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UV Exposure, Vitamin D, and Prostate Cancer Risk in African Americans

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DEDICATION

This dissertation is dedicated to my wife and my daughter.

It could not be done without your love, your patience, and your understanding.

I appreciate your continuous support throughout my education.

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ABSTRACT

Genetics and epidemiological studies have shown that genes and environment interaction play strong roles in prostate cancer (PCa) etiology. PCa incidence and mortality are disproportionately high among African-American (AA) men.

In order to explore the effects of UV exposure, serum Vitamin D, skin color , and vitamin D receptor (VDR) polymorphisms on PCa risk in African-American men; ninety-one affected African-American men with histologically diagnosed adenocarcinoma of the prostate, PSA of > 2.5 ng/ml and a positive digital rectal exam were recruited. Ninety one ethnicity matched controls were also recruited. The mean age of cases and controls was 64.53 and 58.7 years respectively. The mean of serum vitamin D level was 29 ng/ml and 26.75 ng/ml in control and PCa patients respectively. Using the independent samples t-test and Mann-Whitney test there was no significance difference in mean and median of vitamin D serum levels between PCa patients and controls (P=0.42). Interestingly, the mean of tanning potential (relative difference between facultative and constitutive skin pigmentation) was 36.51% and 30.32% among control and PCa subjects, respectively, and, there was moderate association between tanning potential and decreased risk of prostate cancer (OR= 0.707, P=0.0626). There was an association between total UV exposure, outdoor UV exposure, recreational UV exposure, and decreased risk of PCa in age matched samples. However the association of decreased prostate cancer risk with total UV exposure and outdoor UV exposure was not significant. These results indicated that when subjects aged 0-5 and 6-11 years old were highly exposed to ultraviolet radiation (UVR) , this had significant association with reduced risk of PCa. Moderate UV exposure in all age groups had inverse association with the PCa risk while higher exposure to UVR in advanced age did not protect against PCa. Lower skin pigmentation (higher tanning potential) was associated

significantly with reduced risk of PCa. The findings showed that the risk of PCa did not vary significantly by serum concentration of 25(OH)D. Seven polymorphisms in VDR sequence were identified after sequencing. Single nucleotide polymorphisms (SNPs) c.278-69G>A, c.1025-49G>T (ApaI), and c.1025-56A>G were associated with an increasing risk of prostate cancer (OR=1.285, OR=1.22, and OR=2.616 respectively), although these associations were not statistically significant, P>0.05. The less likelihood of prostate cancer risk were found in subjects with SNP c.755+25G>A (OR, 0.256; 95% CI, 0.090 -0.729), c.907+75C>T (OR, 0.175; 95% CI, 0.093 -0.331), c.1025-95G>A (OR, 0.038; 95% CI, 0.005 -0.286), and c.1056T>C (TaqI) (OR, 0.652; 95% CI, 0.338 -1.257). These associations were significant although only c.1056T>C was not significant, p=0.201. Collectively, it is possible that genetic variants may mediate PCa risk via a mechanism involving availability of 1, 25(OH)2 D. Vitamin D protects against risk of PCa. UV exposure, adequate vitamin D uptake, and lighter skin along with advantageous VDR polymorphism as modulators of vitamin D can improve this protection. Our results require further corroboration in large statistically powerful sample collections.

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25-OH D3	Previtamin D
µg/ml	Micrograms per milliliter
1,25(OH)$_2$D	1,25- didyroxyvitamin D
25OHvitamin D	25-hydroxyvitamin D
7-DHC	7-dehydrocholestrol
AR	Androgen receptor
BPH	Benign prostatic hypertrophy
CAPB	Carcinoma prostate brain locus
CCD	Charged-coupled device camera
CEU	Utah residets with ancestry from northern and western Europe
CHB	Han Chinese in Beijing, China
CYP17	Cytochrome p450 17 gene
CYP24	25-hydroxyvitamin D 24-hydroxylase
CYP27B1	25-Vitamin-D3-1α-hydroxylase
CYP3A4	Cytochrome p450 A4 gene
DBP	Vitamin D binding protein
DHC	Dihydroxycholestrol
DHPLC	denatured high performance liquid chromatography
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DRE	Digital rectal examinations
E	Erthyma
EIA	Enzyme immune assay
ELAC2/HPC2	Heredatory prostate caner 2
FBS	Fetal bovine serum
FFQ	Food frequency questionnaire
HPC	Hereditary prostate cancer
HPCX	Hereditary prostate cancer X-linked
HUCC	Howard University Cancer Center
HUH	Howard University Hospital
M	Melanin
ng/ml	Nanograms per milliliter

PCa	Prostate Cancer
PCR	Polymerase chain reaction
PSA	Prostate specific antigen
RXR	Retinoid X Receptor
SNPs	Single nucleotide polymorphisms
SRD5A2	5α-reductase
UVQ	Ultraviolet radiation exposure questionnaire
VDR	Vitamin D receptor
VDRE	Vitamin D response element
YRI	Yruba in Ibadan, Nigeria
$\chi 2$	Chi square

CHAPTER 1: INTRODUCTION

Humans are necessarily exposed to ultraviolet radiation from the sun and over time have developed phenotypes that mediate the potentially harmful and beneficial effects of exposure in different environments. Thus, it is believed that darkly pigmented humans evolved in Africa and 40000–50000 years ago migrated worldwide. In northern Europe, individuals with genetic variants that conferred skin with relatively little melanin and, therefore, low pigmentation were at an advantage, because they could more readily synthesize vitamin D in conditions of limited exposure to sunlight [1]. However, individuals with light-colored skin are also more susceptible to the harmful effects of ultraviolet radiation (UVR), and in recent years public health agencies have emphasized these effects in an attempt to reduce the increasing frequency of skin cancer. In parallel, it has been proposed that indoor lifestyles have resulted in widespread hypovitaminosis D [2]. These research project findings may have potentially important long-term clinical consequences. It is the purpose of this research project to consider these issues.

Prostate Cancer

Prostate cancer is a complex disease with both genetic and environmental components. The disease is marked by diverse rates of progression, responses to therapies, and age of onset. Sporadic prostate cancer may be controlled by several genes at different loci. While much is still not known about the relative contributions of these genes, it is assumed that multiple genetic variants may exhibit

different effects in diverse environments. Different genes may be causal depending on the grade and efficiency of the cancer. For instance for the highly aggressive types of tumours, the genetic signal may come from the interplay of more than one genetic loci. In addition, there can be strong genetic interactions with environmental factors such as diet and UV exposure [3-6].

Epidemiological data reveal that African-Americanmen have the highest incidence and mortality rates for prostate cancer [7-11]. For unknown reasons, prostate cancer incidence and mortality rates for African-Americanmales are among the highest in the world. It is unlikely that the large population differences in prostate cancer risk and mortality can be explained completely by differences in diet or other lifestyle characteristics. A growing body of evidence suggests a striking interrelationship between ethnicity and genomic variation and susceptibility to disease. Despite its high prevalence among African Americans, very little is known regarding genetic predisposition and environmental influences on prostate cancer. One of the critical steps to clarifying the relationship is to further characterize environmental and genomic variation across the diverse spectrum of human populations. While significant progress to date is being made in the identification and characterization of environmental and genomic variation in populations of European descent, comparable progress is not being made in assessing the levels of variation among African Americans. This is important because environmental factors such as diet and UV exposure are modifiable. Thus, knowledge of population differences in environmental and genetic factors is

fundamental to the rational design and implementation of strategies for diagnosis, management, and treatment of common, complex diseases. The new and rapidly growing field of pharmaco-genomics testifies to the significance of finding genes and environmental factors involved in human disease. Particularly intriguing is the interaction of UV exposure and modifiers of Vitamin D levels in the serum (e.g. skin color, genes and diet). In this research project it is proposed that the increased incidence of prostate cancer and mortality in African Americans involves a dynamic interplay of environmental factors such as UV exposure and diet in addition to genetic factors.

Prostate Cancer Risk Factors

Sporadic prostate cancer is one of the most common cancers in men in Western countries. In the United States, African-Americans have the highest incidence and mortality rate compared to other ethnicities [11]. Sadly, the pathogenesis of sporadic prostate cancer is poorly understood for all populations. Although environmental factors including diet, occupation and latitude appear to affect risk their relative importance and combined effects are unclear [12, 13].

Genetics

Epidemiologic studies suggest prostate cancer risk is determined by interactions between environment and genetic predisposition. Studies have provided evidence for familial clustering of prostate cancer, indicating that the disease may have a genetic component [14]. The relative risk of men with a first-degree relative affected with prostate cancer is twice that of men without an affected relative

[15]. The risk is even higher for men with several affected relatives, particularly if the relatives were young at the time of diagnosis [16]. Familial clustering in conjunction with segregation analyses consistent with a pattern of Mendelian inheritance in some prostate cancer families suggest the existence of at least one highly penetrant autosomal dominant susceptibility locus in some men with prostate cancer. Rare high-risk alleles are thought to account for at least 40% of early onset prostate cancer and 9% of all prostate cancers [14]. Genomic-wide linkage analyses conducted with large multiple-case prostate cancer families were instrumental in mapping the first major susceptibility locus for hereditary prostate cancer (HPC) to the long arm of chromosome 1 [17, 18]. Other genomic regions have also been implicated with HPC. These include HPCX at Xq27-28 [19] PeaP at 1q42-43 [20], CAPB at 1p36 [21], and the HPC2/ELAC2 gene on chromosome 17 [22, 23]. Single nucleotide polymorphisms (SNPs) within several candidate genes involved in the synthesis and metabolism of testosterone have also been implicated with prostate cancer risk. These include SRD5A2 [24], CYP3A4 [25, 26], and CYP17 [27-30]. Several polymorphisms in the Vitamin D receptor (VDR) gene have been identified. This include a Poly-A microsatallite in the 3′ flanking region [31, 32], a change in intron VIII that generates BmsI and TaqI restriction enzyme site [33], a synonymous change at codon 352 in exon IX that generates a TaqI restriction enzyme site [34] and a 5′ FokI site in exonII that alters the start codon. The functional significance of these variants remains unknown; however, several

studies have addressed their relationship with prostate cancer [4, 28, 31, 35-47].

Age

Age is a risk factor for prostate cancer, especially men age 50 and older. More than 70 percent of all prostate cancers are diagnosed in men over the age of 65 [48]. Mortality from prostate cancer is two to three times greater among African-American men between the ages of 40 and 70 years than among similarly age American Caucasian men [49].

Race

Prostate cancer is nearly 60 percent more common among African-American men than it is among Caucasian-American men[50]. Japanese and Chinese men native to their country have the lowest rates of prostate cancer[51]. Interestingly, when Chinese and Japanese men immigrate to the United States, they have an increased risk and mortality rate from prostate cancer, when compared to their native populations. In Japan, the incidence of prostate cancer has increased as Western diets and lifestyles have been adopted.

Diet

Epidemiological data suggests that the diet consumed in Western industrialized countries may be one of the most important contributory factors for developing prostate cancer [52].

Obesity

Obesity not only contributes to diabetes and high cholesterol, but has also been associated with some common cancers, including

hormone-dependent tumors such as prostate, breast, and ovarian cancer [53].

Chemical Exposures

Some studies show an increased chance for prostate cancer in men who are farmers, or those exposed to the metal cadmium while making batteries, welding, or electroplating [54]. Additional research is needed in this area to confirm whether this is a true association.

UV Exposure and Skin Color

As previously mentioned, environmental factors such as diet [6] and ultraviolet radiation (UVR) may also affect prostate cancer risk [3-5, 55]. French et al. (2001) found that increased UV exposure was associated with reduced risk for prostate cancer. Luscombe et al.'s (2001) finding showed that UV influences prostate cancer risk and implicates several candidates as potential modifying factors, namely skin color and Vitamin D. Skin color is largely determined by melanins (eumelanin and to a lesser degree phaeomelanin). Melanogenesis occurs in melanocytes and is dependent on the enzyme tyrosinase. Melanin is synthesized in a multistep biochemical pathway operating within organneles called melanosomes [56]. This pathway, called the Raper-Mason pathway [57] is under genetic control. Although the numbers of melanocytes are the same among ethnic groups, the number, size, shape and distribution of melanosomes vary [56]. It is important to note that constitutive pigmentation (pigmentation levels measured in unexposed areas of the skin) is affected by the environment to a much lesser extent than other areas of skin. Most

importantly, skin pigmentation can be readily and precisely measured as a quantitative trait by reflectometry [58].

Vitamin D

Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. There are two different forms of Vitamin D, named D3 and D2, which are very similar in structure. The D2 is a synthetic product, which is predominantly absorbed through by fortified foods. Physiological Vitamin D3 levels result not only from dietary uptake but also by biosynthesis of 7-dehydrocholesterol and UV-light in skin because of sun exposure. In the liver, the vitamin is hydroxylated to 25-hydroxyvitamin D (25-OH Vitamin D), the major circulating metabolite of Vitamin D. Although 1,25-(OH)2 Vitamin D portrays the biological active form of Vitamin D, which is synthesized in the kidney, it is widely accepted that the measurement of circulating 25-OH Vitamin D provides better information with respect to patients Vitamin D status and allows its use in diagnosing hypovitaminosis. The concentration of 25-OH Vitamin D decreases with age and a deficiency is common among elderly persons [59].

Vitamin D is also an important candidate implicated in prostate cancer risk [60-62]. While the exact mechanism is unclear, it was shown that serum 1,25 (OH)2 vitamin D levels affect tumour cell proliferation, differentiation and spread [63-69]. Normal and malignant prostate cells contain VDRs which mediate the antiproliferative action of 1,25(OH)2D [70-72]. 1,25(OH)2D, the biologically active form of vitamin D, binds to nuclear VDRs in the

epithelial cells of the prostate. It has a specific affinity for specific DNA sequences, namely vitamin D response elements [73]. 1,25(OH)2D is capable of acting through both non genomic signaling pathways involving a membrane associated receptor and genomic pathways involving the nuclear VDR. The binding of vitamin D to these response elements evokes a cascade of genetic events, thus becoming responsible for the formation of proteins [74]. 1,25(OH)2D diffuses passively into the cells to bind to the receptor causing a conformational change in the VDR, allowing dimerization with the retinoid X receptor (RXR).

Dimerization enables interaction with the vitamin D response element on target genes, initiating transcription. Vitamin D response element is also found on genes related to cellular differentiation and proliferation, including p21, transforming growth factor-b2, fibronectin, urokinase plasminogen activator and b integrin. These gene products interact with and inhibit cyclin dependent kinases, preventing uncontrolled progression through the cell cycle. Vitamin D response elements have also been identified as the promoters of insulin-like growth factor-binding protein-3 and insulin receptor genes. 1,25(OH)2D, therefore, plays an important role in the growth and function of the normal prostate, as well as in prostate carcinogenesis[75]. The reason is that most of the actions of vitamin D are mediated through an intracellular receptor in the prostate that has a much higher affinity for 1,25(OH)2D than for other metabolites. In addition to the antiproliferative action of 1,25(OH)2D, it can cause apoptosis[76], induce differentiation [77], inhibit telomerase

expression [78],inhibit tumor cell invasiveness [79], and suppress tumor-induced angiogenesis [80, 81] (Figure 1).

Endogenous vitamin D status is the sum of dietary intake and endogenous synthesis. Up to 95% is attributable to synthesis from cholesterol in the skin with sunlight exposure [64]. Vitamin D3 or cholecalciferol is formed from the precursor steroid 7-dehydrocholesterol (7-DHC), which is concentrated in the plasma membrane of the basal keratinocytes in the skin [82]. Upon stimulation by sunlight (UVB, 290-310 nm), 7-DHC is converted to previtamin D3 (Figure 2).

