-54.
164. Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol 1991;11:2057-65.
165. Qiu Y, Robinson D, Pretlow TG, Kung HJ. Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. Proc Natl Acad Sci U S A 1998;95:3644-9.
166. Hirano T, Taga T, Yasukawa K, et al. Human B-cell differentiation factor defined by an anti-peptide antibody and its possible role in autoantibody production. Proc Natl Acad Sci U S A 1987;84:228-31.

167. Belluco C, Olivieri F, Bonafe M, et al. -174 G>C polymorphism of interleukin 6 gene promoter affects interleukin 6 serum level in patients with colorectal cancer. Clin Cancer Res 2003;9:2173-6.
168. Vairaktaris E, Yiannopoulos A, Vylliotis A, et al. Strong association of interleukin-6 -174 G>C promoter polymorphism with increased risk of oral cancer. Int J Biol Markers 2006;21:246-50.
169. Nogueira de Souza NC, Brenna SM, Campos F, Syrjanen KJ, Baracat EC, Silva ID. Interleukin-6 polymorphisms and the risk of cervical cancer. Int J Gynecol Cancer 2006;16:1278-82.
170. Garg R, Wollan M, Galic V, et al. Common polymorphism in interleukin 6 influences survival of women with ovarian and peritoneal carcinoma. Gynecol Oncol 2006;103:793-6.
171. Cozen W, Gebregziabher M, Conti DV, et al. Interleukin-6-related genotypes, body mass index, and risk of multiple myeloma and plasmacytoma. Cancer Epidemiol Biomarkers Prev 2006;15:2285-91.
172. Siegall CB, Schwab G, Nordan RP, FitzGerald DJ, Pastan I. Expression of the interleukin 6 receptor and interleukin 6 in prostate carcinoma cells. Cancer Res 1990;50:7786-8.
173. Okamoto M, Lee C, Oyasu R. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells in vitro. Cancer Res 1997;57:141-6.
174. Chung TD, Yu JJ, Spiotto MT, Bartkowski M, Simons JW. Characterization of the role of IL-6 in the progression of prostate cancer. Prostate 1999;38:199-207.
175. Smith PC, Hobisch A, Lin DL, Culig Z, Keller ET. Interleukin-6 and prostate cancer progression. Cytokine Growth Factor Rev 2001;12:33-40.
176. Giri D, Ozen M, Ittmann M. Interleukin-6 is an autocrine growth factor in human prostate cancer. Am J Pathol 2001;159:2159-65.
177. Twillie DA, Eisenberger MA, Carducci MA, Hseih WS, Kim WY, Simons JW. Interleukin-6: a candidate mediator of human prostate cancer morbidity. Urology 1995;45:542-9.
178. Drachenberg DE, Elgamal AA, Rowbotham R, Peterson M, Murphy GP. Circulating levels of interleukin-6 in patients with hormone refractory prostate cancer. Prostate 1999;41:127-33.
179. Wise GJ, Marella VK, Talluri G, Shirazian D. Cytokine variations in patients with hormone treated prostate cancer. J Urol 2000;164:722-5.
180. Nakashima J, Tachibana M, Horiguchi Y, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res 2000;6:2702-6.
181. Shariat SF, Andrews B, Kattan MW, Kim J, Wheeler TM, Slawin KM. Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. Urology 2001;58:1008-15.
182. Michaud DS, Daugherty SE, Berndt SI, et al. Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. Cancer Res 2006;66:4525-30.
183. Sun J, Wiklund F, Hsu FC, et al. Interactions of sequence variants in interleukin-1 receptor-associated kinase4 and the toll-like receptor 6-1-10 gene cluster increase prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15:480-5.
184. Tan D, Wu X, Hou M, et al. Interleukin-6 polymorphism is associated with more aggressive prostate cancer. J Urol 2005;174:753-6.
185. Sun J, Hedelin M, Zheng SL, et al. Interleukin-6 sequence variants are not associated with prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2004;13:1677-9.

186. Elson GC, Graber P, Losberger C, et al. Cytokine-like factor-1, a novel soluble protein, shares homology with members of the cytokine type I receptor family. J Immunol 1998;161:1371-9.
187. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095-147.
188. Dinarello CA. Interleukin-1 Family (IL-1F1, F2). 4th ed. London: Elsevier Science Ltd; 2003.
189. Nicklin MJ, Weith A, Duff GW. A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. Genomics 1994;19:382-4.
190. Bazan JF, Timans JC, Kastelein RA. A newly defined interleukin-1? Nature 1996;379:591.
191. Thornberry NA, Bull HG, Calaycay JR, et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature 1992;356:768-74.
192. Bird TA, Gearing AJ, Saklatvala J. Murine interleukin-1 receptor: differences in binding properties between fibroblastic and thymoma cells and evidence for a two-chain receptor model. FEBS Lett 1987;225:21-6.
193. Heguy A, Baldari CT, Macchia G, Telford JL, Melli M. Amino acids conserved in interleukin-1 receptors (IL-1Rs) and the Drosophila toll protein are essential for IL-1R signal transduction. J Biol Chem 1992;267:2605-9.
194. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. J Biol Chem 1995;270:13757-65.
195. Colotta F, Re F, Muzio M, et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science 1993;261:472-5.
196. McMahan CJ, Slack JL, Mosley B, et al. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J 1991;10:2821-32.
197. Bristulf J, Gatti S, Malinowsky D, Bjork L, Sundgren AK, Bartfai T. Interleukin-1 stimulates the expression of type I and type II interleukin-1 receptors in the rat insulinoma cell line Rinm5F; sequencing a rat type II interleukin-1 receptor cDNA. Eur Cytokine Netw 1994;5:319-30.
198. Arend WP, Malyak M, Smith MF, Jr., et al. Binding of IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. J Immunol 1994;153:4766-74.
199. Lang D, Knop J, Wesche H, et al. The type II IL-1 receptor interacts with the IL-1 receptor accessory protein: a novel mechanism of regulation of IL-1 responsiveness. J Immunol 1998;161:6871-7.
200. Kopp E, Medzhitov R, Carothers J, et al. ECSIT is an evolutionarily conserved intermediate in the Toll/IL-1 signal transduction pathway. Genes Dev 1999;13:2059-71.
201. Fan XM, Wong BC, Lin MC, et al. Interleukin-1beta induces cyclo-oxygenase-2 expression in gastric cancer cells by the p38 and p44/42 mitogen-activated protein kinase signaling pathways. J Gastroenterol Hepatol 2001;16:1098-104.
202. Ricote M, Garcia-Tunon I, Bethencourt F, et al. The p38 transduction pathway in prostatic neoplasia. J Pathol 2006;208:401-7.

203. Ricote M, Garcia-Tunon I, Bethencourt FR, Fraile B, Paniagua R, Royuela M. Interleukin-1 (IL-1alpha and IL-1beta) and its receptors (IL-1RI, IL-1RII, and IL-1Ra) in prostate carcinoma. Cancer 2004;100:1388-96.
204. Giri D, Ittmann M. Interleukin-1alpha is a paracrine inducer of FGF7, a key epithelial growth factor in benign prostatic hyperplasia. Am J Pathol 2000;157:249-55.
205. Hoosein NM. Neuroendocrine and immune mediators in prostate cancer progression. Front Biosci 1998;3:D1274-9.
206. Xu J, Lowey J, Wiklund F, et al. The interaction of four genes in the inflammation pathway significantly predicts prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:2563-8.
207. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002;94:252-66.
208. Sandler RS, Halabi S, Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med 2003;348:883-90.
209. Gupta S, Adhami VM, Subbarayan M, et al. Suppression of prostate carcinogenesis by dietary supplementation of celecoxib in transgenic adenocarcinoma of the mouse prostate model. Cancer Res 2004;64:3334-43.
210. Krieg M, Weisser H, Tunn S. Potential activities of androgen metabolizing enzymes in human prostate. J Steroid Biochem Mol Biol 1995;53:395-400.
