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THE IMPACT OF INFLAMMATORY AND IMMUNE RESPONSE-RELATED
GENETIC VARIANTS ON PROSTATE CANCER RISK AMONG MEN OF
AFRICAN DESCENT

By

Dominique Zilpha Jones
B.A., Smith College, 2010

A Thesis
Submitted to the Faculty of the
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University of Louisville
Louisville, Kentucky

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DEDICATION

This thesis is dedicated to my beloved mother, Annette Jones, my godmother, Ernestine

Hill, my family and friends

who are my source of inspiration for my interest in cancer research.

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I would like to thank my mentor, Dr. LaCreis Kidd, for her guidance throughout my academic career and research at the University of Louisville. I would also like to thank the other members of my thesis committee, Dr. Geoffrey Clark, Dr. Leila Gobeshjivili, Dr. Shirish Barve, Dr. Carolyn Klinge, and Dr. Guy Brock, for their guidance, encouragement and assistance over the past years. I would like to thank my wonderful fiancée, Joseph Reed, for his encouragement, understanding and patience with me during my graduate program. I also would like to thank my family in New Jersey (Thomas Jones, Sakina Jones), North Carolina (Paula Borden, Peggy and Lovest Alexander, Dion Hill) and friends for all of their support as well.

ABSTRACT

THE IMPACT OF INFLAMMATORY AND IMMUNE RESPONSE-RELATED GENETIC VARIANTS ON PROSTATE CANCER RISK AMONG MEN OF AFRICAN DESCENT

Dominique Zilpha Jones

October 16, 2014

Background: In the U.S., prostate cancer (PCA) metastasis is associated with a 5-yr survival rate of 29% and disproportionately affects men of African descent. Single nucleotide polymorphisms (SNPs) may serve as genetic markers to identify individuals at high risk of developing PCA.

Methods: We examined main and joint effects of 119 inflammatory and immune response-associated SNPs among men of African descent (535 controls, 279 cases) via logistic regression (LR) and entropy-based-multi-factor dimensionality reduction (MDR) modeling.

Results: MDR yielded highly synergistic [(*TLR10*, *TLR6* and *IRF3*), (*TLR2*, *IRAK4*), (*IL1R2*, *IL10*, *IL10RA*)] interaction models as the best predictors of PCA risk based upon informative information gain scores ($p \leq 0.003$). Interestingly, *CCL5* [*rs2107538* (AA, GA+AA), *rs3817655* (GA, AA)] SNPs were strongly associated with a 43-56% decrease in PCA risk.

Conclusion: *CCL5* (*rs2107538*, *rs3817655*) reduces PCA risk by > 50% and may serve as potential targets of PCA treatment among men of African descent.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGMENTS.....	iv
ABSTRACT.....	v
LIST OF TABLES.....	vii-ix
LIST OF FIGURES.....	x
CHAPTER 1: Introduction, Hypothesis, Specific Aims.....	1-53
CHAPTER 2: Methodology	54-78
CHAPTER 3: Toll-like Receptor Signaling	79-99
Results.....	80-92
Discussion.....	93-99
CHAPTER 4: Chemokine Signaling.....	100-122
Results.....	101-114
Discussion.....	115-122
CHAPTER 5: Cytokine Signaling	123-141
Results.....	124-136
Discussion.....	137-141
CHAPTER 6: Summary, Conclusion.....	142-146
REFERENCES.....	147-174
CURRICULUM VITAE.....	175-183

LIST OF TABLES

TABLE	PAGE
1. Table 1. Inflammatory and Immune related gene-gene interactions in Prostate cancer.....	41
2. Table 2. Current Inflammatory and Immune Response Gene Selective Drugs in Clinical Trials.....	49
3. Table 3. Study population characteristics among men of African descent from the U.S. and Jamaica.....	55
4. Table 4. Baseline Characteristics among men of African Descent from the U.S.....	56
5. Table 5. Baseline Characteristics among men from Jamaica.....	57
6. Table 6. Functional consequence of TLR-associated sequence variants.....	60-62
7. Table 7. Functional Consequence of Chemokine-Associated Sequence Variants.....	63-65
8. Table 8. Functional Consequence of Inflammatory-Associated Sequence Variants.....	66-71
9. Table 9a. Relationship between TLR-associated SNPS and PCA among men of African descent.....	83
10. Table 9b. Relationship between TLR-associated SNPs and PCA risk stratified by racial ethnic group	84

11. Table 10a. Interactions and main effects of TLR-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. and Jamaican men of African Descent.....	87
12. Table 10b. Interactions and main effects of TLR-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. men of African Descent.....	89
13. Table 10c. Interactions and main effects of TLR-associated SNPs as predictors of PCA using age-adjusted MDR among Jamaican men of African Descent.....	91
14. Table 11a. Relationship between Chemokine-associated SNPs and PCA Risk among men of African Descent.....	103
15. Table 11b. Relationship between Chemokine-associated SNPs and PCA stratified by racial ethnic group.....	106-107
16. Table 12a. Interactions and main effects of Chemokine-associated SNPs as predictors of PCA using age-adjusted MDR among men of African Descent.....	110
17. Table 12b. Interactions and main effects of Chemokine-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. men of African Descent.....	112
18. Table 12c. Interactions and main effects of Chemokine-associated SNPs as predictors of PCA using age-adjusted MDR among Jamaican men of African Descent.....	113

19. Table 13a. Relationship between Inflammatory-associated SNPs and PCA Risk among men of African Descent.....	126-127
20. Table 13b. Relationship between Inflammatory-associated SNPs and PCA Risk stratified by racial ethnic group.....	129-130
21. Table 14a. Interactions and main effects of Inflammatory-associated SNPs as predictors of PCA using age-adjusted MDR among men of African Descent.....	133
22. Table 14b. Interactions and main effects of Inflammatory-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. men of African Descent.....	135
23. Table 14c. Interactions and main effects of Inflammatory-associated SNPs as predictors of PCA using age-adjusted MDR among Jamaican men of African Descent.....	136

LIST OF FIGURES

FIGURE	PAGE
1. Figure 1. Progression of prostatic inflammation to Prostate Cancer.....	4
2. Figure 2a. Acute Inflammation in Prostate Tumorigenesis	10
3. Figure 2b. Chronic Inflammation in Prostate Tumorigenesis	11
4. Figure 3. Toll-like receptor signaling in innate immunity.....	14
5. Figure 4. The effect of IL1R2 on immune cells and Prostate Cancer.....	36
6. Figure 5. Graphical model of gene-gene interactions.....	76
7. Figure 6. Entropy graph of gene-gene interactions.....	78
8. Figure 7. Entropy graph of TLR gene-gene interactions among U.S. and Jamaican men of African Descent.....	88
9. Figure 8. Entropy graph of TLR gene-gene interactions among U.S. men of African Descent.....	90
10. Figure 9. Entropy graph of TLR gene-gene interactions among Jamaican men of African Descent.....	92
11. Figure 10. Entropy graph of Chemokine gene-gene interactions among U.S. and Jamaican men of African Descent.....	111
12. Figure 11. Entropy graph of Chemokine gene-gene interactions among Jamaican men of African Descent.....	114
13. Figure 12. Entropy graph of Cytokine gene-gene interactions among U.S. and Jamaican men of African Descent.....	134

CHAPTER 1

INTRODUCTION

Prostate cancer as a public health problem

Prostate cancer (PCA) is a slow growing disease and one of the leading causes of cancer-related deaths among men in the U.S. and Caribbean (e.g., Jamaica). In the U.S., 1 in 6 men will develop prostate cancer in their lifetime [1]. When the disease is detected at early and treatable stages, there is a 100% 5-year survival rate among U.S. men diagnosed with localized or regional disease [2]. However, the five-year survival rates drop drastically to 29% among U.S. men diagnosed with metastatic PCA. Among prostate cancer patients with metastatic disease, 90% and 65% of the cancer spreads to the bone and liver, respectively [3]. Metastatic PCA is aggressive and becomes non-responsive to conventional treatments such as hormonal therapy [4, 5]. Among PCA patients who undergo a radical prostatectomy, 20-40% of them will experience recurrent disease [6, 7]. Although, the 5-year survival rates for regional or distant prostate cancer among Jamaican men is unknown, the 74% five-year biochemical recurrence disease free survival rate for Jamaican men with clinically organ-confined prostate cancer (tumor stage 1-2) is comparable to rates found among African-American men [8]. In addition to poor disease prognosis attributed to detection of the disease at later stages and delays in treatment, PCA is associated with a great economic burden in the United States. National

expenditure for prostate cancer care was approximately \$11.9 billion [9], according to the 2010 U.S. National Cancer Institute (NCI) Cancer Trends Progress Report. After diagnosis, the average medical cost for U.S. patients was \$48,808, which included a direct cost of \$29,417 for prostate cancer related procedures. Medical expenses for PCA patients with Medicare insurance were slightly lower than patients on other types of insurance within the U.S. Over a 4-year follow-up period, the average medical cost was \$18,168 per prostate cancer patient on Medicare [10]. According to the PharMetrics database, patients who experienced watchful waiting care incurred a cost of \$7,595; whereas, those who underwent treatment had a direct cost of \$38,745[11, 12]. In particular, treatment options for prostate cancer patients 40 years or older between years 2000 and 2005 incurred the following cost: \$17,753 for chemotherapy, \$29,984 for hormone therapy, \$31,666 for surgery, and \$42,554 for radiation therapy [11, 12].

Determinants of Prostate Cancer

Although the pathogenesis of prostate cancer requires further elucidation, there are a number of established and plausible PCA risk factors. Established risk factors for prostate tumorigenesis, include age, family history of prostate cancer and race [1, 13]. Naturally, as men become older their risk for PCA increases as well. Race is a major determinant of prostate cancer as detailed in the “**Prostate Cancer Health Disparities**” section below.

Plausible risk factors include: hormonal imbalances (e.g., androgen, testosterone); a sedentary lifestyle (e.g., obesity); environmental exposures (e.g., intake of red meat, high-fat dairy products, polyunsaturated fats); exposure to meats-derived carcinogens detected in meats prepared well done via grilling or pan-frying [14-18]; the inheritance of genetic polymorphisms within inflammatory and immune response (e.g., toll-like

receptor, cytokine, chemokine); chronic inflammation [14, 19, 20]. In terms of chronic inflammation, it is estimated that approximately 20% of all human cancers, including prostate cancer (PCA), are associated with chronic inflammation [1, 21, 22]. In fact, epidemiological studies revealed a higher prostate cancer susceptibility among patients with a history of prostatic inflammation compared to those without a history [23, 24].

Chronic inflammation is known to contribute to the various disease states leading to prostate cancer, including prostatic inflammation, proliferative inflammatory atrophy and prostatic intraepithelial neoplasia, as depicted in **Figure 2**. Prostatic inflammation may occur as the result of infection by microbial pathogens, chemical irritation caused by urine reflux, hormone imbalance, diet or environmental exposures, genetic alterations, age and autoimmunity.

Prostatic inflammation is separated into four categories: (1) acute bacterial prostatitis; (2) chronic bacterial prostatitis; (3) chronic pelvic pain syndrome; and (4) asymptomatic inflammatory prostatitis [14]. Both acute and chronic bacterial prostatitis are caused by a bacterial infection of the prostate from *Escherichia coli*, *Enterococcus* spp. (species) or sexually transmitted infections (STIs). Symptoms of bacterial prostatitis include an increase in urination, pain in the genital area, fever, chills, and burning during urination [25]. Although, the cause of chronic pelvic pain syndrome is unknown, it is categorized as either non-inflammatory or inflammatory based on the absence or presence of white blood cells in prostatic fluid [26]. Asymptomatic inflammatory prostatitis is diagnosed based on the presence of white blood cells in prostatic tissue [26].

Figure 1. Progression of prostatic inflammation to prostate cancer. Chronic inflammation of the prostate may result from bacterial infection, age, hormonal imbalance, genetic alterations or environmental exposures (e.g., carcinogenic agents, diet). During inflammation, immune cells (e.g., mast cells, macrophages) travel to the site of inflammation and secrete cytokines and chemokines to recruit more immune cells. The accumulation of immune cells may lead to the formation of PIA lesions. PIA lesions may transition into PIN, which is characterized by epithelial cells with large nucleoli. High grade PIN occurs in 20-30% of prostate cancer and is regarded as a possible precursor to prostate carcinoma.

Prostate Cancer Health Disparities

Men of African descent suffer disproportionately from PCA. African-American and Caribbean men have the highest prostate cancer incidence and mortality rates compared to their Caucasian counterparts [1, 8]. African-American men are 1.6 times more likely to receive a prostate cancer diagnosis and 2.4 times more likely to succumb to this disease than their European-American counterparts [27]. Similarly, Jamaican men have a high incidence rate of prostate cancer in comparison to European-American men [1, 13, 27, 28]. In a prostate cancer survey with more than 3,000 men on the island of Tobago in the Caribbean (i.e., ~60% of male population), the prevalence of prostate cancer among men over 59 years of age was 3 times higher than European-American men [29]. In a recent report on global patterns of prostate cancer, Rebbeck and co-workers (2013) observed that African-Americans had the highest incidence rate according to the age-standardized estimates for prostate cancer compared to Caribbean and African men [30]. However, the higher PCA mortality rates observed among Caribbean men relative to African-American men may be partially attributed to limited access to early PCA detection screening protocols as well as availability of medical care and treatment. Likewise, men from African countries such as South Africa and Senegal had the largest percentage of cancer with stage T3/T4 tumors, due to limited access to healthcare and treatment [30, 31]. Due to the limited availability of cancer statistics for African and Caribbean countries and misclassification of racial/ethnic background, some studies may not accurately depict the PCA incidence and mortality rates among men of African and Caribbean descent.

Differences in prostate cancer screening behavior, family history, access to health care, and cancer treatment regimens cannot fully explain the tumor burden that men of African descent suffer. Some studies speculate biological differences may help to explain the higher PCA incidence among African-American men relative to European Americans. In a retrospective study, Eastham and colleagues (1998) observed that African-American men had significantly higher levels of serum PSA (p-value = 0.00003), PSA densities (p-value = 0.000009) and inflammation in the prostate than European-American men [32]. Additionally, Winters and co-workers (1998) speculated higher levels of total testosterone and sex hormone binding globulin (SHBG) among young African-American men may predispose them to a higher risk of developing PCA than their Caucasian counterparts [33]. This increased risk may be attributed to enhanced cellular division in the prostate epithelium [33]. Moreover, increased apoptotic blockage via Bcl-2 expression, coupled with enhanced cellular proliferation may contribute to increased tumor volume and burden among African-American men relative to Caucasians. In fact, deVere and colleagues (1998) showed a significant relationship between Bcl-2 immunopositivity and high proliferation among African American paraffin-embedded prostatic tumor tissue (p-value= 0.012) but not European-Americans [34].

Our lab and other researchers have speculated that prostate cancer health disparities may be partially attributed to dysregulation within important biological pathways involved in inflammatory and immune response signaling [35-40]. The inflammatory and immune-response signaling pathway is triggered by the detection of pathogens or damaged cells (e.g., tumor cells) via toll-like receptors (TLRs).

Consequently, production of cytokines and chemokines from immune cells via TLRs occur to recruit more immune cells to the site of infection or tissue damage. In fact, Wallace and colleagues (2008) observed significant differences in the mRNA expression of chemokine-related genes in prostate tumor of African- and European-American men compared to surrounding non-tumor prostate tissue [41]. For instance, the mRNA expression of *CXCR4*, *CCR7*, and *CCL5* were higher in prostate tumors collected from African-Americans compared to European-American men. Moreover, Reams and associates (2009) showed the gene expressions of *CXCL2* and *IRF4* were statistically under-expressed by 4-fold in African-American prostate tumor tissue compared to European-American tumors normalized to snap frozen non-tumor matched controls (p-value ≤ 0.05) [42]. The differences shown in gene expression profiles between African- and European-American men may lead to an explanation for the PCA health disparity observed among men of African descent.

Role of Inflammation and immune response in prostate cancer

Prostate tumorigenesis is largely driven by recurrent acute or chronic inflammation. During acute/chronic inflammation, inflammatory cells (e.g., mast cells, polymorphonuclear leukocytes, macrophages) produce pro- and anti-inflammatory cytokines and chemokines to launch an inflammatory response to repair tissue injury, remove pathogens and fight against infectious agents or foreign cells. Chemokines, function as chemoattractants that send signals to prostate cancer cells to migrate and metastasize to other organs in the body. In particular, the inflammatory response driven by recurrent acute or chronic inflammation can target malignant or tumor cells as emphasized in **Figure 2a and 2b**. Chronic inflammation results from an imbalance in the

regulation of pro- and anti-inflammatory signals. This imbalance may favor pro-inflammatory signaling to trigger chronic inflammation of the prostate and ultimately lead to PCA development [43].

Acute inflammation is the immediate response of the immune system triggered by the movement of plasma and leukocytes from blood to the site of inflammation to help clear pathogens in the body, as depicted in **Figure 2a**. Second, chronic inflammation is a state of prolonged inflammation where active inflammation, tissue injury and repair occur simultaneously, as depicted in **Figure 2b**. Following exposure to viral or chemical insults to normal prostate epithelium, viral or chemical carcinogens may induce somatic changes in normal prostate cells that introduce genomic alterations such as deletions mutations, and rearrangements of oncogenes and tumor suppressor genes. In response to these DNA changes, cellular death and repair mechanisms are initiated to fix damaged cells in DNA replication and cellular proliferation. These damaged cells may produce cytokines and chemokines to recruit inflammatory cells for their destruction leading to the onset of acute inflammation. Inflammatory cells serve as the frontline of defense for the immune system during inflammation and the release of factors (e.g., VEGF) that enhance the angiogenic phenotype of tumors. During acute inflammation, inflammatory cells such as tumor associated macrophages (TAMs) release reactive oxygen and nitrogen species that induce oxidative DNA damage. Additionally, mast cells, neutrophils and leukocytes release cytokines and chemokines to recruit more inflammatory cells to the inflamed prostatic tissue (**Figure 2a**). In some cells, the damage may exceed the capacity of the cellular repair mechanism and these cells transition into permanently damaged cells with uncontrolled DNA replication and cellular proliferation otherwise known as tumor cells.

Tumor cells also produce cytokines and chemokines that attract inflammatory cells to their location. Inflammatory cells attempt to infiltrate inflamed prostatic tissue to destroy the tumor cells. Immature dendritic cells become activated and mature by engulfing antigens from the tumor cells. Mature dendritic cells travel to the lymph nodes to present tumor antigens and recruit lymphocytes to the inflamed prostatic tissue. Macrophages and lymphocytes express macrophage inhibitory factor (MIF), which is a potent cytokine that suppresses the transcriptional activity of p53. Persistent tissue damage and regeneration via reactive nitrogen and oxygen species released from inflammatory cells as well as the growing number of damaged cells leads to chronic inflammation.

During chronic inflammation, macrophages and lymphocytes such as T cells, B cells and natural killer (NK) cells are recruited to infiltrate inflamed prostatic tissue (**Figure 2b**). Moreover, chronic inflammation may lead to the accumulation of oncogenic mutations and promote prostate carcinoma. Unlike acute inflammation, chronic inflammation contributes to the development of an estimated 20% of adult cancers via bacterial infections, chronic non-infectious inflammatory diseases and environmental factors [14].

Figure 2a. Acute inflammation in prostate tumorigenesis. During acute inflammation, immune cells such as mast cells, macrophages, neutrophils and leukocytes detect tumor cells through their TLRs, which recognize DAMPs (damage-associated molecular patterns) secreted from tumor cells. TLR signaling induces cytokine and chemokine production in the immune cells (e.g., mast cells, leukocytes, macrophages), which results in an increase recruitment of immune cells needed to target damaged prostatic cancer cells (tumor cells). Tumor associated macrophages secrete ROS and RNS to induce DNA damage. Immature dendritic cells engulf tumor cells to mature and travel to the lymph nodes to present tumor cell antigen to lymphocytes.

Figure 2b. Chronic Inflammation in Prostate Tumorigenesis. Primarily lymphocytes (B, T, NK cells) and macrophages are active in chronic inflammation. Cytokines and chemokines attract these immune cells to infiltrate prostatic cells and target damaged cells (tumor cells).

Chronic inflammation of the prostate may lead to the development of PIA (proliferative inflammatory atrophic) followed by the progression of PIA to PIN (prostatic intraepithelial neoplasia) and ultimately the initiation of prostate cancer. PIA, attributed to repetitive tissue injury and damaged-induced cellular proliferation in prostate epithelium, is characterized by the formation of lesions in the peripheral zone of the prostate gland. The PIA lesions are related to increases in anti-apoptotic (e.g., Bcl-2) and proliferative signals as well as the population of mononuclear (e.g., lymphocyte, monocyte) and polymorphonuclear (e.g., neutrophils, mast cells) cells in the prostate epithelium and stromal tissue [44, 45]. IL17 positive monocytes/macrophages and other inflammatory cells accumulate in PIA lesions of radical human radical prostatectomy specimens [45]. The aforementioned immune cells exasperate PIA due to the overexpression of inflammatory cytokines. For instance, IL17 positive monocytes/macrophages accumulate in PIA lesions of human radical prostatectomy specimens [45]. IL17 plays a dual role in acute and chronic inflammation through the stimulation of IL6 (acute/chronic inflammation) and IL8 (acute inflammation) production [46]. As chronic inflammation persists in the prostate, PIA lesions can transition into PIN, a potential precursor of prostate cancer [44, 47]. High-grade PIN lesions contain abnormal cells with a large nucleus, detected in a number of prostate biopsies, and are associated with a 20-30% increase in the risk of prostate cancer development [47, 48].

Innate and Adaptive Immunity Signal Pathways in Response to Pathogens

Chronic or recurrent exposure to pathogens or infectious agents can lead to prostatic inflammation followed by PIA, PIN and ultimately prostate carcinoma. In response to pathogens or infectious agents, the immune system has two defense

mechanisms, innate and adaptive immunity. Innate immunity immediately triggers the activation of macrophages. During innate immunity, macrophages and mast cells non-specifically target pathogens through the recognition of their antigens. Next, these inflammatory cells secrete soluble inflammatory mediators such as cytokines, chemokines, matrix remodeling proteases and reactive oxygen species (ROS) that attract other inflammatory cells (e.g., epithelial cells, neutrophils, monocytes, natural killer (NK) cells, dendritic cells, basophils, eosinophils) to engage pathogens at the site of infection [49, 50]. Activated macrophages secrete cytokines (e.g., chemokines), which initiates the inflammatory response of the immune system. In addition, immature dendritic cells ingest pathogens and present receptors on their surface that recognize many common pathogens. Immature dendritic cells migrate through the lymph nodes to interact and activate lymphocytes. NK cells destroy certain groups of immature dendritic cells and promote the maturation of other dendritic cells [49]. During innate immunity, T- and B-lymphocytes of adaptive immunity are exposed to foreign antigens via mature dendritic cells and develop receptors that are antigen-specific. Adaptive immunity is a complex antigen-specific defensive response to pathogens or infection and retains memory of specific antigens for future infections or invasion of pathogens. However, the effect of adaptive immunity is observed after approximately 4-7 days of infection [50]. Innate and adaptive immunity leads to the inflammatory response involved in pathogen or infectious agent induced prostate cancer development and progression. However, the focus of this study is restricted to innate immunity signaling, which involves TLRs, chemokines, and cytokines.

Figure 3. Toll-like receptor signaling in innate immunity. TLRs play a critical role in innate immunity through their recognition of pathogen antigens. TLR signaling induces apoptosis, and elevate the expression of MMPs, cytokines and chemokines via the activation of NFκB and IRAK4. However, TOLLIP and IRAK3 can inhibit IRAK4 signaling.

Role of TLRs, Cytokines and Chemokines in Inflammatory and Immune Response

Toll-like receptors in relation to the inflammatory and immune response: The pattern recognition receptor (PRR) family, which includes toll-like receptors (TLRs), plays an essential role in regulating innate immune responses against harmful pathogens, as depicted in **Figure 3** [31]. With the assistance of extracellular accessory proteins (e.g., CD-14, MD-2), TLRs are activated through their recognition of pathogen-associated molecule patterns (PAMPs) of pathogens as well as damage-associated molecular patterns (DAMPs) released from injured epithelial cells [51]. Some human TLRs (e.g., TLR1, TLR2, TLR4, TLR5, and TLR6) are located on the cell surface and recognize lipid or protein ligands; whereas, others located within the intracellular compartments (e.g., TLR3, TLR7, and TLR9) recognize viral genetic material [31, 52].

Once activated, TLRs form complexes with adapter molecules (e.g., MyD88, TRIM, TRIF) to recruit downstream targets (e.g., IRAK4 and TRAF6), which lead to the activation of transcription factors [e.g., nuclear factor- κ B (NF- κ B) and interferon regulatory factors (IRFs)] via mitogen-activated protein kinase (MAPKs) signaling [31]. Overall, the TLR signaling cascade initiates local inflammation, which involves the secretion of inflammatory mediators, chemokines, cytokines, antibodies, major histocompatibility complex class II (MHCII) and interferons [31]. In addition, TLR signaling leads to the maturation of dendritic cells. Immature dendritic cells phagocytize pathogens and present fragments of the pathogen on its cell surface using MHC molecules. Mature dendritic cells induce the adaptive immune response, which is a more complex defense system against pathogens.

Chemokines in relation to the inflammatory and immune response: Chemokines (e.g., CC, CXC, XCL, XC and CX3C), secreted via intracellular signaling through the TLR signaling pathway (i.e., TLR2/TLR4), play a pivotal role in chemotaxis, lymphocyte development, angiogenesis, host response to infection, chronic inflammation, trafficking of dendritic and lymphocytes and ultimately tumorigenesis [35]. These molecules mediate their actions through 7-transmembrane G-protein coupled receptors and serve three major physiological functions. First, chemokines are involved in the maturation and homeostasis of the immune system, as well as trafficking of memory T cells, lymphocytes, monocytes, and neutrophils to the inflammatory site. Chemokines function in a chemotactic manner to attract lymphocytes, monocytes, and neutrophils to the site of infection and into pre-malignant tissue. Chemokine receptors located on the surface of innate immune cells include neutrophils (e.g., CXCR1, CXCR2), immature dendritic cells (e.g., CCR1, CCR5) and NK cells (e.g., CCR2, CCR4, CCR5, CCR7 and CCR8) and activated matured dendritic cells (e.g., CCR7) [53, 54]. The occurrence of chemokine production in pre-malignant tissue may increase cell survival, tissue remodeling, and angiogenesis (e.g., CXCL1, 2, 3, 5, 6, 7) to contribute to tumor development [35].

Cytokines in relation to the inflammatory and immune response: Cytokines promote tissue repair or healing with anti-inflammatory activity and exacerbate inflammation via TLR signaling pathways. Several cytokines are associated with chronic inflammation, including IL1, IL2, IL3, IL4, IL5, IL6, IL7, IL9, IL10, IL12, IL13, IL17, interferon (IFN), TGF β , and tumor necrosis factor (TNF) α and β [36, 46]. For innate immunity, inflammatory immune cells secrete cytokines such as TNF α , TNF β , IL1 β , IL6 IL10 IL12,

IL17, IL18, IL22, IL23, IL25, and IFN α , to eliminate pathogens detected within the host [55]. The subset of cytokines involved in the adaptive immunity such as IL4, IL5, IL6, IL7, IL13 induce the production of antibodies from B-lymphocytes, which destroy microorganisms and restrict area of infection. Moreover, many cytokines (e.g., IL1, IL2, IL3, IL4, IL7, IL9, IL10, IL12, interferon, transforming growth factor- β , tumor necrosis factor α and β) induce cellular responses, such as the mobilization of dendritic cells, monocytes, and T-lymphocytes, within adaptive immunity to eliminate pathogens [46, 56]. Collectively, humoral and cellular responses play an integral role in chronic inflammation, which significantly contributes toward to prostate cancer development [14, 24]. The dysregulation of inflammatory and immune response signaling pathways governed by the aforementioned toll-like receptors, chemokines, and cytokine contributes to inflammation and immune response via promotion of uncontrolled cellular proliferation, growth, invasion, and migration and ultimately leading to tumor development.

Role of inflammatory and immune response pathways in relation to PCA

TLR signaling pathway and prostate cancer tumorigenesis: TLRs are a family of transmembrane receptors that play a pivotal role in prostate cancer progression through its influence on the innate immune system via expression on immune cells and the induction of chemokine and cytokine production. In a gene expression study, human TLRs (TLR1-10) had detectable gene expression levels in normal prostate tissue [57]. In relation to prostate cancer, Varambally and co-workers (2005) observed down regulation of TLR1 gene expression in metastatic prostatic tissue compared to benign and primary localized prostate cancer tissue from the University of Michigan Prostate Specialized

Programs of Research Excellence (SPORE) [58]. Chin and colleagues (2010) observed suppression of prostate cancer tumor growth in IFNAR1 $+/+$ (type I interferon) and TLR3 $+/+$ mice implanted with TRAMP C2 cells (i.e., transgenic mouse prostate cancer epithelial cells) compared to IFNAR1 $-/-$ and TLR3 $-/-$ mice, respectively [59]. Moreover, tumor suppression was observed after treatment with polyinosinic-polycytidylic acid (polyI:C), a natural agonist of TLR3 or immunostimulant, on orthotopic prostate tumors in mice. This lead to the recruitment of T-lymphocytes and NK cells via type I IFN-dependent signaling. The study suggests that TLR3 agonists induce tumor suppression through the activation of the NF- κ B, MAPK and IRF3 signaling pathways via TRIF (Toll/IL-1R domain-containing adaptor inducing IFN) in immune cells. These mediators bind to receptors on the immune cells and promote immune cells to target prostate tumor cells [59].

Galli and co-workers (2010) observed that *TLR2*, *TLR3*, *TLR4*, *TLR5*, and *TLR6* genes were expressed in PC3 cells (human metastatic prostate cancer cell line); however, both *TLR3* and *TLR5* gene and protein expression was present in LNCaP, Du-145, and PC3 cell lines (human metastatic prostate cancer cell lines) [52]. Nonetheless, *TLR7*, *TLR8* and *TLR9* genes were not expressed in PC3, Du-145 and LNCaP cell lines [52]. Unfortunately, this study fail to compare TLR expression in PCA cell lines to normal prostate cells. Stimulation of LNCaP and Du145 cells with poly (I:C) induce the production of chemokines (e.g., CXCL10, CCL5, CCL3 and IL8) via TLR3 and TLR5 and recruitment of leukocytes. This demonstrates that TLR signaling plays a role in chemokine production in PCA cell lines. Gonzalez-Reyes and associates (2011) observed high gene expression of TLR3, TLR4, and TLR9 in prostatic tumor tissue of 133

European men compared to benign tissue [60]. TLR3 and TLR9 mRNA expression had a significant positive association with prostate cancer biochemical recurrence ($p = 0.039$). The study speculates that elevated *TLR3* and *TLR9* mRNA expression in prostate cancer tissue may induce the production of cytokines and chemokines to recruit immune cells and stimulate the release of more cytokines. The continual stimulation of cytokine and chemokine production could feed into a state of immune tolerance and initiation of a tumor microenvironment.