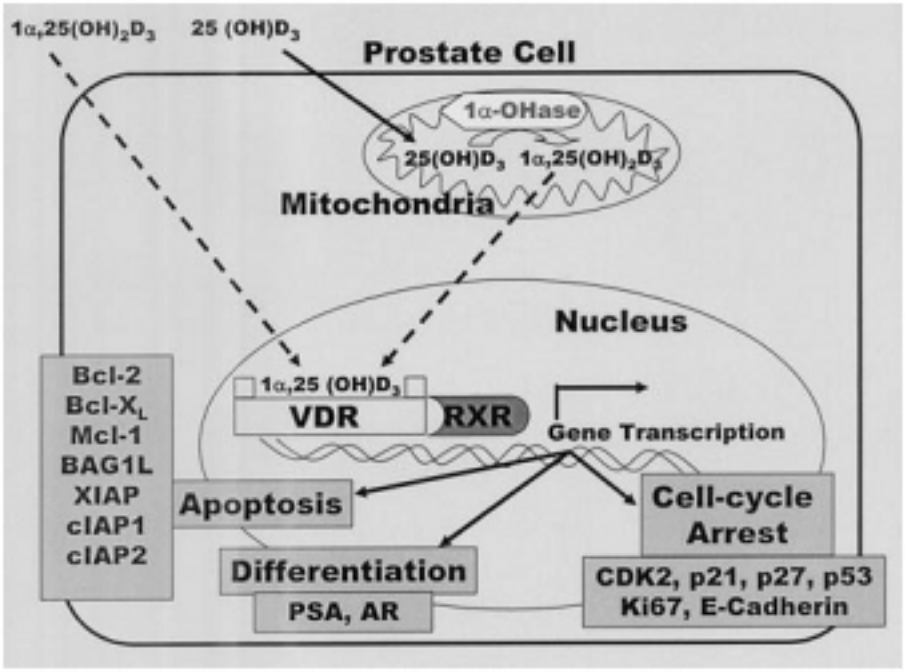

Figure 1. 1,25(OH)2D regulates prostate cell growth by interacting with its nuclear VDR to alter the expression of genes that regulate cell cycle arrest, apoptosis and differentiation [83].

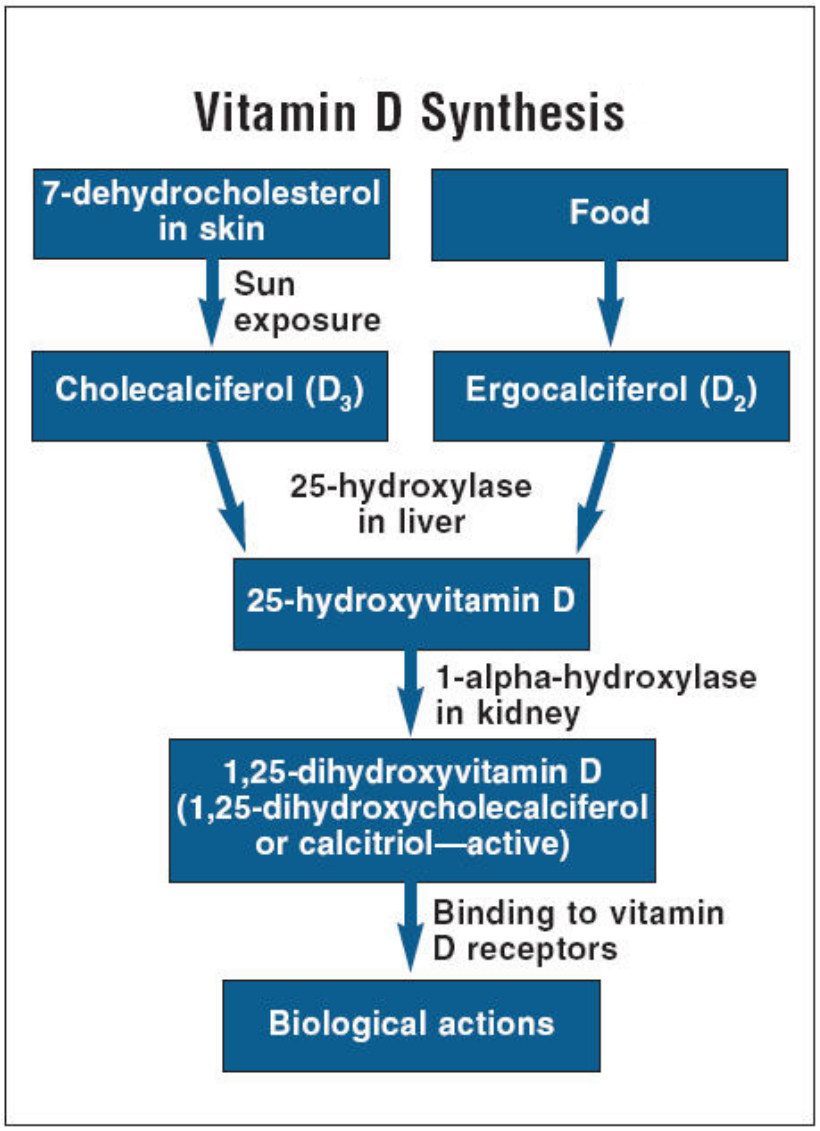

Figure 2. Vitamin D Metabolism.

After several other enzymatic conversions, vitamin D3 is formed. Vitamin D3 enters the circulation and binds with high specificity to vitamin D binding protein (DBP) to exert its systematic actions [84].The physiological and environmental factors that modify the supply of cutaneous vitamin D3 are levels of UV exposure, skin pigmentation, and genes involved in vitamin D synthesis and metabolism. Studies have shown large geographic gradients for vitamin D status [85, 86] due to latitudinal differences in UV exposure. In general, latitude, and season of the year affects the cutaneous photosynthesic process in a highly coordinated mutually dependent manner [87].Since melanin also absorbs the UVR wavelength (~300nm), it has a significant effect on the synthesis of vitamin D3. The highest vitamin D3 response to UVR is seen in whites followed by Orientals and then African Americans [88]. Furthermore, a more recent study has also shown that increased pigmentation will result in reduced UV-mediated synthesis of vitamin D in skin [89].In addition to skin pigmentation, sequence variation within certain genes affects vitamin D synthesis, metabolism, and action. These genes include 1-alpha-hydroxylase, the vitamin D binding protein (DBP) and the vitamin D receptor (VDR) which are highly polymorphic among different human populations [31, 90]. Very little is known about the role of genetic variations at the 1-alpha-hydroxylase and DBP genes in prostate cancer risk. However, there were many genetic studies which have examined the VDR gene and prostate cancer risk [31, 36, 38, 39, 41, 83, 91-94]. Interestingly, those studies lack a consensus on how significantly, if at all, variation in the VDR gene contributes to prostate cancer [45]. There

may be many reasons for the lack of consensus. First, issues such as skin color and ethnicity were not taken into account. In addition serum vitamin D was not measured. The novelty of [4, 5], study of 210 prostate cancer cases and 155 controls was the use of pigmentation genes (melanocortin receptor 1 and tyrosinase) in European males to stratify analyses and determine that the highest risk for prostate cancer was associated with darker skinned European males (skin type 4) who had the lowest UV exposure.

Review of Literature

Vitamin D in the Fight Against Prostate Cancer

In 1990, Schwartz proposed that vitamin D deficiency may underlie the major risk factors for prostate cancer, including advanced age, black race, and northern latitudes. He pointed out that all these factors are associated with decreased synthesis of vitamin D. Mortality rates from prostate cancer in the U.S. are inversely correlated with ultraviolet radiation, the principal source of vitamin D[95].

Sunlight and Vitamin D

In 1992, Hanchette and Schwartz proposed that sunlight and vitamin D may play a role in prostate cancer. They pointed out that men in the United States were 10 times more likely to develop prostate cancer than men in Japan, where men consume higher amounts of vitamin D due to their consumption of fatty fish. Although the authors did not mention it, Japanese men also consume soy, which inhibits the breakdown of calcitriol (activated vitamin D) in the tissues.

Furthermore, traditional Japanese men consume higher quantities of omega-3 fatty acids than their American counterparts. These fats are now known to dissociate vitamin D metabolites from their binding protein, thus raising active levels of those metabolites in the blood [96].

Hanchette and Schwartz analyzed American prostate cancer deaths in relation to sunlight and discovered a 0.0001 negative correlation, which is a very significant association. That is, they found that men who received more sunlight were less likely to die from prostate cancer [55]. In the same year, 1992, Schwartz discovered that death rates from prostate cancer were correlated with death rates from multiple sclerosis, another disease known to be associated with lack of sunlight. Also, he proposed that lack of vitamin D may be a causative factor in both diseases [97].

Calcitriol

Although some of the studies found that activated vitamin D (calcitriol) levels in the blood protected against colon cancer, none of the studies showed that low calcidiol levels (25-hydroxyvitamin D) were associated with risk of developing prostate cancer [98, 99]. In 1993, Skowronski and colleagues discovered that all three types of the prostate cancer cell lines they studied possessed a vitamin D receptor and that the active form of vitamin D, calcitriol, "dramatically inhibited" the growth of two of the three cell lines[100].

However, in 1995 Miller and colleagues examined seven prostate cancer cell lines. They found all 7 cell lines had receptors for vitamin D. They also showed that activated vitamin D (calcitriol)

inhibited the growth of four of seven prostatic carcinoma cell lines and found that the more vitamin D concentration, the greater the growth inhibition. Furthermore, they found that the enzyme that breaks down calcitriol in the tissues (24-hydoxylase) reduced that inhibition. That is, the higher the 24-hydroxylase activity, the lesser the cancer cell inhibition by vit D. Not only did this mean that activated vitamin D may retard prostate cancer growth, it suggested that substances which interfere with 24-hydroxylase may also prove useful in treating prostate cancer[86, 101].

Cholecalciferol

In 1998, Gross and colleagues at Stanford conducted the first clinical trial of a vitamin D metabolite in treating advanced prostate cancer. However, instead of raising the tissue levels of activated vitamin D (calcitriol) by supplementing with oral vitamin D (cholecalciferol), they chose to give calcitriol itself. In spite of circumventing the natural system to raise prostate calcitriol levels, they found calcitriol decreased the rate of progression of PSA blood levels in 6 of the 7 patients. Elevations in blood calcium levels (hypercalcemia) seriously limited the use of calcitriol and the cancer eventually progressed. Cholecalciferol has to be given in massive doses (40,000 units) over an extended period of time (months) to cause significant hypercalcemia. In addition, the tissue production of calcitriol is not rate limited, suggesting that oral cholecalciferol is effective in raising tissue levels of calcitriol[102].

Calcidiol

In 1998, Schwartz, the same scientist who had first postulated that vitamin D deficiency played a role in prostate cancer, confirmed that prostate cells, including most prostate cancer cell lines, were able to activate vitamin D. Schwartz and his colleagues concluded that "these data suggest a potential role for 25(OH)D (calcidiol) in the chemoprevention of invasive prostate cancer." As the easiest way to raise calcidiol is through oral supplementation with vitamin D3, this meant Schwartz et al. [103] were suggesting that plain, inexpensive, non-prescription vitamin D may help prostate cancer.

In the year 2000, Ahonen and colleagues conducted a careful study of calcidiol levels in young men and followed them for the development of prostate cancer. Unlike earlier studies, they found a relationship between low vitamin D blood levels and prostate cancer. Ahonen found young men with calcidiol levels below 40 nmol/L (16 ng/mL) were three times more likely to develop prostate cancer than were men with higher levels [66]. Just as important, it has been found that these men were 6 times more likely to develop invasive cancers. This finding implied a treatment effect for vitamin D as the prevention of invasiveness is a key goal of treatment[70].

Barreto and colleagues (2000) found that calcidiol was just as effective as calcitriol in inhibiting growth. They concluded that their findings "support the use of 25(OH) D as a chemotherapeutic agent in the treatment of prostate cancer." As oral cholecalciferol is the best way to raise calcidiol levels, it became clear that another

group of cancer researchers at a major university medical center was calling for the use of vitamin D in prostate cancer[10, 104].

Tuohimaa et al. [105] demonstrated that calcidiol was just as effective as calcitriol in inhibiting growth of prostate cancer cell lines in vitro. They also found that a vitamin D analogue already on the market, one known to cause less hypercalcemia than other analogues (patentable modifications of calcitriol), was also effective in inhibiting cancer growth. However, their findings about calcidiol again emphasized that readily available vitamin D should help fight prostate cancer. In fact, the authors concluded calcidiol might be a good candidate for "human trials in prostate cancer." Now 4 different groups of scientists, from 4 major university medical centers, were calling for the use of vitamin D in prostate cancer [3-5].

UV Exposure, Skin Types, Vitamin D Levels, and Risk of Prostate Cancer

In 2001, Luscombe and colleagues [3-5] published three studies linking ultraviolet exposure and skin type to the development of prostate cancer. They found that cumulative outdoor exposure, outdoor occupations and skin type was associated with reduced risk of advanced stage tumors. They also found that childhood sunburns dramatically reduced the risk of developing prostate cancer, probably because those with fair skin are more likely to burn but also find it easier to make vitamin D in their skin. Furthermore, they found that people who have difficulty making the skin pigment melanin (a natural sunscreen) are much less likely to develop prostate cancer.

In addition, Zhao and Feldman [106] found that the prostate cancer cell line DU145 which is poorly differentiated and derived from

brain metastasis, can be made to respond to calcitriol by adding drugs which inhibit the breakdown of calcitriol. This raised the possibility that prostate cancers which did not respond to vitamin D could be made responsive by the addition of a metabolic inhibitor. Farhan and colleagues [107] showed that the isoflavonoid found in soybeans, called genistein, is a powerful metabolic inhibitor of the enzyme that breaks down calcitriol.

In 2003, Chen and Holick [108] reiterated their call for the use of vitamin D in prostate cancer. After reviewing most of the research on the subject, the authors concluded, "adequate exposure to sunlight or oral supplementation might provide a simple way to increase synthesis of calcitriol in the prostate and, therefore, decrease the risk of prostate cancer." They added, "adequate vitamin D nutrition should be maintained, not only for bone health in men and individuals, but because it might decrease the risk of prostate cancer and mitigate metastatic disease, should it develop.

Also in 2003, Beer and colleagues [109-111] tested calcitriol as a treatment for prostate cancer. They found a significant reduction in the rate of increase in prostate specific antigen (PSA), a marker of the cancer's growth, although no patient achieved the hoped for 50% reduction. Unfortunately, none of the patients received oral vitamin D supplementation, which would have been more effective in raising prostate calcitriol levels. In fact, none of the patients were even tested or treated for vitamin D deficiency.

In 2003, Bodiwala and colleagues [112] studied sun exposure and skin type and found that men who sunbathed or in any way exposed

themselves to sunlight were less likely to develop prostate cancer. They also identified men with various combinations of skin type and reduced sun exposure, which were up to 13 times more likely to develop prostate cancer.

Vitamin D Receptor Gene Polymorphisms and Prostate Cancer

In 1969 the nuclear vitamin D receptor for 1α25(OH)2D3 was discovered [113]. Since then, the role of VDR in the endocrine system and its presence and function in over 30 tissues and organs has been examined. VDR has been shown to be involved in insulin-like growth factor (IGF) signaling, in inflammation and estrogen-related pathways, and in the activation and regulation of vitamin D and calcium. The involvement of VDR in multiple pathways and points of convergence within these pathways indicates the potential importance of VDR in the etiology of cancer. In 1997, Ingles et al. [31] reported an association with variants of the VDR gene and prostate cancer, supporting the hypothesized importance of VDR and the risk of developing cancer. Since the work of Ingles et al. [31], the association of the VDR gene with several cancers has been examined.