211. Farnsworth WE. Physiology of the prostate. New York: Oxford University Press; 2001.
212. Midzak AS, Liu J, Zirkin BR, Chen H. Effect of myxothiazol on Leydig cell steroidogenesis: inhibition of luteinizing hormone-mediated testosterone synthesis but stimulation of basal steroidogenesis. Endocrinology 2007;148:2583-90.
213. Pardridge WM. Serum bioavailability of sex steroid hormones. Clin Endocrinol Metab 1986;15:259-78.
214. Hsing AW, Reichardt JK, Stanczyk FZ. Hormones and prostate cancer: current perspectives and future directions. Prostate 2002;52:213-35.
215. Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell JD, Russell DW. Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. J Clin Invest 1993;92:903-10.
216. Andriole G, Bruchovsky N, Chung LW, et al. Dihydrotestosterone and the prostate: the scientific rationale for 5alpha-reductase inhibitors in the treatment of benign prostatic hyperplasia. J Urol 2004;172:1399-403.
217. Haas GP, Sakr WA. Epidemiology of prostate cancer. CA Cancer J Clin 1997;47:273-87.
218. Pienta KJ, Esper PS. Risk factors for prostate cancer. Ann Intern Med 1993;118:793-803.
219. Parsons JK, Carter HB, Platz EA, Wright EJ, Landis P, Metter EJ. Serum testosterone and the risk of prostate cancer: potential implications for testosterone therapy. Cancer Epidemiol Biomarkers Prev 2005;14:2257-60.
220. Kehinde EO, Akanji AO, Al-Hunayan A, et al. Do differences in age specific androgenic steroid hormone levels account for differing prostate cancer rates between Arabs and Caucasians? Int J Urol 2006;13:354-61.
221. Ross R, Bernstein L, Judd H, Hanisch R, Pike M, Henderson B. Serum testosterone levels in healthy young black and white men. J Natl Cancer Inst 1986;76:45-8.

222. Stattin P, Lumme S, Tenkanen L, et al. High levels of circulating testosterone are not associated with increased prostate cancer risk: a pooled prospective study. International journal of cancer 2004;108:418-24.
223. Wiren S, Stocks T, Rinaldi S, et al. Androgens and prostate cancer risk: a prospective study. Prostate 2007;67:1230-7.
224. Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. Cancer Res 1995;55:1937-40.
225. Giovannucci E, Stampfer MJ, Krithivas K, et al. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. Proc Natl Acad Sci U S A 1997;94:3320-3.
226. Brinkmann AO. Molecular basis of androgen insensitivity. Mol Cell Endocrinol 2001;179:105-9.
227. Brown TR. Androgen Action. Totpwa, NJ: Humana; 2003.
228. Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 1995;16:271-321.
229. Brown TR. Human androgen insensitivity syndrome. J Androl 1995;16:299-303.
230. Lubahn DB, Joseph DR, Sar M, et al. The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. Mol Endocrinol 1988;2:1265-75.
231. Choong CS, Wilson EM. Trinucleotide repeats in the human androgen receptor: a molecular basis for disease. J Mol Endocrinol 1998;21:235-57.
232. Ellerby LM, Hackam AS, Propp SS, et al. Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. J Neurochem 1999;72:185-95.
233. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991;352:77-9.
234. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12:241-53.
235. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 2000;92:2009-17.
236. Coetzee GA, Ross RK. Re: Prostate cancer and the androgen receptor. J Natl Cancer Inst 1994;86:872-3.
237. Hsing AW, Gao YT, Wu G, et al. Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. Cancer Res 2000;60:5111-6.
238. Russell DW, Wilson JD. Steroid 5 alpha-reductase: two genes/two enzymes. Annu Rev Biochem 1994;63:25-61.
239. Barista I. Epidemiolgy and etiology of prostate cancer. 2nd ed. New York: Oxford University Press; 2001.
240. Price P, Wass JA, Griffin JE, et al. High dose androgen therapy in male pseudohermaphroditism due to 5 alpha-reductase deficiency and disorders of the androgen receptor. J Clin Invest 1984;74:1496-508.