Interestingly, the immunohistochemical staining for TLR4 was significantly lower in high gleason score (Gleason score 4-6) prostatic adenocarcinoma stromal epithelial cells compared to normal and low gleason score (Gleason score 7-10) tissue [61]. After activation of TLR4 with LPS treatment, the gene expression of NF- κ B regulated pro-inflammatory cytokines (e.g., IL1 β) and chemokines (e.g., IL8) was up-regulated, which contributes to an inflammatory state in Du145 cells. Moreover TLR4 signaling may increase the risk of prostate cancer via chronic inflammation. Pronounced TLR7 gene expression has been observed in normal prostate epithelial RWPE1 cells, but TLR7 expression is absent in PC3 and Du145 cells [62]. Consisted with the in vitro findings, protein expression of TLR7 was decreased in high grade PIN and adenocarcinoma of mouse prostatic tissue compared to normal prostatic tissue in mice.

Downstream signaling in the TLR pathway also plays a role in prostate cancer. IRAK4 plays a critical role in the innate immune system by relaying signals from TLRs via recruitment of IRAK1, which activates NF- κ B induce pro-inflammatory response and caspases induce apoptosis. IRAK4 expression was decreased in doxetaxel resistant Du145 and PC3 compared to non-resistant Du145 and PC3 cells [63]. Similarly, down-

regulation of IRAK4 resulted in the resistance of U251 cells, a glioma cell line, to Temozolomide treatment [64]. Both studies suggest that IRAK4 expression modulates the sensitivity of cancer cells to chemotherapy and may serve as a good target for cancer treatments. Zhu and co-workers (2012) demonstrated in HEK293T (human embryonic kidney epithelial cell line) cells that TOLLIP inhibits the TGF β signaling pathway, a key biological pathway in prostate tumorigenesis [65]. In prostate cancer, TGF β functions as a tumor suppressor in the early stages of prostate cancer via suppression of cycle dependent kinases and cMyc. However, in the advanced stages, TGF β transitions into a tumor promoter via induction of VEGF expression to promote tumor progression[66-68]. Varambally and associates (2005) observed higher gene expression of TOLLIP in metastatic prostate tissue compared to benign tissue [58]. In another study, overexpression of TOLLIP was shown to accelerate the degradation of TGF β receptor type 1 (TGF β R1) protein [65]. In addition, TOLLIP interacts with ubiquitinated TGF β R1 via SMAD7, an inhibitory SMAD, which results in the suppression of TGF β signaling. TOLLIP gene expression was observed in androgen sensitive (e.g., LNCaP, MDA-PCA-2a) and insensitive (e.g., PC3, Du145) prostate cancer cell lines as well as prostatic cancer tissue [69]. TOLLIP may participate in the decrease of TGF β receptor protein expression commonly seen in prostate cancer (i.e., LNCaP) and malignant prostate cancer archival tissue compared to benign tissue [70]. Collectively the aforementioned studies suggest that TOLLIP may play a central role in prostate tumorigenesis however, *in vitro* and *in vivo* studies are needed to determine its role. However, no published reports have identified a strong relationship between the expression levels of TLR1 TLR6, TLR10 and IFR3 in PCA cell lines and their subsequent impact on prostate tumorigenesis.

Chemokines, their receptors and prostate cancer : Chemokines are signaling molecules involved in the regulation of inflammation, trafficking of inflammatory cells (e.g., leukocytes, dendritic cells, neutrophils) and immune cell differentiation. These signaling molecules also play a role in cellular behaviors associated with prostate tumorigenesis. Kato and co-workers (2013) reported CCL5 treatment activates the ERK and Rac signaling pathways via one of its receptors (e.g., CCR1) in CCR1 knockdown paclitaxel-resistant PC3 cells (PC3PR) compared to negative control transfected PC3PR [71]. The activation of CCR1 promotes cell invasion in PC3PR cells by increasing mRNA expression and secretion of matrix metalloproteinases, namely *MMP2* and *MMP9* [71]. Matrix metalloproteinases degrade the extracellular matrix, which is an essential step of metastasis [72]. In another study, Vaday and colleagues (2006) observed high levels of pro-inflammatory chemokines CCL5 and CCR5 in radical prostatectomy specimens compared to invasive ductal breast carcinoma as a positive control [73]. In fact, overexpression of CCL5 and CCR5 is strongly associated with aggressive prostate cancer, presumably by triggering leukocyte production and promoting cell survival, proliferation, invasion and metastasis [22, 73, 74]. CCL5 also induced cell migration and proliferation in LNCaP cells through the activation of CCR5 [73]. Moreover, another study observed that *CCL5* and *CCR5* are highly expressed genes in prostate cancer tissue and may play a role in prostate tumorigenesis [75].

It is speculated that CCR7 gene/protein expression may mediate the cellular migration of tumor cells to the lymph nodes in various cancers including prostate cancer [76-79]. In addition to the CCL5-CCR1-CCR5 axes, some chemokines such as CXCL1 and CXCL2 influence cellular behaviors such as cell growth in prostate cancer cells.

Killian and colleagues (2012) observed that silencing these pro-inflammatory chemokines (i.e., CXCL1 and CXCL2) inhibits cell growth and induces apoptosis in PC-3, a metastatic prostate cancer cell line [80]. Similarly, Benelli and associates (2013) showed that CXCL1 transfected TRAMP-C2 cells, a transgenic mouse epithelial prostatic cell line, inhibits tumor growth in mice compared to an empty vector [81]. This report further suggests that CXCL1 may induce senescence to inhibit tumor and cell growth via CXCR2 in TRAMP-C2 cells [81]. CXCR7, another pro-inflammatory chemokine that binds to CXCL11 and CXCL12 (SDF-1a), is elevated in various cancers, including prostate cancer [82-86]. Singh and Lokeshwar (2011) demonstrated high mRNA and protein expression levels of CXCR7 in PC-3 and LNCaP relative to RWPE1 cells (normal immortalized prostate epithelial cell line) [85]. In addition, this study showed that CXCR7 overexpression increased cell proliferation by 23% in RWPE1 cells. Depletion of CXCR7 in C4-2B cells, a derivative of the LNCaP cell line with androgen sensitivity, reduced tumor growth in nude mice and cell proliferation in PCA cell lines (e.g., PC3, LNCaP) [85-87]. CXCR7 promoted cell proliferation via phosphorylation of EGFR and Erk1/2 in RWCX7 cells, derivatives of RWPE1 cells that overexpress CXCR7 [85]. Singh and co-workers (2004) showed that *CCR9* mRNA levels were highly expressed in LNCaP and PC-3 cell lines in comparison to PrEC (normal prostate epithelial cell line) [88]. Treatment of LNCaP and PC-3 cells with CCL25, a known ligand of CCR9, resulted in an increase in cellular migration and invasion, as shown by flow cytometry. After CCL25 treatment, gene expression of metalloproteinases, *MMP2* and *MMP13*, was elevated in PC-3 and LNCaP cell lines compared to untreated PC-3 and LNCaP cells. The increased expression of metalloproteinases, induced by CCL25 was

essential to cellular invasion and migration *in vitro*. Chemokines contribute to prostate cancer phenotypes, including increased cellular proliferation, migration and invasion of prostate cancer cells.

Cytokines and in prostate cancer: The cytokine family, which includes chemokines, interleukins, interferons, and growth factors, plays an essential role in the development and progression of prostate cancer. Cytokines are soluble proteins, peptides or glycoproteins secreted from immune cells as signaling molecules in response to infection, foreign pathogens, and tumor cells [89-91]. Several studies reveal pro-inflammatory cytokines, involved in chronic inflammation, participate in prostate cancer progression. Salman and colleagues (2012) observed elevated secretion of pro-inflammatory cytokines from peripheral blood mononuclear cells (PBMCs) incubated with PC-3 cells (IL1 β , IL6, IL10) or LNCaP cells (IL6) compared to the incubation of PBMCs with no cancer cells [92]. The presence of prostate cancer cells induces the production of cytokines via the immune system. TGF- β 1 is a highly expressed cytokine in tumor prostate epithelial cells that was significantly associated with high vascular density and bone metastasis in prostate cancer [93]. In particular, Wikstrom and associates (1998) observed that overexpression of TGF β 1 in 73 paraffin-embedded tumors was associated with short survival rates among European PCA patients who did not receive any treatment relative to normal prostatic tissue [93]. Rodriguez-Berriguete and colleagues (2013) revealed that high protein expression of cytokines in the tumor necrosis factor family [TNF α , TNFR1, TNF receptor-associated factor 2 (TRAF2)] were associated with high pathological prostatic tumor stage in prostatic tumor tissue relative to normal prostatic tissue [94]. In a pilot study of 93 prostate cancer patients who underwent a radical prostatectomy,

cytokines, IL1 β (HR = 0.431; 95% CI = 0.180-1.029) was marginally linked with a 57% decrease in biochemical progression [94]. IL6, a major cytokine, participates in chronic inflammation and prostate tumorigenesis. For instance, Chung and co-workers (1999) demonstrated a decrease in cell growth after treating hormone independent PCA cell lines (Du145, TSU and PC3) with a neutralizing polyclonal goat anti-human IL6 antibody compared with untreated cells [95]. Moreover, an independent study revealed that autocrine production of IL-6 induces a high gene and protein expression of IFR9, which is critical to the exertion of the IFN α 2 anti-proliferative effect on prostate cancer cells (i.e., PC-3 and MDA PCA 2b cells) [96]. These studies confirm cytokines play a critical role in the progression of prostate cancer via cellular behaviors. In the aforementioned studies, inflammatory and immune response-associated genes and proteins were shown to participate in prostate tumorigenesis via promotion of cellular migration, proliferation and growth, which are critical biological pathways in prostate cancer.

Epidemiology evidence of the relationship between variant inflammatory and immune response related sequence variants and prostate cancer

Over the past decade, many epidemiological studies have shown that genetic variants in inflammatory and immune response-related genes contribute to the dysregulation of critical biological pathways and ultimately leads to cancer development or disease progression. The inheritance of genetic variants in these mediators may impede their function to properly regulate the inflammatory and immune response. Some of the inflammatory and immune response-related sequence variants have been previously reported to play a role in several carcinomas, including carcinoma PCA. Although the relationship between genetic alterations and PCA is controversial [97-99], the majority of

published reports typically focus on men of Asian or European descent. Little or no data addresses the impact of cytokine, chemokine and toll-like receptor-related sequence variants on PCA risk among men of African descent, even though this population suffers disproportionately from this disease [1, 14, 27, 100-102].

TLR-associated sequence variants and PCA

TLR-related sequence variants have been evaluated in relation inflammatory and immune response-related diseases including prostate cancer. Stevens and co-workers (2008) showed that polymorphisms in *TLR1* *rs4833095* CC (OR = 0.64; 95% CI = 0.47-0.86), *TLR1* *rs5743595* CC (OR= 0.63; 95% CI=0.42-0.93), *TLR10* *rs11096955* CC (OR = 0.78; 95% CI = 0.61-0.99), *TLR10* *rs11096957* CC (OR = 0.78; 95% CI = 0.61-0.99) and were linked with a 22-58% decrease in prostate cancer susceptibility among 1,414 cases and 1,414 controls of Latin, European, and Asian descent (p-trend = 0.0022-0.011) [97]. In six observational studies, *TLR4* (*rs1927911* CC, *rs2149356* TT), *TLR10* *rs4274855* AG+AA were linked to a 1.12-6.68 fold increase in the risk of developing PCA among men of European and Asian descent [97, 98, 103-106]. In another study, inheritance of 1 copy of the risk allele for and *TLR4* *rs2149356* (OR = 0.66; 95% CI = 0.51-0.86) was linked to a 13-34% reduction in PCA susceptibility among European Americans (700 cases, 700 matched controls) [107]. Unfortunately, there was no relationship between prostate cancer susceptibility and possession of variant TLR signaling associated alleles *TLR4* (*rs1927906*), *TLR6* (*rs1039559*, *rs3821985*, *rs5743810*), and *IRAK4* *rs4251545* with [97, 99, 103, 104, 106-109].

The relationship between innate immunity genetic anomalies and prostate cancer susceptibility has not been met without controversy [98, 99, 103-105, 107, 108]. Under

the recessive genetic model for *TLR4 rs2149356* (i.e., TT vs. GG + GT), Chen and co-workers (2005) observed a 36% decrease in risk of developing prostate cancer (OR = 0.64; 95% CI = 0.45-0.91) among European men (700 cases, 700 matched controls) [107]; however, this relationship was insignificant in 3 other studies [103-105].

Moreover, inheritance of the *TLR4 rs1927911* CC genotype was linked with a 6.68-fold increase in the risk of developing prostate cancer but had a null effect in other studies [98, 103, 107]. Possession of the *TLR10 rs11096955* CC, and *TLR10 rs11096957* CC genotypes were associated with a 9-21% decrease in prostate cancer susceptibility in two studies but associated with 1.20-1.25-fold increase in prostate cancer risk in another report among men of European descent [97, 106, 109]. However, these findings were null in relation to prostate cancer risk with the exception of *TLR10 rs11096955* CC genotype in one study [106]. In addition, several of the aforementioned studies that evaluated several SNPs simultaneously did not adjust their risk estimates for multiple hypotheses testing [98, 99, 107, 108]. Failure to adjust for multiple comparisons may lead to an over estimation of prostate cancer susceptibility among carriers of putative high-risk alleles.

Moreover, a majority of the previously mentioned studies failed to include men of African descent, despite the fact that men of African descent suffer disproportionately from prostate cancer relative to men of European and Asian descent [98, 99, 103-105, 107, 108]. In addition, some studies utilized different allelic discrimination assays such as Taqman, MassARRAY and multiplex PCR to genotype SNPs [104, 105, 107].

Furthermore, several of the aforementioned reports had evaluated different genotypes in relation to each SNP and varied in the frequency distributions across the genotypes [99, 105-107].

In a recent published report from our lab, we addressed some of the aforementioned concerns that often plague most genetic epidemiology studies. First, we evaluated the role of innate immunity sequence variants among a relatively large subgroup of men of African descent (270 cases, 535 controls). Our sample size enabled us to have adequate statistical power to observe reasonable protective ($OR < 1.00$) and risk ($OR > 1.4$) effects. Lastly, all of our relationships were adjusted for multiple hypothesis testing using the false discovery rate (FDR), in order to adjust for false positive results. Our study was restricted to polymorphisms detected within the genes of TLRs (1, 4, 6, 10), TOLLIP and IRAK4 genes among 814 men of African descent [31]. We revealed that *TLR6* rs2381289 GA ($OR=1.46$; 95% CI=1.02-2.09) and *TOLLIP* rs5743899 AG ($OR=1.59$; 95% CI=1.06-2.38), AG+GG ($OR=1.49$; 95% CI=1.02-2.18) genotypes modestly modified PCA risk among men of African descent from the U.S. and Jamaica. However, these sequences variants did not survive multiple hypothesis testing ($FDR > 0.05$). After stratification by racial/ethnic group and age-adjustment, six TLR-related sequence variants detected were modestly linked to PCA susceptibility among men of African descent from the U.S. Inheritance of *IRAK4* (rs4251545 A and rs4251473 A) and *TLR6* rs5743818 C variants were associated with a 40-66% reduction in PCA risk among U.S. men (χ^2 p-value = 0.099). On the contrary, possession of both *IRAK4* rs4251545 GG referent genotype and *TLR1* CT+CC genotype was associated with a 1.36-fold increase (95% CI = 1.09-1.69) in prostate risk among men of European descent (p-value < 0.01) [108], but *IRAK4* rs4251545 had a null finding in another report [110]. However, the *TOLLIP* rs5743899 recessive genetic model (GG vs. AA+AG) was modestly associated with a 1.14-fold increase in PCA susceptibility ($OR = 1.14$; 95% CI = 1.12-1.18). Among

Jamaicans, possession of *TLR6* *rs2381289* GA+AA ($OR_{age-adj} = 2.05$; 95% CI = 1.10-3.78) and *TLR6* *rs5743818* AC+CC ($OR_{age-adj} = 1.10$; 95% CI = 1.06-1.14) genotypes was linked to a 1.10-2.05-fold increase of PCA development. In contrast, *IRF3* *rs2304206* GG genotype was related to a 68% reduction ($OR = 0.32$; 95% CI=0.11-0.98) in PCA susceptibility. Notably, inheritance of the variant innate immunity alleles among Jamaicans (*TLR6* *rs2381289* A, *IRF3* *rs2304206* G) and U.S. men (*IRAK4* *rs4251545* A) for U.S. men did not survive multiple hypothesis testing (Bonferroni Correction p-value > 0.0016).

Chemokine-associated sequence variants and PCA

Genetic alterations in chemokines and their receptors have been linked to the susceptibility, development and progression of numerous cancers, including prostate cancer [111-115]. The *CCL5* *rs2107538* variant contributed to an increased susceptibility for different cancers [116-118]. In terms of PCA, *CCL5* *rs2107538* GA+AA genotype ($OR=1.44$; 95% CI=1.09-2.38) was associated with an increase in prostate cancer risk among European men (296 cases, 311 controls) [116]. In a pilot study, the *CXCL12* *rs1801157* ($OR = 1.58$; 95% CI = 1.03-2.43) GA+AA genotype was significantly associated with a 1.58-fold increase in prostate cancer risk among Japanese men (167 cases and 167 matched controls) [112]. However, there are no published reports on the main effect of *CCR7* (*rs3136685*, *rs3136687*) and *CCR5* *rs1799988* sequence variants in relation to PCA susceptibility. Some of the aforementioned studies failed to adjust for multiple hypothesis testing [116-119], and perform power calculations to specific odd ratio detection limits [116-118, 120]. Furthermore, prior to our 2012 published report, there were no published findings on the relationship between chemokine associated

sequence variants and PCA among African-American men.

Our lab showed that five chemokine-related sequence variants were significantly associated with prostate cancer development among men of African descent (279 cases and 535 controls) [35]. The inheritance of *CCL5* (*rs2107538* and *rs3817655*) GA, AA and GA+AA genotypes were linked with a 34-44% reduction in PCA risk in the age adjusted LR models among men of African descent [false discovery rate (FDR) $\leq$ 0.0493]. However, the inheritance of the *CCR5 rs1799988* (OR_{age adjusted} = 1.52; 95% CI = 1.02-2.26) recessive genetic model, *CCR7 rs136685* (OR_{age adjusted} = 1.66; 95% CI = 1.09-2.54) AG+GG, and *CCR7 rs136687* (OR_{age adjusted} = 1.14; 95% CI = 1.12-1.16) AG+GG genotypes were associated with a significant 1.14-1.66 fold increase in PCA risk within the age adjusted LR models. The *CCL5* (*rs2107538*, *rs3817655*) dominant (FDR p-value $\leq$ 0.015) and *CCR5 rs1799988* recessive genetic models (FDR p-value = 0.049) remained significant even after adjusting for multiple hypothesis testing.

In a stratified analysis, several chemokine-chemokine receptor markers were linked to PCA susceptibility among men of African descent from the U.S. Inheritance of the *CCR5 rs1799988* GG locus under the recessive genetic model (OR_{unadjusted} = 1.62; 95% CI = 1.04-2.42) and the *CXCR7 rs1045879* AG+GG genotype (OR_{unadjusted} = 1.54; 95% CI = 1.07-2.22) was associated with a 1.54-1.62-fold increase in PCA risk among unadjusted risk models. However, possession *CCL5 rs2107538* GA+AA (OR_{unadjusted} = 0.52; 95% CI = 0.36-0.76), *CCL5 rs2280789* AG+GG (OR_{unadjusted} = 0.60; 95% CI = 0.41-0.89), *CCL5 rs3817655* GA+AA (OR_{unadjusted} = 0.46; 95% CI = 0.32-0.68), *CCL25 rs2032887* AG+GG (OR_{unadjusted} = 0.66; 95% CI = 0.46-0.96) genotypes was linked with a $\geq$ 34% reduction in PCA susceptibility. After adjusting for age, the risk estimates of the

previously mentioned genotypes risk estimates for *CCL5* (*rs2107538*, *rs3817655*) remained significant. However among Jamaican men, *CCR5 rs1799987*, *CCR5 rs1799988*, *CCR7 rs3136685* and *CCR9 rs1488371* loci were linked with PCA risk. In unadjusted risk models, *CCR5 rs1799987* AA (OR_{unadjusted} = 2.18; 95% CI = 1.04-4.58), *CCR5 rs1799988* GG (OR_{unadjusted} = 2.25; 95% CI = 1.08-4.71) and *CCR7 rs3136685* AG+GG (OR_{unadjusted} = 2.30; 95% CI = 1.05-5.07) genotypes were linked with a 2-fold increase in PCA susceptibility (P-trend = 0.034-0.086). On the contrary, inheritance of *CCR9 rs1488371* CA+AA (OR_{unadjusted} = 0.46; 95% CI = 0.23-0.94) genotype was associated with a 54% reduction in PCA risk among unadjusted risk models. After adjusting for age, the risk estimates of the previously mentioned genotypes for *CCR5 rs1799987*, and *CCR7 rs3136685* remained significant among men of African descent. However, only *CCL5* (*rs2107538*, *rs3817655*) sequence variants survived multiple hypothesis testing among U.S. men of African descent (Bonferroni Correction p-value ≤ 0.0012). Our study revealed strong associations between genetic variants in *CCL5* among men of African descent that warrant further investigation in future studies.

Unfortunately, our findings were not in agreement with cancer-related published reports. We observed that the inheritance of *CCL5 rs2107538* GA+AA genotype was associated with a 41-48% reduction in PCA susceptibility (OR = 0.59; 95% CI = 0.44-0.80 and OR_{unadjusted} = 0.52; 95% CI = 0.36-0.76) among men of African descent; however, Saenz-Lopez (2008) observed that the *CCL5 rs2107538* GA+AA genotype was associated with an increase in prostate cancer risk (OR = 1.44; 95% CI = 1.09-2.38) [116, 118]. This variant was also an important predictor of pancreatic cancer risk (OR = 2.3; 95% CI = 1.00-5.30) for a mixed population (i.e., European, African and Asian descent)

and oral cancer risk among Asians (OR = 2.23; 95% CI = 1.07-4.66) [116, 118] [117]. Moreover, we observed an increased PCA susceptibility affiliated with inheritance of the CCR7 (rs3136685 AG+GG, rs3136687 GA+AA) genotypes; however, a couple of studies reported a 60-62% reduction in cancer susceptibility for multiple myeloma and chronic lymphocytic leukemia, among individuals of European descent [119, 120]. However, majority of the aforementioned studies in relation to prostate cancer susceptibility failed to include men of African descent and adjust risk estimates for multiple hypothesis testing [116, 118]. Furthermore, the discrepancies between our study and the aforementioned reports may be attributed to differences in cancer type evaluated in relation to innate immunity genetic variants, limited sample size, genotype frequency distribution, allelic discrimination assays (i.e., PCR–RFLP) and ethnic groups included in each study population sample size and ethnic group [116-120]. In addition, a couple of epidemiological studies evaluated SNPs in relation to cancer susceptibility in populations that included women as well [117, 120].

Cytokine-Associated Sequence Variants and PCA

The production of cytokines can be influenced by single nucleotide polymorphisms (SNPs) in pro- and anti-inflammatory genes. Observed differences in the spectrum of cytokines expressed in an inflammatory environment or level of antitumor response within an individual can be explained by genetic variations, which leads to high- or low-level production of a given cytokine [46]. Epidemiological studies evaluated the relationship between prostate cancer and several cytokine-associated gene variants, such as *TNF rs1800629* (-308 G>A) among Indian men as well as *IL10 rs1800871* (-819 C>T) among European American men [101, 121-128]. For instance, Berhane and associates

(2012) showed that *TNF rs1800629* AA genotype was associated with a 1.79-fold (95% CI = 1.29-2.49) increase in the risk of developing prostate cancer among North Indian men (150 cases, 150 BPH controls) [121]. In another study, *TNF rs1800629* has also been implicated in several other carcinomas (e.g., lymphoma, cervical, gastric) [129-132]. However, some studies have concluded that *TNF rs1800629* was not associated with prostate cancer or other cancers among individuals of European, African and South American descent [101, 121, 133-135]. Kesarwani and associates (2009) observed that *IL10 rs1800896* GA genotype (OR = 2.8; 95% CI = 1.26-6.14) was associated with a 2.8-fold increase in PCA risk among North Indian men (259 cases, 159 controls) [126]. Possession of the *IL1B rs1143627* CC genotype (OR = 0.54; 95% CI = 0.36-0.81) was linked with a 46% reduction in susceptibility of PCA recurrence among U.S. men (524 matched case-control pairs) [122]. Zabaleta and associates (2008) observed *IL10 rs1800871* CT (OR = 0.72; 95% CI = 0.53-0.98), *IL10 rs1800872* CA (OR = 0.70; 95% CI = 0.52-0.93) and CA+AA (OR = 0.74; 95% CI = 0.56-0.98) 26%-30 reduction in PCA risk among men of European descent (889 men) [101]. However in the same study, *IL10 rs1800871* TT (OR = 0.18; 95% CI = 0.05-0.72) genotype was associated with a 82% decrease in PCA susceptibility among men of African descent. However in a report from our lab, *IL10 (rs1800871, rs1800872)* and *IL10 rs1800896* were found to have no association to PCA risk among U.S. men of African descent (193 cases and 666 controls) [40]. Inheritance of *IL1A rs17561* (+4845 G>T) had a protective effect against ovarian cancer risk (OR = 0.90, 95% CI = 0.83–0.97) among European American women (930 cases, 1,037 controls) [136]. In addition, Balasubramanian and colleagues (2006) also showed this sequence variant had a protective effect against breast cancer risk in

European American women (521 cases, 895 controls) as well (p-value = 0.05) [137]. Sequence variant, *IL1A* rs2856836, CT (OR = 2.88; 95% CI = 1.58-5.27) and CC (OR = 8.17; 95% CI = 2.23-29.9) genotypes were associated with a ≥ 2.0 -fold increase in breast cancer risk among Korean women (209 cases, 209 controls) [138]. Although the *IL1A* loci (*rs17561*, *rs2856836*) were associated with increased susceptibility to hormone-related cancers among individuals of European and Asian descent, our lab did not observe a significant association between these markers and PCA risk among men of African descent. Furthermore, some of these studies fail to adjust for multiple hypothesis testing [121, 136, 137], and perform power calculations [121, 136, 137, 138].

Moreover in a recent published report, our lab observed modest associations between prostate cancer risk and the inheritance of *IL1R2* rs11886877, *IL8RB* rs11574752, *TNF* (*rs1800629* and *rs673*) among men of African descent (279 cases and 535 controls) [36]. Among men of African descent, *IL8RB* rs11574752 AA (OR_{age-adjusted} = 38.4; 95% CI = 3.86-382.8), *IL1R2* rs11886877 AA (OR_{age-adjusted} = 1.92; 95% CI = 1.11-3.32), *TNF* rs1800629 GA (OR_{age-adjusted} = 1.54; 95% CI = 1.06-2.24), and *TNF* rs673 GA (OR_{age-adjusted} = 1.50; 95% CI = 1.04-2.16) genotypes in adjusted risk estimates were associated with PCA susceptibility. However, these sequence variants did not survive multiple hypothesis testing according to the new significance level ($\alpha = 0.0011$) (Bonferroni Correction p-value > 0.0011). Upon stratification, intronic *IL1R2* rs11886877 AA (OR_{age-adjusted} = 2.75; 95% CI = 1.38-5.50), GA+AA (OR_{age-adjusted} = 1.82; 95% CI = 1.14-2.88) genotypes and recessive genetic model (OR_{age-adjusted} = 2.05; 95% CI = 1.10-3.80) among U.S. men were linked to a 1.82-2.75 fold increase in risk of developing PCA within age-adjusted risk estimates. However, possession of *RNASEL*

rs12135247 AG genotype ($OR_{\text{age-adjusted}} = 2.10$; 95% CI = 1.04-4.24) was associated with PCA susceptibility among Jamaican men. We adjusted all associations for multiple hypothesis testing and perform power calculations to determine odd ratio limits. Notably, none of the evaluated cytokine-associated sequence variants were associated with PCA risk (Bonferroni Correction $p\text{-value} > 0.0011$). Although we reported null findings, *IL1R2 rs11886877* AA and GA+AA genotypes were associated with significant risk estimates among both the total population and U.S. men in age-adjusted LR models. The *IL1R2 rs11886877* A allele may favor pro-inflammatory signals and mute the protective effect of this receptor against the tumorigenic activity of $IL1\alpha$ and $IL1\beta$. *IL1R2* is a 68kD receptor pre-dominantly expressed on monocytes, B-lymphocytes, polymorphonuclear (PMN) leukocytes and prostatic tumor cells [139, 140]. Under normal conditions, this receptor functions in an anti-inflammatory or immunosuppressive manner by binding to $IL1\alpha$ or $IL1\beta$ to lower pro-inflammatory cytokine production from immune cells (e.g., lymphocyte, macrophage), as shown in **Figure 3** [139, 141, 142]. The pro-inflammatory signal of $IL1$ may be decreased, due to its binding to *IL1R2*. However, the introduction of a sequence variant such as *rs11886877* within the *IL1R2* gene may render the *IL1R2* protein non-functional due to instability of mRNA/protein expression. In addition, the pro-inflammatory signal of $IL1$ may be elevated via production of pro-inflammatory cytokines from immune cells leading to chronic inflammation (**Figure 4**). Furthermore, chronic inflammation may develop into PIA, which can transition to high grade PIN (HPIN) and ultimately initiate PCA development (**Figure 4**). Nonetheless, subsequent studies are needed to evaluate the influence of the aforementioned sequence variant on prostate tumorigenesis. Consequently, *in vitro* studies may be utilized to determine

whether the *IL1R2* rs11886877 AA genotype in normal prostate cells will inhibit the anti-inflammatory and immunosuppressive activity of IL1R2 via IL1 β or IL1 α induction. Overexpression of *IL1R2* or inheritance of the rs11886877 AA genotype in prostate cancer cell lines will provide information on whether or not IL1R2 is protective against cancer cells. These studies will provide more insight about the biological function of IL1R2 in prostate cells and whether IL1R2 may serve as a potential target for prostate cancer treatment.