Most studies of VDR and cancer have focused on six polymorphisms: 1) the rs10735810 or Fok1 polymorphism on exon 2, 2) rs1544410 or Bsm1 on intron 8, 3) rs731236 or Taq1 on exon 9, 4) rs7975232 or Apa1 on intron 8, 5) rs757343 or Tru91 on intron 8, and 6) the poly (A) mononucleotide repeat at the 3′-UTR section of the gene. Different polymorphisms may have different functions depending on the location [114]. The Fok1 polymorphism is near the 5′-UTR region of the gene within the DNA-binding domain. The other polymorphisms are close to

the 3′-UTR region of the gene, the ligand-binding domain, and may have different functions than Fok1 based on their location. The polymorphisms examined near the 3′-UTR region of the gene appear to be in linkage disequilibrium, and the allele frequencies for these polymorphisms appear to vary by race [115]. Most studies of VDR and cancer have been conducted predominantly in non-Hispanic white populations. Ingles et al. [31] reported over a 4-fold increased risk of prostate cancer among men with more than 20 repeats of the poly(A) genotype; however, Andersson et al. [93] reported over a 4-fold increased risk among those with short repeats. Cicek et al. [116] observed that polymorphisms at both the 5′-UTR and 3′-UTR regions of the genes were associated with a 40% to 70% reduced risk of prostate cancer, and that the fbaT haplotype was associated witha 50% reduced risk of prostate cancer. Xu et al. [44] showed that the ff genotype of FokI was associated with more aggressive prostate tumors. Some studies have not shown an association between the VDR polymorphisms and prostate cancer [39, 117].

Role of UV Exposure and its Modifiers in Prostate Cancer Development: a Hypothesis.

In this research project UV exposure is considered as the major factor, with nutrition, skin pigmentation, and VDR gene polymorphisms as potential modifiers. Therfpre, a model is designed for UV exposure and modifiers of prostate cancer risk (Figure 3). There is a direct relationship between exposure to UV and formation of vitamin D in the skin. It is assumed that serum levels of the vitamin D will increase following high levels of UVR exposure. Enriched vitamin D nutrition

will also increase the serum vitamin D levels. Transport of the vitamin D to prostate cells will reduce proliferation and maintain morphology (either preventing or slowing malignant change). As indicated, men with darker skin require more UV exposure to effect increased formation of vitamin D, than men with lighter skin. As a result, the protective effect of any level of exposure to UV is expected to be lower in men with darker skin. Also presence of risk associated VDR variants in individual will decrease this protection.

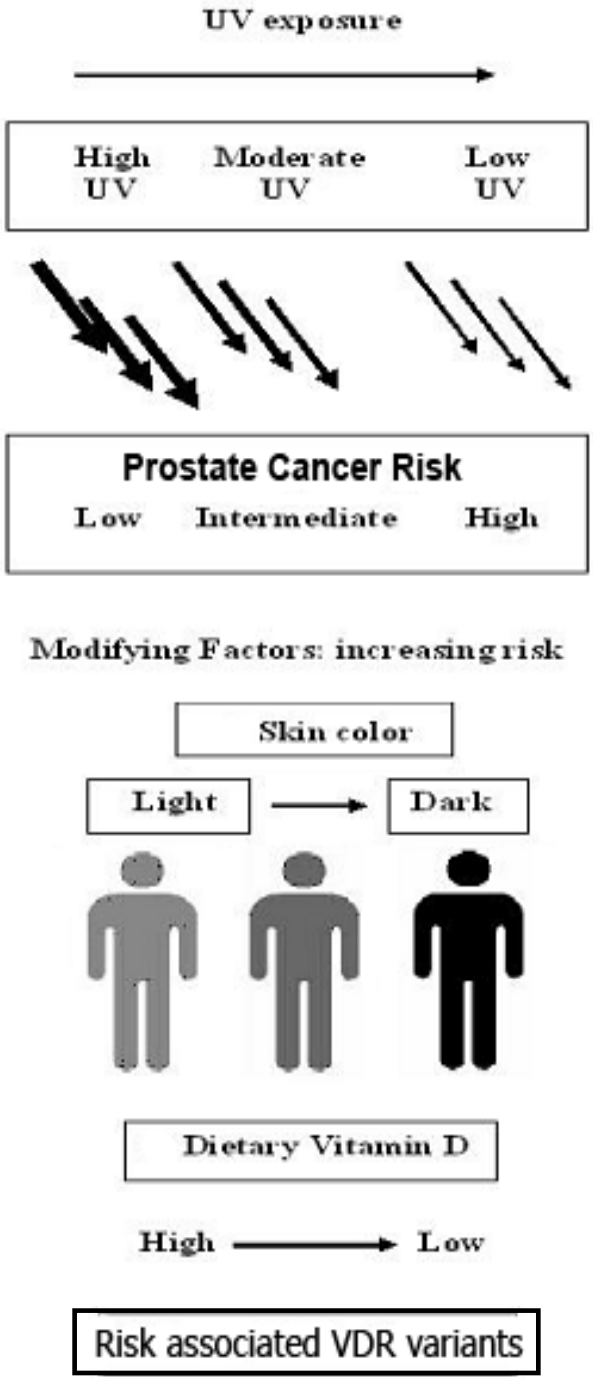

Figure 3. UV Exposure and Modifiers of Prostate Cancer Risk

CHAPTER 2: SPECIFIC AIMS AND HYPOTHESES

Specific aims

The goal of this study is to explore the effects of UV exposure, serum Vitamin D, skin color, and genetic variations on prostate cancer risk in a case-control study of African-Americanmen age > 40 years from the Washington, DC area. Genomic DNA was collected for analyses of genes involved in vitamin D metabolism. The generated genetic data was used along with the epidemiological data to test hypotheses of gene-environment interactions.

To assess UV exposure in African American prostate cancer patients and matched controls.

Ninty one prostate cancer cases and 91 age and race matched controls were recruited. The UV questionnaire was used to assess UV exposure in African-Americanmales.

To study the modifying factors of UV exposure (skin color, serum 25-OH Vitamin D, and genes involved in Vitamin D metabolism.

1. Serum circulating levels of 25-OH Vitamin D were measured by Enzyme Immunoassay (EIA) for all participants. A standardized Food Frequency Questionnaire was used to assess the intake of dietary Vitamin D.
2. Constitutive skin color in all participants was measured using the Dermaspectrophotometer.

To conduct SNPs discovery, characterization, and assessment of variation for genes involved in vitamin D metabolism.

SNPs were detected within the vitamin D receptor (VDR) gene using Denaturing High Performance Liquid Chromatography (DHPLC) technology with combination of sequencing of heterodimer regions resulting from DHPLC.

To utilize the generated data to determine if UV exposure and modifying factors act alone or interact to affect prostate cancer risk in African Americans.

Likelihood ratio contingency, analysis of variance, and logistic regression analyses, were used to test hypotheses on prostate cancer risk.

Hypotheses

The main hypothesis is that increased incidence of prostate cancer and mortality in African Americans involves a dynamic interplay of environmental factors such as diet and UV exposure in addition to genetic factors, some of which directly influence serum vitamin D levels. Individual risk of prostate cancer will increase with low cumulative UV exposure and dark skin. Correspondingly, low risk will be associated with high cumulative exposure and light skin. The relative effects of these parameters on individual risk will in turn be modulated by other environmental factors, e.g., diet and genetic effects.

Hypothesis 1

High UV exposure protects against the risk of prostate cancer. A inverse association would indicate that UV exposure is a protective factor for prostate cancer.

Hypothesis 2

Variation among men for skin pigmentation is associated with prostate cancer risk.

Hypothesis 3

Variation among men for serum 25-(OH) vitamin D levels is associated with prostate cancer risk.

Hypothesis 4

Polymorphisms in VDR gene are associated with prostate cancer risk.

Hypothesis 5

There is interaction between serum 25-(OH) vitamin D, skin color, and VDR gene polymorphisms that alters the risk for prostate cancer.

Scope of Study

Most common diseases appear to result from complex, poorly understood interactions between genetic and environmental factors. Relatively few factors have been unequivocally linked with disease risk or outcome. Evidence from various studies using different experimental approaches has been interpreted as showing that, apart from its harmful effects on the pathogenesis of the common skin cancers, ultraviolet radiation (UVR) may exert a beneficial effect on

development of various internal cancers and other pathologies. This concept is supported by parallel studies showing that hypovitaminosis D is linked with increased risk of various diseases including insulin resistance and multiple sclerosis. These findings suggest that, first, host factors such as skin pigmentation that affect UVR-induced synthesis of vitamin D and, second, polymorphism in genes that mediate the effectiveness of vitamin D action are susceptibility candidates for a variety of diseases. Collectively, these data suggest the hypothesis that, via effects on vitamin D synthesis, UVR exposure has beneficial effects on susceptibility and outcome to a variety of complex diseases.

The major reason proposed for an association of sun exposure with a protective effect in the development of cancer and improved survival is that vitamin D synthesis is a critical component of cellular networks that inhibit cellular proliferation and encourage apoptosis. Therefore this research has focused on measures of sun exposure, serum vitamin D levels (and associated metabolites), skin pigmentation, and genetic variants that may affect vitamin D metabolism.

Few studies have combined all four measures. Although supportive data are available, the concept is unproven. Indeed, other explanations are possible. However, given the potentially important public health implications of the hypothesis and the potential for the development of novel therapeutic modalities, the concept is worthy of further investigation.

CHAPTER 3: METHODOLOGY

Human Subjects

Affected African Americanmen > 40 years of age with histologically diagnosed adenocarinoma of the prostate were recruited through the HUH Urology Department in collaboration with Drs. Augustine Mireku-Boateng and Aaron Jackson, and/or from ongoing HUH/HUCC free prostate cancer screening programs at Howard University Cancer Center (HUCC) and/or from the NBC4 Health and Fitness Expo each January and other numerous community sponsored events. Participants' recruitment was in accordance with Institutional Review Board approval (IRB-02-MED-42).

Eligibility Criteria for Cases

1. Histo-pathological evidence of prostate cancer with clinical staging.
2. A PSA of > 2.5 ng/ml and a positive digital rectal exam (DRE).
3. Full informed consent.

Exclusion Criteria for Cases

1. Age more than 85 years.
2. A chemotherapy, radiation therapy, and/or hormones therapy

Healthy unaffected volunteers with PSA levels < 2.5 ng/ml and normal DREs were also recruited from the HUH urology division, by family practitioners collaborator Dr. Robert Williams and from ongoing HUH/HUCC free prostate cancer screening programs at Howard University Cancer Center.

Eligibility Criteria for Controls

1. Healthy men regularly screened
2. Not related to cases
3. No diagnosis of prostate cancer
4. No family history of prostate cancer among first-degree relatives
5. PSA levels < 2.5 ng/ml
6. DRE negative
7. Matched to cases by age and race

For each prostate cancer patient and matched (If patient between 40-59 years of age, 5 years from patient's age was added or substracted; if patient is 60 years and older, 10 years from patient's age was added or substracted) control information on personal and family history was obtained, and blood samples for candidate gene testing were collected.

Also, personal history including ethnicity, alcohol and tobacco intake, occupation exposures, height and weight, medical history and physical activity were obtained. Each participant answered questions from the UV exposure questionnaire (UVQ)[5]. This questionnaire is designed to elucidate their exposure to UV from childhood (> 12 years) until the present. The UVQ was shown to be valid and reproducible, in skin cancer [118] and renal transplant studies [119]. Additionally, each participant was given the standardized food frequency questionnaire (FFQ) [120] and their constitutive skin color was

measured using the Dermaspectrophotometer. Since there is no further contact with the participants, there were minimal physical, psychological, social, or legal risks involved in this study. Confidentiality of the participants was fully protected. No personal identifiers were recorded and transmitted with the blood samples and clinical data. All personal information on participants was kept separate in a locked cabinet by the dissertation advisor, Dr. Yasmine Kanaan.

All participants signed informed consent, and the Howard University Research Institutional Review Board approved study forms and procedures.

Measuring Skin Pigmentation Using Derma-spectrophotometer

Measurement of skin pigmentation was done using the computerized narrow-band reflect meter called Derma-Spectrophotometer (Minolta Chromameter, Courage and Khazaka Mercameter) to objectively measure skin color in African-Americanpatients and controls. Using two wave lengths, the Derma-Spectrophotometer records the reflectance of light emitted on the skin. The results are expressed in terms of erythema (E) and melanin (M) indices (0 to 100%). This instrument and others were used in numerous studies on skin reflectance [121, 122]. In order to measure constitutive skin pigmentation (pigmentation levels measured in unexposed area of the skin) the inner arm was used as the measured site. And also facultative pigmentation (forehead and back of the hand) were measured. Multiple measurements of E and M were taken on the inner arm, forehead, and the back of the hand. The tanning potential (sun exposure index) was calculated as the relative

difference between the two measurements (i.e., facultative pigmentation minus constitutive pigmentation divided by constitutive pigmentation multiplied by 100) [123].

Assessment of UVR Exposure

A validated questionnaire was used to assess ultraviolet radiation questionnaire (UVQ) [119]. This questionnaire is self-administered and was used to determine several measures of sun exposure, including residential solar radiation, outdoor activity, according to the following:

1. Childhood sun burning, defined as erythema for more than 48 hours or blistering (scored yes/no);

2. Sunbathing score which recorded as never, occasional, and frequent (scored 0, 1, and 2 units, respectively) in age categories (20-39, 40-59 and >60 years). The cumulative score is obtained by adding the units from the three age categories;

3. Foreign holidays recorded as at least one holiday each year in a sunny country over the last 10 years (scored yes/no);

4. Cumulative sun exposure in years (weekdays and weekends considered separately in the three age categories above and combined);

5. The extent of occupation spent outdoors is expressed in units (proportion of time spent outdoors x10) with odds ratios relating to a 10% change in the proportion of outdoor working.

Assessment of Diet

Self-administered Food Frequency Questionnaires (FFQ) was used for dietary assessment. The FFQ is an appropriate epidemiologic method

for dietary assessment and is designed to obtain qualitative and descriptive information about usual food consumption and vitamins intake patterns. Specifically, the 98.2 item Block Brief 2000 questionnaire was administered. The Block questionnaire was developed using previously described methods [120], with a food list designed to cover greater than 90% of the average intakes of over 30 nutrients in Whites, African-Americans, and Hispanic Americans. The questionnaire is self administered and reports how often food is consumed as number of times per day, week, months or year. The usual portion size is reflected as small, medium, or large with a picture representation of these sizes. The Block FFQ was validated and used to assess dietary intake in an African-Americanpopulation [124]. The completed FFQs sent to the Block Dietary Systems in Berkeley, CA for analysis.