241. Reichardt JK, Makridakis N, Henderson BE, Yu MC, Pike MC, Ross RK. Genetic variability of the human SRD5A2 gene: implications for prostate cancer risk. Cancer Res 1995;55:3973-5.
242. Makridakis N, Ross RK, Pike MC, et al. A prevalent missense substitution that modulates activity of prostatic steroid 5alpha-reductase. Cancer Res 1997;57:1020-2.
243. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med 2003;349:215-24.
244. Stoner E. The clinical development of a 5 alpha-reductase inhibitor, finasteride. J Steroid Biochem Mol Biol 1990;37:375-8.
245. Laroque PA, Prahalada S, Gordon LR, et al. Effects of chronic oral administration of a selective 5 alpha-reductase inhibitor, finasteride, on the dog prostate. Prostate 1994;24:93-100.
246. Stattin P, Bylund A, Rinaldi S, et al. Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. J Natl Cancer Inst 2000;92:1910-7.
247. Dahiya R, Lee C, Haughney PC, Chui R, Ho R, Deng G. Differential gene expression of transforming growth factors alpha and beta, epidermal growth factor, keratinocyte growth factor, and their receptors in fetal and adult human prostatic tissues and cancer cell lines. Urology 1996;48:963-70.
248. Chan JM, Stampfer MJ, Giovannucci E, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science 1998;279:563-6.
249. Culig Z, Hobisch A, Cronauer MV, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 1994;54:5474-8.
250. Martini F. Bone Formation and Growth. In: Berriman L, ed. Fundamentals of Anatomy and Physiology. San Fransisco: Pearson Education Inc.; 2006.
251. Rauch F. Bone accrual in children: adding substance to surfaces. Pediatrics 2007;119 Suppl 2:S137-40.
252. Mora S, Gilsanz V. Establishment of peak bone mass. Endocrinol Metab Clin North Am 2003;32:39-63.
253. Hadjidakis DJ, Androulakis, II. Bone remodeling. Ann N Y Acad Sci 2006;1092:385-96.
254. Rosen CJ. Pathogenesis of osteoporosis. Baillieres Best Pract Res Clin Endocrinol Metab 2000;14:181-93.
255. Cummings SR, Bates D, Black DM. Clinical use of bone densitometry: scientific review. JAMA 2002;288:1889-97.
256. Li H, Cuartas E, Cui W, et al. IL-1 receptor-associated kinase M is a central regulator of osteoclast differentiation and activation. J Exp Med 2005;201:1169-77.
257. Moffett SP, Zmuda JM, Cauley JA, et al. Association of the G-174C variant in the interleukin-6 promoter region with bone loss and fracture risk in older women. J Bone Miner Res 2004;19:1612-8.
258. Kimble RB. Alcohol, cytokines, and estrogen in the control of bone remodeling. Alcohol Clin Exp Res 1997;21:385-91.
259. Faulkner KG. Update on bone density measurement. Rheum Dis Clin North Am 2001;27:81-99.

260. Versluis RG, Vismans FJ, van de Ven CM, Springer MP, Petri H. Radiographic absorptiometry of the phalanges as a screening instrument to detect osteoporosis of the hip. Acta Radiol 1999;40:418-21.
261. Bunker CH, Zmuda JM, Patrick AL, et al. High bone density is associated with prostate cancer in older Afro-Caribbean men: Tobago prostate survey. Cancer Causes Control 2006;17:1083-9.
262. Zhang Y, Kiel DP, Ellison RC, et al. Bone mass and the risk of prostate cancer: the Framingham Study. Am J Med 2002;113:734-9.
263. Nelson RL, Turyk M, Kim J, Persky V. Bone mineral density and the subsequent risk of cancer in the NHANES I follow-up cohort. BMC Cancer 2002;2:22.
264. Farhat GN, Taioli E, Cauley JA, et al. The association of bone mineral density with prostate cancer risk in the Osteoporotic Fractures in Men (MrOS) Study. Cancer Epidemiol Biomarkers Prev 2009;18:148-54.