Figure 4. The effect of IL1R2 on immune cells and prostate cancer. Inheritance of *IL1R2* rs11886877 reference allele G does not modify prostate cancer susceptibility among men of African descent. However, the inheritance of the *IL1R2* rs11886877 A allele was associated with an increase in prostate cancer risk. We proposed that rs11886877 A allele will impede function of the IL1R2 as a decoy receptor for IL1 on immune cells and allow the IL1 pro-inflammatory signal to induce the production of cytokines. Eventually immune cells will infiltrate normal epithelial prostate cells to initiate chronic inflammation that feeds into →PIA→ HPIN (high grade PIN) and ultimately prostate cancer development.

To further investigate the impact of these sequence variants on prostate cancer, we will examine the biological significance of variants through *in vitro* and *in vivo* studies in relation to prostate cancer therapies, cellular behavior (e.g., cellular proliferation, migration, invasion) and tumorigenesis. We will evaluate the gene and protein expression of *CCL5*, *CCR5*, *CCR6*, *CCR7*, *CCR9*, *TLR2*, *TLR6*, *TLR10*, *IRF3*, *IRAK4* and *IL1R2* in standard prostate cancer cell lines (e.g., PC3, Du-145, LNCaP). Next, we will evaluate the effect of target gene mimics or inhibitors on cellular behaviors in normal and cancer cell lines. Lastly, we will perform functional studies for selected genes significantly impacted by sequence variants through knockout and knockin *in vivo* models. Such studies will enable us to evaluate the effect of overexpression or inhibition of these genes on tumor growth in response to selected current prostate cancer therapies using state-of-art imaging techniques (e.g., Multispectral optoacoustic tomography).

Combined effects and gene-gene interactions on prostate cancer susceptibility

Gene-gene interactions aid researchers to model the complex involvement of biological pathways, and environmental factors in the etiology of prostate cancer as well as identify susceptible genes. High order interactions are difficult to detect and may lead to countless contingency tables with inadequate observations, inaccurate risk estimates and extremely wide 95% CIs. Only 4 of these published reports focused on PCA. However, 3 of the gene-gene interaction studies used logistic regression to detect interactions, and failed to predict high order (e.g., >2) gene-gene interactions in relation to PCA cancer risk. Moreover, two of the PCA genetic epidemiology studies did not have adequate statistical power to observe associations. Statistical power is the probability to correctly reject a false null hypothesis and dependent on sample size as well as size of

effect. High statistical power decreases the probability of type II error to occur, which is the detection of no effect when there is actually an effect. Linear modeling limits logistic regression to effectively model high-order interactions with ample statistical power.

Therefore, many researchers including our lab evaluate gene-gene interactions using multi-locus prediction tools [e.g., multivariate adaptive regression spline (MARS Logit), classification and regression trees (CART), computational evolution system (CES), modified logistic regression, and multifactorial dimension reduction program (MDR)] to perform intensive data mining for multivariate analyses.

Multi-locus prediction tools have both advantages and drawbacks. For instance, MARS Logit is a non-parametric method used to predict gene-gene interactions, but analyses are limited to large datasets with a bias toward the contribution of the additive genetic model to disease susceptibility [143]. CART is a nonparametric statistical method used to detect mutually exclusive and exhaustive subgroups based on common traits of a population [144]. Interestingly, CART is user friendly, provides 95% confidence intervals and the results are easy to interpret. However, the CART analysis is unable to estimate the effect of an independent variable, which may lead to data dredging and multi-level classification trees that are difficult to interpret. CES (computational evolution system) models are biologically sound nonlinear interactions of high-dimensional genetic data in 3D; however the interactions are governed by conventional mathematical models [145]. MDR is an intensive non-parametric method that randomly shuffles data to detect unbiased 1-4 factor interaction models on disease susceptibility[146]. This technique provides a cross validation consistency (CVC), and average testing accuracy (ATA or prediction score) percentages as well as permutation

testing p-values to adjust for multiple hypothesis testing. In addition, the current version of MDR does not require an equal number cases and controls to predict high order interactions. Unlike CART, MARS Logit, logistic regression, and CES, MDR is able to adjust analyses for multiple hypothesis testing. However, MDR does not provide risk estimates and associated 95% confidence intervals.

Among peer-reviewed articles discussed in this report, 20% (9 out of 45) of the published reports evaluated gene-gene interactions among inflammatory and immune-related sequence variants in relation to cancer susceptibility [40, 101, 102, 108, 112, 118, 147-149]. Genetic variants within inflammatory and immune response-related genes in relation to prostate cancer have been observed in various gene-gene interaction studies (**Table 1**). For instance, Sun and associates (2006) showed that the gene-gene interaction involving IRAK4 polymorphisms and TLR1 CT+CC genotype had a 1.37-9.68 fold increase in risk of developing prostate cancer among Swedish men using logistic regression [108]. Xu and colleagues (2005) reported a statistically significant four-factor gene-gene interaction model between IL10, IL1RN, TIRAP and TLR5 linked with prostate cancer risk among Sweden men (Permutation testing p-value = 0.019) using MDR [147]. Although this study controlled for multiple hypothesis testing, Xu and associates only evaluated gene-gene interactions among men of European descent. In the literature, a report by Duell and co-authors (2006) was one of the few studies on cytokine gene-gene interactions (e.g., CCL5-CCR5, CCL5-TNF α , CCR5- TNF α) [118]. Unfortunately, these gene-gene interactions were in relation to pancreatic cancer and not prostate cancer. Zabaleta and colleagues (2008) observed a significant two-factor gene-gene interaction involving IL1B-511 and IL10-592 with a 2.56-fold (95% CI = 1.09-6.02)

increase in prostate cancer risk among African-Americans using MARS Logit [101]. In another study, Zabaleta and colleagues (2009) showed an increase of prostate cancer risk among individuals who possessed both the *IL-1B* CC and *IL-10* GG genotypes (OR=3.38; 95% CI=1.70–6.71) among European American men using MARS Logit [102]. However, the authors were unable to determine gene-gene interactions in relation to prostate cancer risk among African-Americans due to limited sample size. Most of the previous studies evaluated gene-gene interaction models among men of European and Asian descent, and limited analyses were performed among men of African descent.

Table 1. Inflammatory and Immune related gene-gene interactions in Prostate cancer

Gene	Cases/ Controls	Ethnicity	Disease	Multiple Hypothesis Testing p-value	Reference
IL1B, IL10	54/109	African American	Prostate cancer	MARS; p=0.032	Zabaleta, 2008 [101]
IL1B, IL10	68/378	European American	Prostate cancer	MARS; p=n/a	Zabaleta, 2009 [102]
IL10, IL1RN, TRAP, TLR5	1,444/866	Swedish	Prostate cancer	MDR; p=0.019	Xu, 2005[147]
IRAK4, TLR1	1,383/780	Swedish	Prostate cancer	LR; p=0.03	Sun, 2006 [108]
TLR10, TLR6, IRF3	279/535	African American, Jamaican	Prostate cancer	MDR; p=0.001	Rogers, 2013 [31]
TLR2, IRAK4	279/535	African American	Prostate cancer	MDR; p=0.001	Rogers, 2013 [31]
CCR7, CCL5, CCR9	279/535	African American, Jamaican	Prostate cancer	MDR; p=0.001	Jones, 2011 [150]
CCR6, CCL5, CCR9	279/535	African American	Prostate cancer	MDR; p=0.001	Kidd, 2012 Unpublished data
CCR6, CCR4, CXCR7	279/535	Jamaican	Prostate cancer	MDR; p=0.017	Kidd, 2012 Unpublished data

n/a: Not available

To overcome the challenges and gaps of the aforementioned studies, our lab evaluated complex interactions among 119 inflammatory and immune response sequence variants in relation to PCA among men of African descent. We evaluated 1-, 2- and 3-factor gene-gene interaction models among 119 inflammatory and immune response-related sequence variants using a supercomputer with 24 computer processing units (CPUs). Gene-gene interactions were examined using MDR to identify interaction models with high cross validation consistency values, ranging between 80-100%, and accurate prediction scores (e.g., $ATA \geq 50\%$). As previously reported, our lab used multifactor-dimensionality reduction coupled with entropy graphs to identify, validate and visualize joint effects of inflammatory and immune response sequence variants as PCA predictors. MDR is a non-parametric method designed to detect higher order gene-gene interactions involving 2 or more loci with relatively small sample sizes (≥ 200 cases, ≥ 200 controls) for case-control studies, which is beyond the capacity of LR. It also has adequate statistical power to detect significant 2-4-factor gene-gene interactions in the presence of small sizes (200 cases, 200 controls). MDR analyses can be adjusted for multiple hypothesis testing using permutation testing, which minimizes spurious findings due to chance. In addition, this non-parametric method has the capacity to adjust for co-founders (e.g., age) and utilizes entropy graphs to interpret the effect of gene-gene interactions. Entropy graphs indicate which factors within a gene-gene interaction is synergistic or not through connecting color-coded lines between each SNP. The color scheme is as follows: red (highly synergistic), orange (moderately synergistic), green (moderately redundant), blue (highly redundant), and gold (neither synergistic/redundant).

The current version of MDR does not require an equal number cases and controls to predict high order interactions. For instance, our lab reported on gene-gene interactions among inflammatory and immune response-related sequence variants among men of African descent [31]. We observed that the age-adjusted three-factor (*TLR10 rs11096957*, *TLR6 rs2381289*, *IRF3 rs2304206*) interaction model was the best predictor of prostate cancer risk due to its high prediction accuracy score (ATA) of 65% and cross validation value (CVC) of 100% among men of African descent from the U.S. and Jamaica (Permutation testing p-value = 0.001) [31]. The synergy of this three-factor model was primarily driven by the information gain scores of *TLR6 rs2381289* (IG = 1.71%) and the pairwise interaction between *TLR10 rs11096957* and *IRF3 rs2304206* (IG = 1.75%) (see **Chapter 3 Results**). However when MDR analysis was restricted to U.S. men, the age-adjusted 2-factor model, *IRAK4 rs4251545* and *TLR2 rs1898830*, was found to be the best predictor of prostate cancer risk based upon 100% CVC and 62% ATA values (Permutation testing p-value = 0.001). The MDR-based entropy graph displayed a highly synergistic pairwise interaction between *IRAK4 rs4251545* and *TLR2 rs1898830* with an information gain (IG) score of 2.33%. Due to the information score exceeding the mutual information gain scores of each loci, this information gain score was regarded as informative. For the Jamaican men, only the one-factor model of *TLR6 rs2381289* survived multiple hypothesis testing and therefore selected as the best predictor of PCA susceptibility. MDR analysis for chemokine-associated sequence variants showed that the age-adjusted three-factor model (*CCR7 rs3136685*, *CCL5 rs3817655*, *CCR9 rs41289608*) was selected as the best interaction model based upon 100% CVC and 67% ATA values among men of African descent (see **Chapter 4 Results**). However within this interaction,

the MDR entropy-based graph revealed no strong synergistic interaction due to none of the pairwise interactions yielding informative information gain scores. Similarly, the age-adjusted three-factor model (*CCR6* rs3093024, *CCL5* rs3817655, *CCR9* rs41289608) among U.S. men were statistically significant with 69% ATA score and 100% CVC value in relation to prostate cancer (Permutation testing p-value = 0.001). Moreover, the age-adjusted three-factor model (*CCR6* rs3093024, *CCR4* rs6550178, *CXCR7* rs7559855) was associated with a 90% CVC value and 70% ATA score among Jamaican men (Permutation p-value = 0.017). Within this interaction, the MDR entropy-based graph showed a moderately synergistic interaction between SNPs in *CCR6* and *CXCR7* with an informative pairwise interaction IG score of 3.41% and *CCR4* rs6550178 with the highest individual IG score of 8.2%.

Among the cytokine-associated gene-gene interactions, a three-factor model (*IL1R2* rs11886877, *IL10* rs1800896, *IL10RA* rs4252243) was selected as the best predictor of PCA risk among men of African descent based upon a 61% ATA score (Permutation testing p-value = 0.003) (see **Chapter 5 Results**). This model displayed a highly synergistic interaction with an informative IG score of 0.86% for the pairwise interaction between *IL1R2* rs11886877 and *IL10* rs1800896. MDR analysis restricted to U.S. men showed a significant three-factor interaction model (*IL1B* rs1143627, *IL1A* rs1800587, *IL1RN* rs315951) with a 100% CVC value and 67% ATA score (Permutation testing p-value = 0.001). The synergy of this three-factor interaction model was based upon an informative mutual information gain score for *IL1B* rs1143627 and *IL1A* rs1800587 pairwise interaction (IG = 1.93%) (Data not shown). Among Jamaican men, the two-factor model (*IL1RN* rs315951 and *IL1R1* rs949963) was selected as the best

predictor of PCA risk with a 100% CVC value, 65% ATA score, permutation testing p-value of 0.008 and informative pairwise mutual information gain score of 4.77% (Data not shown). However, interaction models predicted in relation to prostate cancer for Jamaican men must be further validated due to limited sample size of Jamaican population.

Gene-gene interactions among genetic variants are essential to understanding the complex genetic architecture of prostate cancer. Further investigation of these interactions using a pooled multi-center study may lead to the identification of polymorphisms related to PCA risk within racially/ethnically diverse subgroups and disease progression. Such efforts may lead to the detection of validation of SNP signatures that may help to explain the higher PCA tumor burden among men of African descent relative to their Caucasian counterparts. To further investigate whether modification of a combination of gene targets (e.g., CCL5, CCR5) may impact PCA behavior (e.g., cell migration, proliferation, invasion, metastasis) using appropriate *in vitro* and *in vivo* studies. Moreover, it will also be very valuable to assess the effect of altered expression (i.e., overexpression or knockout) of inflammatory and immune response associated gene products (e.g., TLR, chemokine, cytokine) in prostate tumorigenesis. For instance silencing of CXCL1 and CXCL2 inhibits cell growth and induces apoptosis in PC3 cells [80]. Ultimately, the investigation of inflammatory and immune-response associated genes will help to understand how these genes play a role in prostate tumorigenesis.

Significance

Currently, physicians diagnose patients with prostate cancer based upon tumor biopsies (e.g., needle or TUR), pathological analyses of tumor traits (e.g., tumor size, grade, stage), a blood test (e.g., PSA levels) and digital rectal exams. However, these standard methods fail to accurately distinguish between non-aggressive and aggressive disease. The inability of physicians to determine which patients should receive a biopsy and aggressive therapy will lead to unnecessary and costly clinical management of prostate cancer. The identification of high-risk alleles associated with inflammatory and immune-response genetic variants may help to improve early detection of PCA and ultimately aggressive disease. In addition, genetic variants may be used to develop a genetic kit, which consist of a high risk SNP panel, to identify high-risk individuals who should receive aggressive prostate cancer therapy. The development of genetic kits will be very marketable for translational medicine and may prove to replace or enhance current diagnostic techniques. In addition, scientists will want to understand the effect of conventional drugs on people with high-risk alleles. Ultimately, genetic kits may help reduce the use of invasive diagnostic techniques (e.g., tissue biopsy) for cancer diagnosis, improve the decisions of physician about treatment options for patients, and guide the discovery of new therapeutic targets.

Over the years, cancer researchers have utilized genetic alterations associated with inflammatory and immune response genes to identify potential therapeutic agents and treatment strategies for cancer. Drug agonists and antagonists of toll-like receptors, chemokines, and cytokines are being evaluated in various clinical trials as potential anti-cancer therapeutic strategies, as summarized in **Table 2**. TLR signaling plays a critical role in immune response surveillance, which detect pathogens and tumor cells. TLR

agonists have been studied as anti-tumor drugs in relation to tumorigenesis. For instance, Sohn and associates (2014) observed that TLR7 agonist, imiquimod, induces apoptosis in the squamous cell carcinoma cell line, SCC12, through the suppression of A20 protein expression and activation of JNK [151]. TLR7 agonists, imiquimod and loxoribine, stimulate pro-inflammatory cytokine (IL6) and chemokine (CXCL1, CXCL2, CCL2) production in TRAMP-C2 cells (mouse prostate cancer cell line) via elevation of TLR7 gene and protein expression [62]. In particular, loxoribine was shown to inhibit the growth of TRAMP-C2 cells primarily through TLR7 signaling. Thus, TLR7 agonists may possess anti-tumorigenic activity against prostate cancer tumorigenesis. TLR9 is a member of the innate immune system, which makes it a great target for candidate anti-cancer therapeutic agents. Wakayama Medical University conducted a Phase I and II clinical trial to treat advanced recurrent esophageal cancer patients with a combination of novel tumor specific epitope peptides and TLR9 agonist, CpG7909 (Trial #: NCT00669292). Several studies have shown elevated TLR9 protein expression in poorly differentiated prostatic tumors and strongly associated with poor prognosis of prostate cancer [152, 153]. Moreover, the TLR9 agonist, CpG-ODN, stimulates PC3 cells to secrete immunosuppressive cytokines, IL8 and TGF β 1[154].

The National Center of Tumor Diseases in Heidelberg, Germany has a Phase I clinical trial in progress studying the effect of CCR5 blockage on tumor cells in colorectal metastases. CCR5 inhibitor, maraviroc, is used to treat colorectal cancer metastasis in 12 patients with an 8-week follow-up time (Trial #: NCT01736813). Ochoa and co-workers (2013) demonstrated that maraviroc reduces liver injury, weight loss and tumor size in CDE-treated mice with hepatocellular carcinoma [155]. In another study,

Velasco and colleagues (2012) observed that CCR5 antagonists (maraviroc and vicriviroc) decreased both cellular invasion and tumor metastases on the lung in breast cancer metastasis *in vivo* using NOD/SCID mice [156]. In a non-randomized Phase I/II clinical trial completed in 2010, Biokine Therapeutics Ltd evaluated a CXCR4 antagonist, BKT-140, as a novel treatment against multiple myeloma (Trial# NCT01010880). BKT-140 has a synergistic effect with Granulocyte-colony stimulating factor (G-CSF) to mobilize white blood cells and reduce anemic episodes.

Expansion of the knowledge on the role of inflammatory and immune response-related genes in prostate cancer tumorigenesis will provide guidance in the design of personalized medicine for cancer patients. Nonetheless, observational studies must evaluate all possible inflammatory and immune response signaling pathway targets. Further investigation of the inflammatory and immune-response signaling pathway may lead to the identification of novel and more effective targets to improve clinical management of prostate cancer.

Table 2. Current Inflammatory and Immune Response Gene Selective Drugs in Clinical Trials

Target	Compound	Company	Status	Trial #
CCR5	Maraviroc	National Center for Tumor Diseases, Heidelberg	Phase I	NCT01736813
CXCR4	BKT140	Biokine Therapeutics Ltd	Phase I/II	NCT01010880
TLR	gp100, R848 gel, MAGE-3	M.D. Anderson Cancer Center	Phase II	NCT00960752
TLR7	Imiquimod	New York University School of Medicine	Phase I/II	NCT01421017
TLR8	VTX-2337	University of Washington	Phase I	NCT01334177
TLR9	CpG7909	Wakayama Medical University	Phase I/II	NCT00669292
IL15	IL15	National Institutes of Health Clinical Center, National Cancer Institute	Phase I/II	NCT01369888

The following sections of this master's thesis will be organized into chapters 2 through 6. Chapter 2 describes the methods used to analyze main and joint effects of inflammatory and immune response related sequence variants. Next, research findings will be divided into 3 separate chapters for each signaling pathway. Chapter 3 describes the key logistic regression modeling and MDR analysis findings for the toll-like receptor signaling pathway. Chapter 4 highlights the key logistic regression and MDR analysis findings for the chemokine signaling pathway. Chapter 5 describes the key logistic regression modeling and MDR analysis findings for the cytokine signaling pathway. Lastly, Chapter 6 consists of the master's thesis summary, conclusion and clinical relevance.

Research Hypothesis

The proposed research hypothesizes that the inheritance of one or more high-risk alleles in inflammatory and immune response genes involved in the toll-like receptor, chemokine and cytokine signaling pathways will modify prostate cancer susceptibility among men of African descent.

Specific Aims

The following two specific aims will test the proposed research hypothesis:

Specific Aim1: Determine main effects of inflammatory and immune response related sequence variants on prostate cancer susceptibility among men of African descent. Sequence variants within inflammatory and immune response genes may influence the interaction among the toll-like receptor, cytokine-cytokine receptor and chemokine-chemokine receptor signaling pathways. These variants may impact toll-like receptor surveillance in innate immunity, chemokine and cytokine production during chronic inflammation and mobilization of inflammatory immune cells within the inflammatory and immune response signaling pathway. Regulation of the toll-like receptor, chemokine and cytokine signaling pathways are pivotal to the balance between anti- and pro-inflammatory signals released from immune cells. Genetic alterations that interfere with the operation of the inflammatory and immune response pathways may elevate pro-inflammatory signals leading to chronic prostatitis (chronic inflammation) and initiation of prostate cancer development.

Specific Aim 2: Evaluate gene-gene interactions among inflammatory and immune response related sequence variants among U.S. and Jamaican men. The effect of single sequence variants may not fully explain the role of inflammatory and immune response-related genes in prostate cancer development and progression. Therefore, the investigation of multiple sequence variants is needed to identify important PCA predictors. Multi-locus tools such as MDR can detect high order (≥ 2) interactions among sequence variants to elucidate the complexity of the roles these pathways and target genes predispose individual to prostate cancer risk. The interplay between

genes within the inflammatory and immune response pathway will provide insight to the complex mechanisms that feed into prostate cancer development. Gene-gene interactions can negatively or positively influence the translation of protein product and expression on various cell types. Immune cells (e.g., macrophages, leukocytes) express toll-like receptors on the extracellular membrane to detect bacterial infection and pathogens. Cytokine and chemokine receptors are also expressed on immune cells and utilized to detect their respective ligands and direct cell-cell communications. The inheritance of sequence variants within inflammatory and immune response associated genes may render non-functional proteins.

CHAPTER 2

METHODOLOGY

Study Population

The current study consisted of two independent case-control study sets with participants from the Prostate Cancer Clinical Outcome (PC²O) Study and the Prostate Cancer Case-Control study (**Table 3, 4, and 5**). Among all 814 men of African descent, germ-line DNA samples were collected for 279 PCA cases and 535 disease-free men, as shown in Table 1. In the PC²O Study, 603 unrelated men of African descent were recruited from Columbia, South Carolina and Howard University Hospital (HUH) Division of Urology in Washington, D.C. or related PCA screening programs between 2001 and 2005. This population of men of African descent (i.e., self-reported African Americans, East African Americans, West African Americans, and Afro-Caribbean Americans) consisted of 170 incident PCA cases and 433 controls (**Table 4**). Between March 2005 and July 2007, two hundred twenty-one unrelated Jamaican men were recruited and consecutively enrolled into a prostate cancer case-control study (109 prostate cancer cases, 102 controls) during their first time visit at urology clinics (**Table 5**). Details on case and control ascertainment and inclusion criteria for both sub-populations have been detailed elsewhere [157, 158].

Table 3. Study population characteristics among men of African descent from the U.S. and Jamaica.

Characteristics	Cases	Controls	p value ^a
Number of Participants, n	279	535	---
Age at enrollment (yrs), Median (range)	67 (45-91)	53 (27-89)	<0.0001
Family History of Prostate Cancer ^b , n (%)			
Yes	35 (16.1)	21 (12.5)	0.316
No	182 (83.9)	147 (87.5)	
Missing	62 (22.2)	367 (68.6)	
PSA (ng/ml), median (range)	11.7 (0.01-10,000)	0.9 (0.0-4.0)	<0.0001
PSA ^b (ng/ml), n (%)			
< 4	37 (13.8)	517 (99.8)	<0.0001
≥ 4	231 (86.2)	1 (0.2)	
Missing	11 (0.04)	17 (0.03)	
Gleason Score ^b , n (%)			
4	12 (5.6)		
5	14 (6.5)		
6	74 (34.2)		
7	70 (32.4)		
8	18 (8.3)		
9	22 (10.2)		
10	6 (2.8)		
Missing	63 (22.6)		
Global WAA ^b , median (range)	0.79 (0.25-0.94)	0.767 (0.25-0.95)	0.107

Abbreviations: PSA, prostate specific antigen; WAA, West African Ancestry; ^aChi-square test of homogeneity was used to determine whether the prevalence of family history or high PSA levels (PSA ≥ 4 ng/mL) vary between cases and controls; ^bWilcoxon Sum Rank Test was used to examine whether differences exist within the median age (yrs), PSA (ng/ml) and Global WAA between cases and controls.

Table 4. Baseline Characteristics among men of African Descent from the U.S.

Characteristics	Cases	Controls	p value ^a
Number of Participants, n	170	433	---
Age at diagnosis (yrs), Median (range)	65 (45-91)	51 (27-89)	<0.0001
Family History of Prostate Cancer ^b , n (%)			
Yes	18 (16.7)	9 (13.6)	0.592
No	90 (83.3)	57 (86.4)	
Missing	62 (36.5)	367 (84.8)	
PSA ^b (ng/ml), median (range)	7.0 (0.01-5,000)	0.9 (0.0-3.9)	<0.0001
PSA (ng/ml), n (%)			
< 4	37 (23.1)	416 (100.0)	<0.0001
≥ 4	123 (76.9)	0 (0.0)	
Missing	10 (5.9)	17 (1.6)	
Gleason Score ^b , n (%)			
4	12 (11.1)		
5	14 (13.0)		
6	29 (26.9)		
7	32 (29.6)		
8	5 (4.6)		
9	12 (11.1)		
10	4 (3.7)		
Missing	62 (36.5)		
Global WAA ^b , mean (SD)	0.79 (0.25-0.94)	0.77 (0.25-0.94)	0.107

Abbreviations: PSA, prostate specific antigen; ^aDifferences in frequencies were tested by a Chi-square test of homogeneity or Fisher's Exact Test; ^bDifferences in median age (yrs) between cases and controls were tested using the Wilcoxon Sum Rank test.

Table 5. Baseline Characteristics among men from Jamaica

Characteristics	Cases	Controls	p value ^a
Number of Participants, n	109	102	---
Age at diagnosis (yrs), Median (range)	70 (49-80)	60 (40-80)	<0.0001
Family History of Prostate Cancer ^b , n (%)			
Yes	17 (15.6)	12 (11.8)	0.272
No	92 (84.4)	90 (88.2)	
Missing	0 (0.0)	0 (0.0)	
PSA ^b (ng/ml), median (range)	35.0 (4.0-10,000)	1.2 (0.2-4.0)	<0.0001
PSA (ng/ml), n (%)			
< 4	0 (0.0)	101 (99.0)	<0.0001
≥ 4	108 (100.0)	1 (1.0)	
Missing	0 (0.0)	0 (0.0)	
Gleason Score ^b , n (%)			
4	---		
5	---		
6	45 (41.7)		
7	38 (35.2)		
8	13 (12.0)		
9	10 (9.3)		
10	2 (1.8)		
Missing	1 (0.01)		

Abbreviations: PSA, prostate specific antigen; ^aDifferences in frequencies were tested by a Chi-square test of homogeneity or Fisher's Exact Test; ^bDifferences in median age (yrs) between cases and controls were tested using the Wilcoxon sum Rank test.

Criteria for Inflammatory and Immune Response Gene and SNP selection

Inflammatory and immune response-associated genes and SNPs were selected using one or more of following criteria: (1) epidemiological or molecular biological evidence from published reports indicating a relationship between the SNP/gene with cancer or inflammatory/immune response related diseases; (2) commonly studied loci; (3) marked disparities in genotype frequency comparing men of African descent to their Caucasian counterparts (i.e., $\pm 10\%$ change); (4) evidence demonstrating a link between the SNPs and alterations in mRNA expression/stability or protein expression/structure or function using *in silico* tools (e.g., SNPinfo) or published reports (<http://snpinfo.niehs.nih.gov/snpfunc.htm>); and (5) a minor allele frequency $\geq 1\%$ reported in the National Center for Biotechnology Information (NCBI) Entrez SNP, (www.ncbi.nlm.nih.gov). On average, a majority of the SNPs had minor allele frequencies ranging from 22-27%. However, eight SNPs with allele frequencies greater than 1% but less than 5% were included in the analysis to explore whether rare SNPs would lead to substantial gains in effect sizes (i.e., 2-3 fold increases in risk) and contribute to the missing genetic heritability [159, 160]. The SNPinfo webserver enabled us to annotate and/or predict the functional consequence of inflammatory and immune response associated sequence variants on altered alleles relative to referent alleles, as summarized in the **Functional Consequence Tables 6-8**.

Quality Control Assurance and Data Management of Genotype data

At the onset of the project, allelic discrimination focused on inflammatory and immune response associated SNPs among men of African Descent. To minimize misclassification bias, laboratory technicians were blinded to the case status of study

participants. Each batch of up to 96 samples included four non-DNA template controls and eight duplicate samples, which enabled us to calculate the percent contamination (i.e., 0%) and concordance rates (i.e., 100%) per batch and for the entire data set, respectively. Genotype call rates were calculated separately for each SNP and study participant. Lastly, we tested whether the distribution of the genotypes among disease-free individuals had significant departures from the Hardy-Weinberg equilibrium (HWE).

Prior to performing marker statistics, we excluded subjects who had genotype call rates that were <90%. To ensure high quality data, nine SNPs were excluded from the final analysis if: the distribution of the genotypes among controls deviated substantially from the Hardy-Weinberg Equilibrium, using a conservative significance level cut-off value of $P \leq 0.005$; they had a minor allele frequency that was < 1%; or the genotype call rates fell below 95%. Following data clean up, 119 inflammatory and immune-related SNPs were included in the final analysis consisting of 814 men of African descent (279 cases, 535 controls). All quality control analyses and data management was performed using Golden Helix's SNP Variation Software 7.0 (Bozeman, MT).

Genetic Analysis of Variant Inflammatory and Immune Response-Associated SNPs

De-identified germ-line DNA was obtained from incident PCA male cases (n = 279) and controls (n = 535). SNPs detected in *TLRs* (1, 2, 4, 6, and 10), *IRAK4*, *TOLLIP*, *IRF3*, *CCLs* (1,2,5,7,11,17,21,24,25, and 26), *CCRs* (4,5,6, 7, and 9), *CXCL12*, *CXCRs* (5 and 7), *ILs* (1, 8, 10), *TNF*, *RNASEL* were genotyped using Illumina's GoldenGate genotyping assay system combined with Veracode Technology (Illumina, Inc., San Diego, CA). Allelic discrimination was performed using a BeadXpress Reader (Illumina, Inc., San Diego, CA) according to the manufacturer's instructions [161].

Table 6. Functional consequence of TLR-associated sequence variants^a.