Serum Separation

Three tubes of 10ml blood collected from participants. Serum was separated from 10 ml blood collected in BD Vacutainer tubes. The blood samples were inverted slowly 8 to 10 times to mix with protease inhibitors and coagulants (SST gel), and were centrifuged at 1850 x g for 10 min at room tempreture. The upper layer serum in 1 ml aliquots was transferred to cryo-preservative tubes and preserved in -70^0 C freezer for determination of serum vitamin D concentration

Lymphocyte Isolation

The lymphocytes were separated from the other blood collection tube containing anticoagulant for both cases and controls. The blood was centrifuged at 300 x g for 10 min at room temperature, 15 ml of

the middle layer (buffy coat) was carefully transferred into a new clean tube; the blood was diluted by addition of an equal volume (15 ml) of 0.9% sodium chloride (NaCl). The diluted blood was carefully layered over 15 ml of Lymphoprep solution (Life Technologies) in a centrifuge tube without mixing the blood and separation fluid. The tube was capped to prevent the formation of aerosols, and centrifuged for 30 min at 500 x g. The lymphocyte cells were removed from the interface using a Pasteur pipette without removing the upper layer into a new 50 ml conical tube and diluted 3 times volume with 0.9% NaCl to reduce the density of the solution and pellet the cells by centrifugation for 10 min at 500 x g. The pellet was suspended with 4.5 ml of cold RPMI Mediatech CellGro + 20% Fetal Bovine Serum (FBS) (Gibco BRL) and 0.9 ml of cold (50% Dimethyl sulfoxide (DMSO) + 50% FBS). The suspension was divided into 3 tubes and saved at –70˚C.

Genomic DNA Extraction

DNA from 10 ml blood of patient and control was extracted using QiAmp DNA Blood Maxi kit (Qiagene cat.# 51194). Ten ml anticoagulant treated blood was transferred into 50 ml centrifuge tube. Five hundred ul Qiagen protease stock and 12 ml of buffer AL was added to each tube and then was mixed using vortex at least 3 times at 5 seconds each time. Mixture was incubated at 70^0 C for 30 minutes while shaking. Ten ml of 100% ethanol was added to each tube and was mixed thoroughly using vortex. Sample was filtrated by using Qiamp Maxi column which was located in a fresh 50 centrifuge tube and was centrifuged at 1850 x g for 3 minutes. Filtrate was discarded and column was placed on a centrifuge tube and 5 ml buffer AWI was added directly to the column.

Sample was centrifuged at 4500 x g for 1 minute. Five ml of buffer AW2 was added to the column and the column was centrifuged for 15 minutes.

To elute the extracted DNA, the column was placed in a fresh 50 centrifuge tube and 1 ml buffer AE, which was equilibrated to room temperature was added directly onto the membrane. The column was incubated for 5 minutes at room temperature. The column was centrifuged at 4500 x g for 5 minute. DNA concentration in the eluate was measured using Nanodrop ND-1000 spectrophotometer (Nanodrop co.) according to manufacturer instruction. DNA stock was diluted to final concentration of 100 ng/µl with TE (10mM Tris and 1 mM EDTA, pH 8.4) buffer, aliquot, and stored at –70˚C.

Serum 25-OH Vitamin D Assay

An Enzyme Immunoassay (EIA) from Immunodiagnostic Systems Ltd (ADS Ltd, AZ) was used according to manufacturers' instructions. The ADS 25-Hydroxy Vitamin D EIA kit intended for the quantitative determination of 25-hydroxyvitamin D (25-OH D) and other hydroxylated metabolites in human serum or plasma. Serum concentration of 25-OH D is considered to be the most reliable measure of overall vitamin D status and thus can be used to determine whether a patient is vitamin D sufficient [59, 125].

Calibrators, controls and samples were diluted (1:40) with biotin abeled 25-OH D. The diluted samples were incubated in microtitre wells which are coated with a highly specific sheep 25-OH D antibody for 2 hours at room temperature before aspiration and washing. Twenty µl of enzyme (horseradish peroxidase) labeled avidin, was added and it binds

to biotin complexe and, following a further wash step, color was developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures was read in a microtitre plate reader (450 nm), and the developed color intensity was inversely proportional to the concentration of 25-OH D. Seven calibrators were used as standards for plotting the vitamin D standard curve. Controls with known 25-OH D was used to insure day- to- day validity of results.

Calculation of Results

The percent binding of each calibrator, control and unknown sample were calculated as follows:

B/B0%= (mean absorbance) x 100 / (mean absorbance for '0' calibrator), and a calibration curve were prepared by plotting B/B0% on the ordinate against concentration of 25 – hydroxyvitamin D on the abscissa. Calculated B/B0% for each unknown sample and read values of the curve in nmol/L (nM).

The actual vitamin D concentration was calculated based on the relationship of 1 ng/ml=2.5 nmol/L. The dose response curve (standard curve) of the absorbance unit vs. concentration was generated using the results obtained from the calibrators.

SNP Detection Within the VDR Gene, Using Denaturing High Performance Liquid Chromatography (DHPLC) Technology.

DHPLC analysis is a chromatographic mutation analysis method that relies on the formation and separation of double-stranded DNA fragments that contain mismatched pairs from a pool of PCR amplified DNA fragments known as heteroduplex DNA. A pair of primers is designed

to generate a PCR product of up to 400 bp spanning the sequence of interest. When the PCR product is heated to 95^{0}C and then slowly cooled, the DNA strands separate and randomly reanneal to form a mixture of three species: a mutant homoduplex, a heteroduplex, and a wild-type homoduplex. Individuals who are heterozygous in a single-nucleotide mutation or polymorphism have a 1:1 ratio of wild type and mutant DNA. The heteroduplex DNA fragments form as a result of base pairing of the single-stranded mutated DNA with single-stranded, wild type DNA. The two strands will not form hydrogen bonds at the mutation site because the base pairs are mismatched, thus giving the heteroduplex different melting properties than the homoduplex. At a critical temperature (partially-denaturing conditions), the mismatched bases in the heteroduplexes begin to separate, while the matched bases of the homoduplexes remain intact. The percentage of organic mobile phase that disrupts the interactions between DNA fragments and the column matrix is lower for heteroduplex DNA strands than homoduplex DNA strands. Therefore, the heteroduplex DNA fragments elute earlier in the gradient. The denaturation leads to a reduction in the double-stranded portion of the PCR fragment. Single-stranded DNA fragments elute earlier than double-stranded fragments at elevated temperatures. Analysis on the Wave system is performed at a temperature sufficient to partially denature (melt) the DNA heteroduplexes. DHPLC is based upon heteroduplex detection; the heteroduplex profiles are identified by visual inspection of the chromatograms on the basis of the appearance of additional earlier eluting peaks. Corresponding homozygous profiles show only one peak (Figure 4).

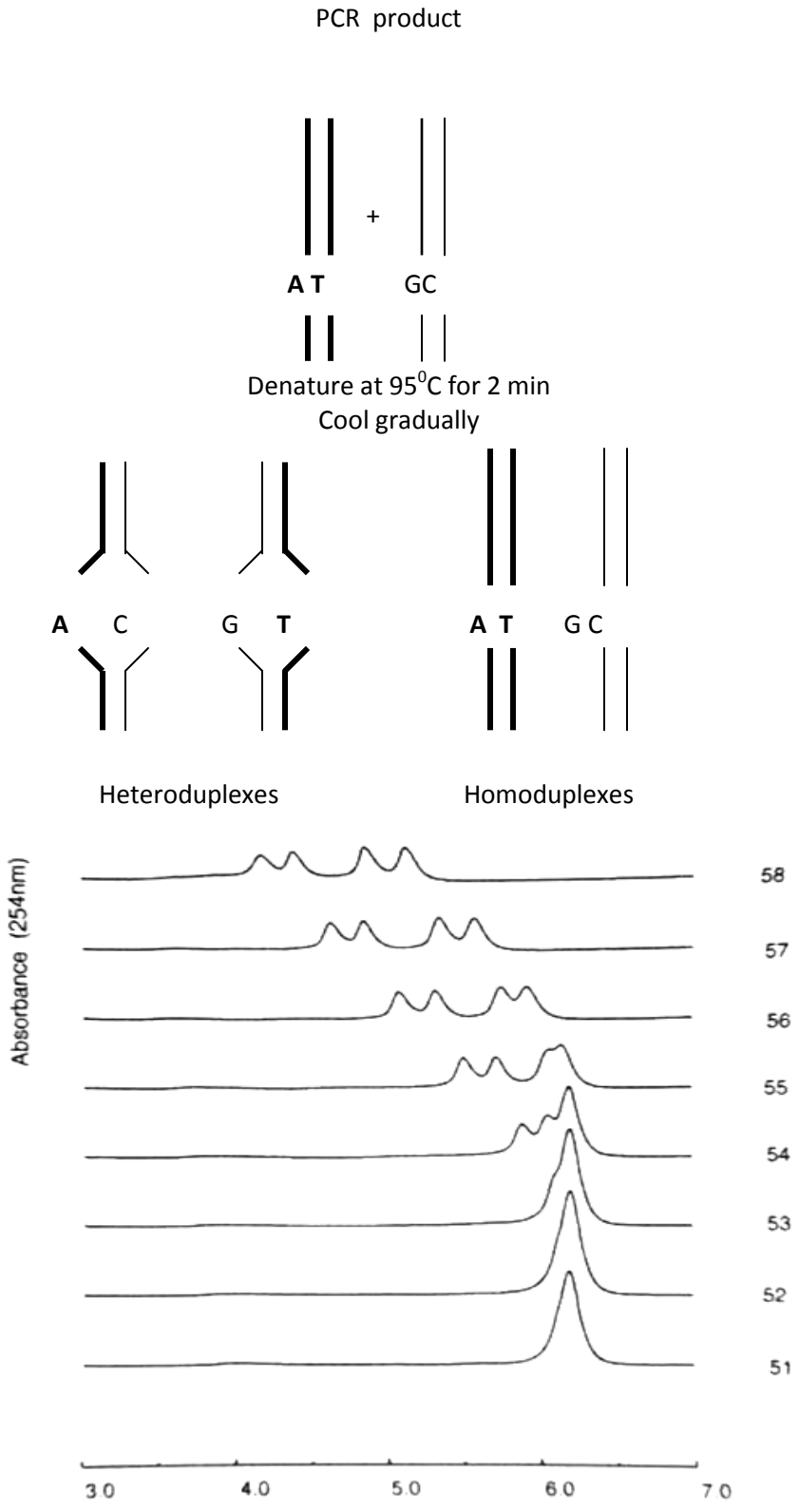

Figure 4. Detection of Mutations and Polymorphisms on the WAVE Nucleic Acid Fragment Analysis System (DHPLC).

Sixteen hundred base pairs upstream of the promoter region from the transcription start site of the VDR (amplicons P-1 through P-6) gene was amplified using polymerase chain reaction (PCR). Different primer sets were used for amplification of VDR promoters, and exons fragments.

Although the exact number and location of reported promoter, exons, and introns in VDR genes are controvercial the regions according to the majority of resources has been selected from http://www.ensembl.org/Homo_sapiens/Transcript/Summary?g=ENSG00000111424;t=ENST00000395324 (Figure 5).

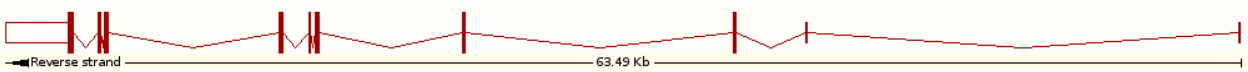

Figure 5. Transcript: VDR-202 (ENST00000395324)

Therefore, nine exon regions (E1-E9) whitin VDR gene have been sequenced to be studied for polymorphisms (Table 1).

Table 1. Primer sequences corresponding to promoter and Exon chromosomal regions of VDR gene used for PCR amplification and sequencing.

Chromosomal Region [a]	Region Length bp(Location)[b]	Fragment length (bp)	Primer sequence
P1	345 (10441907-10442251)	345	5' CCAAGTCAAGATGGTTGC 3' 5' GACCTCAGTAGCCAAGTTTAC 3'
P2	418 (10442191-10442608)	418	5' GTAACAGGTTGGCGAGCG 3' 5' CCCACAGGTCCAGTCCTCTC 3'
P3	388 (10442565-10442952)	388	5'CAGTCAAGGGAAGCAGAGAATAAC 3' 5' CCGCACGAATGGGAAATC 3'
P4	432 (10442886-10443317)	432	5'CTGTCTCAGAAATGGTTCAGAG 3' 5' TGGATGGCTGCGGAAAAC 3'
P5	423 (10443166-10443588)	423	5' CCATCCATCAGACTGGCAGG 3' 5' GGCTCAGAGGGACAAGGTG 3'
P6	372 (10443879-10443508)	372	5'GCTGTGAAAAAAGACTAACTCTC 3' 5' TGATTGAACTTGGGAATGGAC 3'
E1	244 (10419697-10419940)	230	5’ CGTGCCCACTTCCTTAGAGACTG 3’ 5’ CCACCACCTTCTTATGCCCCT 3’
E2	338 (10416006-10416343)	396	5’ GATGCCCACCCTTGCTGAG 3’ 5’ TGCTTCTTCTCCCTCCCTTTC 3’
E3	307 (10402027- 10402333)	395	5’ TCCGTGATGACAGGGTGAGG 3’ 5’ TACAGAGGAAGGGCAGGCAGA 3’
E4	494 (10394476-10394969)	348	5’ GTGCCCAGCCTAGAGGTGAGA 3’ 5’ CGTCCCTACCCCAGTTCTGTTC 3’
E5	314 (10394129-10394442)	349	5’ GCCTTCCTGTAGACCTTCCTCAA 3’ 5’ ACCTCCTTCCATCCAGCAGC 3’
E6	444 (10393022-10392579)	333	5’ ACCTGTGGAGTCACTGTGGGATTC 3’ 5’ AGCCTGCGTGACAGAGCAAGA 3’
E7	434 (10383626-10384059)	403	5’ GAACACTCTTGTCCCTTCCAGCC 3’ 5’ TCTCTCCCTGTTGGTGCCTAACTC 3'
E8	348 (10383355-10383702)	353	5’ AGATTCTGGCTCCACCCGTC 3’ 5’ CAGCAGGTCTTTGTCCTTCATACTC 3’
E9	525 (10381743-10382267)	402	5’ AGTCACTGGAGGGCTTTGGG 3’ 5’ TGAGGAGGGCTGCTGAGTAGC 3’

[a] P= promoter, E= Exon [b] Positions are based on the genomic sequence from the chromosome 12 contig NT029419 NCBI Build 34 (www.ncbi.nlm.nih.gov).

Collected genomic DNA from individuals (cases and controls) were amplified in a 25-µl PCR reaction containing 1X PCR buffer II, 30 ng of genomic DNA, 20 pmol each of forward and reverse primers, 2 mM $MgCl_2$, 0.2 mM dNTP, and 2µl AmpliTaq Gold® DNA polymerase (Applied Biosystems). The PCR reaction was performed for 35 cycles of the following stages:

1. Denaturation at 94˚C for 20 sec,
2. Annealing for 40 sec at optimized temperature per each sequence,
3. Extension at 72˚C for 20 sec,
4. Incubation 10 minute at 72˚C in a Perkin Elmer thermal cycler.

Alternative condition for improving of amplification was used according to following.