265. Kim JG, Lim KS, Ku SY, Kim SH, Choi YM, Moon SY. Relations between interleukin-1, its receptor antagonist gene polymorphism, and bone mineral density in postmenopausal Korean women. J Bone Miner Metab 2006;24:53-7.
266. Keen RW, Woodford-Richens KL, Lanchbury JS, Spector TD. Allelic variation at the interleukin-1 receptor antagonist gene is associated with early postmenopausal bone loss at the spine. Bone 1998;23:367-71.
267. Ferrari SL, Ahn-Luong L, Garnero P, Humphries SE, Greenspan SL. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. J Clin Endocrinol Metab 2003;88:255-9.
268. Nemetz A, Toth M, Garcia-Gonzalez MA, et al. Allelic variation at the interleukin 1beta gene is associated with decreased bone mass in patients with inflammatory bowel diseases. Gut 2001;49:644-9.
269. Yamada Y, Ando F, Niino N, Shimokata H. Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. J Clin Endocrinol Metab 2003;88:3372-8.
270. James L, Onambele G, Woledge R, et al. IL-6-174G/C genotype is associated with the bone mineral density response to oestrogen replacement therapy in post-menopausal women. Eur J Appl Physiol 2004;92:227-30.
271. Ota N, Hunt SC, Nakajima T, et al. Linkage of interleukin 6 locus to human osteopenia by sibling pair analysis. Hum Genet 1999;105:253-7.
272. Birmann BM, Tamimi RM, Giovannucci E, et al. Insulin-like growth factor-1- and interleukin-6-related gene variation and risk of multiple myeloma. Cancer Epidemiol Biomarkers Prev 2009;18:282-8.
273. El-Omar EM, Rabkin CS, Gammon MD, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology 2003;124:1193-201.
274. Hoffmann SC, Stanley EM, Cox ED, et al. Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant 2002;2:560-7.
275. Lai CQ, Tucker KL, Choudhry S, et al. Population admixture associated with disease prevalence in the Boston Puerto Rican health study. Hum Genet 2009;125:199-209.

276. Freedman ML, Haiman CA, Patterson N, et al. Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. Proc Natl Acad Sci U S A 2006;103:14068-73.
277. Bidwell J, Keen L, Gallagher G, et al. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1999;1:3-19.
278. Hassan MI, Aschner Y, Manning CH, Xu J, Aschner JL. Racial differences in selected cytokine allelic and genotypic frequencies among healthy, pregnant women in North Carolina. Cytokine 2003;21:10-6.
279. Girnita DM, Webber SA, Ferrell R, et al. Disparate distribution of 16 candidate single nucleotide polymorphisms among racial and ethnic groups of pediatric heart transplant patients. Transplantation 2006;82:1774-80.
280. Bridges SL, Jr., Jenq G, Moran M, Kuffner T, Whitworth WC, McNicholl J. Single-nucleotide polymorphisms in tumor necrosis factor receptor genes: definition of novel haplotypes and racial/ethnic differences. Arthritis Rheum 2002;46:2045-50.
281. van den Velden PA, Reitsma PH. Amino acid dimorphism in IL1A is detectable by PCR amplification. Hum Mol Genet 1993;2:1753.
282. Ness RB, Haggerty CL, Harger G, Ferrell R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. Am J Epidemiol 2004;160:1033-8.
283. Pociot F, Molvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992;22:396-402.
284. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
285. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225-9.
286. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Height, body weight, and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev 1997;6:557-63.
287. Engeland A, Tretli S, Bjorge T. Height, body mass index, and prostate cancer: a follow-up of 950000 Norwegian men. Br J Cancer 2003;89:1237-42.
288. Zuccolo L, Harris R, Gunnell D, et al. Height and prostate cancer risk: a large nested case-control study (ProtecT) and meta-analysis. Cancer Epidemiol Biomarkers Prev 2008;17:2325-36.
289. Dorgan JF, Albanes D, Virtamo J, et al. Relationships of serum androgens and estrogens to prostate cancer risk: results from a prospective study in Finland. Cancer Epidemiol Biomarkers Prev 1998;7:1069-74.