Gene dbSNPID Location	Allele	Chr	Nucleotide Change	Amino acid change	Predicted Functional Consequence	Major	Minor	NCBI EA	NCBI AA	Current MAF Study Total Population	Current MAF Study U.S.	Current MAF Study Jamaican
IRAK4 rs4251467 Intron 3	C/T	12	C>T			T	C	0.932	0.926	0.062	0.058	0.078
IRAK4 rs4251473 Intron 5	A/C	12	A>C			C	A	0.951	0.76	0.202	0.204	0.195
IRAK4 rs4251520 Intron 9	C/T	12	C>T			T	C	0.88	0.812	0.184	0.176	0.220
IRAK4 rs4251545 Exon 8	A/G	12	A>G	Ala>Thr	Splice Site benign	G	A	0.937	0.625	0.300	0.304	0.282
IRF3 rs2304206 5'UTR		19	G>A		TFBS			0.727	0.421	0.384	0.388	0.368
IRF3 rs968457 Near Gene'5	T/C	19	T>C		Splice Site nsSNP, benign	C	T	1	0.85	0.080	0.070	0.123
TLR1 rs3923647 Exon 1	T/A	4	T>A		nsSNP probably damaging	T	A	0.913	0.917	0.068	0.058	0.109
TLR1 rs4624663 3'UTR	T/C	4	T>C			T	C	0.973	0.938	0.068	0.064	0.089
TLR1 rs4833095 Exon 1	C/T	4	C>T		nsSNP	C	T	0.273	0.646	0.198	0.208	0.152
TLR1 rs5743595 Intron 2	G/A	4	G>A			A	G	0.753	0.958	0.029	0.033	0.010
TLR1 rs5743604 Intron 3	G/A	4	G>A			A	G	0.729	0.542	0.450	0.470	0.368
TLR2 rs1898830 Intron 1	A/G	4	A>G			A	G	0.628	0.833	0.136	0.359	0.327
TLR2 rs3804099 Exon 1	C/T	4	C>T			C	T	0.586	0.354	0.382	0.256	0.181
TLR2 rs7656411 Near Gene'3	G/T	4	G>T			G	T	0.792	--	0.423	0.017	0.015

Gene dbSNPID Location	Allele	Chr	Nucleotide Change	Amino acid change	Predicted Functional Consequence	Major	Minor	NCBI EA	NCBI AA	Current MAF Study Total Population	Current MAF Study U.S.	Current MAF Study Jamaican
TLR2 rs7656411 Near Gene'3	G/T	4	G>T			G	T	0.792	--	0.423	0.017	0.015
TLR4 rs2149356 Intron 3		9	T>G					0.707	0.304	0.242	0.370	0.340
TLR4 rs1927906 Near Gene'3	T/C	9	T>C			T	C	0.94	0.604	0.449	0.191	0.186
TLR4 rs1927911 Intron 1	G/A	9	G>A			G	A	0.743	0.542	0.353	0.256	0.196
TLR4 rs4986791 Exon 3	C/T	9	C>T		nsSNP possibly damaging	C	T	0.959	0.938	0.017	0.070	0.083
TLR4 rs5030710 Exon 3	C/T	9	C>T		Splice Site	T	C	1.000	0.729	0.149	0.097	0.025
TLR6 rs1039559 Intron 1	G/A	4	G>A		TFBS	A	G	0.513	0.771	0.190	0.227	0.255
TLR6 rs2381289 3'UTR	G/A	4	G>A			G	A	0.587	0.771	0.245	0.494	0.435
TLR6 rs3821985 Exon 1	G/C	4	G>C			C	G	0.722	0.423	0.364	0.493	0.436
TLR6 rs5743810 Exon 1	G/A	4	G>A		nsSNP benign	G	A	0.588	0.907	0.072	0.031	0.015
TLR6 rs5743818 Exon 1	C/A	4	C>A			A	C	0.761	0.839	0.083	0.032	0.025
TLR10 rs10776483 Exon 1	G/A	4	G>A			A	G	0.707	--	0.232	0.134	0.142
TLR10 rs11096955 Exon 1	T/G	4	T>G		nsSNP benign	T	G	0.408	--	0.492	0.382	0.382
TLR10 rs11096957 Exon 1	T/G	4	T>G		nsSNP possibly damaging	G	T	0.408	--	0.493	0.424	0.421
TLR10 rs11466640 Intron 1	G/A	4	G>A			G	A	0.757	0.957	0.028	0.098	0.079

Gene dbSNPID Location	Allele	Chr	Nucleotide Change	Amino acid change	Predicted Functional Consequence	Major	Minor	NCBI EA	NCBI AA	Current MAF Study Total Population	Current MAF Study U.S.	Current MAF Study Jamaican
TLR10 rs4274855 5'UTR	T/C	4	T>C			C	T	0.747	0.958	0.031	0.455	0.425
TOLLIP rs3168046 3'UTR	G/A	11	G>A		miRNA	G	A	0.568	0.568	0.422	0.428	0.397
TOLLIP rs5743867 Intron 1	G/A	11	G>A			A	G	0.92	0.854	0.201	0.202	0.197
TOLLIP rs5743899 Intron 1	T/C	11	T>C			T	C	0.892	0.652	0.428	0.419	0.465

Abbreviations: Chr, chromosome; UTR, untranslated region; TFBS, transcription factor binding site, miRNA, micro RNA; nsSNP, nonsynonymous SNP; EA, European-American; AA, African-American; ^aTLR-related SNPs were analyzed among men of African Descent.

Table 7. Functional Consequence of Chemokine-Associated Sequence Variants^a.

Gene dbSNP ID Location	Chr	Location	Nucleotide	Amino Acid Change	Predicted Functional Consequence	Current Study MAF
CCR6 rs1012656	6	5'UTR			TFBS	C=19.5
CCL2 rs1024611	17	Near gene 5'			TFBS	C=19.7
CXCR7 rs1045879	2	Exon 1	T>C	Leu266Leu		G=30.7
CCL17 rs11076191	16	Intron 1			TFBS	A=2.3
CCL21 rs11574914	9	Near gene 5'			TFBS	A=9.1
CCL21 rs11574915	9	5'UTR			TFBS, Splicing	C=10.6
CCL21 rs11574916	9	3'UTR			TFBS, miRNA	A=5.5
CCR9 rs12721497	3	Exon 3	G>A	Met284Val	nsSNP, benign	G=1.9
<i>CCR9</i> <i>rs1488371</i>	3	<i>Intron 2</i>				<i>A=15.6</i>
CCR6 rs1556413	6	Near gene 5'			TFBS	A=20.2
CCL7 rs17809012	17	Near gene 5'			TFBS	G=34.6
CXCL12 rs17880777	10	Near gene 5'			TFBS	C=17.4
CCR5 rs1799987	3	Intron 1			TFBS	A=40.3
<i>CCR5</i> <i>rs1799988</i>	3	<i>5'UTR</i>			<i>TFBS</i>	<i>G=41.9</i>
CCR5 rs1800024	3	Intron 2			TFBS	A=19.8
CXCL12 rs1801157	10	3'UTR	-	-	<u>miRNA</u>	A=6.3
CCR6 rs2023305	6	Near gene 5'			TFBS	A=16.8

Gene dbSNP ID Location	Chr	Location	Nucleotide	Amino Acid Change	Predicted Functional Consequence	Current Study MAF
CCL25 rs2032887	19	Exon 3	G>A	His101Arg	Splicing, probably damaging	G=27.4
CCL5 rs2107538	17	Near gene 5'			TFBS	A=46.6
CCR5 rs2227010	3	Near gene 5'			TFBS	G=19.8
CCL17 rs223895	16	Intron 1				A=29.9
CCL5 rs2280789	17	Intron 1			TFBS	G=22.3
CCL1 rs2282691	17	Intron 2				A=46.2
CCR9 rs2286486	3	Near gene 5'			TFBS	G=25.9
CCL24 rs2302004	7	Intron 1			TFBS	G=41.9
CCL26 rs2302009	7	3'UTR			miRNA	C=40.7
CXCL12 rs266093	10	3'UTR			miRNA	G=42.9
CCL21 rs2812378	9	Near gene 5'			TFBS	G=41.9
CXCL12 rs2839685	10	Near gene 5'			TFBS	A=31.4
CXCL12 rs2839695	10	3'UTR			miRNA	G=17.5
CCR6 rs3093023	6	Near gene 5'				A=13.8
CCR6 rs3093024	6	Intron 1				A=32.3
CCR7 rs3136685	17	Intron 1				G=49.5

Gene dbSNP ID Location	Chr	Location	Nucleotide	Amino Acid Change	Predicted Functional Consequence	Current Study MAF
CCR7 rs3136687	17	Intron 1				A=44.4
CCL5 rs3817655	17	Intron 2			TFBS	A=46.5
CCR9 rs41289608	3	5'UTR			TFBS, Splicing	A=36
CCL11 rs4795896	17	Near gene 5'			TFBS	G=4.8
CXCR5 rs523604	11	Intron 1				A=34.9
CCR4 rs6550178	3	Intron 1			TFBS	A=17.2
CCL25 rs7259568	19	Near gene 5'			TFBS	A=9.3
CXCR7 rs7559855	2	Intron 1				A=38.5
CCR9 rs7613548	3	Near gene 5'			TFBS	A=44
CCR4 rs7632357	3	Near gene 5'			TFBS	C=41.6

Abbreviations: Chr, chromosome; UTR, untranslated region; TFBS, transcription factor binding site, miRNA, micro RNA; nsSNP, nonsynonymous SNP; ^aForty-three chemokine-related SNPs were analyzed among men of African Descent. SNPs denoted in bold had significant risk estimates associated with PCA risk in the total population. Italicized SNPs were significantly associated with PCA risk in the Jamaican population. Columns shaded in gray symbolize SNPs that were significantly associated with PCA risk in the U.S. population.

Table 8. Functional Consequence of Inflammatory-Associated Sequence Variants.

Gene dbSNPID Location Functional Consequence	NCBI Nucleotide Change (Major> Minor allele)	NCBI AA MAF (%)	NCBI AA Major/Major, Major/Minor, Minor/Minor Genotypes n(%)	Current Study Nucleotide Change (Major> Minor allele)	Current Study AA MAF (%)	Current Study AA Major/Major, Major/Minor, Minor/Minor Genotypes n (%)	Overall χ^2 p-value NCBI AAs vs Current Study AAs ^{††}
IL10RB rs1058867* 3'UTR miRNA	G>A	A=37.9	26 (42.0) 25 (40.3) 11 (17.7)	G>A	A=33.9	239 (44.6) 229 (42.9) 67 (12.5)	0.514
IL1B rs1071676* 3'UTR miRNA (miRanda and Sanger)	G>C	C=14.6	19 (79.2) 3 (12.5) 2 (8.30)	G>C	C=16.1	378 (70.7) 142 (26.5) 15 (2.80)	0.106
IL1R2 rs1123902* Intron 1	A>C	C=31.8	10 (45.5) 10(45.5) 2 (9.10)	A>C	C=30.7	258 (48.2) 225 (42.1) 52 (9.70)	0.949
IL8RB rs1126579* 3'UTR miRNA	C>T	T=14.5	46 (74.2) 14 (22.6) 2 (3.20)	G>A [†]	A=13.8	402 (75.1) 118 (22.1) 15 (2.80)	0.886
IL1B rs1143627* Near gene 5' TFBS	C>T	T=37.5	12 (50.0) 6 (25.0) 6 (25.0)	G>A [†]	A=39.6	194 (36.3) 256 (48.2) 83 (15.5)	0.063
IL1B rs1143634*	C>T	T=12.9	47 (75.8) 14 (22.6) 1 (1.60)	G>A [†]	A=15.5	381 (71.2) 142 (26.5) 12 (2.30)	0.833
IL8RB rs11574752* 3'UTR miRNA	G>A	A=10.4	19 (79.2) 5 (20.8) 0 (0.00)	G>A	A=9.40	435 (81.3) 99 (18.5) 1 (0.20)	0.798
IL1R2 rs11886877 Intron 1	---	---	---	G>A	A=35.8	211 (39.4) 265 (49.5) 59 (11.1)	

Gene dbSNPID Location Functional Consequence	NCBI Nucleotide Change (Major> Minor allele)	NCBI AA MAF (%)	NCBI AA Major/Major, Major/Minor, Minor/Minor Genotypes n(%)	Current Study Nucleotide Change (Major> Minor allele)	Current Study AA MAF (%)	Current Study AA Major/Major, Major/Minor, Minor/Minor Genotypes n (%)	Overall χ^2 p-value NCBI AA vs Current Study AA ^{††}
RNASEL rs12135247** 3'UTR TFBS, miRNA	T>C	C=16.3	33 (67.3) 16 (32.7) 0 (0.00)	A>G [†]	G=17.9	368 (68.8) 142 (26.5) 25 (4.70)	0.226
IL1R2 rs12328606** Near gene 5' TFBS	C>T	T=11.2	38 (77.6) 11 (22.4) 0 (0.00)	G>A [†]	A=13.5	405 (75.7) 116 (21.7) 14 (2.60)	0.798
IL1A rs1304037* 3'UTR miRNA	A>G	G=39.6	8 (33.3) 13 (54.2) 3 (12.5)	A>G	G=41.1	191 (35.7) 248 (46.4) 96 (17.9)	0.760
IL1B rs16944* Near gene 5' TFBS	A>G	G=39.0	18 (30.5) 36 (61.0) 5 (8.50)	A>G	G=45.1	156 (29.2) 275 (51.4) 104 (19.4)	0.108
IL1A rs17561* Exon 4 Splicing (ESE or ESS), nsSNP, benign	G>T	T=15.3	44 (71.0) 17 (27.4) 1 (1.60)	C>A [†]	A=18.6	358 (66.9) 155 (29.0) 22 (4.10)	0.698
TNF rs1799964* Near gene 5' TFBS	T>C	C=12.9	46 (74.2) 16 (25.8) 0 (0.00)	A>G [†]	G=16.5	374 (69.9) 145 (27.1) 16 (3.00)	0.492
IL1A rs1800587* 5'UTR TFBS, Splicing (ESE or ESS)	C>T	T=39.1	9 (39.1) 10 (43.5) 4 (17.4)	G>A [†]	A=41.8	183 (34.2) 257 (48.0) 95 (17.8)	0.885

Gene dbSNPID Location Functional Consequence	NCBI Nucleotide Change (Major> Minor allele)	NCBI AA MAF (%)	NCBI AA Major/Major, Major/Minor, Minor/Minor Genotypes n(%)	Current Study Nucleotide Change (Major> Minor allele)	Current Study AA MAF (%)	Current Study AA Major/Major, Major/Minor, Minor/Minor Genotypes n (%)	Overall χ^2 p-value NCBI AA vs Current Study AA ^{††}
TNF rs1800629* Near gene 5' TFBS	G>A	A=13.7	46 (74.2) 15 (24.2) 1 (1.60)	G>A	A=16.9	368 (68.8) 153 (28.6) 14 (2.60)	0.752
IL10 rs1800871* Near gene 5'TFBS	C>T	T=36.3	28 (45.2) 23 (37.1) 11 (17.7)	G>A [†]	A=40.7	188 (35.0) 258 (48.0) 89 (17.0)	0.219
IL10 rs1800872** Near gene 5' TFBS	C>A	A=38.8	19 (38.8) 22 (44.9) 8 (16.3)	C>A	A=40.7	188 (35.0) 258 (48.0) 89 (17.0)	0.874
IL10 rs1800893* Near gene 5' TFBS	G>A	A=37.1	22 (35.5) 34 (54.8) 6 (9.70)	G>A	A=36.4	216 (40.4) 248 (46.4) 71 (13.2)	0.419
IL10 rs1800896* Near gene 5' TFBS	A>G	G=40.5	7 (33.3) 11 (52.4) 3 (14.3)	A>G	G=33.3	243 (45.4) 228 (42.6) 64 (12.0)	0.491
IL1R1 rs2192752* Near gene 5' TFBS	A>C	C=4.80	56 (90.3) 6 (9.70) 0 (0.00)	A>C	C=5.60	477 (89.1) 56 (10.5) 2 (0.40)	1.000
IL8 rs2227532* Near gene 5' TFBS	T>C	C=9.70	50 (80.6) 12 (19.4) 0 (0.00)	A>G [†]	G=8.60	448 (83.7) 82 (15.3) 5 (1.00)	0.690
IL8 rs2227538* 5'UTR TFBS, Splicing (ESE or ESS)	C>T	T=17.7	41 (66.1) 20 (32.3) 1 (1.60)	G>A [†]	A=23.2	323 (60.4) 176 (32.9) 36 (6.70)	0.291

Gene dbSNPID Location Functional Consequence	NCBI Nucleotide Change (Major> Minor allele)	NCBI AA MAF (%)	NCBI AA Major/Major, Major/Minor, Minor/Minor Genotypes n(%)	Current Study Nucleotide Change (Major> Minor allele)	Current Study AA MAF (%)	Current Study AA Major/Major, Major/Minor, Minor/Minor Genotypes n (%)	Overall χ^2 p-value NCBI AA vs Current Study AA ^{††}
IL8 rs2227545* 3'UTR miRNA	A>C	C=8.70	19 (82.6) 4 (17.4) 0 (0.00)	A>C	C=8.50	449 (83.9) 81 (15.1) 5 (1.00)	0.812
IL10RA rs2229113* Exon 7 nsSNP, probably damaging	G>A	A=20.5	14 (63.7) 7 (31.8) 1 (4.50)	G>A	A=18.8	355 (66.4) 159 (29.7) 21 (3.90)	0.782
IL10RB rs2834167* Exon 2 Splicing (ESE or ESS), nsSNP, benign	A>G	G=16.9	44 (71.0) 15 (24.2) 3 (4.80)	A>G	G=11.0	423 (79.1) 106 (19.8) 6 (1.10)	0.050
IL1A rs2856836* 3'UTR miRNA	T>C	C=17.4	16 (69.6) 6 (26.1) 1 (4.30)	A>G [†]	G=18.6	358 (66.9) 155 (29.0) 22 (4.10)	1.000
IL10RA rs3135932* Exon 5 Splicing (ESE or ESS), nsSNP, benign	A>G	G=2.10	23 (95.8) 1 (4.20) 0 (0.00)	A>G	G=2.60	508 (95.0) 26 (4.80) 1 (0.20)	1.000
IL1RN rs315951* 3'UTR miRNA	C>G	G=47.9	8 (33.3) 9 (37.5) 7 (29.2)	C>G	C=48.0	119 (26.9) 268 (50.1) 123 (23.0)	0.482
RNASEL rs3738579* 5'UTR TFBS, Splicing (ESE or ESS)	T>C	C=16.7	14 (66.7) 7 (33.3) 0 (0.00)	A>G [†]	G=12.3	411 (76.8) 116 (21.7) 8 (1.50)	0.474

Gene dbSNPID Location Functional Consequence	NCBI Nucleotide Change (Major> Minor allele)	NCBI AA MAF (%)	NCBI AA Major/Major, Major/Minor, Minor/Minor Genotypes n(%)	Current Study Nucleotide Change (Major> Minor allele)	Current Study AA MAF (%)	Current Study AA Major/Major, Major/Minor, Minor/Minor Genotypes n (%)	Overall χ^2 p-value NCBI AA vs Current Study AA ^{††}
IL1R1 rs3917225* Near gene 5' TFBS	A>G	G=12.3	49 (80.3) 9 (14.8) 3 (4.90)	A>G	G=9.10	438 (81.9) 97 (18.1) 0 (0.00)	0.001
IL8 rs4073* Near gene 5' TFBS	A>T	T=26.6	34 (54.8) 23 (37.1) 5 (8.10)	T>A	A=20.9	335 (62.6) 176 (32.9) 24 (4.50)	0.262
IL1R2 rs4141134** Near gene 5' TFBS	T>C	C=11.2	39 (79.6) 9 (18.4) 1 (2.00)	A>G [†]	G=13.7	399 (74.6) 125 (23.4) 11 (2.00)	0.660
IL1RN rs4251961* Near gene 5' TFBS	T>C	C=20.2	37 (59.7) 25 (40.3) 0 (0.00)	A>G [†]	G=17.9	367 (68.6) 145 (27.1) 23 (4.30)	0.035
IL10RA rs4252243* Near gene 5' TFBS	C>T	T=32.5	9 (45.0) 9 (45.0) 2 (10.0)	G>A [†]	A=27.5	271 (50.7) 234 (43.7) 30 (5.60)	0.522
IL8RB rs4674257** Near gene 5' TFBS	G>A	A=25.0	14 (58.3) 8 (33.3) 2 (8.30)	G>A	A=20.1	347 (64.9) 161 (30.10) 27 (5.00)	0.468
IL8RB rs4674259* 5'UTR TFBS	A>G	G=23.9	12 (52.2) 11 (47.8) 0 (0.00)	A>G	G=20.0	349 (65.0) 158 (30.0) 28 (5.00)	0.176
RNASEL rs486907* Exon 1 nsSNP, benign	G>A	A=16.7	16 (66.7) 8 (33.3) 0 (0.00)	G>A	A=13.2	402 (75.1) 125 (23.4) 8 (1.50)	0.528

Gene dbSNPID Location Functional Consequence	NCBI Nucleotide Change (Major> Minor allele)	NCBI AA MAF (%)	NCBI AA Major/Major, Major/Minor, Minor/Minor Genotypes n(%)	Current Study Nucleotide Change (Major> Minor allele)	Current Study AA MAF (%)	Current Study AA Major/Major, Major/Minor, Minor/Minor Genotypes n (%)	Overall χ^2 p-value NCBI AA vs Current Study AA ^{††}
L1R2 rs6726713** Near gene 5' TFBS	C>T	T=11.2	38 (77.6) 11 (22.4) 0 (0.00)	G>A [†]	A=12.1	417 (78.0) 106 (19.8) 12 (2.20)	<i>0.689</i>
TNF rs673* Near gene 5' TFBS	G>A	A=13.7	45 (72.6) 17 (27.4) 0 (0.00)	G>A	A=17.4	364 (68.0) 156 (29.2) 15 (2.80)	<i>0.546</i>
IL10RB rs8178433* Near gene 5' TFBS	T>G	G=12.9	46 (74.2) 16 (25.8) 0 (0.00)	A>C [†]	C=12.4	408 (76.3) 121 (22.6) 6 (1.10)	<i>0.811</i>
IL1R1 rs949963* Near gene 5' TFBS	G>A	A=31.1	31 (50.8) 22 (36.1) 8 (13.1)	G>A	A=33.1	249 (46.5) 218 (40.8) 68 (12.7)	0.771
IL10RA rs9610* 3'UTR miRNA	A>G	G=41.9	20 (32.3) 32 (51.6) 10 (16.1)	A>G	G=33.9	237 (44.3) 233 (43.5) 65 (12.2)	0.184
IL10RB rs999788* Near gene 5' TFBS	C>T	T=19.5	40 (67.8) 15 (25.4) 4 (6.80)	G>A [†]	A=12.4	410 (76.6) 117 (21.9) 8 (1.50)	<i>0.026</i>

[†]The nucleotide change may vary relative to that reported in NCBI depending on whether the genotyping was performed using the sense or anti-sense DNA strand.

^{††}The chi-square test was used to assess differences in the overall genotype frequencies comparing men of African descent as reported in NCBI to those in the total population from the current study. P-values generated from the Fisher's exact test (in italics) were used when expected genotype counts were < 5 for either cases or controls. Abbreviations: UTR, untranslated region; TFBS, transcription factor binding site; nsSNP, non-synonymous coding SNP; miRNA, microRNA binding site;; MAF, Minor Allele Frequency NCBI, National Center for Biotechnology Information Entrez SNP

*NCBI AFR1 or African American Population Panel

**NCBI ASW Population Panel

Ancestry markers

Cases and controls were genotyped with a set of 100 genome-wide ancestry informative markers to correct for potential population stratification among our admixed population of self-reported African-Americans, West African-Americans, East African-Americans, Afro-Caribbean-Americans, as previously described [162, 163]. Individual genetic ancestry was determined using 100 ancestry identification markers (AIMs) for West African and European genetic ancestry as well as genotype data using the Bayesian Markov Chain-Monte Carlo method implemented in the STRUCTURE 2.1 program [162, 164]. Study participants were grouped from lowest to highest genetic West African Ancestry, with scores ranging from 0-100%. These 100 markers were assembled using DNA from self-identified African-Americans (Coriell Institute for Medical Research, n = 96), Yoruban West Africans (HapMap, n = 60), West Africans (Bantu and Nilo Saharan speakers, n = 72), Europeans (New York City, n = 24), and CEPH Europeans (HapMap Panel, n = 60), as previously reported [163]. Individuals with a West African ancestry (WAA) score $\geq 25\%$ and available inflammatory and immune response associated sequence variants were included in the final analysis.

Statistical Analysis for Single Gene Effects

Logistic regression (LR) modeling analyses were used to evaluate the relationship between 119 inflammatory and immune response SNPs among men of African descent and PCA risk. For each SNP, we examined the frequency differences in the distribution of homozygous major, heterozygous, or homozygous minor genotypes between cases and controls using the chi-square (χ^2) test of homogeneity. Odds ratios (OR) and corresponding 95% confidence intervals (CI) for PCA risk in association with

inflammatory and immune response-related SNPs were estimated using unconditional multivariate after adjusting for potential confounders (e.g., age, PSA, WAA).

Confounders were kept in the LR model if they significantly changed the risk estimates by $\pm 20\%$ compared to the unadjusted model. LR analyses for genetic variants and PCA risk were conducted using the major or common genotype as the referent category. Chi-square test and LR analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC) and SVS software 7.0 (Golden Helix, Inc., Bozeman, MT). Adjustment for multiple hypothesis testing was achieved using false discovery rate (FDR) or Bonferroni Correction with a significance level of 0.05 and 0.001, respectively.

Statistical Power for Single Gene Effects

We calculated the odds of developing PCA among carriers of at least one or more minor allele among 119 inflammatory and immune response-associated sequence variants based on the average MAF 22-27%, a PCA disease prevalence of 0.740%, a significance level (α) of 5%, and 100% linkage disequilibrium (LD) between the causal and predisposing variant. We assumed the causal SNP was in complete linkage disequilibrium with the predisposing variant ($r^2 = 1.0$) and a co-dominant genetic model with 1 degree of freedom (df). According to our sample size for the combined population (279 cases, 535 controls), U.S. (170 cases, 433 controls) and Jamaican men (109 cases, 102 controls), we had >80% power to detect odds ratios (ORs) of ≥ 1.4 , ≥ 1.6 , ≥ 1.9 for TLRs, ≥ 1.5 , ≥ 1.55 , ≥ 2.0 for chemokines and ≥ 1.4 , ≥ 1.6 , ≥ 1.8 for cytokines in relation to PCA risk, respectively. Statistical power calculations were performed using Power for Genetic Association Version 2 Software, as described previously [165].

Analysis of Gene-Gene Interactions Using Multi-factor Dimensionality Reduction

To evaluate the single- and joint- modifying effects of 119 candidate inflammatory and immune response sequence variants within a large dataset is computationally challenging. In order to overcome this problem, open source and freely available Multi-factor Dimensionality Reduction (MDR) 2.0 was used to detect and characterize all possible one-, two-, and three-way interaction models in relation to PCA (<http://www.epistasis.org/>) [166]. To reduce computation time needed to process thousands of SNP combinations in relation to PCA risk, we distributed MDR on a workstation with 12 hyper-threaded cores across two central processing units (total of 24 simultaneous threads of execution) and 24 GB of RAM. Although MDR has been described elsewhere, for convenience we provide a brief summary. With MDR, genetic information is reduced to a one-dimensional multi-locus genotype variable shown in **Figure 5** [167, 168]. Information from various disease loci were grouped and labeled as “high risk” or “low risk” based on whether or not the control ratio met or exceeded a particular threshold. Subsequently, the resulting one-dimensional multi-locus genotype variable was examined for its capacity to categorize and predict disease outcome through cross-validation and permutation testing procedures. A 10-fold cross-validation was achieved by dividing the entire dataset into a training set and an independent testing set. The training set involved $9/10^{\text{th}}$ of the data; the remaining $1/10^{\text{th}}$, known as the independent testing set, was evaluated against the training set. Evaluation of each independent testing set predicted average testing accuracy values for each MDR model. The greatest cross validation consistency (i.e., $\text{CVC} \geq 8/10$) and highest prediction accuracy score [i.e., Average Testing Accuracy (ATA)] were selected as the best

predictors of disease outcome. Sensitivity and specificity were determined as functions of true negatives (TN), false positives (FP), and false negatives (FN). Sensitivity, specificity and balanced accuracy values were calculated as follows: sensitivity = $(TP)/(TP+FN)$; specificity = $(TN)/(FP + TN)$; and balanced accuracy = $(\text{sensitivity} + \text{specificity})/2$. ATAs were averaged across all 10 pieces of the data, in order to provide an estimate of the predictive ability of the loci in relation to the outcome of interest. We used cross-validation consistency (CVC) to determine the degree to which the same best MDR model was selected across the 10 divisions of the data. Models with a CVC of $\geq 8/10$ using a 10-fold cross-validation were considered more carefully. CVCs and ATAs were calculated across 1,000 random seeds to ensure reproducibility in model selection. If the MDR model met the CVC criteria, we selected models that had the highest ATAs. Multiple hypothesis testing was controlled by CVC in combination with permutation testing. Permutation testing results ≤ 0.05 , generated using random seed 500, were considered statistically significant. Age-group covariate effects were removed by integrating over- and under-sampling methods.

Figure 5. Graphical model of gene-gene interactions. MDR analysis reduces genetic data into a one-dimensional variable. The graphical model is an example of a 1-, 2- and 3-factor interaction graphical models using Random Seed 500. For each model, the major/major homozygous genotype (0), heterozygous genotype (1) and minor/minor homozygous genotypes (2) are shown. In each square, darker gray bars represent cases and lighter gray bars represent controls.

Visualization of Interaction Models Using Hierarchical Interaction Entropy Graphs

Hierarchical interaction entropy graphs, based on information theory, were used to visualize and interpret complex interactions among selected SNPs and PCA risk [169-172]. With this approach, individual and all possible pairwise loci are assigned a joint or mutual information percentage score based on disease risk, respectively. Joint mutual information and mutual information gain scores are based on a number system, ranging from 0-100%. However, these scores rarely exceed 5-6%. When the pairwise or joint mutual information exceeds the mutual information gain scores, then the pairwise interaction is considered more informative in relation to prostate cancer risk when compared to each locus considered separately. Potential interactions are assessed using interaction entropy graphs, which uses a color-coding system to depict redundant or synergistic interactions. Within the entropy graph, lines depicted between SNP pairs that are color-coded red, orange, green, blue, and gold represent highly synergistic, moderately synergistic, moderately redundant, highly redundant, and neither synergistic/redundant pairwise interaction models, respectively, as depicted in **Figure 6**. All entropy-based analyses were conducted using Orange software [173].