94˚C for 20 seconds; exon specific temprature ˚C for 40 seconds and 72˚C for 20 seconds for 5 cycles at each increment

94˚C for 20 seconds; optimum temperature ˚C for 40 seconds and 72˚C for 20 seconds for 35 cycles

VDR Exons	**Increment annealing temprature**
VDR Exon 1	53.6,55.6, 57.6, 59.6
VDR Exon 2	54.9, 56.9, 58.9, 60.9
VDR Exon 3	54.7, 56.7, 58.7, 60.7
VDR Exon 4	55. 57, 59,61
VDR Exon 5	53.6,55.6, 57.6, 59.6
VDR Exon 6	52.5, 54.5, 56.5, 58.5
VDR Exon 7	53.8, 55.8, 57.8, 59.8
VDR Exon 8	54.6, 56.6, 58.6, 60.6
VDR Exon 9	56.1, 58.1, 60.1, 62.1

Prior to the detection of polymorphisms in the VDR gene using denaturing high performance liquid chromatography (DHPLC), samples were heated at 95˚C for 4–8 min, removed from the thermal cycler, and cooled at room temperature for 20 minutes. The samples were loaded

into the DHPLC instrument (WAVE® DNA Fragment Analysis System, TRANSGENOMICS, Omaha, NE) and run according to the preset condition icluding; an initial gradient of 45% buffer A (0.1 M tri-ethyl-ammonium acetate (TEAA) solution, pH 7.0) and 55% buffer B (0.1 M TEAA containing 25% acetonitrile, pH 7.0), followed by a final gradient of 36% buffer A and 64% buffer B, using an acquisition time of 8.7 minutes. DNASep® cartridge column was used, which normal operating pressure of the system is between 1100 and 2800 psi at 0.9 ml/min flow rate. The cartridge is packed with C18 alkalylated, polystyrene-divinylbenzene polymeric beads that allow for analysis under a wide range of pH 2 to 13 and temperature 40°C to 80°C conditions. A positively charged ion-pairing reagent, TEAA allows the negatively charged DNA backbone to interact with the hydrophobic DNASep cartridge matrix.

Majority of Samples demonstrating two or more heteroduplex peaks were sequenced using an ABI 377 DNA sequencer (ABI, Foster City, CA) to confirm the presence of SNPs in both direction order to identify any polymorphisms within the PCR fragment, the florescent labeled Big-dye terminator cycle sequencing kit (Applied Biosystems) were used[59].

DNA Sequencing

Genomic DNA was amplified as described and purified with Qiagen column (QIAquick PCR purification Kit 50) for sequencing. In order to eliminate Taq polymerase errors, at least two samples representing five or more independent amplifications were pooled for sequencing

bidirectionally with the Applied Biosystems Taq Dye Deoxy terminator cycle sequencing Kit according to the manufacture's instructions. Polymerase chain reaction for sequencing was performed using 8 μl of Terminator ready reaction mix and conditions in a 20 μl reaction volume containing 100 ng of PCR product as a template, 3.2 pmole PCR primers and newly designed primers. PCR was performed in a GeneAmp 9700 thermal cycler as follows: denaturation at 94◦C for 4 min, 25 cycles of 30 sec at 94◦C, 30 sec at 50◦C, 4 min at 60◦C and final extension for 7 min at 60◦C. The extension product was purified by Centriflex gel filtration cartridge (Edge Biosystems, Inc.) to remove excess terminators[61].

Modification of the above methods gave better results: PCR was performed as follows: 25 cycles of denaturation at 96◦C for 10 sec, annealing at 50◦C for 5 sec, and extension at 60◦C for 4 min. To some samples DMSO added to increase the sensitivity of Taq polymerase. MgCl2 concentratiom has been optimized for high CG contents fragments. The purification of extension products (PCR) was performed by adding 2 μl of 3 M sodium acetate pH 4.6, 50 μl of 95% ethanol, shaking vigorously and centrifugating for 30 min at 14,000 rpm. The ethanol solution was aspirated with a micropipetter and the pellet was washed with 250 μl of 70% ethanol without disturbing the pellet. The alcohol solution was aspirated with a micropipetter, and a KimWipe was used to remove any alcohol from the sides of the tube. The pellet was air dried and resuspended in 25 μl of Template Suppression Reagent (PE; P/N 401674); heated at 95◦C for 2 minutes to denature, placed on ice

until ready to load. Samples were sequenced commercially by ACGT Incorporation (Wheeling, IL) using DNA cycle sequencing.

Polymorphisms Screening

In order to compare and align the forward and reverse DNA sequences in any amplified regions of VDR gene with wild type (reference) sequences, generated data from gene analyzer along with wild type sequence corresponding to each region were imported to Sequencher Ver. 4.8 (Gene Codes Corporation) software. And presence of possible polymorphism was visually inspected and chromosomal position of those polymorphisms was reported. SNPs were identified from the International Hapmap project (http//:www.hapmap.org). Samples with the same heteroduplex pattern on DHPLC were considered to have the same polymorphisms.

Statistical analyses

A binary logistic regression model was considered appropriate for analyzing the data, because: a) in a binary logistic regression model, the dependent variable can be predicted based on both continuous and categorical independents. b) through the binary logistic regression model, it can be determined how much variance in the dependent variable can be explained by the independents, whether there is any interaction effect between variables, which independent is of more importance. Two factors environmental and genetics were analyzed separately. First, environmental factors were applied as independent variables to predict the dependent variable, prostate cancer. The equation for the logit model is given below [126]:

$$\Pr(\text{Prostate Cancer} = 1) = \frac{1}{1 + e^{-f(x)}}$$

Where f(x) = β_0 + β_1*UV Exposure + β_2* Serum vitamin D + β_3* Tanning potential + β_4* Diet vitamin D + β_5*Supplemental vitamin D + β_6* Age

In this equation, Pr (Prostate Cancer=1) means the probability of having a prostate cancer. A linear model including all the predictors was used to represent the logit of the probability, so that through this model, the data were used to predict the probability of prostate cancer risk of a patient considering their environmental factors.

Gene-environment interaction was evaluated on a multiplicative scale by adding an interaction term to the model containing the main effect variables. Possible confounding effects of age was assessed and found that appreciably altered risk estimates, and thus, they were included as covariates. Differences in allele frequencies between case subjects and control subjects were tested for each SNP with the use of logistic regression analysis. Allelic odds ratios (ORs) and 95% confidence intervals (95% CI) were estimated on the basis of a multifactorial model. Data were analysed using logistic regression to calculate ORs as estimates of relative risk of prostate cancer associated with SNP. Unconditional logistic regression modeling was used to calculate ORs and 95% CI associated with sun exposure measures and VDR SNPs. To determine whether the association with VDR SNPs is modified by factors affecting vitamin D status, the analyses were stratified by tertiles of the sun exposure index. Logistic regression

analysis was used to compare the frequencies of the VDR SNPs in the cases and controls and derive age-corrected odds ratios. It was first determined whether the frequencies of individual SNPs were significantly different in cases and controls. Second, it was determined whether prostate cancer risk was associated with combinations of VDR variants represented by combinations of SNPs. Thus, the proportions of men who had particular combinations of VDR SNPs were compared in cases and controls. To determine whether the association of VDR variants with prostate cancer risk was modified by the extent of UVR exposure, the association of VDR variants with risk in cases and controls stratified into those with high and low levels of exposure (above and below the median) was studied. Tests of interaction were performed by including cross-product terms in the logistic models and conducting a one degree of freedom Wald test.

The logistic regression model was performed by using SAS v9.0 software (SAS Institute Inc.) and SPSS v16.0 (IBM Corp. Chicago) was used for the frequency count. All reported P values are based on two sided test and $P \leq 0.05$.

CHAPTER 4: RESULTS

Introduction

The main objective of the current study is to determine the effects of UV exposure, serum Vitamin D, skin color, and genetic variations on prostate cancer risk in a case-control study. The interaction between these factors would also be analyzed. In line with this, research questions were formulated:

1. Does high UV exposure protect people against the risk of having a prostate cancer?
2. Is variation among men for skin color associated with prostate cancer risk?
3. Is variation among men for serum 25-(OH) vitamin D levels associated with prostate cancer risk?
4. Is there any association between dietary vitamin D, supplemental vitamin D and risk of prostate cancer?
5. Are polymorphisms in VDR gene associated with prostate cancer risk?

Prior to answering the five research questions, a titest and Mann-Whitney was used to compare the descriptive statistics of each factor. To answer the first three research questions, a Point-Biserial Correlation Coefficient was computed in order to determine the relationship between the factors. The results of the computation of Point-Biserial Correlation Coefficient are presented in the section after the descriptive. For the fourth research question the odd ratio was computed so that the association between the groups was

determined. A binary Logistic Regression Model then was employed to verify the correlation coefficient.

Description of the Human Participants

In order to explore the effects of UV exposure, serum Vitamin D, and skin color on prostate cancer risk in African-Americanmen, a comprehensive data base was built to explore the interactions of vitamin D levels, UV exposure, and diet in African-Americanmen with and without prostate cancer. Ninety one affected African-Americanmen with histologically diagnosed adenocarcinoma of the prostate (Table 3), age of onset (mean 64.53 years, range 40-89), Gleason score (range 4-10) and PSA level (range 4 - >100 ng/ml) and ninety one age and ethnicity matched control subjects, with mean age 58.7 years (range 40-89) and PSA level (range 0.4-3.5) were obtained from the Urology Department at Howard University Hospital and the Prostate Screening program at Howard University Cancer Center. Cases and controls did not significantly differ in terms of residence and age. This study is executed with approval from the Howard University Institutional Review Board and participant's informed consent.

The prostate cancer cases were ≤ 65 years of age at the time of diagnosis. At the time of recruitment, cases were slightly older than controls (average 64.53 and 58.67 years, respectively). Most of the cases were in age groups 60-69 years (46.15%) whereas age group 50-59 years was the highest (42.40%) among controls. The number of patients with PSA level ≤ 4.0 ng/ml was higher in controls than cases (89.20 % and 48.80 % respectively). The highest PSA level in cases was ≥100 ng/ml whereas PSA level of 10.0-19.9 ng/ml was the highest among

controls. The majority of cases had moderate grade [Gleason score 6 (49.20 %)] tumors.

Table 2. Clinical and Demographic Characteristics of the Subjects. [a]

Characteristic	Case Subjects (N=91)	Control subjects (N=91)
Age --yr		
Mean Age	64.53± 8.97	58.67± 9.51
Age at Diagnosis -- no. (%)		
≤65	49 (53.85)	NA
>65	42 (46.15)	NA
Age Group – no. (%)		
40-49	4 (4.4)	15 (16.3)
50-59	20 (22)	39 (42.4)
60-69	42 (46.15)	24 (26.09)
≥70	25 (27.48)	14 (15.22)
PSA level --- no. (%) [b]		
No. of subjects	86	65
≤ 4.0 ng/ml	42 (48.8)	58 (89.2)
4.1-9.9 ng/ml	24 (27.9)	6 (9.2)
10.0-19.9 ng/ml	6 (7.00)	1 (1.5)

CONTINUED

Table 2. (Continued)

Characteristic	Case Subjects (N=91)	Control subjects (N=91)
PSA level --- no. (%) [b]		
20.0-49.9 ng/ml	5 (5.8)	0 (0)
50.0-99.9 ng/ml	4 (4.7)	0 (0)
≥100 ng/ml	5 (5.8)	0 (0)
Missing data	5	26
Gleason score for biopsy ---- no. (%) [c]		
No. of subjects	61	
≤ 4	3 (4.90)	NA
5	6 (9.80)	NA
6	30 (49.20)	NA
7	16 (26.20)	NA
8	3 (4.90)	NA
9	2 (3.30	NA
10	1 (1.6)	
Missing data	30	NA

[a]Plus-minus values are means ±SD.

[b]Prostate specific antigen (PSA) levels were obtained at the time of diagnosis for case subjects and at the time of study enrollment for control subjects.

[c]The Gleason score ranges from 2 to 10, with higher scores indicating more aggressive disease.

Cumulative UV Exposure

Cutaneous production of vitamin D greatly contributes to systemic vitamin D levels [83]. Occupational and physical activities as surrogate of sunlight exposure have been used; those who are engaged in work with walking, labor work, or hard work or those who did not engaged in any outdoor physical activities.

The mean was computed for each of the factors together with the sub-factors as shown in table 2. A t-test and Mann-Whitney were also used to determine if there is a significant difference between cases and controls. The lifetime cumulative sun exposures were 25379.54 hrs in cancer cases and 26453.42 hrs in controls (Table 4). There was no significant difference in cumulative sun exposures between cases or controls (P =0.73).

The outdoor UV exposure was significantly higher in control group when compared to case group, P=0.00. All other factors didn't have any significant differences between the groups; however, mean of total UV exposure, serum vitamin D levels, supplemental vitamin D, diet vitamin D, and tanning potential were higher in controls than cases.

In order to eliminate the effect of confounder factor (age), further analysis was done for 53 age matched controls and cases (Table 5). The association significantly changed after further adjusting for the variable factor, age.

Table 3. Comparison of Mean for the Environmental Factors in Prostate Cancer Cases and Controls (N = 182)

Characteristics	Cases (mean)	Controls (mean)	*P* (t-test)	*P* (Mann-Whitney)
Age--yr	64.53	58.67	0.001	0.0001
Total UV exposure--hr	25379.54	26453.42	0.073	0.0785
Profession	9023.305	7596.882	0.54	0.4780
Outdoor	1786.57	5017.189	0.001	0.0007
Recreation	12960.22	12638.8	0.79	0.2498
Residence	1609.187	1201.122	0.61	0.4498
Serum vitamin D –ng/ml	26.75	29.0615	0.291	0.2079
Supplemental vitamin D—mg/day	129.7	169.51	0.174	0.0791
Diet vitamin D—mg/day	140.329	155.001	0.42	0.4157
Tanning potential--%	30.32	36.51	0.16	0.3982

Table 4. Comparison of Mean for the Environmental Factors in Prostate Cancer Cases and Controls (N = 106) age matched[a]

Characteristics	Cases no. (mean)	Controls no. (mean)	*P(* t-test)	*P* (Mann-Whitney)
Age--yr	60.90	60.90	1.0000	0.9975
Total UV exposure--hr	20013.94	28041.97	**0.003**	**0.0083**
Profession	5633.64	8112.5	0.123	0.2000
Outdoor	1846.98	6117.44	**0.003**	**0.0442**
Recreation	11417.74	13254.08	**0.031**	**0.0324**
Residence	1115.58	1007.81	0.893	0.3340
Serum vitamin D—ng/ml	28.27	29.72	0.618	0.7006
Supplemental vitamin D—mg/day	147.25	171.99	0.516	0.3168
Diet vitamin D—mg/day	156.67	146.06	0.689	0.8571
Tanning potential--%	24.77	39.5	**0.01**	**0.0266**

[a]Control's age matched to exact case's age

Skin Pigmentation (Tanning Potential)

Skin is the major source of vitamin D; 90-95% of most people's vitamin D requirements come from casual sun exposure. Melanin is an effective sunscreen and decreases vitamin D production in the skin. It was suggested that skin pigmentation evolved to prevent excess production of vitamin D in the skin [83]. To determine the effect of increased skin pigment on the cutaneous production of vitamin D3, circulating vitamin D concentrations were determined. The mean of tanning potential was 30.32% and 36.51% (Table 4) among prostate cancer and control subjects, respectively. There was no significant difference between the controls and cases (P=0.16). In order to eliminate the effect of a confounder factor (age), further analysis was done for 53 age matched controls and cases (Table 5). Interestingly, there was a statistically significant difference between the control and cases (P=0.01).

Dietary and Supplement Vitamin D

Overall, the estimated supplemental and dietary vitamin D from the FFQ (Table 4), was lower among prostate cancer than control participants (Supplement: 129.7 mg/day and 169.51 mg/day; Diet: 140.329 mg/day, 155 mg/day) respectively. There were no significant differences between the controls and cases (P=0.42, P=0.174). In order to eliminate the effect of a confounder factor (age), further analysis was done for 53 age matched controls and cases (Table 5). There was no statistically significant difference in mean supplemental and/or dietary vitamin D between the control and cases (P=0.516; P=0.689).