290. Miller EA, Stanford JL, Hsu L, Noonan E, Ostrander EA. Polymorphic repeats in the androgen receptor gene in high-risk sibships. Prostate 2001;48:200-5.
291. Janne OA, Palvimo JJ, Kallio P, Mehto M. Androgen receptor and mechanism of androgen action. Ann Med 1993;25:83-9.
292. Bennett CL, Price DK, Kim S, et al. Racial variation in CAG repeat lengths within the androgen receptor gene among prostate cancer patients of lower socioeconomic status. J Clin Oncol 2002;20:3599-604.
293. Sartor O, Zheng Q, Eastham JA. Androgen receptor gene CAG repeat length varies in a race-specific fashion in men without prostate cancer. Urology 1999;53:378-80.

294. Compston JE. Sex steroids and bone. Physiol Rev 2001;81:419-47.
295. Vanderschueren D, Bouillon R. Androgens and bone. Calcif Tissue Int 1995;56:341-6.
296. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest 2006;116:1186-94.
297. Mellstrom D, Johnell O, Ljunggren O, et al. Free testosterone is an independent predictor of BMD and prevalent fractures in elderly men: MrOS Sweden. J Bone Miner Res 2006;21:529-35.
298. Chen Z, Arendell L, Aickin M, Cauley J, Lewis CE, Chlebowski R. Hip bone density predicts breast cancer risk independently of Gail score: results from the Women's Health Initiative. Cancer 2008;113:907-15.
299. Cauley JA, Lucas FL, Kuller LH, Vogt MT, Browner WS, Cummings SR. Bone mineral density and risk of breast cancer in older women: the study of osteoporotic fractures. Study of Osteoporotic Fractures Research Group. JAMA 1996;276:1404-8.
300. Douchi T, Yamamoto S, Nakamura S, Oki T, Maruta K, Nagata Y. Bone mineral density in postmenopausal women with endometrial cancer. Maturitas 1999;31:165-70.
301. McGlynn KA, Gridley G, Mellemkjaer L, et al. Risks of cancer among a cohort of 23,935 men and women with osteoporosis. International journal of cancer 2008;122:1879-84.
302. Kubricht WS, 3rd, Williams BJ, Whatley T, Pinckard P, Eastham JA. Serum testosterone levels in African-American and white men undergoing prostate biopsy. Urology 1999;54:1035-8.
303. Svartberg J, Agledahl I, Figenschau Y, Sildnes T, Waterloo K, Jorde R. Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip. International journal of impotence research 2008;20:378-87.
304. Lou YR, Qiao S, Talonpoika R, Syvala H, Tuohimaa P. The role of Vitamin D3 metabolism in prostate cancer. J Steroid Biochem Mol Biol 2004;92:317-25.
305. Hannan MT, Litman HJ, Araujo AB, et al. Serum 25-hydroxyvitamin D and bone mineral density in a racially and ethnically diverse group of men. J Clin Endocrinol Metab 2008;93:40-6.
306. Pilz S, Dobnig H, Nijpels G, et al. Vitamin D and mortality in older men and women. Clin Endocrinol (Oxf) 2009.
307. Cashman KD, Wallace JM, Horigan G, et al. Estimation of the dietary requirement for vitamin D in free-living adults >=64 y of age. Am J Clin Nutr 2009.
308. Leder BZ, Araujo AB, Travison TG, McKinlay JB. Racial and ethnic differences in bone turnover markers in men. J Clin Endocrinol Metab 2007;92:3453-7.
309. Bid HK, Manchanda PK, Mittal RD. Association of interleukin-1Ra gene polymorphism in patients with bladder cancer: case control study from North India. Urology 2006;67:1099-104.
310. Konwar R, Gara R, Singh M, Singh V, Chattopadhyay N, Bid HK. Association of interleukin-4 and interleukin-1 receptor antagonist gene polymorphisms and risk of benign prostatic hyperplasia. Urology 2008;71:868-72.
311. Sehouli J, Mustea A, Konsgen D, Katsares I, Lichtenegger W. Polymorphism of IL-1 receptor antagonist gene: role in cancer. Anticancer Res 2002;22:3421-4.