Figure 6. Entropy graph of gene-gene interactions. MDR analysis produce an entropy graph to demonstrate if a gene-gene interaction has a synergistic effect or not. The lines between SNPs display a pairwise mutual information gain score representative of the interaction and are color-coded in the following manner: red (highly synergistic), orange (moderately synergistic), green (moderately redundant), blue (highly redundant), and gold (neither synergistic/redundant). Each SNP displays the mutual information gain score underneath its name.

CHAPTER 3

TOLL-LIKE RECEPTOR-ASSOCIATED SEQUENCE VARIANTS AND PROSTATE CANCER RISK AMONG MEN OF AFRICAN DESCENT

Among inflammatory and immune response regulators, toll-like receptors (TLRs) have a critical role in innate immune responses against chronic/recurrent inflammation, a major contributor to PCA development. However, it remains unclear whether sequence variants within TLR genes influence PCA risk among men of African descent. Therefore, we evaluated the impact of 32 TLR associated single-nucleotide polymorphisms (SNPs) on PCA risk among African Americans and Jamaicans. SNP profiles of 814 subjects were evaluated using Illumina's Veracode genotyping platform. Single and combined effects of SNPs in relation to PCA risk were assessed using age-adjusted logistic regression and entropy-based multifactor dimensionality reduction (MDR) models. Seven sequence variants detected in TLR6, TOLLIP (Toll-interacting protein), IRAK4 (interleukin-1 receptor-associated kinase 4) and IRF3 (interferon regulatory factor 3) were marginally associated with PCA [31]. However, none of these effects remained significant after adjusting for multiple hypothesis testing. Nevertheless, MDR modeling revealed a complex interaction between IRAK4 rs4251545 and TLR2 rs1898830 as a significant predictor of PCA risk among US men (Permutation testing p-value = 0.001). However, these genetic susceptibilities detected within the *TLR* signaling pathway require further assessment and validation to provide a better understanding of their influence on PCA risk among men of African descent.

Results

Population Description

The demographic and other pertinent characteristics of cases and controls for the entire study population and each study center are summarized in **Table 3-5**. Overall, men diagnosed with prostate cancer were 14 years older and had higher PSA levels than controls ($P < 0.0001$). Among controls, Jamaican men were about 9 years older and had higher PSA levels ($P < 0.0001$) and as well as higher median Gleason scores ($P = 0.018$) than U.S. men. As summarized in **Table 3**, there were no significant differences in family history of PCA with respect to the following: (1) cases to controls from the total population ($P = 0.316$) (**Table 3**), U.S. alone ($P = 0.592$) (**Table 4**), or Jamaica alone ($P = 0.272$) (**Table 5**), and (2) controls ($P = 0.757$) or cases ($P = 0.830$) comparing the two study centers (data not shown). Among African-Americans, the degree of West African ancestry did not vary by disease-status, as shown in **Table 4** [162-164]; however, no such data was collected for the Jamaican men.

Minor allele and genotype frequency among men of African descent from the U.S. and Jamaica

Overall, the average minor allele frequency (MAF) for *TLR*-associated SNPs among disease-free U.S. and Jamaican men combined was 23.3% [standard deviation (SD) = 15.8]. When stratified by study site, the MAFs for U.S. and Jamaican men were 23.3% (SD = 16.1) and 21.9% (SD = 14.9), respectively. When the *TLR*-associated SNPs among the total population (i.e., U.S. and Jamaican men combined) were analyzed, 87.5% had minor allele frequencies $\geq 5\%$.

Association between TLR-associated sequence variants and prostate cancer risk

Three *TLR*-associated markers (*TLR6* rs2381289, *TOLLIP* rs3168046, and *TOLLIP* rs5743899) were modestly linked with PCA susceptibility for the total population under the age-adjusted LR models, as shown in **Table 9a**. In particular, possession of the *TLR6* rs2381289 GA or *TOLLIP* rs5743899 AG+GG genotypes was associated with a 1.46-1.49 fold increase in the risk of developing PCA. In contrast, *TOLLIP* rs3168046 AA carriers had a significant 42% reduction in PCA susceptibility ($OR_{\text{age-adjusted}} = 0.58$; 95%CI = 0.35, 0.98). However, none of these markers remained significant after adjusting for multiple hypothesis testing (FDR p-value > 0.05).

Upon stratification by study center, six of the 32 candidate *TLR*-associated SNPs were identified as modestly significant determinants of PCA risk after adjusting for age, as shown in **Table 9b**. Within the U.S. population, a nominal 40-66% reduction in PCA susceptibility was observed among men who possessed one or more *IRAK4* (rs4251545 A and rs4251473 A) or *TLR6* rs5743818 C minor alleles; however, the relationship was strongest for carriers of the *IRAK4* rs4251545 AA genotype ($OR_{\text{age-adjusted}} = 0.34$; 95%CI = 0.13, 0.91). Moreover, the *TOLLIP* rs5743899 locus, under the recessive genetic model (GG versus AG+AA), was modestly associated with a 1.14 fold increase in the risk of developing PCA ($OR_{\text{age-adjusted}} = 1.14$; 95%CI = 1.12, 1.18). Among Jamaican men, *IRF3* rs2304206, *TLR6* rs2381289, and *TLR6* rs5743818 were marginally associated with PCA risk. For instance, inheritance of the *IRF3* rs2304206 GG genotype ($OR_{\text{age-adjusted}} = 0.32$; 95%CI = 0.10, 0.98) was linked to a modestly significant 68% reduction in PCA susceptibility. On the other hand, there was a 1.10-2.0-fold increase in PCA risk associated with inheriting the *TLR6* rs2381289 GA+AA ($OR_{\text{age-adjusted}} = 2.05$; 95%CI =

1.10, 3.78) or *TLR6* rs5743818 AC+CC GA ($OR_{\text{age-adjusted}} = 1.10$; 95% CI = 1.06, 1.14) genotypes. Notably, additive genetic models for *IRF3* rs2304206 and *TLR6* rs2381289 were significantly related to prostate cancer risk, which was modestly suggestive of a significant dose-response effect in relation to the number of inherited minor alleles (P-trend ≥ 0.0193). According to the new significance level ($\alpha=0.0016$), these modest associations did not persist after adjustments for multiple hypothesis testing among U.S. men and Jamaican men, respectively (Bonferroni Correction p-value ≥ 0.033 , p-value ≥ 0.014).

Table 9a. Relationship between TLR-associated SNPS and prostate cancer risk among men of African descent.

Gene (Allele, position, function)	Genotype	Cases n (%)	Controls n(%)	Unadjusted OR (95% CI)	Age Adjusted OR (95% CI)	Chi-square (χ^2) p-value*	p-trend	FDR p-value
<i>TLR6</i> <i>rs2381289</i> 3'UTR	GG	140 (50.4)	302 (56.7)	1.00 (referent)	1.00 (referent)	0.164	0.198	1.000
	GA	124 (44.6)	200 (37.7)	1.33 (0.98, 1.80)	1.46 (1.02, 2.09)	0.062		
	AA	14 (5.04)	30 (5.63)	1.00 (0.52, 1.96)	1.20 (0.52, 2.76)	0.984		
	GA+AA	138 (49.64)	230 (43.33)	1.28 (0.96, 1.72)	1.43 (1.00, 2.00)	0.088		
	AA vs (GG+AG)			0.88 (0.46, 1.70)	1.02 (0.46, 2.29)	0.724		0.853
<i>TOLLIP</i> <i>rs3168046</i> 3'UTR miRNA	GG	104 (37.3)	189 (35.4)	1.00 (referent)	1.00 (referent)	0.363	0.261	0.798
	GA	131 (46.9)	239 (44.8)	1.00 (0.72, 1.40)	0.85 (0.58, 1.25)	0.981		
	AA	44 (15.8)	106 (19.9)	0.75 (0.49, 1.15)	0.58 (0.35, 0.98)	0.194		
	GA+AA	175 (62.7)	345 (64.7)	0.92 (0.68, 1.24)	0.76 (0.54, 1.10)	0.596		1.000
	AA vs (GG+AG)			0.76 (0.51, 1.11)	0.64 (0.40, 1.02)	0.155		1.000
<i>TOLLIP</i> <i>rs5743899</i> Intron 1	AA	85 (30.8)	187 (35.2)	1.00 (referent)	1.00 (referent)	0.208	0.686	1.000
	AG	140 (50.7)	235 (44.2)	1.31 (0.94, 1.82)	1.59 (1.06, 2.38)	0.109		
	GG	51 (18.5)	110 (20.7)	1.02 (0.67, 1.55)	1.29 (0.78, 2.14)	0.926		
	AG+GG	191 (69.2)	345 (64.9)	1.22 (0.89, 1.66)	1.49 (1.02, 2.18)	0.215		0.884
	GG vs (AA+AG)			0.87 (0.60, 1.26)	0.98 (0.64, 1.53)	0.458		0.756

The chi-square (χ^2) test p-values were used to determine the difference in the genotype frequencies between cases and controls. *Fisher's p-values were calculated when expected genotype counts were <5 for either cases and controls. Significant odd ratios (ORs) and 95% confidence interval (CI) are indicated in boldface. Abbreviations: UTR, untranslated region; miRNA, microRNA binding site; FDR, false discovery rate.

Table 9b. Relationship between TLR-associated SNPs and PCA risk stratified by racial/ethnic group.

Gene (Allele, position, function)	Genotype	Unadjusted OR (95%CI) U.S. Men	Unadjusted OR (95%CI) Jam. Men	Age Adjusted OR (95%CI) U.S. Men	Age Adjusted OR (95%CI) Jam. Men	χ^2 p-value* U.S. Men	p-trend U.S. Men	χ^2 p-value* Jam. Men	p-trend Jam. Men
<i>IRAK4</i> <i>rs4251473</i> Intron 5	CC	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.270*	0.101	1.000*	0.955
	CA	0.76 (0.51, 1.14)	1.02 (0.55, 1.88)	0.61 (0.38, 0.99)	0.92 (0.47, 1.79)	0.181		0.950	
	AA	0.57 (0.21, 1.56)	0.92 (0.28, 2.98)	0.34 (0.09, 1.21)	1.42 (0.32, 6.31)	0.274		0.887	
	CA+AA	0.74 (0.50, 1.08)	1.00 (0.56, 1.78)	0.58 (0.36, 0.92)	0.97 (0.52, 1.84)	0.119		0.997	
	AA vs (CC+CA)	0.62 (0.22, 1.68)	0.91 (0.28, 2.92)	0.40 (0.11, 1.40)	1.46 (0.34, 6.38)	0.347		0.878	
<i>IRAK4</i> <i>rs4251545</i> Exon 15 Splicing (ESEor ESS)	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.072	0.023	0.685	0.473
	GA	0.71 (0.48, 1.04)	1.04 (0.58, 1.86)	0.60 (0.38, 0.94)	1.07 (0.56, 2.04)	0.076		0.909	
	AA	0.50 (0.23, 1.08)	1.47 (0.60, 3.56)	0.34 (0.13, 0.91)	1.97 (0.70, 5.52)	0.079		0.391	
	GA+AA	0.68 (0.47, 0.96)	1.13 (0.66, 1.94)	0.56 (0.36, 0.86)	1.22 (0.67, 2.24)	0.033		0.663	
	AA vs (GG+GA)	0.58 (0.28, 1.23)	1.45 (0.62, 3.40)			0.158		0.390	
<i>IRF3</i> <i>rs2304206</i> Intron 1 TFBS	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.767	0.558	0.079	0.037
	AG	1.15 (0.78, 1.70)	0.74 (0.42, 1.32)	1.22 (0.76, 1.97)	0.65 (0.34, 1.22)	0.475		0.314	
	GG	1.12 (0.65, 1.94)	0.32 (0.11, 0.90)	1.30 (0.68, 2.49)	0.32 (0.10, 0.98)	0.666		0.031	
	AG+GG	1.14 (0.80, 1.66)	0.65 (0.38, 1.12)	1.24 (0.80, 1.95)	0.57 (0.31, 1.06)	0.470		0.120	
	GG vs (AA+AG)	1.04 (0.63, 1.70)	0.37 (0.14, 1.00)	1.15 (0.64, 2.07)	0.40 (0.13, 1.16)	0.881		0.051	
<i>IRF3</i> <i>rs968457</i> 5' Near Gene Splicing (ESE or ESS) nsSNP Benign	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.699*	0.485	0.133*	0.069
	GA+AA	0.92 (0.53, 1.58)	0.68 (0.30, 1.54)	0.85 (0.43, 1.67)	0.62 (0.25, 1.54)	0.750		0.359	
	GA+AA	0.86 (0.50, 1.49)	0.56 (0.26, 1.18)	0.77 (0.40, 1.51)	0.50 (0.22, 1.18)	0.609		0.128	
	AA vs (GG+GA)		0.18 (0.02, 1.56)		0.14 (0.01, 1.60)	0.987		0.120	
<i>TLR6</i> <i>rs2381289</i> 3' UTR	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.770*	0.801	0.043*	0.019
	GA	1.13 (0.78, 1.64)	1.98 (1.12, 3.50)	1.28 (0.82, 2.00)	2.00 (1.06, 3.76)	0.510		0.017	
	AA	0.88 (0.40, 1.96)	2.12 (0.48, 9.30)	0.92 (0.34, 2.50)	2.57 (0.50, 13.2)	0.768		0.318	
	GA+AA	1.10 (0.76, 1.56)	1.99 (1.51, 3.46)	1.23 (0.80, 1.90)	2.05 (1.10, 3.78)	0.607		0.014	
	AA vs (GG+GA)	0.84 (0.38, 1.82)	1.58 (0.36, 6.82)	0.82 (0.31, 2.20)	1.92 (0.38, 9.64)	0.663		0.535	
<i>TLR6</i> <i>rs5743818</i> Exon 1	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.170*	0.176	0.056*	0.077
	AC+CC	0.64 (0.38, 1.08)	2.62 (0.90, 7.65)	0.54 (0.28, 1.01)	1.10 (1.06, 1.14)	0.099		0.077	
<i>TOLLIP</i> <i>rs3168046</i> UTR'3 miRNA	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.326	0.166	0.220	0.811
	GA	0.77 (0.52, 1.14)	1.56 (0.85, 2.83)	0.73 (0.45, 1.18)	1.15 (0.58, 2.26)	0.201		0.149	
	AA	0.73 (0.44, 1.20)	0.89 (0.38, 2.06)	0.63 (0.34, 1.16)	0.56 (0.21, 1.46)	0.220		0.789	
	GA+AA	0.76 (0.52, 1.10)	1.37 (0.80, 2.41)	0.70 (0.45, 1.08)	0.99 (0.52, 1.88)	0.139		0.283	
	AA vs (GG+AG)	0.84 (0.53, 1.32)	0.69 (0.32, 1.46)	0.74 (0.42, 1.30)	0.52 (0.22, 1.22)	0.449		0.333	
<i>TOLLIP</i> <i>rs5743899</i> Intron 1	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.239	0.490	0.559	0.534
	AG	1.40 (0.93, 2.10)	1.11 (0.59, 2.08)	1.67 (1.02, 2.73)	1.35 (0.66, 2.74)	0.103		0.740	
	GG	1.10 (0.66, 1.86)	0.75 (0.35, 1.61)	1.30 (0.69, 2.41)	1.12 (0.47, 2.66)	0.697		0.465	
	AG+GG	1.31 (0.89, 1.92)	0.98 (0.54, 1.76)	1.55 (0.98, 2.46)	1.28 (0.66, 2.48)	0.166		0.952	
	GG vs (AA+AG)	0.90 (0.58, 1.43)	0.70 (0.36, 1.37)	1.14 (1.12, 1.18)	0.93 (0.44, 1.96)	0.678		0.306	

The chi-square (χ^2) test p-values were used to determine the difference in the genotype frequencies between cases and controls. *Fisher's p-values were calculated when expected genotype counts were <5 for either cases and controls. Significant odd ratios (ORs) and 95% confidence interval (CI) are indicated in boldface. Abbreviations: UTR, untranslated region; TFBS, transcription factor binding site; ESE exonic splicing enhancer; ESS, exonic splicing silencers; miRNA, microRNA binding site; nsSNP, non-synonymous coding SNP; Jam, Jamaican.

Analysis of gene-gene interactions using Multi-factor Dimensionality Reduction

MDR modeling was used to efficiently assess and validate age-adjusted gene-gene interactions in relation to PCA risk for the total population, U.S. men alone, and Jamaican men alone [31]. The top one-, two-, and three-way interaction models for the total population, involving U.S. and Jamaican men combined, displayed 100% cross validation consistency (CVC) values, 57-65% average testing accuracy (ATA) scores, and permutation p-values = 0.001 (**Table 10a**). However, the three-way interaction among *TLR6 rs2381289*, *TLR10 rs11096957*, and *IRF3 rs2304206* was selected as the best PCA predictor for men of African descent, since this model had the highest average testing accuracy (ATA = 0.6505). This three-way interaction was primarily driven by a highly synergistic relationship between *TLR10 rs11096957* and *IRF3 rs2304206* due to its pairwise mutual information gain score (IG = 1.32%), which was higher than the mutual IG scores for each variant (**Figure 7**).

CVC and ATA scores for all 1-, 2-, and 3-factor models among U.S. men were significant and characterized as 90-100% and 57-62%, respectively, as described in **Tables 10b**. However, interaction between *TLR2 rs1898830* and *IRAK4 rs4251545* was chosen as the best PCA predictor, based on a stronger CVC (100%) value as well as ATA (61.94%) score and lower permutation testing value (Permutation testing p-value = 0.001) relative to the best three-factor model (Permutation p-value = 0.015). Moreover, the two-way interaction was highly synergistic since the pairwise mutual information gain score (2.33%) exceeded the mutual information gain scores for *TLR2 rs1898830* alone (0.04%) and *IRAK4 rs4251545* alone (1.30%), as shown in **Figure 8**.

For the Jamaican population, the one-way model containing the *TLR6* rs2381289 loci was the best PCA-related MDR model based on a 62.7% prediction accuracy score (Permutation testing p-value = 0.018). Although the three- factor model had high CVC scores ($\geq 80\%$), this model failed to reach statistical significance (**Table 10c**).

Table 10a. Interactions and main effects of TLR-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. and Jamaican men of African Descent.

Best Model (dbSNPID#)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i>-value
<u>One Factor</u> <i>TLR6 rs2381289</i>	32	10/10	0.572	0.001
<u>Two Factor</u> <i>TLR10 rs11096957</i> <i>TLR6 rs2381289</i>	496	10/10	0.611	0.001
<u>Three Factor</u> <i>TLR10 rs11096957</i> <i>TLR6 rs2381289</i> <i>IRF3 rs2304206</i>	4960	10/10	0.650	0.001

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Figure 7. Entropy graph of TLR gene-gene interactions among U.S. and Jamaican men of African Descent. MDR analysis revealed a significant three-factor interaction (TLR10, TLR6 and IRF3) model with a pairwise mutual information gain score of 3.57%, 100% CVC and 65% ATA values (Permutation p-value=0.001). A highly synergistic relationship was observed for TLR6 rs2381289-TLR10 rs1109657 and TLR10 rs1109657-IRF3 rs2304206 interactions represented by a red line.

Table 10b. Interactions and main effects of TLR-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. men of African Descent.

Best Model (dbSNPID#)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i>-value
<u>One Factor</u> <i>IRAK4 rs4251473</i>	32	10/10	0.574	0.179
<u>Two Factor</u> <i>TLR2 rs1898830</i> <i>IRAK4 rs4251545</i>	496	10/10	0.619	0.001
<u>Three Factor</u> <i>TLR6 rs3821985</i> <i>TLR4 rs1927906</i> <i>TLR2 rs3804099</i>	4960	9/10	0.618	0.015

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Figure 8. Entropy graph of TLR gene-gene interactions among U.S. men of African Descent. MDR analysis revealed a significant and highly synergistic (red line) two-factor interaction (TLR2 and IRAK4) model with a pairwise information gain score of 2.33%, 100% CVC and 61.9 % ATA values. The three-factor interaction between TLR6 rs3821985, TLR4 rs1927906 and TLR2 rs3804099 was shown as a good predictor of prostate cancer risk with a 90% and 61.8% ATA values (p=0.015).

Table 10c. Interactions and main effects of TLR-associated SNPs as predictors of PCA using age-adjusted MDR among Jamaican men of African Descent.

Best Model dbSNPID #	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i>-value
<u>One Factor</u> <i>TLR6 rs2381289</i>	32	10/10	0.627	0.018
<u>Two Factor</u> <i>TLR6 rs2381289</i> <i>TOLLIP rs3168046</i>	496	7/10	0.646	0.203
<u>Three Factor</u> <i>TLR10 rs11096955</i> <i>TLR4 rs1927911</i> <i>TOLLIP rs5743899</i>	4960	10/10	0.639	0.174

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Figure 9. Entropy graph of TLR gene-gene interactions among Jamaican men of African Descent. MDR analysis revealed a significant one-factor (TLR6) model with a mutual information gain score of 5.08%, 100% CVC and 62.7 % ATA values. However, two- and three- factor interaction models did not survive multiple hypothesis testing.

Discussion

Dysregulation and genetic alterations in immune system function are linked to many cancers. In particular, it is estimated that approximately 20% of all human cancers, including prostate cancer (PCA), are associated with chronic inflammation [174, 175]. TLR activation, a key initiator of inflammation and dysregulation of TLR-responsive pathways, has been associated with cancer susceptibility. Consequently, we evaluated 32 *TLR*-associated sequence variants to determine their individual and joint modifying effects on PCA risk among 279 cases and 535 disease free men of African descent [31]. We evaluated the impact of select polymorphic *TLR*-related genes (i.e., *TLR1*, *TLR 2*, *TLR 4*, *TLR 6*, *TLR10*, *IRAK4*, *IRF3*, and *TOLLIP*) on PCA susceptibility among men of African descent. Out of the 32 minor variant alleles, 3 loci (*TLR6* *rs2381289*, *TLR6* *rs5743818*, *TOLLIP* *rs5743899*) displayed a 1.14-2.05 fold increase in PCA risk after adjusting for age; whereas, 4 markers (*TOLLIP* *rs3168046*, *IRAK4* *rs4251473*, *IRAK4* *rs4251545*, *IRF3* *rs2304206*) were linked to a 39-68% reduction in PCA risk. Among U.S. men, there was a nominal 40-66% reduction in PCA susceptibility among those who possessed one or more *IRAK4* (*rs4251545* A, *rs4251473* A) or *TLR6* *rs5743818* C minor alleles. Similar to the total population, the *TOLLIP* *rs5743899* SNP under the recessive genetic model (GG versus AG+AA) was modestly associated with a 1.14 fold increase in the risk of developing PCA ($OR_{\text{age-adjusted}} = 1.14$; 95% CI = 1.12, 1.18) among men of African descent from the U.S. The *TOLLIP* *rs3168046* GA was unique to the total population; whereas, 3 markers [*IRAK4* *rs4251473* (CA, CA+AA), *IRAK4* *rs4251545* (GA, AA GA+AA), *TLR6* *rs2381289* (GA, GA+AA)] were unique to the U.S. men. Among Jamaicans, both the *IRF3* *rs2304206* and *TLR6* *rs2381289* loci were significant

under the additive genetic model. *IRF3* rs2304206 GG genotype was linked to a 68% reduction ($OR_{\text{age-adjusted}}=0.32$; 95%CI=0.10, 0.98) in PCA risk among Jamaican men (**Table 9b**). In addition, inheritance of *TLR6* rs2381289 GA and GA+AA genotypes were associated to a 2-fold increase in PCA susceptibility among Jamaican men (χ^2 p-value = 0.014-0.017). However, *TLR6* (rs2381289, rs5743818), *IRAK4* rs4251473, *TOLLIP* (rs3168046, rs5743899), and *IRF3* (rs2304206, rs968457) have never been studied in relation to cancer in the literature. Nonetheless, selected TLR-related sequence variants in the current study did not survive multiple hypothesis testing according to the new significance level ($\alpha = 0.0016$) (Bonferroni Correction p-value > 0.0016).

Collectively, 10 studies evaluated 14 out of the 32 SNPs examined in our study [97-99, 103-109]. In the Cancer of the Prostate Study (CAPS), inheritance of one or more *TLR1* rs4833095 (OR = 1.28; 95% CI = 1.12-1.44) and *TLR10* rs11096955 (OR = 1.16; 95% CI = 1.02-1.30), *TLR10* rs11096957 (OR = 1.16; 95% CI = 1.02-1.30) minor alleles were associated with an increase in prostate cancer risk among European Americans [99, 105]. However, the American Cancer Society Cancer Prevention II Nutrition Cohort (CPS-II) study reported protective effects for *TLR1* rs4833095 CC (OR = 0.64; 95% CI = 0.47-0.86), *TLR10* rs11096955 CC (OR = 0.78; 95% CI = 0.61-0.99) and *TLR10* rs11096957 CC (OR = 0.78; 95% CI = 0.61-0.99) genotypes [97]. In the Health Professionals Follow-up Study (HPFS) [99, 109], and Prostate, Lung, Colon and Ovarian Cancer Screening Trial (PLCO) Study [99], inheritance of *TLR10* (rs11096955, rs11096957) had no association with PCA risk among European Americans. However, there were null findings for *TLR1* rs4833095, *TLR10* (rs11096955, rs11096957) in relation to PCA susceptibility among men of African descent in the PLCO study.

Consistent with our study, five independent studies reported null findings in relation to prostate cancer risk and possession of *TLR1* *rs46224663* and *TLR6* (*rs5743814*, *rs5743810*, *rs1039559*, *rs3821985*) variant alleles [97, 99, 106, 109]. Inheritance of one or more minor alleles was associated with either an increase [*TLR1* *rs5743604* (OR = 1.28; 95% CI = 1.12-1.44), *TLR10* *rs4274855* (OR = 1.21; 95% CI = 1.03-1.39)] [99, 106] or decrease [*TLR1* *rs5743595* CC (OR = 0.63; 95% CI = 0.42-0.93), *TLR1* *rs2149356* (OR = 0.83; 95% CI = 0.67-0.99), *TLR4* *rs1927911* (OR = 0.79; 95% CI = 0.62-0.96)] [97, 99, 107] in the risk of developing prostate cancer among Caucasians in three separate observational studies. However, several studies including our study found that the previously mentioned sequence variants were not significantly related to prostate cancer among men of African and European descent [99, 103, 105, 109]. Song and associates (2009) observed that the *TLR4* *rs1927911* CC genotype was linked to an increase in prostate cancer risk in a Korean population case-control study (143 controls, 157 cases) [98]; whereas, another study did not reveal a statistically significant relationship among Korean men (223 controls, 240 cases) [104]. Discrepant findings for these two studies may be attributed to differences in methods used for allelic discrimination [98, 104]. For instance, the RFLP-PCR method used in the 2009 study [98] may not have the same capacity to discern between homozygous and heterozygous genotypes, relative to fluorescence polarization detection, a more advance method used by Kim and co-workers (2012) [104]. Other explanations for differences in prostate cancer risk estimates for the aforementioned *TLR*-related SNPs may include: inadequate statistical power to detect significant differences between prostate cancer cases and controls among Koreans [98, 104]; failure to adjust for multiple hypothesis testing [97-

99, 104-109]; and unknown environmental exposures/lifestyle factors (e.g., pathogens, environmental toxins, antioxidants) [97-99, 103-109].

The functional impact and biological activity of TLR genes are greatly affected by the inheritance of their associated sequence variants. For instance, *TLR6 rs2381289* located in 3'UTR and *TLR2 rs1898830* located in intron 1 may affect transcriptional regulation of *TLR* genes. *TLRs* 2 and 6 are controlled by the master regulatory transcription factor p53 [176]. *TLR*-associated SNPs may alter p53 interactions with *TLR* genes. *In silico* tools revealed that *IRAK4 rs4251545* codes for a change from Ala to Thr in mRNA splicing, which in turn may influence mRNA stability of IRAK4 kinase activity, as well as TLR signaling protein-protein interactions. Such alterations may lead to biochemical conditions that favor cell death, decreases in matrix metalloproteinases linked to cell migration, decreases in pro-inflammatory cytokines/chemokines and ultimately abrogation of tumor growth. Yeyeodu and associates (2013) identified that *IRAK4 rs4251545* resides in a transcription enhancer/silencer region and codes for a phosphorylation site and non-synonymous mutation at position 428 [177]. It was speculated inheritance of the *IRAK4 rs4251545* TT genotype was associated with a 4.5 fold increase in breast cancer susceptibility among African-American women relative to those with the referent genotype[177]. In addition, this high susceptibility may have been attributed to alteration of IRAK4 phosphorylation. This change may influence the transcriptional level of IRAK4 and impair TLR3 regulated apoptosis in breast cancer cells [177].

Lastly, we examined the combined effects of *TLR*-associated SNPs as predictors of prostate cancer using age-adjusted MDR analysis to determine the best gene-gene

interaction models. In the total population, MDR analysis revealed a significant three-factor (*TLR10 rs11096957-TLR6 rs2381289-IRF3 rs2304206*) interaction model as a good predictor of PCA risk (Permutation testing p-value = 0.001). Among U.S. men of African descent, the best predictor of PCA risk was the two-factor gene-gene interaction model between *IRAK4 rs4251545* and *TLR2 rs1898830* (Permutation testing p-value = 0.001). However, only the *TLR6 rs2381289* SNP for the Jamaican population remained statistically significant after adjusting for age and multiple hypothesis testing (Permutation testing p-value = 0.018). However, there are no published reports on the TLR-associated sequence variants observed in our gene-gene interaction study.

The strengths and limitations of our approach were considered in our research findings. Although nominal relationships between TLR-associated SNPs and PCA risk were observed, we cannot rule out the possibility that other TLR sequence variants within the innate immune pathway may still influence PCA risk and disease prognosis. Consequently, future studies will enable us to evaluate the relationship between *TLR* SNPs and Gleason score, tumor stage, biochemical/disease recurrence, as well as overall/disease specific mortality. Multi-center pooled genetic studies with thousands of cases and controls may enable us to confirm and validate our findings. Moreover, PCA susceptibility may also be influenced by polymorphisms of some genes even further downstream of the TLR signaling pathway, including caspases (*CASP* 3, 7, 8, and 10), mitogen-activated protein kinases (*MAPKs*), inflammatory cytokines, and chemokines. In fact, selected cytokine- and chemokine-associated sequence variants have been evaluated in relation to PCA susceptibility among men of African descent in published reports by our lab [35, 36].