Level of Vitamin D in Sera

The measurement of the major circulating form of vitamin D, 25(OH)D, is the gold standard for determining the vitamin D status of a patient. The normal range, which is typically 25-37.5 nmol/L (10-15 ng/ml) to 137.5-162.5 nmol/L (55-65 ng/ml) by most commercial assays, is not truly reflective of whether a patient is vitamin D deficient or intoxicated (17). The mean serum vitamin D level was 26.75 ng/ml and 29.0615 ng/ml in prostate cancer patients and controls respectively (Table 4). Using the independent samples t-test and Mann-Whitney, there was no significance difference in mean serum level between prostate cancer patients and control (P=0.291) before or after age matched controls and cases (P=0.618) (Table 5).

Summary

Based from table 3 it can be observed that the mean total UV exposure, outdoor exposure, recreational exposure, and tanning potential have a significant difference between the case and control group in age matched samples since it has a p-value of less than 0.05.

Mean of total UV exposure was significantly higher in controls than cases 28041.96 hr and 200013.96 hrs respectively. Mean of professional UV exposure in controls was higher than cases but was not statistically significant, P=0.123. Mean of recreational UV exposure in age matched controls was significantly higher than cases, 13254.08 hrs and 11417.74 hrs, respectively. Differences in mean of residential UV exposure in cases and controls was not statistically significant, P=0.896. Supplemental vitamin D in controls was 171.93 mg/day which is slightly higher than 147.25 mg/day in cases however it was not

statistically different, P=0.516. Mean of dietary vitamin D didn't reflect any differences between cases and controls, 156.67 mg/day and 146.06 mg/day, respectively, P=0.689.Interestingly the mean of tanning potential (UV exposure index) was strongly higher in controls when it was compared to cases, 39.5 % and 24.7%, respectively, P=0.01.

Association of environmental factors and risk of prostate cancer

Using conditional logistic regression analysis all age groups (50-59 years, 60-69 years, and 70≥ years) showed strong association with increased risk of prostate cancer, Ors; 2.008, 9.126, and 8.734 respevtively (Table 5). None of the variables (environmental factors) were significant, P>0.05 (Table 5). Interstingly outdoor sun exposure and Tanning potential indicated significant association with decreased risk of prostate cancer, ORs; 0.707, and 0.310 respectively.

Table 5. Association of Environmental Factors, Age Groups and Prostate Cancer Risk Using Conditional Logistic Regression

Variable	Regression Coefficient	Standard Error	Odds Ratio[a]	95% CI	*P*
Intercept	-1.5252	0.6051			0.0117
Age -- yr					
40-49			1		Reference
50-59	0.6972	0.6785	2.008	(0.531-7.592)	0.3041
60-69	2.2111	0.6768	**9.126**	(2.422-34.382)	**0.0011**
≥ 70	2.1673	0.7142	**8.734**	(2.154-35.416)	**0.0024**
Tanning potential[b]	-0.3465	0.1861	**0.707**	(0.491-1.018)	**0.0626**
Total sun exposure[b]	0.7145	0.6774	2.043	(0.542-7.707)	0.2915
Outdoor[b]	-1.1704	0.3732	**0.310**	(0.149-0.645)	**0.0017**
Recreation	-0.2673	0.3341	0.765	(0.398-1.473)	0.4237
Professional	-0.5292	0.5963	0.589	(0.183-1.895)	0.3748
Diet Vitamin D[b]	0.0917	0.1960	1.096	(0.746-1.609)	0.6400
Supplemental Vitamin D[b]	-0.0508	0.1808	0.950	(0.667-1.355)	0.7788
Serum Vitamin D[b]	-0.0934	0.1773	0.911	(0.643-1.289)	0.5982

[a] Odds ratio adjusted for age

[b] Variables are standardized

Association of Environmental Factors and Risk of Prostate Cancer for Age Matched Cases and Controls

The means of total UV exposure, outdoor, recreation, and tanning potential were significantly different between controls and cases (Table 4). In order to test the association between these factors and risk of prostate cancer a binary logistic regression analysis was performed (Table 6).

The significant variables were recreational UV exposure and the tanning potential ($P< 0.05$). Since the coefficient was negative for all factors, it was concluded that increase of any of the environmental variables decreases the occurrence of prostate cancer, although they were not statistically significant. Furthermore, it was shown that the odds ratio for tanning potential wad slighly lower compared to other environmental factors; hence it can be concluded that the more tanning potential the lower risk for prostate cancer.

Table 6. Association of Environmental Factors and Risk of Prostate Cancer in Age Matched Cases and Controls

Variable	Coefficient	Odds Ratio[a]	P-Value	95% CI
Constant	3.0068		0.001	
Total UV exposure	-0.0000153	0.99	0.485	(0.99-1)
Outdoor UV exposure	-0.0000958	0.99	0.091	(0.99-1)
Recreational UV exposure	-0.0001296	0.99	0.05	(0.99-1)
Tanning potential	-0.0209306	0.98	0.015	(0.96-1)

[a]Logistic regression analyses was used to determine odd ratios

Protective effect of Early- Life Sun Exposure

Risk of prostate cancer in relation to stage of life from ≥5 years to ≤ 40 years was assessed by self-report. Each life stage was assigned a solar radiation level and classified as low, medium, or high. Sun bathing scores of 0 for low exposure, 1 for moderate exposure, and 2 for high exposure were given to each category (Table 7). The less likelihood of prostate cancer risks were found among men in 0-5 years (OR, 0.17; 95% CI, 0.03-0.744), and 6-11 years (OR, 0.28; 95% CI, 0.076-1.058) with high sun exposure. Interestingly this inverse association between prostate cancer risks and high sun exposure intensity was seen among men in 12-17 years (OR, 0.41; 95% CI, 0.086-1.946), but was not statistically significant.

Non-significant inverse associations were found among men with moderate sun exposure in all age groups, whereas non-significant direct association could be found between risk of prostate cancer and high sun exposure levels in men 18-29 years (OR, 1.54; 95% CI, 0.395-6.033), 30-39 years (OR, 1.05; 95% CI, 0.288-3.849), and 40≥ years (OR, 1.33; 95% CI, 0.320-5.480).

Table 7. Age Groups Stratified by Sunbathing Score and Risk of Prostate Cancer

Age period (years)	Cases No. (%)	Controls No. (%)	Odds Ratio	[95% CI]	*p* Value
0–5 (years)					
Low exposure[a]	22 (25.88)	24 (30)	1		Reference
Moderate exposure[b]	36 (42.35)	30 (37.5)	0.44	(0.113 - 1.714)	0.237
High exposure[c]	27 (31.76)	26 (32.5)	0.17	(0.03 - 0.744)	0.019
6-11 (years)					
Low exposure	11 (12.94)	9 (11.11)	1		Reference
Moderate exposure	35 (41.18)	32 (39.51)	0.76	(0.241 - 2.408)	0.644
High exposure	39 (45.88)	40 (49.38)	0.28	(0.076 - 1.058)	0.061
12-17 (years)					
Low exposure	6 (6.98)	4 (4.94)	1		Reference
Moderate exposure	33 (38.38)	30 (37.04)	0.74	(0.169 - 3.235)	0.69
High exposure	47 (54.65)	47 (58.02)	0.41	(0.086 - 1.946)	0.262
18-29 (years)					
Low exposure	7 (8.14)	7(8.64)	1		Reference
Moderate exposure	33 (38.37)	40 (49.38)	0.83	(0.194 - 3.545)	0.8
High exposure	46 (53.49)	34 (41.98)	1.54	(0.395 - 6.033)	0.533
30-39 (years)					
Low exposure	14 (16.28)	10 (12.35)	1		Reference
Moderate exposure	38 (44.19)	46 (56.79)	0.44	(0.128 - 1.508)	0.192
High exposure	34 (39.53)	25 (30.86)	1.05	(0.288 - 3.849)	0.947
40 =< (years)					
Low exposure	21 (24.42)	19 (23.46)	1		Reference
Moderate exposure	38 (44.19)	44 (54.32)	0.49	(0.170 - 1.426)	0.191
High exposure	27 (31.4)	18 (22.22)	1.33	(0.320 - 5.480)	0.697

[a] sunbathing score=0, [b] sunbathing score=1, [c] sunbathing score=2

Genetics Analysis

VDR Polymorphisms Screening

In order to determine whether there were novel single nucleotide polymorphisms (SNPs) within the transcriptional regulatory regions or coding regions of vitamin D receptor (VDR) 6 promoter sites and 9 exons were analyzed.

SNPs were located in participants using Genbious version 4.6.0 software (Biomatters LTD), Figure 9-12. SNPs nomenclature was assigned according to publication of the latest manuscript on this issue by JT den Dunnen et al. 2000 [127].

Six (c.278-69G>A, c.755+25G>A, c.1025-95G>A, c.907+75C>T, c.1025-56A>G, and c.1025-49G>T) distinct polymorphisms (which occur at a frequency of greater than 1 in 100 chromosomes) in non coding regions; and one (c.1056T>C) distinct polymorphisms in the coding region, have been detected in VDR among the 182 African Americans (Table 8). When compared to Entrez database SNP (dpSNP) only two of these polymorphisms, c.907+75C>T and c.1025-56A>G, had not been previously reported and may be unique to African Americans. Reference SNP accession ID (rs ID) assigned for all previously reported SNPs using BLAST SNP, http://www.ncbi.nlm.nih.gov/SNP/snp_blastByOrg.cgi (Table 8).

The prevalence of the variant alleles within c.278-69G>A, c.755+25G>A, c.1025-95G>A, c.907+75C>T, c.1056T>C, c.1025-56A>G, and c.1025-49G>T among controls were 40.6%, 5.5%, 1.1%, 30.77%, 23%, 5.5%, and 43.95% respectively; and 35%, 18.7%, 23%, 72.5%, 31.87%, 2.2%, and

39.5% among prostate cancer patients respectively. These SNPs were not located in evolutionarily conserved regions or known splicing sites. There was no SNP located in VDR promoter region. Only one nonsense polymorphism, c.1056T>C, was detected in coding region.

HapMap Genome Browser (Phase 1, 2 & 3 - merged genotypes & frequencies) was used to investigate the genotype and allele frequencies of the SNPs in different populations (http://hapmap.ncbi.nlm.nih.gov/index.html.en).

Genotype and allele frequencies of rs1168266, rs11574114, rs731236, and rs7975232 were found in hapmap database (Table 9-12).

Frequencies of altered alleles in for SNPs rs1168266, rs11574114, rs731236, and rs797532 (0.32, 0.01, 0.27, and 0.20 respectively) in African American were lower than their European counterparts, 0.605, 0.027, 0.44, and 0.578 respectively (Table 13). Also the heterozygosis frequencies of African American were different than European ones.

Figure 6. c.273-67G>A was Located in Case Number 6091.

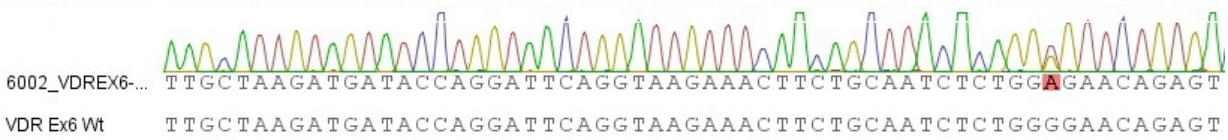

Figure 7. c.755+25G>A was Located in Case Number 6002.

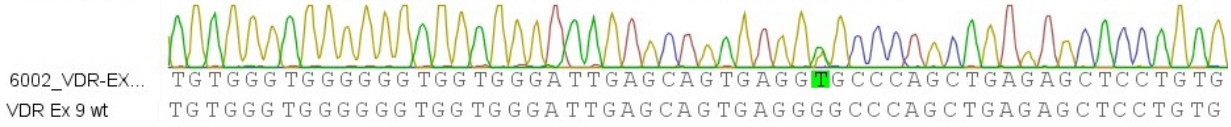

Figure 8. c.1025-49G>T was located in Case Number 6002.

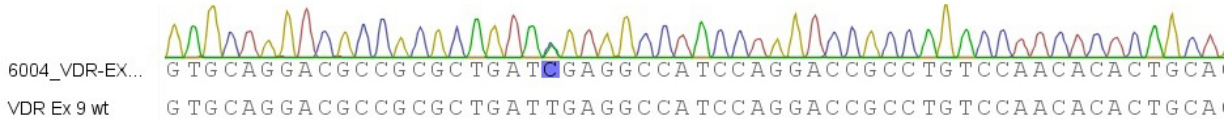

Figure 9. c.1056T>C was Located in Case Number 6004.

Table 8. VDR Polymorphisms[a] in Prostate Cancer Cases and Controls

SNP[b]	dbSNP Identifier	Effect	Cases no. (%)[c]	Controls no. (%)	AA case heterozygosity[d]	AA control heterozigosity
c.278-69G>A	rs11168266	noncoding	32(35)	37(40.6)	0.17 (32/184)	0.20 (37/182)
c.755+25G>A	rs61614728	Noncoding	17(18.7)	5(5.5)	0.09 (17/184)	0.03 (5/182)
c.1025-95G>A	rs11574114	Noncoding	21(23)	1(1.1)	0.11 (21/184)	0.01 (1/182)
c.907+75C>T	not reported	Noncoding	66(72.5)	28(30.77)	0.36 (66/184)	0.15 (28/182)
c.1056T>C	rs731236	coding	29(31.87)	21(23)	0.16 (29/184)	0.12 (21/182)
c.1025-56A>G	not reported	Noncoding	2(2.2)	5(5.5)	0.01 (2/184)	0.03 (5/182)
c.1025-49G>T	rs7975232	noncoding	36(39.5)	40(43.95)	0.20 (36/184)	0.22 (40/182)

[a]Polymorphisms, variants occurring with the frequency of ≥1 out of 100 chromosomes.

[b]Nucleotide numbering starting with the first nucleotide (nucleotide 1/A) in the ATG-translation initiation codon of VDR coding seguence. Beginning of the intron; the number of the last nucleotide of preceding exon, a plus sign and the position in the intron. End of the intron; the number of the first nucleotide of the following exon, a minus sign and the position upstream in the intron. c = coding DNA reference sequence used for numbering. > = original nucleotide change direction [127].

[c]Allele frequency.

[d]The hetrozygosity was determined by dividing the diallelic variant chromosomes by the total chromosomes (numbers in parenthesis).

Table 9. Genotype and Allele Frequencies of rs1168266 in Different Populations

Population	Genotype frequencies									
							Ref-allele		Other allele	
	genotype	freq	genotype	freq	genotype	freq	allele	freq	allele	freq
CEU	G/G	0.211	A/G	0.368	A/A	0.421	G	0.395	A	0.605
CHB	G/G	0.650	A/G	0.225	A/A	0.125	G	0.762	A	0.237
JPT	G/G	0.385	A/G	0.462	A/A	0.154	G	0.615	A	0.385
YRI	G/G	0.071	A/G	0.500	A/A	0.429	G	0.321	A	0.679

Population descriptors:

YRI: Yoruba in Ibadan, Nigeria
JPT: Japanese in Tokyo, Japan
CHB: Han Chinese in Beijing, China
CEU: CEPH (Utah residents with ancestry from northern and western Europe)

Table 10. Genotype and Allele Frequencies of rs11574114 in Different Populations

Population	Genotype frequencies									
							Ref-allele		Other allele	
	genotype	freq	genotype	freq	genotype	freq	allele	freq	allele	freq
CEU	G/G	0.945	A/G	0.055	A/A	0	G	0.973	A	0.027
CHB	G/G	1.000	A/G	n/a	A/A	0	G	1.000	A	0
JPT	G/G	1.000	A/G	n/a	A/A	0	G	1.000	A	0
YRI	G/G	0.491	A/G	0.439	A/A	0.070	G	0.711	A	0.289

Population descriptors:

YRI: Yoruba in Ibadan, Nigeria
JPT: Japanese in Tokyo, Japan
CHB: Han Chinese in Beijing, China
CEU: CEPH (Utah residents with ancestry from northern and western Europe)

Table 11. Genotype and Allele Frequencies of rs731236 in Different Population

Population	Genotype frequencies									
							Ref-allele		Other allele	
	genotype	freq	genotype	freq	genotype	freq	allele	freq	allele	freq
CEU	C/C	0.224	C/T	0.431	T/T	0.345	T	0.560	C	0.440
CHB	C/C	n/a	C/T	0.022	T/T	0.978	T	0.989	C	0.011
JPT	C/C	n/a	C/T	0.250	T/T	0.750	T	0.875	C	0.125
YRI	C/C	0.069	C/T	0.379	T/T	0.552	T	0.741	C	0.259

Population descriptors:

YRI: Yoruba in Ibadan, Nigeria
JPT: Japanese in Tokyo, Japan
CHB: Han Chinese in Beijing, China
CEU: CEPH (Utah residents with ancestry from northern and western Europe)

Table 12. Genotype and Allele Frequencies of rs7975232 in Different Population

Population	Genotype frequencies									
							Ref-allele		Other allele	
	genotype	freq	genotype	freq	genotype	freq	allele	freq	allele	freq
CEU	G/G	0.241	G/T	0.362	T/T	0.397	G	0.422	T	0.578
CHB	G/G	0.500	G/T	0.357	T/T	0.143	G	0.679	T	0.321
YRI	G/G	0.117	G/T	0.517	T/T	0.367	G	0.741	T	0.259

Population descriptors:

YRI: Yoruba in Ibadan, Nigeria
CHB: Han Chinese in Beijing, China
CEU: CEPH (Utah residents with ancestry from northern and western Europe)

Table 13. Comparison of Frequency of Alleles and Genotypes in African American and European

	Freq. of Altered Allele		Freq. of Heterozygosity	
SNP ID	**AA**	**CEU**	**AA**	**CEU**
rs1168266	0.32	0.605	0.37	0.368
rs11574114	0.01	0.027	0.12	0.055
rs731236	0.27	0.44	0.28	0.431
rs7975232	0.20	0.578	0.42	0.362

CEU: CEPH (Utah residents with ancestry from northern and western Europe)

AA: African American

Mono-variate regression analysis of VDR polymorphisms and Risk of prostate cancer

Based on the odds ratios and related P values (Table 14), which assume an additive genetic model, the SNPs c.278-69G>A, c.1025-49G>T, and c.1025-56A>G were associated with an increasing risk of prostate cancer (OR=1.285, OR=1.22, and OR=2.616 respectively), although these associations were not statistically significant, P>0.05. The less likelihood of prostate cancer risk were found in subjects with SNP c.755+25G>A (OR, 0.256; 95% CI, 0.090 -0.729), c.907+75C>T (OR, 0.175; 95% CI, 0.093 -0.331), c.1025-95G>A (OR, 0.038; 95% CI, 0.005 -0.286), and c.1056T>C (OR, 0.652; 95% CI, 0.338 -1.257). These associations were significant although only c.1056T>C was not significant, p=0.201.

Table 14. Odds Ratio for each Type of Polymorphism with the Risk of Prostate Cancer (N = 182)

SNP	Alternative Alleles	Associated Allele[a]	Odds Ratio	95% CI	P-Value
c.278-69G>A	G, A	A	1.285	(0.706 -2.339)	0.412
c.755+25G>A	G, A	A	0.256	(0.090 -0.729)	0.011
c.907+75C>T	C, T	T	0.175	(0.093 -0.331)	0.000
c.1025-95G>A	G, A	A	0.038	(0.005 -0.286)	0.002
c.1025-49G>T	G, T	T	1.220	(0.677 -2.198)	0.66
c.1056T>C	T, C	C	0.652	(0.338 -1.257)	0.201
c.1025-56A>G	A, G	G	2.616	(0.494 -13.846)	0.258

[a]These alleles assumed to be associated with risk of prostate cancer.

Logistic Regression Analysis of VDR Polymorphisms and Risk of Prostate Cancer (Age Adjusted)

SNPs c.755+25G>A, c.907+75C>T, c.1025-95G>A, and c.1025-49G>T were all significant predictors for prostate cancer risk, $p < 0.05$ (Table 15). It can be observed that polymorphism c.755+25G>A, c.907+75C>T and c.1025-95G>A were associate with the decrease risk of prostate cancer (OR=0.233, 0.132, and 0.0213 respectively), while polymorphism c.1025-49G>T had significant direct association to the risk of prostate cancer (OR=4.822; P=0.004). However, polymorphism 123G/A, c.1056T>C, and c.1025-56A>G showed non significant inverses association with risk of prostate cancer (OR=1.787, 1.425, and 1.731 respectively $p>0.05$).

Table 15. Binary Logistic Regression Analysis for Association of VDR Polymorphisms and Risk of Prostate Cancer (Age Adjusted)

SNPs	Correlation coefficient	OR	P value
c.278-69G>A	0.58098	1.787	0.148
c.755+25G>A	-1.4541	0.233	0.020
c.907+75C>T	-2.0247	0.132	0.000
c.1025-95G>A	-3.845	0.021	0.001
c.1025-49G>T	1.57326	4.822	0.004
c.1056T>C	0.35481	1.425	0.565
c.1025-56A>G	0.54925	1.731	0.605
Constant	0.54479		0.047

Joint Effect of Different VDR Gene polymorphisms and Their Associations with Risk of Prostate Cancer

In order to explore whether the combination of polymorphisms has additive effect in the prediction of prostate cancer binary logistic regression analysis was applied (Table 16).

Combination percentage of SNPs c.755+25G>A and c.907+75C>T, c.907+75C>T and c.1025-95G>A, c.907+75C>T and c.1056T>C, - c.278-69G>A and c.1025-49G>T were significantly associated with decreasing risk of prostate cancer (OR=0.256, 0.043, and 0.314 respectively). While, combination of presence of SNPs c.278-69G>A and c.1025-49G>T significantly increased risk of prostate cancer (OR=2.614, P=0.021).

A non significant inverse association between risk of prostate cancer and combination of presence of SNPs c.755+25G>A and c.1056T>C, c.755+25G>A and c.907+75C>T and c.1056T>C, and c.907+75C>T and c.1025-95G>A and c.1056T>C was observed (OR=0.750, 0.494, and 0.000 respectively). Also a non significant association between combine SNP c.278-69G>A and c.1025-56A>G and increasing risk of prostate cancer was observed (OR=1.534; P=0.64).

Table 16. Combination of VDR Gene Polymorphisms and Risk of Prostate Cancer in Cases and Controls

Combination of Polymorphisms	Regression Coefficient	Odds Ratio	*P*-Value
c.755+25G>A and c.907+75C>T	-1.362	0.256	0.021
c.755+25G>A and c.1025-95G>A	0.000	1.000	0.000
c.755+25G>A and c.1056T>C	-0.288	0.750	0.712
c.907+75C>T and c.1025-95G>A	-3.154	0.043	0.002
c.907+75C>T and c.1056T>C	-1.158	0.314	0.003
c.1025-95G>A and c.1056T>C	-14.437	0.000	0.957
c.755+25G>A and c.907+75C>T and c.1025-95G>A	0.000	1.000	0.000
c.755+25G>A and c.907+75C>T and c.1056T>C	-0.704	0.494	0.423
c.907+75C>T and c.1025-95G>A and c.1056T>C	-14.423	0.000	0.959
c.755+25G> C.907+75C>T and c.1025-95G>A and c.1056T>C	0.000	1.000	0.000
c.278-69G>Aand c.1025-49G>T	0.961	2.614	0.021
c.278-69G>Aand c.1025-56A>G	0.428	1.534	0.644
c.1025-49G>T and c.1025-56A>G	0.000	1.000	0.000
c.278-69G>Aand c.1025-49G>T and c.1025-56A>G	0.000	1.000	0.000

VDR Genotypes Stratified by UV Exposure

To determine whether the association of VDR variants with prostate cancer risk was mediated by the extend of UV exposure , the cases and controls were stratified to high and low exposure groups using the median value of total UV exposure (20800 hr).

Interaction terms between UV exposure and VDR genotypes was created and that whether the odds radio for the association of the genotype with prostate cancer risk was mediated by the level of exposure was determined (Table 17).

To compare VDR variants frequencies in men stratified by exposure the association between prostate cancer risk and VDR polymorphism using logistic regression analysis was studied. Table 15 shows numbers and percentage of cases and controls with or without certain VDR variants as well as OR and P value for likelihood of prostate cancer for each polymorphism in all samples and stratified by exposure groups.

Polymorphisms c.278-69G>A, c.1025-56A>G, and c.1025-49G>T (OR=1.285, 2.616, and 1.220 respectively) increased the risk of prostate cancer whereas, polymorphisms c.1056T>C, c.755+25G>A, C.907+75C>T, and c.1025-95G>A (OR = 0.652, 0.256, 0.175, and 0.083 respectively) decreased the risk of prostate cancer. Although the association of prostate cancer risk with polymorphisms c.278-69G>A, c.1025-56A>G, c.1056T>C, and c.1025-49G>T didn’t achieve significance (P = 0.412, 0.258, 0.201, and 0.508 respectively).

Moreover, c.278-69G>A, c.1025-56A>G, c.1056T>C, c.755+25G>A, and c.1025-49G>T polymorphism frequencies were not significantly different

in cases and controls. However, in the group below the median c.1025-56A>G, c.755+25G>A, C.907+75C>T and c.1025-49G>T polymorphisms were associated with increase risk of prostate cancer, OR= 1.228, 4.871, 6.824, and 1.228 respectively) although only association with C.907+75C>T polymorphism was significant, P= 0.00. It was found that c.1056T>C, c.1025-95G>A polymorphism were associated with decreased risk of prostate cancer in men with mean UV exposure below the median (OR = 0.567 and 0.072 respectively) although c.1056T>C was not significantly associated, P = 0.24. In the group above the median, c.278-69G>A, c.1056T>C, and c.1025-95G>A polymorphism frequency were associated with decrease risk of prostate cancer (OR = 0.654, 0.735, and 0.00 respectively), although these associations were not significant. In this group c.1025-56A>G, c.755+25G>A, and IVS9 -286 C/T polymorphism were associated with increased risk of prostate cancer (OR = 1.389, 2.778, and 5.053 respectively) although only C.907+75C>T association was significant, P = 0.00. In the group above the median UV exposure no association was found between risk of prostate cancer and c.1025-49G>T polymorphism, OR = 1.043.

Table 17. VDR SNPs, UVR Exposure, and Prostate Cancer Risk in Cases and Controls

	Total study Group				<20800 hr*		>20800 hr	
SNP	Cases no. (%)	Controls no. (%)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
c.278-69G>A								
No	60 (65.2)	54 (59.3)	1 (refrence)	0.412	1 (refrence)	0.931	1 (refrence)	0.332
Yes	32 (34.8)	37 (40.7)	1.285 (0.706, 2.339)		0.963 (0.407, 2.279)		0.654 (0.71, 1.54)	
c.1025-56A>G								
No	90 (97.8)	86 (94.5)	1 (refrence)	0.258	1 (refrence)	0.999	1 (refrence)	0.748
Yes	2 (2.2)	5 (5.5)	2.616 (0.494, 13.846)		1.228		1.389 (0.19, 10.3)	
c.1056T>C								
No	63 (68.5)	70 (76.9)	1 (refrence)	0.201	1 (refrence)	0.244	1 (refrence)	0.515
Yes	29 (31.5)	21 (23.1)	0.652 (0.338, 1.257)		0.567 (0.219, 1.472)		0.735 0(.29, 1.86)	
c.755+25G>A								
No	75 (81.5)	86 (94.5)	1 (refrence)	0.011	1 (refrence)	0.065	1 (refrence)	0.143
Yes	17 (18.5	5 (5.5)	0.256 (0.090, 0.729)		4.781 (.909, 2.279)		2.778 0(.71, 10.9)	
c.907+75CT								
No	26 (28.3)	63 (69.2)	1 (refrence)	0.001	1 (refrence)	0.001	1 (refrence)	0.001
Yes	66 (71.7)	28 (30.8)	0.175 (0.093, 0.331)		6.824 (2.654, 17.546)		5.053 (2.04, 12.5)	
c.1025-49G>T								
No	71 (77.2)	90 (98.9)	1 (refrence)	0.002	1 (refrence)	0.015	1 (refrence)	0.999
Yes	21 (22.8)	1 (1.1)	0.038 (0.005, 0.286)		0.072 (0.009, 0.605)		0	
c.1025-49G>T								
No	56 (60.9)	51 (56)	1 (refrence)	0.508	1 (refrence)	0.632	1 (refrence)	0.923
Yes	36 (39.1)	40 (44)	1.220 (0.677, 2.198)		1.228 (0.530, 2.844)		1.043 (0.44, 2.46)	

CHAPTER 5: DISCUSSSION

The genetics and epidemiology of prostate cancer has developed new theories with advancement of research, yet there is a lot to be learned about the specific factors that alter prostate cancer susceptibility in individuals. The improved techniques for early diagnosis and subsequently prevention of prostate cancer will be certainly led by the pursuit for identification of fundamental biomarkers that can help predict who are at highest risk. The results from the dissertation project described herein showed that it is possible to assess and predict the ways in which vitamin D metabolism and VDR SNPs are associated with prostate cancer risk; further enabling us to study prostate cancer and its associations within the context of the UVR hypothesis.

Associations between UV Exposure and the Risk of Prostate Cancer

The first aim of this study was to determine the relationship between UV exposure and the risk of prostate cancer in African American. The hypothesis is that there is a significant negative association between UV exposure and the risk of prostate cancer. Conversely, in non age matched participants no significant difference in cumulative sun exposure between case and controls was found. Interestingly, the outdoor UV exposure was significantly higher in controls compared to cases whereas none of the other stratified UV exposure categories; recreational, professional, and residential, showed significance differences between cases and controls. However,

we demonstrated that the mean of total UV exposure in cases was significantly lower than controls in age matched samples. Also recreational UV exposure was significantly higher among controls when it was compared to cases whereas there were no significant differences in professional and residential UV exposure between the two groups. There was inverse association between total, recreation, residence, profession, and outdoor UV exposure and prostate cancer risk although it was not significant. In this study, there was an association between total, outdoor, recreational UV exposure, and decreased risk of prostate cancer in age matched participants, although only total UV exposure was not significant.

This hypothesis is supported by the findings that within the United States and worldwide, the risk of prostate cancer is correlated inversely with the availability of UV radiation, the principal source of vitamin D [11, 55, 94, 95, 97] . In this study, sun exposure data was collected by administering a questionnaire. This method can only be used for short periods of time and recalled data must still be used to estimate lifetime exposure. Various questionnaires have been used to assess UVR exposure but none has gained universal acceptance, implying it is a difficult parameter to measure. The exposure data is dependent on recall bias in often, elderly men. The questionnaire records various aspects of exposure. In this study, adult cumulative exposure per year and sunbathing score was selected. Adult cumulative exposure per year is a marker of chronic UV exposure that provides a measure of occupational and recreational exposure. This continuous variable allows the possibility of thresholds to be investigated.