Future studies will assess whether selected TLR-related SNPs may alter TLR signaling, miRNA and/or protein expression or relative expression activity of downstream targets. The genetic admixture among African-American men, as documented by other published reports, may modify the relationship between *TLR* SNPs and prostate cancer risk. Adjustment of our risk models for West African Ancestry ultimately had no significant bearing on calculated risk estimates among African-American men. Our gene-gene interaction analyses were controlled for multiple hypothesis testing with permutation testing. Given the high prediction accuracy (i.e., 61.9%) between *TLR2* and *IRAK4*, our study findings require replication within independent study sets. However, recent simulation studies demonstrate that even modest disparities in genotype frequencies among study participants of independent study sets may interfere with the capacity to replicate complex interactions [178]. Consequently, to replicate our findings, it is critical that future replicate studies have the same genetic architecture (i.e., ancestry identification markers and *TLR* SNPs) as the African-Americans in the current study. Caution is recommended in the interpretation of our study findings due to a modest marginal effect between *TLR* signaling sequence variants and PCA risk. However, enthusiasm for the relationship between PCA and the innate immune signaling pathway was slightly elevated in our exhaustive 2- and 3-way interactions. In particular, our exploratory analysis revealed a synergistic relationship between *IRAK4* and *TLR2* as significant PCA markers among men of African descent in the U.S. (**Table 10b**). We speculate genetic variations in *TLR*-related genes may influence the PCA risk by modulating cell survival, proliferation and/or inflammation. To corroborate these findings and explore the functional consequences of *TLR*-related SNPs

on PCA development, we plan to overexpress both IRAK4 and TLR2, individually and IRAK4-TLR2 in combination in PCA cell lines. Such studies may help us understand the role of the IRAK4-TLR2 axis in the PCA phenotype, including cell death, proliferation, migration, colony formation and invasion. In addition, these modified cell lines could be implanted into mice to observe their effect on PCA tumor growth and metastasis. The combination of our genetic variation analysis of *TLR*-related polymorphisms with biological studies will aid in the accurate prediction and eventual offset of genetic risk factors that predispose men of African descent to PCA.

CHAPTER 4

CHEMOKINE LIGAND 5 (CCL5) AND CHEMOKINE RECEPTOR(CCR5) GENETIC VARIANTS AND PROSTATE CANCER RISK AMONG MEN OF AFRICAN

Chemokine and chemokine receptors play an essential role in tumorigenesis. Chemokine-associated single nucleotide polymorphisms (SNPs) are associated with various cancers. However, their impact on prostate cancer (PCA) among men of African descent is unknown. Consequently, we evaluated main and combined effects of 43 chemokine-associated SNPs in germ-line DNA samples from 814 African-American and Jamaican men (279 PCA cases and 535 controls) in relation to PCA risk using the Illumina's Goldengate genotyping system, multivariate logistic regression and entropy-based MDR. We hypothesized that inheritance of variant chemokine-associated alleles may lead to alterations in PCA susceptibility, presumably due to variations in antitumor immune responses. For the total population, inheritance of *CCL5* *rs2107538* (AA, GA+AA) and *rs3817655* (AA, AG, AG+AA) genotypes were linked with a 34-48% reduction in PCA risk [35]. The recessive and dominant models for *CCR5* *rs1799988* and *CCR7* *rs3136685* were associated with a 1.52-1.73 fold increase in PCA risk. Upon stratification, only *CCL5* *rs3817655* and *CCR7* *rs3136685* remained significant for the Jamaican and U.S. subgroups, respectively [35]. In summary, *CCL5* (*rs2107538*, *rs3817655*) and *CCR5* *rs1799988* sequence variants significantly modified PCA susceptibility among men of African descent, even after adjusting for age and multiple

comparisons. Our findings require further evaluation and validation in relation to prostate cancer risk and ultimately disease progression, biochemical/disease recurrence and mortality in larger high-risk subgroups. Such efforts will help to identify genetic markers capable of explaining disproportionately high prostate cancer incidence, mortality, and morbidity rates among men of African descent.

Results

Prevalence of minor alleles/genotype frequency comparing men of African Descent from the U.S. and Jamaica

Overall, the chemokine-related SNPs were fairly common among disease-free individuals from the entire sub-population of U.S. and Jamaica, with average minor frequencies of 26-27% and a standard deviation of 14%, respectively (data not shown). Thirty-eight SNPs had minor allele frequencies $\geq 5\%$. For exploratory purposes, five rare SNPs (*CCR9* rs12721497, *CCL17* rs11076191, *CCL11* rs4795896, *CCL21* rs11574916, *CXCL12* rs1801157) were analyzed with minor allele frequencies ranging between 0.015-0.490. The minor allele frequency comparing controls from the U.S. and Jamaica were strongly correlated ($R^2 = 0.957$). Only 5 out of the 43 SNPs analyzed were discordant comparing men of African descent from the U.S. to men from Jamaica (p-value ≤ 0.0487), namely *CCL17* rs11076191, *CCL21* rs11574916, *CCR7* rs3136685, *CCR7* rs3136687, and *CCR9* rs12721497 [35].

Relationship between chemokine sequence variants and prostate cancer risk

Among all men of African descent, five sequence variants were significantly associated with the risk of developing prostate cancer, as summarized in **Table 11a**. Possession of the *CCL5* rs2107538 GA+AA ($OR_{unadjusted} = 0.59$; 95%CI = 0.44, 0.80) or

CCL5 *rs3817655* GA+AA ($OR_{unadjusted} = 0.54$; 95%CI = 0.40, 0.74) genotype was linked with a 41-46% reduction in PCA risk in the unadjusted LR models. These effects remained significant for both *CCL5* SNPs after adjusting for age. The recessive genetic model for *CCR5* *rs1799988* ($OR_{age-adjusted} = 1.52$; 95%CI = 1.02, 2.26) as well as the dominant models for *CCR7* *rs3136685* ($OR_{age-adjusted} = 1.66$; 95%CI = 1.09, 2.54) and *CCR7* *rs3136687* ($OR_{age-adjusted} = 1.14$; 95%CI = 1.12, 1.16), respectively, were associated with a significant 1.14-1.66 fold increase in PCA risk within the age adjusted LR models. After controlling for multiple comparisons, the dominant genetic models for the two *CCL5* SNPs (*rs2107538*, *rs3817655*) remained significant with false-discovery rates ($FDR \leq 0.015$); whereas, the recessive model for *CCR5* *rs1799988* was statistically significant ($FDR = 0.0494$).

Table 11a. Relationship between Chemokine-associated SNPs and PCA Risk Among men of African Descent.

Gene (Allele, position, function)	Genotype	Cases n (%)	Controls n (%)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	Chi-square (χ^2) p-value*	p-trend	FDR
<i>CCR5</i> <i>rs1799988</i> 5'UTR TFBS	AA	85 (30.7)	194 (36.7)	1.00 (referent)	1.00 (referent)	0.0050	0.004	0.068
	AG	107 (38.6)	227 (43.9)	1.08 (0.76, 1.52)	0.83 (0.55, 1.25)	0.6760		
	GG	85 (30.7)	108 (20.4)	1.80 (1.23, 2.63)	1.38 (0.87, 2.17)	0.0030		
	AG+GG	192 (69.3)	335 (63.3)	1.31 (0.96, 1.78)	1.01 (0.70, 1.46)	0.0900		
	GG vs (AA+AG)			1.73 (1.24, 2.40)	1.52 (1.02, 2.26)	0.0013		0.482 0.049
<i>CCL5</i> <i>rs2107538</i> 5' near gene TFBS	GG	111 (39.8)	150 (28.1)	1.00 (referent)	1.00 (referent)	0.0020	0.001	0.049
	GA	124 (44.4)	270 (50.6)	0.62 (0.45, 0.86)	0.72 (0.49, 1.06)	0.0040		
	AA	44 (15.8)	114 (21.4)	0.52 (0.34, 0.80)	0.53 (0.32, 0.89)	0.0030		
	GA+AA	168 (60.2)	384 (71.9)	0.59 (0.44, 0.80)	0.66 (0.46, 0.96)	0.0007		
	AA vs (GG+GA)			0.83 (0.57, 1.20)	0.74 (0.47, 1.16)	0.0570		0.015 0.474
<i>CCL5</i> <i>rs3817655</i> Intron 2 TFBS	GG	114 (41.0)	147 (27.5)	1.00 (referent)	1.00 (referent)	0.0040	0.002	0.019
	GA	115 (41.4)	278 (52.0)	0.53 (0.38, 0.74)	0.57 (0.38, 0.84)	0.0002		
	AA	49 (17.6)	110 (20.5)	0.57 (0.38, 0.87)	0.54 (0.32, 0.89)	0.0009		
	GA+AA	164 (59.0)	388 (72.5)	0.54 (0.40, 0.74)	0.56 (0.39, 0.81)	0.0001		
	AA vs (GG+GA)			0.83 (0.57, 1.20)	0.74 (0.47, 1.16)	0.3170		0.004 1.000
<i>CCR7</i> <i>rs3136685</i> Intron 1	AA	55 (19.7)	151 (28.3)	1.00 (referent)	1.00 (referent)	0.0290	0.031	0.308
	AG	139 (49.8)	237 (44.4)	1.61 (1.11, 2.34)	1.86 (1.18, 2.92)	0.0120		
	GG	85 (30.5)	146 (27.3)	1.60 (1.06, 2.40)	1.39 (0.85, 2.28)	0.0240		
	AG+GG	224 (80.3)	383 (71.7)	1.61 (1.13, 2.28)	1.66 (1.09, 2.54)	0.0080		
	GG vs (AA+AG)			1.16 (0.85, 1.60)	0.92 (0.62, 1.35)	0.3480		0.599 0.161
<i>CCR7</i> <i>rs3136687</i> Intron 1	GG	84 (30.1)	173 (32.4)	1.00 (referent)	1.00 (referent)	0.0410	0.458	0.352
	GA	153 (54.8)	249 (46.5)	1.26 (0.91, 1.76)	1.45 (0.97, 2.16)	0.1611		
	AA	42 (15.1)	113 (21.1)	0.77 (0.49, 1.19)	0.96 (0.57, 1.62)	0.2340		
	GA+AA	195 (69.9)	362 (67.6)	1.11 (0.81, 1.52)	1.14 (1.12, 1.16)	0.5162		
	AA vs (GG+GA)			0.66 (0.45, 0.98)	0.76 (0.49, 1.20)	0.0372		0.716 0.509

The chi-square (χ^2) test p-values were used to determine the difference in the genotype frequencies between cases and controls.

*Fisher's p-values were calculated when expected genotype counts were <5 for either cases and controls. Significant odd ratios (ORs) and 95% confidence interval (CI) are indicated in boldface. Abbreviations: TFBS, transcription factor binding site; FDR, false discovery rate.

In an exploratory analysis, we evaluated risk estimates for all 43 chemokine targets for each racial/ethnic group, as depicted in **Table 11b**. Among U.S. men, *CCR5 rs1799988*, *CCL5 (rs2107538, rs2280789, rs3817655)*, *CCL25 rs2032887*, and *CXCR7 rs1045879* were associated with PCA risk. Inheritance of the *CCL25 rs2032887* AG+GG ($OR_{unadjusted} = 0.66$; 95% CI = 0.46, 0.96), *CCL5 rs2107538* GA+AA ($OR_{unadjusted} = 0.52$; 95% CI = 0.36, 0.76), *CCL5 rs2280789* AG+GG ($OR_{unadjusted} = 0.60$; 95% CI = 0.41, 0.89), and *CCL5 rs3817655* GA+AA ($OR_{unadjusted} = 0.46$; 95% CI = 0.32, 0.68) genotypes were significantly associated with a 34-54% reduction in the risk of developing PCA with chi-square p-values ranging from 0.0001-0.027. Although the magnitude of the reduction in PCA risk for *CCL5 rs2107538* GA+AA, *CCL5 rs3817655* GA+AA and *CCL25 rs2032887* AG+GG genotypes remained practically unchanged after adjusting for age, the findings only remained significant for *CCL5 rs3817655* SNP after the adjustment ($OR_{unadjusted} = 0.46$, $OR_{age-adjusted} = 0.51$). The 1.5-1.6 fold increase in PCA susceptibility was associated with the *CCR5 rs1799988* recessive model ($OR_{unadjusted} = 1.62$; 95% CI=1.08, 2.42) and the *CXCR7 rs1045879* AG+GG genotype ($OR_{unadjusted} = 1.54$; 95% CI = 1.07, 2.22; $P = 0.02$) was lost in the age adjusted risk models.

For the Jamaican population, there was a two-fold increase in PCA susceptibility associated with the *CCR5 rs1799987* AA ($OR_{unadjusted} = 2.18$; 95% CI=1.04, 4.58), *CCR5 rs1799988* GG ($OR_{unadjusted} = 2.25$; 95% CI = 1.08, 4.71), and *CCR7 rs3136685* AG+GG ($OR_{unadjusted} = 2.3$; 95% CI = 1.05, 5.07) genotypes, with corresponding chi-square P-values ranging from 0.02-0.037. Additionally, a 54% reduction in PCA risk was observed for individuals who possessed the *CCR9 rs1488371* CA+AA genotype ($OR_{unadjusted} = 0.46$; 95% CI = 0.23, 0.94). Out of the 4 markers, the *CCR7 rs3136685*

SNP remained significant after adjusting for age. Notably, the magnitude of PCA risk estimates did not change for the *CCR5*, *CCL5*, and *CCR9* SNPs, comparing the adjusted and unadjusted risk models. However, only *CCL5* (*rs2107538*, *rs3817655*) sequence variants survived multiple hypothesis testing among U.S. men of African descent (Bonferroni Correction p-value < 0.0012).

Table 11b. Relationship between Chemokine-associated SNPs and PCA Risk stratified by racial ethnic group.

Gene (Allele, position, function)	Genotype	Unadjusted OR (95%CI) US Men	Unadjusted OR (95%CI) Jamaican Men	Age-Adjusted OR (95%CI) US Men	Age-Adjusted OR (95%CI) Jam Men	χ^2 p-value* US Men	p-trend US Men	χ^2 p-value* Jam Men	p-trend Jam Men
<i>CCR5</i> rs1799988 5'UTR TFBS	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.063	0.076	0.085	0.034
	AG	0.95 (0.62, 1.44)	1.26 (0.66, 2.40)	0.68 (0.42, 1.12)	1.23 (0.60, 2.52)	0.808		0.484	
	GG	1.58 (0.99, 2.49)	2.25 (1.08, 4.71)	1.06 (0.60, 1.85)	2.23 (0.99, 5.00)	0.053		0.031	
	AG+GG	1.15 (0.79, 1.68)	1.56 (0.86, 2.82)	0.80 (0.52, 1.26)	1.55 (0.81, 2.98)	0.453		0.142	
	GG vs (AA+AG)	1.62 (1.08, 2.42)	1.96 (1.04, 3.70)	1.28 (0.78, 2.12)	1.98 (0.98, 3.98)	0.020		0.037	
<i>CCL5</i> rs2107538 5' near gene TFBS	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.003	0.002	0.286	0.116
	GA	0.54 (0.36, 0.80)	0.75 (0.40, 1.40)	0.67 (0.42, 1.08)	0.82 (0.40, 1.64)	0.003		0.375	
	AA	0.48 (0.28, 0.82)	0.53 (0.24, 1.16)	0.53 (0.28, 1.01)	0.52 (0.22, 1.22)	0.007		0.116	
	GA+AA	0.52 (0.36, 0.76)	0.68 (0.38, 1.23)	0.63 (0.40, 0.99)	0.72 (0.37, 1.40)	0.001		0.204	
	AA vs (GG+AG)	0.68 (0.42, 1.11)	0.64 (0.32, 1.26)	0.66 (0.37, 1.20)	0.58 (0.28, 1.24)	0.125		0.192	
<i>CCL5</i> rs2280789 Intron 1 TFBS	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.039	0.018	0.508	0.823
	AG	0.60 (0.40, 0.90)	1.20 (0.66, 2.18)	0.60 (0.37, 0.97)	1.48 (0.76, 2.89)	0.015		0.549	
	GG	0.62 (0.24, 1.56)	0.59 (0.18, 1.89)	1.00 (0.34, 2.99)	0.42 (0.12, 1.46)	0.309		0.373	
	AG+GG	0.60 (0.41, 0.89)	1.06 (0.61, 1.86)	0.64 (0.40, 1.01)	1.18 (0.63, 2.20)	0.011		0.82	
	GG vs (AA+AG)	0.72 (0.28, 1.82)	0.55 (0.18, 1.80)	1.14 (1.12, 1.18)	0.36 (0.10, 1.27)	0.488		0.313	
<i>CCL5</i> rs3817655 Intron 2 TFBS	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.0002	0.003	0.275	0.11
	GA	0.44 (0.29, 0.65)	0.72 (0.38, 1.34)	0.49 (0.30, 0.80)	0.74 (0.36, 1.50)	<0.0001		0.302	
	AA	0.56 (0.34, 0.92)	0.53 (0.24, 1.16)	0.54 (0.29, 1.02)	0.50 (0.22, 1.20)	0.022		0.115	
	GA+AA	0.46 (0.32, 0.68)	0.66 (0.36, 1.20)	0.51 (0.32, 0.80)	0.66 (0.34, 1.28)	<0.0001		0.167	
	AA vs (GG+GA)	0.88 (0.55, 1.40)	0.66 (0.34, 1.28)	0.80 (0.46, 1.42)	0.61 (0.28, 1.27)	0.577		0.22	
<i>CCL25</i> rs2032887 Exon 3 ESE/ESS, nsSNP probably damaging missense R>H	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.084	0.034	0.474	0.379
	AG	0.67 (0.46, 0.99)	1.42 (0.80, 2.48)	0.70 (0.44, 1.11)	1.54 (0.82, 2.88)	0.404		0.225	
	GG	0.61 (0.29, 1.28)	1.10 (0.36, 3.38)	0.57 (0.22, 1.42)	0.84 (0.24, 2.88)	0.196		0.86	
	AG+GG	0.66 (0.46, 0.96)	1.37 (0.80, 2.36)	0.68 (0.44, 1.05)	1.42 (0.78, 2.60)	0.027		0.253	
	GG vs (AA+AG)	0.71 (0.34, 1.47)	0.93 (0.32, 2.75)	0.65 (0.26, 1.60)	0.68 (0.20, 2.24)	0.357		0.898	
<i>CCR5</i> rs1799987 Intron 1 TFBS	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.197	0.951	0.107	0.041
	GA	1.29 (0.88, 1.92)	1.28 (0.68, 2.44)	0.89 (0.56, 1.44)	1.25 (0.61, 2.56)	0.191		0.442	
	AA	0.84 (0.48, 1.48)	2.18 (1.04, 4.58)	0.58 (0.29, 1.14)	2.20 (0.98, 4.94)	0.558		0.039	
	GA+AA	1.18 (0.80, 1.70)	1.56 (0.86, 2.82)	0.81 (0.52, 1.26)	1.55 (0.81, 2.98)	0.399		0.142	
	AA vs (GG+GA)	0.72 (0.44, 1.21)	1.88 (0.99, 3.56)	0.61 (0.32, 1.15)	1.92 (0.96, 3.88)	0.222		0.051	
<i>CCR7</i> rs3136685 Intron 1	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.381	0.731	0.103	0.086
	AG	1.32 (0.86, 2.02)	2.24 (0.98, 5.16)	1.58 (0.94, 2.66)	2.78 (1.09, 7.08)	0.197		0.056	
	GG	1.07 (0.65, 1.76)	2.38 (1.02, 5.58)	1.02 (0.56, 1.86)	2.52 (0.97, 6.52)	0.778		0.045	
	AG+GG	1.23 (0.82, 1.84)	2.30 (1.05, 5.07)	1.36 (0.83, 2.21)	2.66 (1.10, 6.42)	0.305		0.037	
	GG vs (AA+AG)	0.90 (0.59, 1.36)	1.28 (0.74, 2.25)	0.76 (0.46, 1.26)	1.16 (0.62, 2.15)	0.622		0.372	
<i>CCR7</i> rs3136687	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.022	0.294	0.229	0.218
	GA	1.81 (1.16, 2.80)	0.98 (0.55, 1.75)	1.73 (1.03, 2.93)	1.13 (0.60, 2.14)	0.008		0.459	

Intron 1	AA	1.27 (0.74, 2.18)	0.46 (0.18, 1.18)	1.18 (0.63, 2.20)	0.74 (0.25, 2.17)	0.380		0.106	
	GA+AA	1.63 (1.07, 2.49)	0.85 (0.49, 1.48)	1.53 (0.96, 2.53)	1.06 (0.57, 1.95)	0.023		0.573	
	AA vs (GG+GA)	0.86 (0.55, 1.32)	0.46 (0.19, 1.14)	1.15 (1.12, 1.18)	0.69 (0.25, 1.91)	0.482		0.092	
<i>CCR9</i> <i>rs1488371</i> Intron 2	CC	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.013	0.902	0.003	0.018
	CA	0.94 (0.62, 1.40)	0.52 (0.26, 1.08)	0.88 (0.54, 1.42)	0.60 (0.26, 1.31)	0.747		0.08	
	AA	1.57 (0.50, 4.90)		0.92 (0.22, 3.80)		0.435		0.984	
	CA+AA	0.98 (0.66, 1.44)	0.46 (0.23, 0.94)	0.88 (0.54, 1.40)	0.48 (0.22, 1.04)	0.901		0.034	
	AA vs (AA+CA)	1.60 (0.52, 4.97)		0.95 (0.23, 4.00)		0.414		0.984	
<i>CXCR7</i> <i>rs1045879</i> Exon 1 synSNP L>L	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.065	0.038	0.459	0.762
	AG	1.54 (1.06, 2.26)	1.38 (0.77, 2.48)	1.30 (0.82, 2.04)	1.30 (0.68, 2.50)	0.025		0.279	
	GG	1.53 (0.84, 2.79)	0.86 (0.34, 2.16)	1.02 (0.47, 2.20)	0.74 (0.27, 2.04)	0.166		0.756	
	AG+GG	1.54 (1.07, 2.22)	1.24 (0.72, 2.14)	1.24 (0.80, 1.93)	1.15 (0.62, 2.10)	0.02		0.431	
	GG vs (AA+AG)	1.21 (0.69, 2.13)	0.76 (0.31, 1.84)	0.88 (0.42, 1.84)	0.67 (0.25, 1.80)	0.499		0.538	

The chi-square (χ^2) test p-values were used to determine the difference in the genotype frequencies between cases and controls.

*Fisher's p-values were calculated when expected genotype counts were <5 for either cases and controls. Significant associations are indicated in boldface. Abbreviations: UTR, untranslated region; TFBS, transcription factor binding site; ESE exonic splicing enhancer; ESS, exonic splicing silencers; synSNP, synonymous coding SNP; Jam, Jamaican.

Analysis of gene-gene interactions using Multi-factor Dimensionality Reduction

MDR modeling was utilized to evaluate and validate gene-gene interactions among 43 chemokine-associated sequence variants in relation to PCA risk. The top age-adjusted one-, two- and three-factor gene-gene interaction models for the total population, including men of African descent from Jamaica and the U.S., were statistically significant with 100% cross validation consistency (CVC), 59-67% average testing accuracy (ATA) and permutation testing p-values of 0.001, as shown in **Table 12a**. The three-factor interaction model consisting of *CCR7 rs3136685*, *CCL5 rs3817655*, and *CCR9 rs41289608* was the best predictor of PCA risk among men of African descent, based upon the highest ATA value of 66.5%. This three-factor interaction model was driven by the mutual information gain score of *CCL5 rs3817655* (IG = 2.8%) (**Figure 10**). However, none of the pairwise interactions of the three-factor interaction model yielded more information than each SNP considered alone.

Analysis of age-adjusted gene-gene interactions among U.S. men of African descent yielded statistically significant one-, two- and three-factor interaction models with 100% CVC, 58-68% ATA values, and permutation testing p-values of 0.001-0.003 (**Table 12b**). In addition, the best one-factor MDR model (*CCL5 rs3817655*) was a persistent predictor of PCA risk in the total population and U.S. men, as shown in **Tables 12a and 12b**, respectively. Yet, the three-factor gene-gene interaction model (*CCR6 rs3093024-CCL5 rs3817655-CCR9 rs41289608*) was selected as the best predictor of PCA risk among U.S. men based upon its high ATA value of 68.7% (Permutation testing p-value = 0.001). However, this 3-way interaction was dominated by *CCL5 rs3817655* (Data not shown).

Upon stratification of age-adjusted MDR analyses for Jamaican men, the three-factor gene-gene interaction model of *CCR6 rs3093024*, *CCR4 rs6550178* and *CXCR7 rs7559855* was statistically significant and the overall best predictor of PCA susceptibility (Permutation testing p-value = 0.017) (**Table 12c**). The three-factor model was selected as the best predictor of risk due to its association with an extremely high ATA score of 70% among Jamaican men. This interaction model was moderately synergistic (orange line) and primarily driven the main effect of *CCR4 rs6550178* (IG = 8.2%) and the pairwise interaction between *CCR6* and *CXCR7* (IG = 3.41%) (**Figure 11**). Thus far, *CCR4 rs6550178* had the highest individual information gain score among all of the sequence variants included in this study.

Table 12a. Interactions and main effects of Chemokine-associated SNPs as predictors of PCA using age-adjusted MDR among men of African Descent.

Best Model (dbSNPID#)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i>-value
<u>One Factor</u> <i>CCL5 rs3817655</i>	43	10/10	0.595	0.001
<u>Two Factor</u> <i>CCL5 rs3817655</i> <i>CCR4 rs6550178</i>	903	10/10	0.628	0.001
<u>Three Factor</u> <i>CCR7 rs3136685</i> <i>CCL5 rs3817655</i> <i>CCR9 rs41289608</i>	12,341	10/10	0.665	0.001

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Figure 10. Entropy graph of Chemokine gene-gene interactions among U.S. and Jamaican men of African Descent. The mutual gain scores for all the pairwise combinations for the best 2- and 3-factor MDR models did not exceed the information gain scores of the individual loci. Consequently the selected gene-gene interaction models were not informative and predominantly driven by a strong single SNP effect from *CCL5* rs3817655 (IG = 2.8%).

Table 12b. Interactions and main effects of Chemokine-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. men of African Descent.

Best Model (dbSNPID#)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i>-value
<u>One Factor</u> <i>CCL5 rs3817655</i>	43	10/10	0.584	0.001
<u>Two Factor</u> <i>CCL5 rs3817655</i> <i>CCR5 rs1799988</i>	903	10/10	0.619	0.003
<u>Three Factor</u> <i>CCR6 rs3093024</i> <i>CCL5 rs3817655</i> <i>CCR9 rs41289608</i>	12,341	10/10	0.687	0.001

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Table 12c. Interactions and main effects of Chemokine-associated SNPs as predictors of PCA using age-adjusted MDR among Jamaican men of African Descent.

Best Model (dbSNPID #)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i>-value
<u>One Factor</u> <i>CCR9 rs2286486</i>	43	10/10	0.606	0.061
<u>Two Factor</u> <i>CCR9 rs2286486</i> <i>CCR7 rs3136687</i>	903	10/10	0.637	0.329
<u>Three Factor</u> <i>CCR6 rs3093024</i> <i>CCR4 rs6550178</i> <i>CXCR7 rs7559855</i>	12,341	9/10	0.704	0.017

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Figure 11. Entropy graph of Chemokine gene-gene interactions among Jamaican men of African Descent. MDR analysis revealed that only the three-factor (*CCR6* rs3093024-*CXCR7* rs7559855-*CCR4* rs6550178) survived multiple hypothesis testing with 90% CVC and 70.4% ATA values (Permutation p-value=0.017). However, a moderately synergistic (orange line) interaction between *CCR6* and *CXCR7* associated with a pairwise mutual information gain score of 3.41%, which is higher than the mutual information gain scores of each SNP. In addition, the three-factor interaction model was predominantly driven by *CCR4* rs6550178 (IG = 8.2%).

Discussion

Chemotaxis is an important process required for tumor growth and metastasis [179, 180]. It is regulated by a complex network of chemokines, chemokine receptors and downstream targets that synergistically regulate immune and inflammatory responses [180]. Recent molecular studies have demonstrated that over expression of selected chemokines and chemokine receptors are related to aggressive cancer phenotypes, including lung, breast and prostate cancer [181-185]. Some observational studies suggest inheritance of susceptibilities detected in chemokine-associated genes may alter the risk of developing cancer [116-120]. However, to our knowledge, there are no published reports on the impact of inheriting multiple functional variants in relation to prostate cancer among men of African Descent. Therefore, the current study evaluated the individual and combined effects of 43 chemokine associated sequence variants on PCA risk among 279 cases and 535 disease-free men of African descent from the U.S and Jamaica using LR and MDR modeling. Five SNPs detected in *CCL5*, *CCR5* and *CCR7* were significantly associated with prostate cancer risk among all study participants; however, only three markers survived adjustments for potential confounders and multiple hypothesis testing [35]. Notably, inheritance of at least one *CCL5* rs3817655 A or *CCL5* rs2107538 A loci was linked with a 34-44% decrease in PCA susceptibility among all men of African Descent. In addition, the recessive genetic model for *CCR5* rs1799988 was associated with a 52-73% increase in PCA risk. We also observed significant main effects for the *CCL5* rs3817655 and *CCR7* rs3136685 SNPs among U.S. and Jamaican men, respectively.

MDR analysis revealed several significant chemokine-associated gene-gene interactions among men of African descent. In the total population, a three factor model interaction model was selected as the best prostate cancer predictor for the total population (*CCR7 rs3136685-CCL5 rs3817655-CCR9 rs41289608*) as well as the African-Americans from the U.S. (*CCR6 rs3093024- CCL5 rs3817655-CCR9 rs41289608*) with CVC = 100% ATA = 66.5-68.7 and Permutation-testing p-value = 0.001. (CVC = 100%; ATA = 66.5% value; Permutation testing p-value = 0.001). However, upon closer inspection, these 3-way interactions were primarily driven by *CCL5 rs3817655* none of the interactions within the three-factor model were informative. Interestingly, a significant three-factor interaction model (*CCR6 rs3093024-CCR4 rs6550178- CXCR7 rs7559855*) for Jamaican men had the highest ATA value (70.4%) compared to the total population and U.S men interaction models (Permutation p-value = 0.017). This interaction model was moderately synergistic due to the major main effect of *CCR4 rs6550178* (IG = 8.2%) and a pairwise interaction between *CCR6 rs3093024* and *CXCR7 rs7559855* (IG = 3.41%). In fact, the pairwise mutual information gain score of *CCR6 rs3093024* and *CXCR7 rs7559855* was higher than information gain scores of the individual variants. However, the other interaction models among Jamaican men did not survive multiple hypothesis testing.

Several cancer cells, including PCA cells, express chemokines and their cell surface bound receptors. Chemokine ligand 5 (RANTES) is a small molecule with a strong capacity to induce cellular migration of inflammatory cells and production of its receptor (CCR5) in human PCA cell lines [73, 75]. The CCL5/CCR5 axis induces PCA cell proliferation and invasion [74]. It's speculated that once CCL5 binds to CCR5, it

serves as an autocrine factor and activates cellular responses involved in cancer progression [73]. In the current study, possession of the *CCL5* rs3817655 A or *CCL5* rs2107538 A loci was linked with a protective effect in relation to prostate cancer risk among all men of African descent from the U.S. and Jamaica combined. The directionality of the risk estimates persisted when we stratified by racial/ethnic group; however, the findings were only statistically significant for the U.S. men. In addition, findings for the total and U.S. subgroups remained significant even after adjusting for age and multiple hypothesis testing. The observed protective effects associated with the two aforementioned *CCL5* SNPs (*rs3817655*, *rs2107538*) may be attributed to a reduction in transcriptional activation, reduced protein levels, and ultimately reduced tumorigenic capacity. To our knowledge, there are no published reports on the impact of the *CCL5* *rs3817655* SNP on prostate cancer susceptibility or its functional consequence on genes/proteins. On the other hand, the *CCL5* *rs2107538* G-403A promoter SNP is associated with a decrease in protein expression detected in serum collected from Type II diabetic and disease-free subjects [186]. In addition, this locus has been evaluated in two independent prostate cancer studies. In a study involving 607 Caucasian male residents of Spain (297 cases, 3011 controls), Saenz-Lopez and co-workers (2008) observed a 1.44-fold increase in PCA risk among carriers of the *CCL5* *rs2107538* GA+AA genotype (p-value = 0.039) [116]. However, this finding did not corroborate with a larger null report consisting of 1553 Caucasian men (i.e., 815 PCA cases, 738 controls) from Australia [187]. Our findings of a protective effect against PCA among our study participants is consistent with other published reports that reveal a decrease in the risk of developing gastric cancer, lymphoma, and type 1 diabetes [113, 186, 188]. Within a

multi-ethnic pancreatic case-control study, the prevalence of the “A” allele was more frequent among disease-free Asian and African-Americans relative to pancreatic cases [118]; however, this allele was more prevalent among Caucasian pancreatic cases relative to controls. Collectively, these findings may suggest inheritance of genetic susceptibilities detected in the *CCL5* gene may vary across different racial/ethnic groups.

The functional impact of *CCL5* sequence variants is complicated by the high degree of linkage disequilibrium within both the promoter and intron 1 region. An and co-workers (2006), evaluated the impact of three SNPs detected in the promoter region (*rs2280788* -28C/G, *rs2107538* -405 G/A) and intron 1 of *CCL5* *rs2280789* [189]. They demonstrated that transcriptional regulation of *CCL5* was primarily governed by an intron 1.1 A/G SNP (*rs2280789*). Intron 1.1 G allele corresponded with a strong decrease in transcriptional activity of RANTES; whereas, the -28G allele had a modest up-regulation in human cell lines. In our stratified analysis, the intron 1.1 *CCL5* *rs2280789* AG or AG+GG genotypes were associated with a marginal 36-41% decrease in PCA risk among men of African descent from the U.S.; however, these findings require further evaluation in a larger study set. The *CCL5* *rs2280788* -28C/G was not evaluated in the current study, since the C allele frequency is 0% for African-American men, as reported in NCBI. The functional consequence of SNPs detected in *CCL5* is further perplexed by their interaction with downstream receptors.

The biological activity mediated by *CCL5* is facilitated through its interaction with chemokine receptors (CCR1, CCR2, CCR3, and CCR5). However, relative to CCR1-3, CCR5 plays a more important role in *CCL5*-mediated cell migration [190]. CCR5, a member of the beta chemokine receptor family, is a seven transmembrane

protein, which is expressed by T cells and macrophages. Over expression of CCR5 has been detected in aggressive prostate cancer tissue relative to benign prostatic hyperplasia [75]. The *CCR5 rs1799988* C allele is significantly associated with viral load set point (i.e., decreased time from asymptomatic HIV⁺ to AIDS and increased infectiousness) and AIDS progression [191]; however, there are no published reports for this 5'UTR SNP in relation to prostate cancer or other inflammatory/immune response-related diseases. In the current study, we observed a 1.52-1.75 fold increase in the risk of developing prostate cancer among all men of African descent who possessed the *CCR5 rs1799988* CC genotype. However, the impact of this SNP in relation to PCA risk was more pronounced among men of African descent from Jamaica relative to U.S. men. This increased risk may have an impact on transcriptional activity, which may result in increased protein levels of CCR5; however, this requires confirmation using *ex vivo*, *in vitro*, and micro-dissected tumor tissue-based assays.

The functional consequence of intronic sequence variants, *CCR6 rs3093024*, *CCR4 rs6550178* and *CXCR7 rs7559855*, is unknown for any cancer. However, our lab observed a significant 3-factor gene-gene interaction (*CCR6 rs3093024-CCR4 rs6550178-CXCR7 rs7559855*) model, which was the best predictor of PCA risk among Jamaican men (Permutation testing p-value = 0.017) . Although these chemokine sequence variants have not been studied in relation to PCA in the literature, the genes in which these variants reside are associated with PCA. Several published reports have implicated that the expression of CCR6, CCR4 and CXCR7 plays a role in prostate cancer progression. Ghadjar and co-workers (2008) showed the CCR6 protein was expressed in over 50% of prostatic tumors and strongly associated with aggressive

prostate cancer, according to gleason score (p-value = 0.003) and tumor stage ($p < 0.0005$) [192]. For CXCR7, Wang and associates (2008) demonstrated that CXCR7/RDC1 protein expression increases gradually from PIN to localized and metastatic PCA [86]. CXCR7/RDC1 targets cadherin-11 and influences the expression of pro-angiogenic VEGF and IL8 in prostate cancer, which may contribute to tumor growth. In addition, CXCR7 is a direct target of hypermethylated in cancer 1 (HIC1), a gene that regulates proliferation migration and invasion [193]. The promoter of HIC1 is hypermethylated in prostate cancer cells. Restoration of HIC1 in prostate cancer cell lines, PC3 and C4-2B, inhibited cellular proliferation, migration and invasion via down regulation of MMP2/3 protein expression. A study by Derhovanessian and colleagues (2009) observed that hormone-resistant prostate cancer patients with IL17+ T cells lacking the expression of CCR4 were strongly associated with a poor prognosis [194]. CCR4 expression may have a protective effect against prostate cancer progression.

The strengths, limitations and future directions were considered in relation to our findings on PCA susceptibility among men of African descent. Forty-three sequence variants were evaluated in relation to prostate cancer risk among men of African descent from the U.S. and Jamaica [35]. A strong correlation between the minor allele frequencies between these two study populations enabled us to pool genetic data to identify relationships that would have remained undetected if we evaluated the populations separately. As a result of pooling, we identified three SNPs (i.e., *CCL5* rs3817655, *CCL5* rs2107538, *CCR5* rs1799988) that were significantly associated with prostate cancer in the total population even after adjusting for age and multiple hypothesis testing. Upon stratification by study center, we cannot rule out the possibility

that race/ethnic specific sequence variants may track with disease progression or prognosis. Consequently, future targeted sequencing will allow us to identify, evaluate and validate novel and commonly reported chemokine-associated SNPs as tumor classification and prognostication tools among African-American, Caribbean, African and European men. Such efforts will require pooled data from multi-center studies that seek to identify the genetic signatures related to prostate cancer health disparities domestically and globally. In addition, molecular biological studies are needed to understand the functional consequence of the *CCL5* and *CCR5* sequence variants on: mRNA expression/stability, or protein expression, structure and/or function. This will require observational studies that allow us to consider lifestyle, geographical, environmental, or cultural differences that may interact with genetic susceptibilities and subsequently modify PCA outcomes. In future studies, we will evaluate the mRNA and protein expression of *CCL5* and *CCR5* in metastatic and non-metastatic prostate cancer cell lines compared to normal prostate cells using qRT-PCR and western blot analysis. We will also examine the impact of coding and non-coding SNPs in *CCL5* and *CCR5* on cellular migration, proliferation and invasion in selected prostate cancer cell lines to determine the functional mechanism of selected SNPs. We will also evaluate the protein expression of *CCL5* and *CCR5* in tumor tissue collected from men of African and European descent to determine any unique expression profiles between ethnic groups. Lastly, study participants self-identified themselves as African-American, Caribbean, African, or Jamaican. Population admixture, which commonly occurs among men of African descent, may bias risk estimates. After adjusting risk estimates for West African Ancestry, risk estimates did not significantly vary when compared to unadjusted risk

models (data not shown). Statistical power greater than 80% was used to observe effect sizes of ≥ 1.5 (or ≤ 0.67) and 1.55 (or ≤ 0.64) for the total and U.S. populations, respectively. All p-values were adjusted for multiple hypotheses testing to minimize false positive results using the false discovery rate and Bonferroni Correction. Despite these efforts, we cannot rule out the possibility that the significant relationships observed for the U.S. (*CCL5* rs3817655) and total populations (*CCL5* rs2107538, *CCL5* rs3817655, *CCR5* rs1799988) are suggestive and warrant further validation in larger studies. Future studies in our laboratory will focus on high-throughput targeted sequencing to evaluate the impact of novel and commonly reported *CCL5* (rs2107538, rs3817655) and *CCR5* (rs1799988) sequence variants on PCA susceptibility and disease prognosis among men of African descent. Even modest variations in genotype allele frequencies among men of African descent can reduce the chances of replicating single SNP effects within and between independent and validation study sets. To ensure reproducibility within future and ongoing studies, extreme care is needed to select study sets with comparable ancestry and genetic backgrounds.

CHAPTER 5

THE IMPACT OF GENETIC VARIANTS IN INFLAMMATORY-RELATED GENES ON PROSTATE CANCER RISK AMONG MEN OF AFRICAN DESCENT

Although case–control studies have evaluated the role of variant inflammatory-related loci in prostate cancer, their impact is virtually unknown among men of African descent. To address this, we evaluated the impact of inflammatory cytokine single nucleotide polymorphisms (SNPs) on prostate cancer risk for men of African descent. Forty-four SNPs in inflammatory cytokine-associated genes in germ-line DNA were evaluated among 814 African-American and Jamaican men (279 prostate cancer cases and 535 controls) using Illumina’s Golden gate genotyping system, logistic regression and entropy-based MDR. Four SNPs were modestly associated with prostate cancer after adjusting for age. In the total population, inheritance of the *IL1R2* *rs11886877* AA, *IL8RB* *rs11574752* AA, *TNF* *rs1800629* GA + AA, and *TNF* *rs673* GA genotypes modestly increased prostate cancer risk by 1.45 to 11.7-fold relative to the referent genotype. Among U.S. men, age-adjusted dominant, recessive and additive genetic models for the *IL1R2* *rs11886877* locus were linked to an increase in prostate cancer susceptibility. However, these main effects did not persist after adjusting for multiple hypothesis testing. Our preliminary data does not strongly support the hypothesis that inflammatory-related sequence variants influence prostate cancer risk among men of African descent. However, further evaluation is needed to assess whether other variant inflammatory-related genes may contribute to prostate cancer risk and disease

progression in larger and ethnically diverse multi-center studies. Findings from our study will help to fill in the gaps in information pertinent to prostate cancer among men of African descent.

Results

Prevalence of inflammatory-associated sequence variants among men of African Descent

Inheritance of variant inflammatory-related loci was fairly common among African-American men in the current study. Specifically, the minor allele frequencies of 44 sequence variants ranged from 3% to 48%, as depicted in **Table 8**. Notably, the observed genotype frequency distribution among controls did not significantly deviate from expected counts according to the Hardy Weinberg equilibrium. With the exception of three loci (*IL1RN* *rs4251961*, *IL10RB* *rs999788*, and *TNF* *rs673*), the observed genotype frequencies in the current study corroborated with values for individuals of African-American/African ancestry, reported in the NCBI's SNP entrez **Table 8** (p-value = 0.081-0.975).

Relationship between inflammatory sequence variants and prostate cancer risk

Seven out of 44 sequence variants detected in inflammatory-related genes were modestly associated with prostate cancer risk among 814 men of African descent (279 cases and 535 controls) [36]. For unadjusted risk models, elevations in prostate cancer susceptibility were observed among carriers of *IL1R2* *rs1188687* (AA, GA+AA), *IL8RB* *rs11574752* (AA, GA+AA), *TNF* *rs1800629* (GA, GA+AA), *TNF* *rs673* (GA), *IL1A* (*rs17561* AA, *rs2856836* GG) and *IL10RA* *rs4252243* AA genotypes with risk estimates ranging from 0.17-11.7 among men of African descent. Only half of these loci remained

significant after adjusting for age, namely the *IL1R2* *rs1188687* (AA, GA+AA), *IL8RB* *rs11574752*AA, and *TNF* *rs673*GA markers (**Table 13a**). Inheritance of *IL1R2* *rs1188687* [AA (OR_{age-adjusted} = 1.92; 95% CI = 1.11-3.32), GA+AA (OR_{age-adjusted} = 1.46; 95% CI = 1.01-2.10)], *IL8RB* *rs11574752* [AA (OR_{age-adjusted} = 38.4; 95% CI = 3.86-382.8), recessive genetic model (OR_{age-adjusted} = 39.2; 95% CI = 3.94-390)], *TNF* *rs1800629* [GA (OR_{age-adjusted} = 1.54; 95% CI = 1.06-2.24), GA+AA (OR_{age-adjusted} = 1.53; 95% CI = 1.06-2.20)] and *TNF* *rs673* [GA (OR_{age-adjusted} = 1.50; 95% CI = 1.04-2.16)] genotypes were associated with an increase in PCA risk among men of African descent (**Table 13a**). However, *IL1R2* *rs1188687* marker was the only genetic susceptibility factor that was significant under the additive genetic model, indicative of a significant dose-response effect in relation to the number of inherited minor allele (P-trend = 0.010). The aforementioned markers were not classified as important prostate cancer predictors after adjusting for multiple comparisons bias using the Bonferroni correction, with a significance cut-off of 0.0011.

Table 13a. Relationship between Inflammatory-associated SNPs and PCA Risk among men of African Descent.

Gene (Allele, position, function)	Genotype	Cases n (%)	Controls n (%)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)	Chi-square (χ^2) p-value*	p-trend	Bonferroni Correction
<i>IL1R2</i> <i>rs11886877</i> Intron 1	GG	87 (31.2)	211 (39.4)	1.00 (referent)	1.00 (referent)	0.034	0.01	NS
	GA	149 (53.4)	265 (49.5)	1.36 (0.99, 1.88)	1.35 (0.92,1.98)	0.058		
	AA	43 (15.4)	59 (11.1)	1.77 (1.11, 2.82)	1.92 (1.11, 3.32)	0.017		
	GA+AA	192 (68.8)	324 (60.6)	1.44 (1.06, 1.95)	1.46 (1.01, 2.10)	0.021		
	AA vs (GG+GA)			1.47 (0.96,2.24)	1.61 (0.98,2.63)	0.074		
<i>IL1A</i> <i>rs17561</i> Exon 4 Splicing nsSNP, benign	CC	195 (69.9)	358 (66.9)	1.00 (referent)	1.00 (referent)	0.025	0.108	NS
	CA	82 (29.4)	155 (29.0)	0.97 (0.70,1.34)	1.01 (0.68,1.48)	0.858		
	AA	2 (0.70)	22 (4.10)	0.17 (0.04,0.72)	0.40 (0.08,1.83)	0.016		
	CA+AA	84 (30.1)	177 (33.1)	0.87 (0.64,1.20)	0.96 (0.66,1.40)	0.388		
	AA vs (CC+CA)			0.17 (0.04,0.72)	0.40 (0.09,1.82)	0.016		
<i>IL8RB</i> <i>rs11574752</i> 3'UTR miRNA (miRanda, Sanger)	GG	230 (82.4)	435 (81.3)	1.00 (referent)	1.00 (referent)	0.011	0.784	NS
	GA	43 (15.4)	99 (18.5)	0.82 (0.55,1.21)	0.90 (0.56,1.40)	0.326		
	AA	6 (2.20)	1 (0.20)	11.3 (1.36, 94.6)	38.4 (3.86, 382.8)	0.009		
	GA+AA	49 (17.6)	100 (18.7)	0.93 (0.64,1.35)	1.08 (0.69,1.70)	0.693		
	AA vs (GG+GA)			11.7 (1.40, 98.0)	39.2 (3.94, 390)	0.008		
<i>TNF</i> <i>rs1800629</i> 5' near gene TFBS	GG	171 (61.2)	368 (68.8)	1.00 (referent)	1.00 (referent)	0.047	0.087	NS
	GA	103 (37.0)	153 (28.6)	1.45 (1.06, 1.97)	1.54 (1.06,2.24)	0.019		
	AA	5 (1.80)	14 (2.60)	0.77 (0.27, 2.16)	1.30 (0.37,4.60)	0.619		
	GA+AA	108 (38.8)	167 (31.2)	1.39 (1.03,1.90)	1.53 (1.06,2.20)	0.032		
	AA vs (GG+GA)			0.68 (0.24,1.91)	1.13 (0.32,3.90)	0.462		
<i>TNF</i> <i>rs673</i> 5' near gene TFBS	GG	171 (61.3)	364 (68.0)	1.00 (referent)	1.00 (referent)	0.009	0.228	NS
	GA	106 (38.0)	156 (29.2)	1.45 (1.06, 2.00)	1.50 (1.04,2.16)	0.018		
	AA	2 (0.70)	15 (2.80)	0.28 (0.06, 1.26)	0.47 (0.09,2.40)	0.097		
	GA+AA	108 (39.1)	171 (32.0)	1.34 (0.99, 1.82)	1.43 (1.00, 2.05)	0.055		
	AA vs (GG+GA)			0.25 (0.06,1.10)	0.41 (0.08,2.07)	0.067		
<i>IL1A</i> <i>rs2856836</i> 3'UTR miRNA (miRanda, Sanger)	AA	196 (70.3)	358 (66.9)	1.00 (referent)	1.00 (referent)	0.024	0.089	NS
	AG	81 (29.0)	155 (29.0)	0.96 (0.69,1.32)	0.99 (0.67,1.45)	0.776		
	GG	2 (0.70)	22 (4.10)	0.17 (0.04, 0.71)	0.40 (0.09,1.82)	0.016		
	AG+GG	83 (29.7)	177 (33.1)	0.86 (0.63,1.17)	0.94 (0.65,1.36)	0.333		
	GG vs (AA+AG)			0.17 (0.04, 0.72)	0.40 (0.09,1.82)	0.016		
<i>IL10RA</i> <i>rs4252243</i> 5' near gene TFBS	GG	134 (48.4)	268 (50.4)	1.00 (referent)	1.00 (referent)	0.066	0.168	NS
	GA	115 (41.5)	234 (44.0)	0.98 (0.72,1.32)	0.83 (0.58,1.18)	0.893		
	AA	28 (10.1)	30 (5.60)	1.86 (1.07, 3.24)	1.62 (0.82, 3.21)	0.028		
	GA+AA	143 (51.6)	264 (49.6)	1.08 (0.81,1.44)	0.91 (0.64,1.28)	0.605		

	AA vs (GG+GA)		1.88 (1.10, 3.21)	1.77 (0.91, 3.43)	0.021	
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The chi-square (χ^2) test p-values were used to determine the difference in the genotype frequencies between cases and controls. *Fisher's p-values were calculated when expected genotype counts were <5 for either cases and controls. Significant odd ratios (ORs) and 95% confidence interval (CI) are indicated in boldface. Abbreviations: UTR, untranslated region; TFBS, transcription factor binding site; ESE exonic splicing enhancer; ESS, exonic splicing silencers; nsSNP, non-synonymous coding SNP; miRNA, microRNA binding site.

Upon stratification by sub-population, modestly significant prostate cancer predictors varied by racial/ethnic group in the unadjusted and adjusted risk models. Possession of the *RNASEL* *rs1213524* AG ($OR_{\text{age-adjusted}} = 2.10$; 95% CI = 1.04-4.24) genotype was associated with a 2.10-fold increase in the risk of developing prostate cancer among Jamaican men (**Table 13b**). However, this loci was not significant in the dominant, recessive or additive genetic models. Similar to the total population, inheritance of sequence variants in *IL1R2*, *IL10RA* and *TNF* among U.S. men were linked with an increase in prostate cancer risk. Among U.S. men, four inflammatory-related sequence variants, *IL1R2* *rs11886877* (GA, GA+AA, AA), *TNF* *rs673* GA, *TNF* *rs1800629* (GA, GA+AA), *IL10RA* *rs4252243* AA were associated with 1.4-2.34 fold increase in prostate cancer risk; whereas, reductions were observed among carriers of *IL1B* *rs1071676* GC+CC, *IL1B* *rs1143634* GA+AA, and *IL1A* *rs1800587* AA genotypes within unadjusted risk estimate models ($p\text{-value} \geq 0.028$). Out of these 7 markers, most of the genetic models for the *IL1R2* loci remained significant after adjusting for age. Possession of *IL1R2* *rs11886877* variant alleles under the dominant/co-dominant [AA ($OR_{\text{age-adjusted}} = 2.75$; 95% CI = 1.38-5.50), GA+AA ($OR_{\text{age-adjusted}} = 1.82$; 95% CI = 1.82-2.88), *IL1R2* *rs11886877* recessive genetic ($OR_{\text{age-adjusted}} = 2.05$; 95% CI = 1.10-3.80)] and *IL10RA* *rs4252243* recessive genetic ($OR_{\text{age-adjusted}} = 2.49$; 95% CI = 1.08-5.72) models were associated with an elevation in PCA susceptibility among U.S. men of African descent. In the stratified analysis, none of the aforementioned markers survived correction for multiple hypothesis testing according to the new significance level among U.S. and Jamaican men (Bonferroni Correction $p\text{-value} \geq 0.004$ and $p\text{-value} \geq 0.018$), respectively.

Table 13b. Relationship between Inflammatory-associated SNPs and PCA Risk stratified by racial ethnic group.

Gene (Allele, location, function)	Genotype	Unadjusted OR (95%CI) US men	Age-Adjusted OR (95%CI) US men	Unadjusted OR (95%CI) Jam men	Age-Adjusted OR (95%CI) Jam men	χ^2 p-value* US men	χ^2 p-value* Jam men	p trend US men	p trend Jam men
<i>IL1B</i> <i>rs1071676</i> UTR'3 miRNA (miRanda, Sanger)	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.050	0.550	0.022	0.276
	GC	0.72 (0.48, 1.10)	0.70 (0.42, 1.14)	1.39 (0.71, 2.70)	1.28 (0.62, 2.64)	0.124	0.338		
	CC	0.16 (0.02, 1.25)	0.19 (0.02, 2.00)	2.02 (0.18, 22.8)	1.15 (0.10, 14.6)	0.081	0.568		
	GC+CC	0.66 (0.44, 1.00)	0.66 (0.40, 1.10)	1.42 (0.74, 2.72)	1.26 (0.62, 2.60)	0.050	0.294		
	CC vs (GG+GC)	0.18 (0.02, 1.36)	0.21 (0.02, 2.18)	1.89 (0.16, 21.1)	1.10 (0.08, 13.7)	0.096	0.606		
<i>IL1B</i> <i>rs1143634</i> Exon 4 Splicing (ESE/ ESS) cds- synonymous	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.051	0.447	0.016	0.203
	GA	0.67 (0.44, 1.01)	0.65 (0.40, 1.06)	1.51 (0.76, 3.00)	1.37 (0.64, 2.90)	0.058	0.243		
	AA	0.21 (0.02, 1.60)	0.24 (0.02, 2.86)	2.05 (0.18, 23.0)	1.16 (0.10, 14.6)	0.131	0.561		
	GA+AA	0.63 (0.42, 0.95)	0.62 (0.38, 1.02)	1.54 (0.78, 3.00)	1.36 (0.65, 2.82)	0.028	0.208		
	AA vs(GG+GA)	0.23 (0.02, 1.80)	0.27 (0.02, 3.20)	1.89 (0.16, 21.1)	1.10 (0.08, 13.7)	0.157	0.606		
<i>IL1R2</i> <i>rs11886877</i> Intron 1	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.007	0.889	0.002	0.631
	GA	1.60 (1.08, 2.40)	1.63 (1.00, 2.64)	0.92 (0.50, 1.68)	0.94 (0.48, 1.80)	0.020	0.782		
	AA	2.34 (1.31, 4.16)	2.75 (1.38, 5.50)	0.82 (0.36, 1.86)	0.94 (0.38, 2.30)	0.004	0.633		
	GA+AA	1.72 (1.18, 2.52)	1.82 (1.14, 2.88)	0.89 (0.50, 1.58)	0.94 (0.50, 1.74)	0.005	0.700		
	AA vs (GG+GA)	1.76 (1.04, 2.96)	2.05 (1.10, 3.80)	0.86 (0.40, 1.80)	0.97 (0.43, 2.20)	0.033	0.691		
<i>RNASEL</i> <i>rs12135247</i> UTR'3 TFBS, miRNA	AA	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.820	0.025	0.909	0.216
	AG	1.06 (0.72, 1.58)	1.14 (0.71, 1.84)	2.17 (1.14, 4.12)	2.10 (1.04, 4.24)	0.756	0.018		
	GG	0.77 (0.30, 1.96)	0.70 (0.24, 2.10)	0.45 (0.08, 2.40)	0.28 (0.04, 1.70)	0.570	0.344		
	AG+GG	1.02 (0.70, 1.50)	1.07 (0.68, 1.68)	1.81 (0.99, 3.30)	1.68 (0.88, 3.24)	0.906	0.053		
	GG vs (AG+AA)	0.76 (0.30, 1.92)	0.67 (0.22, 1.97)	0.36 (0.06, 1.91)	0.22 (0.04, 1.35)	0.555	0.232		
<i>TNF</i> <i>rs1800629</i> 5' near gene TFBS	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.101	0.782	0.113	0.549
	GA	1.50 (1.03, 2.20)	1.52 (0.96, 2.42)	1.21 (0.68, 2.12)	1.41 (0.78, 2.63)	0.034	0.518		
	AA	0.90 (0.28, 2.80)	1.51 (0.36, 6.24)	1.00 (0.06, 16.3)	1.00 (0.06, 17.2)	0.842	1.000		
	GA+AA	1.44 (0.99, 2.10)	1.53 (0.97, 2.40)	1.20 (0.68, 2.10)	1.40 (0.75, 2.60)	0.050	0.525		
	AA vs (GG+GA)	0.78 (0.25, 2.42)	1.32 (0.32, 5.40)	0.94 (0.06, 15.2)	0.88 (0.05, 15.0)	0.666	0.962		
<i>IL1A</i> <i>rs1800587</i> UTR'5 TFBS, Splicing (ESE or ESS)	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.088	0.450	0.028	0.224
	GA	0.75 (0.50, 1.10)	0.68 (0.42, 1.08)	1.38 (0.76, 2.50)	1.42 (0.74, 2.72)	0.119	0.297		
	AA	0.56 (0.32, 0.96)	0.78 (0.40, 1.53)	1.56 (0.69, 3.50)	1.64 (0.70, 4.00)	0.038	0.279		
	GA+AA	0.70 (0.48, 1.00)	0.70 (0.45, 1.10)	1.42 (0.80, 2.50)	1.47 (0.80, 2.72)	0.053	0.222		
	AA vs (GG+GA)	0.66 (0.40, 1.10)	0.96 (0.52, 1.80)	1.30 (0.62, 2.70)	1.34 (0.60, 3.00)	0.105	0.478		
<i>IL10RA</i> <i>rs4252243</i> 5' near gene TFBS	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.062	0.632	0.275	0.329
	GA	0.92 (0.63, 1.33)	0.70 (0.44, 1.10)	1.24 (0.70, 2.20)	1.21 (0.64, 2.28)	0.648	0.448		
	AA	2.02 (1.04, 3.95)	2.10 (0.90, 4.98)	1.50 (0.54, 4.26)	1.04 (0.34, 3.20)	0.038	0.436		
	GA+AA	1.03 (0.72, 1.50)	0.81 (0.52, 1.30)	1.28 (0.74, 2.21)	1.15 (0.64, 2.10)	0.863	0.328		
	AA vs (GG+GA)	2.11 (1.10, 4.02)	2.49 (1.08, 5.72)	1.37 (0.50, 3.74)	1.02 (0.40, 2.98)	0.023	0.539		
<i>TNF</i> <i>rs673</i>	GG	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)	0.027	0.452	0.279	0.874
	GA	1.50 (1.02, 2.20)	1.46 (0.92, 2.30)	1.22 (0.70, 2.14)	1.41 (0.76, 2.62)	0.025	0.498		

5' near gene TFBS	AA	0.24 (0.03, 1.84)	0.54 (0.06, 4.44)	0.33 (0.03, 3.24)	0.40 (0.03, 4.70)	0.178	0.340	
	GA+AA	1.38 (0.95, 2.00)	1.40 (0.88, 2.20)	1.14 (0.66, 2.00)	1.33 (0.72, 2.46)	0.087	0.635	
	AA vs (GG+GA)	0.21 (0.02, 1.60)	0.47 (0.06, 3.91)	0.31 (0.03, 2.98)	0.35 (0.03, 4.08)	0.132	0.308	

The chi-square (χ^2) test p-values were used to determine the difference in the genotype frequencies between cases and controls.

*Fisher's p-values were calculated when expected genotype counts were <5 for either cases and controls. Significant odd ratios (ORs) and 95% confidence interval (CI) are indicated in boldface. Abbreviations: UTR, untranslated region; TFBS, transcription factor binding site; ESE exonic splicing enhancer; ESS, exonic splicing silencers; nsSNP, non-synonymous coding SNP; Jam, Jamaican.

Analysis of gene-gene interactions using Multi-factor Dimensionality Reduction

MDR analyses revealed that the one-, two- and three-factor interaction models survived multiple hypothesis testing with a 90-100% CVC and a 58-61% ATA values among men of African descent, as shown in **Table 14a** (Permutation p-value=0.001-0.013). TNF rs673 was selected as the best one-factor model among men of African descent, based on the highest information gain score relative to the other cytokine-associated sequence variants (IG = 2.08%) (**Figure 12**). For the two-factor interaction model (*IL10RA* rs4252243, *IL10RB* rs8178433), the MDR entropy-based graph revealed no informative pairwise mutual information gain score for the interaction (**Figure 12**). The three-factor gene-gene interaction model (*IL1R2* rs11886877, *IL10* rs1800896, *IL10RA* rs4252243) was selected as the best predictor of PCA susceptibility among men of African descent based on its association with the highest ATA value (ATA = 61%). This three-factor interaction model was primarily driven by a highly synergistic (red line) pairwise interaction between IL10 and IL1R2 associated with a mutual information gain score of 0.86%. This pairwise interaction was more informative than each individual SNP when considered alone (**Figure 12**).

Upon stratification by ethnic group, the one- and three-factor interaction models were statistically significant with 100% CVC and 60-67% ATA values among U.S. men (Permutation testing p-value = 0.001-0.005) (**Table 14b**). *IL10* rs4252243 was selected as the best one-factor predictor of prostate cancer, based on a 100% CVC and 59.7% ATA. Perhaps MDR allows us to detect important prostate cancer predictors that we were unable to observe under the Bonferroni adjusted LR models. The three-factor interaction model (*IL1B* rs1143627, *IL1A* rs1800587, *IL1RN* rs315951) was selected as

the best predictor of PCA risk due to its high CVC value of 100% and ATA score of 67% among U.S. men (Permutation testing p-value = 0.001). The synergy of this interaction model was driven by a highly synergistic interaction between *IL1B rs1143627* and *IL1A rs1800587* with an informative IG score of 1.93% (Data not shown). Among Jamaican men, the two-factor (*IL1RN rs315951* and *IL1R1 rs949963*) interaction model associated with a 100% CVC value and 65% ATA score remained significant after adjustment for multiple hypothesis testing (Permutation testing p-value = 0.008). This two-factor interaction model was highly synergistic due to its informative pairwise mutual information gain score of 4.77% (Data not shown).

Table 14a. Interactions and main effects of Inflammatory-associated SNPs as predictors of PCA using age-adjusted MDR among men of African Descent.

Best Model (dbSNPID #)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i> –value
<u>One Factor</u> <i>TNF rs673</i>	44	10/10	0.580	0.001
<u>Two Factor</u> <i>IL10RA rs4252243</i> <i>IL10RB rs8178433</i>	946	9/10	0.598	0.013
<u>Three Factor</u> <i>IL1R2 rs11886877</i> <i>IL10 rs1800896</i> <i>IL10RA rs4252243</i>	13,244	9/10	0.610	0.003

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Figure 12. Entropy graph of Cytokine gene-gene interactions among U.S. and Jamaican men of African Descent. MDR analysis revealed that 1-, 2- and 3-factor interaction models survived multiple hypothesis testing with a 90-100% CVC and 58-61% ATA values (Permutation p-value=0.001-0.013). The three-factor gene-gene interaction model (IL1R2 rs11886877, IL10 rs1800896, IL10RA rs4252243) was the best predictor of PCA risk among men of African descent. The synergy of this model was based on interaction information gain score of 0.86% for IL10 and IL1R2 that is higher than the mutual information gain scores of each variant.

Table 14b. Interactions and main effects of Inflammatory-associated SNPs as predictors of PCA using age-adjusted MDR among U.S. men of African Descent.

Best Model (dbSNPID #)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing <i>p</i> -value
<u>One Factor</u>				
<i>IL10RA rs4252243</i>	44	10/10	0.597	0.005
<u>Two Factor</u>				
<i>IL10RA rs4252243</i> <i>IL10RB rs8178433</i>	946	10/10	0.627	0.084
<u>Three Factor</u>				
<i>IL1B rs1143627</i> <i>IL1A rs1800587</i> <i>IL1RN rs315951</i>	13,244	10/10	0.665	0.001

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Table 14c. Interactions and main effects of Inflammatory-associated SNPs as predictors of PCA using age-adjusted MDR among Jamaican men of African Descent.

Best Model (dbSNPID #)	Interactions	Cross Validation Consistency (CVC)	Average Testing Accuracy (ATA)	Permutation Testing p-value
<u>One Factor</u> <i>IL1R2 rs4141134</i>	44	10/10	0.577	0.243
<u>Two Factor</u> <i>IL1RN rs315951</i> <i>IL1R1 rs949963</i>	946	10/10	0.653	0.008
<u>Three Factor</u> <i>IL1A rs1304037</i> <i>IL10 rs1800872</i> <i>IL1RN rs315951</i>	13,244	10/10	0.657	0.060

*Analysis was restricted to 279 cases and 535 controls. Interaction models were adjusted for multiple hypothesis testing with 1,000 permutations.

Discussion

Chronic inflammation has been associated with tumor development and metastasis in cancer progression. Inflammatory response is regulated through a complex network of cytokines, cytokine receptors and downstream targets that synergistically regulate innate/humoral immune and inflammatory processes. Recent molecular and genetic epidemiology studies have demonstrated that chronic inflammation and susceptibilities in inflammatory-associated genes are related to aggressive cancer phenotypes, including lymphoma, and gastric and prostate cancer [101, 122, 195-197]. However, to our knowledge, there are limited published reports on the impact of inheriting multiple cytokine-related functional sequence variants in relation to prostate cancer among men of African Descent. Therefore, our current study evaluated the individual and combined effects of 44 inflammatory associated sequence variants on prostate cancer risk among 279 cases and 535 disease-free men of African descent from the U.S and Jamaica [36]. Cytokine-associated sequence variants (*IL1R2* rs11886877 AA, *IL10RA* rs4252243 AA) were associated with a 2.11-2.34-fold increase in risk of developing prostate cancer among men of Descent from the U.S. However, these sequence variants did not survive multiple hypothesis testing. MDR analysis revealed significant and highly synergistic age adjusted two-factor (*IL10RB* rs8178433 and *IL10RA* rs4252243) pairwise and three-factor (*IL1R2* rs11886877, *IL10* rs1800896, *IL10RA* rs4252243) interaction models associated with prostate cancer risk among men of African descent. Nevertheless, the three-factor interaction was selected as the overall best predictor of prostate cancer susceptibility among men of African based upon an informative information gain score and high ATA value (Permutation p-value = 0.003).

Our findings show a modest increase in prostate cancer risk for unadjusted and adjusted logistic regression models for *IL1R2* rs11886877 among men of African descent. Although this sequence variant did not survive multiple hypothesis testing, the additive, dominant and recessive genetic models of this variant were significant. The *IL1R2* rs11886877 SNP is located 2415 base pairs from the transcription start site. Its close proximity to the regulatory region of the *IL1R2* gene suggests it may have a high likelihood of regulating *IL1R2* gene expression. Currently, there are no published reports on the relationship between *IL1R2* rs11886877 and prostate cancer for any population. Although there is no evidence of the impact of this sequence variant on prostate cancer risk among European- and African-American men, the relationship between the *IL1R2* gene expression and prostate cancer has been demonstrated through published reports [140, 198, 199]. However, Ricote and colleagues (2004) observed high protein expression of IL1R2 in high and low gleason score prostatic tumor tissue compared to normal prostatic tissue [140]. The study findings suggest that high expression of IL1R2 is in result of the absence of IL1Ra (antagonist of IL1) to mute the pro-inflammatory effect of IL1 α in prostate cancer. However, IL1 α does not have a high affinity to bind to IL1R2. Additionally, other studies also suggest that IL1R2 may function as a decoy receptor or natural inhibitor of IL1 [139, 140]. Moreover, Leshem and colleagues found that the promoter region of *IL1R2* possesses putative binding motifs for the *TMPRSS2/ERG* fusion gene, which is highly expressed in aggressive prostate cancer [198]. When the expression of *IL1R2* was knocked down using small interfering RNAs, it resulted in the reduction of *ZEB2* mRNA expression in hTERT/shp53/CyclinD-CDK4 overexpressing cells exposed to *TMPRSS2/ERG* [198]. The *TMPRSS2/ERG* fusion gene indirectly up-

regulates *ZEB2*, a facilitator of the epithelial to mesenchymal transition (EMT), by binding to *IL1R2* to increase prostate cancer tumorigenesis [200]. Further investigation of *IL1R2* in relation to PCA risk is needed to determine the effect of *IL1R2* expression on prostate cancer tumorigenesis.

Additionally, MDR analyses revealed other inflammatory-related sequence variants associated with PCA susceptibility. Within a three-factor interaction model, the inheritance of *IL1R2* *rs11886877* combined with *IL10* *rs1800896* and *IL10RA* *rs4252243* was significantly associated with PCA susceptibility based upon an informative IG of 0.86% for the pairwise interaction (*IL1R2* *rs11886877*, *IL10* *rs1800896*), individual IG score of 1.26% (*IL10RA* *rs4252243*) and ATA score of 61% among men of African descent. IL10 is a major anti-inflammatory cytokine and IL10RA (interleukin 10 receptor alpha) is a known mediator of its signaling in immunosuppression. For instance, Stearns and associates (2003) observed that IL10 treatment down-regulates the gene and protein expression of MMP2 and membrane type 1 MMP in non-immortalized primary prostate cancer cells (HPCA-10c) via its receptor IL10R [201]. Moreover, Cardillo and colleagues (2006) observed an increase in the protein expression of IL10 in the epithelium of prostatic tissue compared to normal prostatic tissue [202]. Based upon LR modeling, the inheritance of *IL10* *rs1800896* and *IL10RA* *rs425224* was associated with a marginal increase in PCA susceptibility among men of African from the U.S. men. This increase in PCA risk may be attributed to these genetic alterations within these genes (*IL10* and *IL10RA*), which can impair gene function of these anti-inflammatory cytokines.

Out of 44 inflammatory-related sequence variants, 7 SNPs included in our study were evaluated in relation to prostate cancer outcomes within 4 independent

observational studies [121, 122, 125, 126]. Commensurate with our findings, two observational studies demonstrated that sequence variants detected in *IL10* (*rs1800871*, *rs1800872*) and *IL8* *rs4073* were not significantly related to prostate cancer risk [122, 126]. Inheritance of *TNF* *rs1800629* AA genotype was associated with a significant 1.8 fold increase in prostate cancer risk among North Indian men in a small study (150 cases, 150 controls); however, this marker resulted in null findings in a larger study (484 cases, 484 controls) [121, 122]. In our preliminary analyses inheritance of one or more *TNF* *rs1800629* A alleles was marginally associated with a 1.5-fold increase in prostate risk; however, this relationship did not survive adjustment after multiple hypothesis testing. Lastly, *IL10* *rs1800896* G and *IL1B* *rs1143627* C alleles had protective effects in two separate Caucasian sub-populations. However, neither of these markers served as important prostate cancer predictors among African American men in the current study. Casey and colleagues (2002) showed a 2-fold increase in prostate cancer susceptibility linked to inheritance of the *RNASEL* *rs486907* AA genotype among men of European descent [125]. However, this loci was not related to prostate cancer risk among African Americans in the current study. This may be due to differences in minor allele frequencies, prevalence of the sequence variants, environment exposures, failure to adjust findings for multiple hypothesis testing or inadequate sample size.

We considered the strengths, limitations and future directions of our study. Forty-four sequence variants were evaluated in relation to prostate cancer risk among men of African descent from the U.S. and Jamaica. Upon stratification by study center, we identified race/ethnicity specific inflammatory-associated sequence variants associated with prostate cancer risk. However, selected inflammatory-related sequence variants did

not show a robust relationship with prostate cancer susceptibility. Population admixture, which commonly occurs among men of African descent, may bias risk estimates. However, adjustment of risk estimates by West African Ancestry and/or age did not modify the directionality of observed risk estimates among men from the U.S. (Data not shown). Although, the sample statistical size of this study population is small, we ensured that we had ample power to accurately detect risk estimates. Our findings do not support the hypothesis that inflammatory related sequence variants are associated with prostate cancer among men of African descent. However, we cannot eliminate the possibility that *IL1R2* and other inflammatory-related sequence variants not included in this study may influence the risk of prostate cancer development or aggressive tumor behavior. In larger studies, the impact of individual or interaction between inflammatory cytokine-associated SNPs in relation to prostate cancer tumor grade, biochemical or disease recurrence, and mortality will undergo evaluation using targeted sequencing, *in vitro* studies, *in silico* and bioinformatics tools. Such efforts will help to identify genetic markers linked to disproportionately high prostate cancer incidence, mortality, and morbidity rates among men of African descent.

CHAPTER 6

CONCLUSION AND SUMMARY

The literature on the contribution of chronic inflammation to prostate cancer (PCA) development and progression has increasingly grown over the past decade. Many reports discuss how inflammatory-related genes (e.g., TLRs, chemokines, cytokines) and genetic alterations of these genes play a pivotal role in PCA pathogenesis. Toll-like receptors (TLRs), which are major mediators in innate immunity serve as a surveillance system. In the presence of pathogens, TLRs are activated and trigger the inflammatory response via innate immunity. Inflammation is a critical response in innate immunity that leads to tissue repair and attracts innate immune cells via production of cytokines/chemokines. Repetitive damage to prostatic tissue leads to chronic inflammation in the prostate. Genetic polymorphisms within the inflammatory and immune response pathway may lead to an imbalance in pro-and anti- inflammatory signals and result in prolonged chronic inflammation. Some inflammatory and immune response related sequence variants have been previously studied in relation to PCA risk; however, most of these studies focus on European and Asian sub-populations. Furthermore, the contribution of gene-gene interactions on PCA susceptibility has not been well studied. Failure to include gene-gene interactions may greatly limit the detection of markers that influence PCA susceptibility.

To address these gaps in the literature, we evaluated 119 single nucleotide polymorphisms (SNPs) within inflammatory and immune response-related genes (i.e.,

TLRs, chemokines and cytokines) among men of African descent [31, 35, 36]. Logistic regression (LR) analyses did not reveal any significant associations with PCA susceptibility that survived multiple hypothesis testing among toll-like receptor -related sequence variants. Interestingly after adjustment for multiple hypothesis testing of LR models, possession of *CCL5* (*rs2107538*, *rs3817655*), *CCR5* *rs1799988* and *IL1R2* *rs11886877* sequence variants were strongly and/or marginally associated with PCA susceptibility among men of African descent. *CCL5* [(*rs2107538* (GA, AA), *rs3817655* (GA, AA))] genotypes showed a decrease in PCA risk, primarily driven by the U.S. population. However, the variant alleles of *IL1R2* *rs11886877* A and *CCR5* *rs1799988* G were associated with an increase in PCA susceptibility among men of African descent.

Among the inflammatory and immune response associated gene-gene interactions, we observed several highly synergistic interactions. A highly synergistic three-factor (*TLR10* *rs11096957*, *TLR6* *rs2381289* and *IRF3* *rs2304206*) interaction model was observed with a high CVC value and ATA score among men of African descent from the U.S. and Jamaica (Permutation testing p-value = 0.001). Additionally, this three-factor interaction model was the best predictor of PCA risk was primarily driven by pairwise interaction between *TLR10* *rs11096957* and *IRF3* *rs2304206* (IG = 1.32%) and the main effect of *TLR6* *rs2381289* (IG = 1.71%) . Among U.S. men, the two-factor (*TLR2* *rs1898830*, *IRAK4* *rs4251545*) interaction model was selected as the best predictor of PCA risk, based on an informative pairwise mutual IG score (IG = 2.33%) that exceeded main effects (Permutation testing p-value = 0.001). Chemokine associated interactions resulted in a three-factor (*CCR6* *rs3093024*, *CXCR7* *rs7559855*, and *CCR4* *rs6550178*) interaction model as the best predictor of PCA risk among

Jamaican men (ATA = 70.4%; Permutation testing p-value = 0.017). The synergy of this interaction model was primarily based upon the informative pairwise mutual interaction information gain score between *CCR6 rs309302* and *CXCR7 rs7559855* (IG = 3.41%) and the main effect of *CCR4 rs6550178* (IG = 8.2%) . Lastly, cytokine-associated interaction models yielded a highly synergistic three-factor (*IL1R2 rs11886877*, *IL10 rs1800896*, *IL10RA rs4252243*) interaction model, primarily driven by *IL10RA rs4252243* (IG = 1.26%) coupled with the *IL1R2 rs11886877* -*IL10 rs1800896* axis (IG = 0.86%) among men of African (ATA = 61% ; Permutation testing p-value = 0.003). Collectively, our findings suggest that the inheritance of main [*CCL5 (rs2107538, rs3817655)*, *IL1R2 rs11886877*] and joint [(*TLR10 rs11096957*, *TLR6 rs2381289*, and *IRF3 rs2304206*), (*TLR2 rs1898830*, *IRAK4 rs4251545*), (*CCR6 rs3093024*, *CXCR7 rs7559855*, and *CCR4 rs6550178*)] effects modify the PCA susceptibility among men of African descent.

Further investigation of these sequence variants will further elucidate the role of inflammatory and immune response-related genes on PCA development and aggressive phenotype associated with men of African descent. Future studies will evaluate the following: (1) the impact of *CCL5*, *CCR5* and *IL1R2* expression on prostate cancer tumorigenesis via knockout and/or knockin *in vitro* and *in vivo* models; and (2) the association of inflammatory and immune response-associated sequence variants in relation to PCA tumor grade, biochemical or disease recurrence, and mortality among men of African descent.

In the U.S., 17% of men will develop PCA in their lifetime. Once PCA metastasizes, it is associated with a 5 year survival rate of 29%. Current conventional therapies are not very effective against PCA metastatic disease. Moreover, African-American and Caribbean men are disproportionately affected by this disease compared to their European-American counterparts. Therefore, it is imperative that we study the biological underpinnings responsible for PCA development and progression. Our published reports evaluated the contribution of genetic alterations in the inflammatory and immune response pathway to PCA development. The investigation of sequence variants within inflammatory and immune response-associated genes involved in prostate cancer will help to improve the overall detection, prognosis, diagnosis, and clinical management of PCA among men. For clinicians and physicians, the prostate serum antigen (PSA) test and DRE (digital rectum exam) are unreliable PCA screening prognostication tools. Infact, the U.S. Preventive Services Task Force (USPSTF) and American Urological Association (AUA) highly recommend against the use of the PSA test for PCA detection due to its lack of specificity and sensitivity. Moreover, PCA detection cannot solely depend on DRE findings, since this test must always be performed in combination with another test. Consequently, clinicians and physicians are unable to make reliable PCA prognostic and diagnostic decisions for patients without the use of invasive procedures such as prostate biopsies. Even after dozens of painful needle core biopsies, tumors may still remain undetected. As a result of the USPSTF's and AUA's recommendations, many clinicians and urologists have experienced a significant loss financially due to their inability to highly recommend the PSA test to their patients. The identification of genetic alterations that either reduce or enhance the prostate cancer

aggressive phenotype may serve as a potential solution to the issues associated the PSA test. These genetic alterations will help in the design of PCA specific genetic marker (SNP) panels and therapeutic agents against PCA metastatic disease. Clinicians and physicians may use these panels as non-invasive PCA prognostic and/or diagnostic tools to determine disk risk due to the high detectability of SNPs in DNA isolated from human blood specimens. Sequence may replace the PSA test and DRE as robust and reliable screening tools for PCA detection. Ultimately, genetic markers may improve personalize medicine and explain the disproportionate effect on prostate cancer incidence, mortality, and morbidity rates among men of African descent.

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CURRICULUM VITAE

Dominique Zilpha Jones, B.A.

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A. EDUCATION

University of Louisville, Louisville, KY **2011-Present**

M.S. and Ph.D., Pharmacology and Toxicology

Smith College, Northampton, MA **2006-2010**

B.A., Biochemistry

B. AWARDS

- Alice Eaves Barns Award, University of Louisville **2014**
- Dean's Citation, University of Louisville **2014**
- Southern Regional Education Board Doctoral Scholar Fellowship,
University of Louisville **2012-2015**
- Integrated Programs in Biomedical Sciences Fellowship (IPIBS),
University of Louisville **2011-2012**
- Dean's List, Smith College **2009-2010**
- Jackie Robinson Foundation Scholar **2006-2010**
- American Chemical Society Scholar **2006-2010**

C. RESEARCH EXPERIENCE

Graduate Research Assistant, Department of Pharmacology **08/2011-Present**

and Toxicology

University of Louisville, Louisville, KY

PI: Dr. LaCreis Kidd

- Investigated inflammatory and immune response related genetic variants linked to prostate cancer risk through logistic regression and multi-factor dimensionality reduction (MDR) modeling
- Received training in statistical analysis software (SAS) and Linux operating system programming
- Evaluated miRNA expression profiles in prostate cancer serum and cell lines as
- potential prognostic tools for prostate cancer diagnosis
- Utilized molecular cloning techniques to examine the impact of microRNA expression on cellular behavior in normal prostate and prostate cancer cells.

Summer Intern, James Graham Brown Cancer Center

06/2011-08/2011

University of Louisville, Louisville, KY

PI: Dr. LaCreis Kidd

- Completed Collaborative Institutional Training Initiative (CITI) in Human Research and Health Insurance Portability and Accountability Act (HIPPA) Privacy Training
- Received training on the operation of a Linux Operation System and Multi-factor Dimensionality Reduction program, Statistical Analysis Software (SAS) programming and Golden Helix SNPs Variation Software

- Completed a summer research project titled “Chemokine-associated Genetic Variants as Predictors of Prostate Cancer Risk among men of African Descent”

Research Technician, Biomedical and Biotechnology

11/2010-05/2011

Research Institute

North Carolina Central University, Durham, NC

PIs: Dr. Maxwell Gyamfi and Dr. Sean Kimbro

- Examined concentrations of proteins involved in the synthesis and metabolism of the SAM pathway through a BCA Assay, and Western Blotting Analysis
- Performed a lipid profile to determine the levels of glucose, triglycerides, HDL, LDL, and VLDL in mice serum samples, a lipid extraction technique on hepatic tissue, and RNA extraction
- Received American Association for Laboratory Animal Science (AALAS) certification training in animal care for research and education through the AALAS Learning Library

Undergraduate Research Fellow, Department of Biochemistry

08/2009-05/2010

Smith College, Northampton, MA

PI: Professor Lale Burk

- Observed the biosynthesis of Dehydrodiisoeugenol (DDIE) from eugenol and isoeugenol in an acidic, basic and neutral buffer environment through thin layer chromatography and successfully synthesized coupled product DDIE in a neutral buffer system

- Submitted a Special Studies research paper entitled “Investigation of Natural Products: The Oxidation of Olefins with Horseradish Peroxidase IV in Buffering Environments” to the Department of Biochemistry
- Presented a poster “Felix Haurowitz: His Life and Contributions to Biochemistry” on Haurowitz’s research: first isolation of fetal hemoglobin, the change in crystallized deoxyhemoglobin’s conformation during the absorption of oxygen and the theory of antibody formation
- Analyzed the impact of Haurowitz’s publications (350 papers and 11 books between 1918-1966) on modern biochemistry and immunology

Teacher Assistant, Department of Biochemistry

08/2009-05/2010

Smith College, Northampton, MA

PI: Dr. Amy Burnside

- Prepared laboratory for weekly experiments; amylase purification, activity assay and SDS-gel electrophoresis
- Made chemical stock solutions, distributed and organized laboratory equipment
- Assisted instructor in perfecting lab experiments and students with problem-solving of their laboratory projects and assignments
- Reviewed and offered constructive criticism on laboratory notebooks of students
- Managed laboratory lock-up and clean up

Student Research Fellow, Biomanufacturing Research Institute

07/2009

and Technology Enterprise

Department of Pharmaceutical Sciences

North Carolina Central University, Durham, NC

PI: Dr. Dayami Lopez

- Performed a protein quantitative analysis with BCA on a Beckman Du Spectrophotometer, a Real-time PCR using SYBR Green on the 7500 Fast Real-Time PCR System, and data analysis to determine the effects of polarization and zaragozic acid (ZA) on the expression of the LDL receptor and PCSK9 proteins in hepatocytes
- Prepared cell culture and passed cells for C3A cell line (subclone of HepG2 cell line)
- Audited a North Carolina Central University graduate school course entitled Metabolic Diseases

D. PEER-REVIEWED MANUSCRIPTS

- Kidd L.R., Jones D.Z., Beache S., Rogers E.N., Ragin C., Jackson M., McFarlane-Anderson N., Tulloch-Reid M, Flores-Obando R., Barve S.S., Rudd J.E., and Kimbro K.S.. Chemokine Ligand 5 (CCL5) and Chemokine Receptor (CCR5) Genetic Variants and Prostate Cancer Risk among men of African Descent. *Hereditary Cancer in Clinical Practice*, 10(1):16, 2012.
- Rogers E.N., Jones D.Z., Yeyeodu, S.T, Ragin C., Jackson M.,McFarlane-Anderson N., Tulloch-Reid M, Kimbro K.S., and Kidd L.R. Toll-like Receptor (TLR)-Associated Sequence Variants as Predictors of Prostate Cancer Risk Among Men of African Descent. *Genes and Immunity*, 10.1038/gene.2013.22, 2013.
- Kidd, L.R., Rogers, E.N., Yeyeodu, S.T., Jones, D.Z., Kimbro, K.S. Contribution of Toll-like Receptor Signaling Pathways to Breast Tumorigenesis and Treatment. *Breast Cancer: Targets and Therapy*, 10.2147/BCTT.S29172, 2013.
- Jones D.Z., Ragin C., Jackson M.,Kidd N.C., Flores-Obando R.E.McFarlane-Anderson N., Tulloch-Reid M, Kimbro K.S., and Kidd L.R. The Impact of Genetic

Variants in Inflammatory-related Genes on Prostate Cancer Risk among men of African Descent. *Hereditary Cancer in Clinical Practice*, 11(1):19, 2013.

- Spruiell K., Jones D.Z., Cullen J.M., Awumey E.M., Gonzalez F. J., and Gyamfi, M.A. Role of human pregnane X receptor in high fat diet-induced obesity in pre-menopausal female mice. *Biochemical Pharmacology*, 2014 Apr 7. pii: S0006-2952(14)00207-X. doi: 10.1016/j.bcp.2014.03.019.

E. ABSTRACTS AND POSTER PRESENTATIONS: NATIONAL/LOCAL MEETINGS

- **Jones D.Z.**, Burk L. Felix Haurowitz: His Life and Contributions to Biochemistry. Celebrating Collaborations, Smith College, Northampton, Massachusetts, April 2010.
- **Jones D.Z.**, Ragin C., Jackson M., McFarlane-Anderson N., Morrison S., Flores-Obando F., Kimbro K.S., and Kidd, L.R. Chemokine-associated genetic variants as predictors of prostate cancer risk among men of African Descent. Research Louisville!, University of Louisville, Kentucky, October 11, 2011.
- Kidd L.R., **Jones D.Z.**, Ragin C., Jackson M., McFarlane-Anderson N., Morrison S., Flores-Obando F., Kimbro K.S. Chemokine-associated genetic variants as predictors of prostate cancer risk among men of African Descent. AACR Cancer Health Disparities, Washington, D.C. September 18, 2011.
- Kidd L.R., **Jones D.Z.**, Ragin C., Jackson M., McFarlane-Anderson N., Morrison S., Flores-Obando F., Kimbro K.S. Chemokine-associated Loci and Prostate Cancer Among Men of African Descent. AACR Annual Meeting, Chicago, IL, March 31, 2011.

- **Jones D.Z.**, Anene D., Aloway A., Anene P., Avila D.V., Gobejishvili L., Barve S.S., McNally L.R., and Kidd L.R. Potential micro-RNA Biomarkers Associated with Cell Migration and Metastasis. Research Louisville!, Louisville, Kentucky, September 18, 2012.
- Anene D., **Jones D.Z.**, Aloway A., Anene P., Avila D.V., Gobejishvili L., Barve S.S., McNally L.R., and Kidd L.R. Are Cell Adhesion Associated Micro-RNAs Linked With Metastatic Prostate Cancer? Research Louisville!, Louisville, Kentucky, September 18, 2012.
- Aloway A., **Jones D.Z.**, Anene D., Anene P., Avila D.V., Gobejishvili L., Barve S.S., McNally L.R., and Kidd L.R. Cell Survival miRNAs (29a,29c,and 221) and Pre-metastatic Prostate Cancer ? Research Louisville!, Louisville, Kentucky, September 18, 2012.
- **Jones D.Z.**, Anene D., Aloway A., Anene P., Avila D.V., Gobejishvili L., Barve S.S., McNally L.R., and Kidd L.R. Potential micro-RNA Biomarkers Associated with Cell Migration and Metastasis. James Graham Brown Cancer Center Retreat, Louisville, Kentucky, October 26, 2012.
- Anene D., **Jones D.Z.**, Aloway A., Anene P., Avila D.V., Gobejishvili L., Barve S.S., McNally L.R., and Kidd L.R. Are Cell Adhesion Associated Micro-RNAs Linked With Metastatic Prostate Cancer? The Kentucky Honors Roundtable, Murray State University, Murray, Kentucky, October 27, 2012.
- **Jones D.Z.**, Anene D., Aloway A., Anene P., Avila D.V., Gobejishvili L., Barve S.S., McNally L.R., and Kidd L.R. Reduced Expression of miR-342-3p in stage I, III, and IV Prostate Cancer. AACR Annual Meeting, Washington, D.C., April 8, 2013.

- **Jones D.Z.**, Linder J., Avila D.V., Gobejishvili L., Barker D., Schmidt L., Hobbing K., Clark G., and Kidd L.R. TGF-beta Signaling in prostate cancer cell lines derived from European- and African-American men. Research Louisville!, Louisville, Kentucky, September 24, 2013.
- **Jones D.Z.**, Linder J., Avila D.V., Gobejishvili L., Barker D., Schmidt L., Hobbing K., Clark G., and Kidd L.R. TGF-beta Signaling in prostate cancer cell lines derived from European- and African-American men. James Graham Brown Cancer Center Retreat, Louisville, Kentucky, October 25, 2013.
- **Jones D.Z.**, Hobbing K., Schmidt M.L., Clark G., and Kidd L.R. The Effect of miR-186 Inhibition on Cell Viability and Colony Formation in Prostate Cancer. Research Louisville!, Louisville, Kentucky, September 16, 2014.
- **Jones D.Z.**, Hobbing K., Schmidt M.L., Clark G., and Kidd L.R. The Effect of miR-186 Inhibition on Cell Viability and Colony Formation in Prostate Cancer. James Graham Brown Cancer Center Retreat, Louisville, Kentucky, October 17, 2014.

F. PROFESSIONAL MEMBERSHIP AND LEADERSHIP

- School of Medicine Diversity Committee, Student Member, 2014-Present
Society of Toxicology, 2014-Present
- Black Biomedical Graduate Student Organization (BBGSO), President, 2012-Present
- Golden Key International Honor Society, Member, 2012-Present
- American Association of Cancer Research (AACR), Member, 2012-Present
- Sigma Xi, The Scientific Research Society, Member, 2010

G. SCIENTIFIC MEETINGS, WORKSHOPS, TRAINING

- University of Louisville Pharmacology and Toxicology Departmental Seminars, 2011-Present
- American Association for Cancer Research, National Meeting, 2012-Present
- UofL Grant Writing Academy, Participant, 2013