Although, in this study all the men with very high levels of cumulative exposure had outdoor occupations, as holidays abroad are of relatively short duration they do not generally affect adult cumulative exposure. Time lived abroad in a sunny country may also affect total exposure.

In 1992, Hanchette and Schwartz [55] presented ecologic data from 3073 United States counties showing an inverse association between prostate cancer mortality and UVR, where the mortality was significantly lower in the South. Low sun exposure from self-reported recreational and occupational activities since age 20 years was associated with a three fold increased risk of prostate cancer in an English case-control study [5]. In that study, most men with high cumulative sun exposure had outdoor occupations [11], which are consistent with this study's finding of reduced risk associated with high outdoor activity. In our study, total occupational exposure was associated with a non significant risk reduction for prostate cancer. It is possible that the assessment of occupational exposure as a surrogate measure of sun exposure was not as sensitive as the measure used by Luscombe et al. [5] that asked specifically about sun exposure. Usual residence in a high solar radiation region or being born in the South was associated with reduced risk in the National Health and Nutrition Examination Survey I follow-up study [128]. Similarly, lower mortality rates were associated with high residential solar radiation exposure in a case control study based on a death certificate [129]. Although, this dissertation study has not resulted in finding a significant association with residential sun exposure,

these U.S.-wide studies had a much broader range of exposure than a metropolitan area based study which did not include any men with lifelong residence in a low solar radiation region. These findings are therefore of significant importance as they suggest a public health strategy to reduce the impact of prostate cancer. Further independent support for these findings came from a case-control study designed to compare parameters of acute and chronic exposure in 210 prostate cancer cases and 155 patients with benign prostatic hypertrophy (BPH) [5]. All the men were of Northern European Caucasians decent and residents in North Staffordshire, England (latitude 53.01°N). The BPH patients were chosen as Prostate cancer was fairly common in this population and establishing this diagnosis largely excluded the possibility of concurrent risk [130]. Exposure was assessed using parameters derived from a validated questionnaire [5, 119, 131]. The cumulative lifetime exposure is positively a predictor comprising exposure from weekday and weekend activity, whilst estimates of occupational and recreational exposure was significantly protective (odds ratio = 0.998 per week). Of particular interest were the proportions of cancer and BPH patients in each quartile of exposure. Thus, comparison of the odds of having prostate cancer, between the lowest and highest quartiles, resulted in a significant odds ratio (odds ratio = 3.03). Other parameters of exposure were also linked with reduced risk such as sunbathing score (never, rarely, occasionally, frequently; scored 1, 2, 3 and 4), regular foreign holidays (average weeks abroad per year) and childhood sunburning (yes/no) were protective. Unlikely, in this study, because of the

small size of the sample, findings didn't elaborate any differences in association of the highest and lowest quartile of exposure and the risk of prostate cancer. One previous study showed cases cumulative exposure was associated with age at diagnosis; observed through the data of men with the lowest quartile of exposure developed prostate cancer at a younger age (median 67.7 years) than all other patients (median 72.1 years) ($p = 0.006$, hazards ratio = 1.52) [5]. While these findings support the UVR hypothesis, they were derived from a small exploratory study with the possibility that observed associations are spurious because of multiple significance testing.

In our study, the recreational (history of foreign holiday) exposure had significant association with reducing risk of prostate cancer. Conversely, Bodiwala et al. (2003) found that living abroad in a hot climate for 6 months or more was not linked with risk of prostate cancer [132]. The reason why this parameter was not associated with prostate cancer risk is not clear though it may be related to the relationship between the extent of exposure to UV and cutaneous synthesis of vitamin D. Thus, vitamin D synthesis in skin does not increase linearly with length of exposure; as observed at the equator 15% of cutaneous 7- dehydrocholesterol is converted into previtamin D3 after exposure periods of 30 min or 8 h [87]. This reaction may be a mechanism to limit UV-mediated vitamin D production. The consequence of which is regular short-term exposure will result in adequate vitamin D production, demonstrating that such exposures may be most protective against prostate cancer. Thus, an exposure to

bright sunlight for only 15 min appears sufficient for adequate synthesis of Vitamin D [87, 133].

Other factors that will influence cutaneous vitamin D synthesis include the ability to mount a pigmentation response to UV since increased melanin production will reduce UV-mediated synthesis of previtamin D3 [82, 87]. Adult sunbathing may be an important factor in determining prostate cancer risk because it involves exposure of larger areas of the body, some of which such as the torso will generally be less pigmented than the face. Importantly, exposure of the torso and legs to sub-erythemic doses of UV results in greater increases in serum vitamin D levels than exposure of the head, neck or arms [134]. The different parameters derived from the questionnaire may reflect upon how behavior patterns would result in varied levels of cutaneous vitamin D synthesis. Notably, the Public Health campaigns have for long warned of the damaging effects of UV exposure because of the risk of skin cancer. This advice may need further re-examination since studies are also signifying the importance of UV that can protect against some cancers [129, 135]. Indeed, there is considerable interest in utilizing clinically the anti-proliferative and pro-differentiating effects of 1,25-dihydroxyvitamin D [136, 137]. It was recognized that while the association between UV and prostate cancer risk has now been observed in two groups of Northern European Caucasians from North Staffordshire, there is need for studies in populations from different latitudes that receive more exposure. While there is no corresponding data for central England, studies in Edmonton, Canada, which is on similar latitude to North Staffordshire

(52◦N), demonstrate that photosynthesis of vitamin D ceases by mid-October and does not resume until mid-April. While, in Los Angeles (34◦N) and Puerto Rico (18◦N), vitamin D synthesis continues all year [87]. Another interesting aspect of UVR in association to risk of prostate cancer is seasonal UV index alteration. Colli et al.[138] showed that the correlation between prostate cancer rates and UV indexes for white men was strongest in the fall and winter, moderate in the spring, while weak or nonexistent in the summer. Although the same strategy was not followed in this study the outcomes are still comparable with others. In this study the recruitment was based on the availability of participants in a non seasonal basis manner. However considering Washington DC with altitude (35.83 ◦ N) that confers low UV index is consistent with the findings that low UV exposure is associated with prostate cancer risk. These indicators suggest that vitamin D synthesis from sunlight in the spring and summer might be sufficient to confer protection from prostate cancer for white men in most of the United States, but that the risk is greatly increased from the modest amounts synthesized through the rest of the year. In contrast to the prostate cancer results for white men, the prostate cancer incidence for black men exhibited a statistically significant correlation with the UVB radiation levels only in the summer. Vitamin D synthesis which is low in the winter for whites in most of the United States, [139] is undoubtedly lower for blacks. The predictor for black men with increased skin melanin pigmentation is based on reduced ability to synthesize sufficient vitamin D from ambient UVB radiation exposure in seasons other than summer to affect prostate

cancer progression. An increase in skin melanin pigmentation will absorb solar ultraviolet radiation and significantly reduce the production of vitamin D3 in the skin [140]. Studies of black men and Mexican Americans have indicated lower circulating concentrations of calcidiol [141]. The circulating concentration of calcidiol is reported to reflect the cumulative effects of exposure to sunlight and dietary intake of vitamin D [142]. Because no correlation was found with mortality for black men and UV indexes [143], it is possible that black men cannot synthesize sufficient vitamin D from ambient UVB radiation exposure to affect disease progression after its initiation. Some studies [144-146] although not all [147] have suggested that black men have a more advanced form of prostate cancer at diagnosis, which might reduce the benefits of the lower levels of vitamin D that blacks are able to synthesize from exposure to sunlight. A critical sunlight threshold exists below which the risk of prostate cancer increases, which is lower for white men than for black men. For white men, this threshold is exceeded in the whole country in the summer but is not met in the northern part of the country in the other seasons. Hence, the seasons other than summer are most relevant for white men, and the summer is the most relevant for black men.

Protective Effect of Early -Life Exposure

In this study other parameters of exposure were also found to be linked with reduced risk, such as sun exposure score (never, moderate, high; scored 0, 1, and 2), was protective. These results indicated that when subjects aged 0-5 and 6-11 years old were highly exposed to UVR, this was significantly associated with reduced risk of prostate

cancer. Moderate UV exposure in all age groups had inverse association with the prostate cancer risk. It seems that higher exposure to UVR in advanced age did not protect against prostate cancer. Increasing skin pigmentation by age, or efficiency of impact of early-life exposure may induce these differences. These findings are similar to what others have found, John et al. (2007) indicated high residential solar radiation in the state of birth, a proxy measure for early-life sun exposure, was associated with reduced prostate cancer risk [65]. Among men born in a region of high solar radiation the risk was reduced by 51% with a slightly greater risk reduction noted for fatal than for nonfatal prostate cancer, while among men with frequent recreational sun exposure the risk of fatal prostate cancer was reduced by 53%. The finding of reduced risk associated with early-life sun exposure was consistent with results from a case-control study conducted in England where several indicators of childhood sun exposure, including sun burns and sunbathing were inversely associated with prostate cancer risk [5]. There are few contrary reports [148] that may be the result of the differences between the histories of the populations. For example, in the NHANES I cohort [148], large proportions (80-91%) of men remained in the solar radiation region where they were born, whereas in a case-control study [65], all men eventually moved to California, a state with high solar radiation, and large proportions of cases (75%) and controls (75%) spent 40 or more years in a high solar radiation region before the interview. Unlike the NHANES I follow-up study, the case-control study did not include any men with lifelong low residential sun exposure. A recent study [65] showed the

importance of early-life sun exposure and those from studies of adult sun exposure, are not necessarily in conflict. For example, because many case-control studies found a damaging effect of sunburns early in life on the risk of melanoma, it had been widely believed that susceptibility to melanoma was restricted to a ‘‘critical period’’ in early life. However, subsequent studies have shown that after controlling for sunburns early in life, sunburns during adulthood also confer increased risk [149]. Although based on small numbers, findings in this study suggest similarly that the window of opportunity for sunlight to alter prostate cancer risk is not restricted to adulthood. Although most epidemiologic studies have focused on the role of sunlight/vitamin D exposure in adulthood, it is biologically plausible that exposure to vitamin D in early life also may contribute to reduced risk. In particular, it is known that neonatal prostate cells express VDR and that early-life exposure of rats to high levels of 1,25(OH)2D results in alterations in the cellular composition of the prostate gland [150]. For example, whereas the ratio of epithelial to stromal cells in the normal rodent prostate is 5:1, prepubertal rats exposed to pharmacologic doses of 1,25(OH)2D developed prostate glands that were composed predominantly of stromal cells [151]. Because epithelial cells are the targets of carcinogenesis in the prostate, a reduction in the epithelial cell population is one mechanism whereby exposure to vitamin D in early life could reduce prostate cancer risk.

Association of Skin Pigmentation and Prostate Cancer Risk

In this study, we hypothesized that there is a significant association between variations among men for skin pigmentation and prostate cancer risk. Response to UVR varies markedly and in the context of skin cancer risk there has been much interest in defining host characteristics that protect against the adverse effects of exposure [152]. Ability to tan and susceptibility to burning have attracted particular attention. In Caucasians, the widely used Fitzpatrick scale combines assessment of these characteristics [99] although it can be criticized because there is no simple inverse correlation between burning and tanning [153]. Skin type is a polygenic trait and studies showing associations with polymorphisms in the melanocortin 1 receptor [47] and p53 [154] genes suggest prostate cancer risk will be mediated by allelism in genes that determine this phenotype. Polymorphic variants in the pigmentation-associated melanocortin 1 receptor and tyrosinase genes are linked with prostate cancer risk [55]. While many individuals with sun-sensitive skin will avoid UVR, Kricker et al. [155] found that 22% of subjects with highly sensitive skin who were outdoors on the preceding weekend reported being sunburnt. The relationship between prostate cancer risk, exposure and ability to tan is likely to be complex system, and it still remains unclear if skin type 1 confers increased risk because of sun avoidance or decreased risk because of more effective vitamin D synthesis.

The initial focus of this study was to determine whether exposure to UVR is linked with skin pigmentation. Recent studies on skin

pigmentation have indicated that higher skin pigmentation is associated with less exposure than other types [155], this possibility needs to be examined in prostate cancer patients.

Our study established that the mean of tanning potential in control subjects was higher than case subjects, but was not significant in non age adjusted analysis. After age adjustment, the mean of tanning potential (skin pigmentation) was significantly higher in control subjects than case subjects. Also lower skin pigmentation (higher tanning potential) was associated significantly with reduced risk of prostate cancer. Similar results were observed in studies conducted by Bodiwala et al. (2003). They found that childhood sunburning is associated with reduced prostate cancer risk, which was investigated by including traits linked with response to UVR [112]. They speculated that subjects with skin type 1 are more likely to sunburn and that childhood sunburning is a surrogate for this phenotype. Skin type 1 is described in Caucasians as those skin burns easily and have no ability to tan.; often observed to have fair complexion, blond or red hair and blue eyes. This leads to speculations for conditions of moderate exposure like regions of northern United States and Europe where prostate cancer is common; the risk is lowest in men with skin type 1 since they more easily synthesize vitamin D than men with types 2, 3 or 4 [156], whose risk may be controlled by avoiding UVR to avoid burning [11]. The data was congruent with the view that low levels of UVR exposure increase prostate cancer risk [5, 132]. Partitioning data for low levels of sunbathing (score $\leq$3.0) alone resulted in a group comprising 78.9%

cancer cases, whose score represents essentially no sunbathing in adult life. While, intermediate sunbathing (scores >3.0 ≤8.0) did not differentiate cases of cancer from BPH patients. Similarly, the protective effects of skin type 1 was only observed in men with the lowest levels of sunbathing, indicating that at higher levels sufficient vitamin D is synthesized even in more pigmented men [112]. We used recursive partitioning to examine the hypothesis that the association of skin pigmentation with risk is more evident in men grouped by levels of exposure. This is consistent with Bodiwala's findings [112], where skin type 1 in other ethnicities can be generalized as low skin pigmentation in African American. The control subjects in high UV exposure partition (higher or equal to median) had higher mean of tanning potential than cases. Therefore, there was the additive effect of UV exposure and skin pigmentation in protection of prostate cancer. Due to skin pigmentation being the same in both low exposure cases and controls, the protective effect was a result of UV exposure in this group. It is confirmed by finding that the protective effect of skin type 1 was only observed in men with the lowest levels of sunbathing, indicating that at higher levels sufficient vitamin D is synthesised even in more pigmented men [112].

Conversely, findings in this study are not consistent with other studies that assessed sun exposure based on pigmentation measurements [157], Skin pigmentation measurements, which quantify a biological effect (i.e., skin response to UV radiation), are likely to be more accurate measures of sun exposure than self-reports, which depend on participants' recall. Given the increase in facultative pigmentation

with age, the sun exposure index was proposed as a measure of cumulative lifetime sun exposure [123]. Compared with sun sensitive individuals who burn, those who tan spend more time outdoors [11, 158], and Japanese women residing in high solar radiation regions had darker foreheads than those residing in lower solar radiation regions [159].

Together, these data support the use of the pigmentation-based index as a measure of cumulative sun exposure. Therefore, it was proposed to quantify individuals based on objective measurements of skin pigmentation using the 'sun exposure index' (SEI) [123]. The SEI is calculated as the increase in facultative pigmentation above the constitutive level and is expressed as a percentage of the constitutive level. The SEI appeared to be related to cumulative lifetime UV exposure and may be used in epidemiological research as an objective estimate of UV exposure at different body sites in Caucasians. Unlike the other studies our study was conducted in African American and because of much higher skin pigmentation compared to other ethnicities the variation between the tanning potentials is not the results of UV exposure but the results of skin pigmentation genes. Thus, the cumulative life time sun exposure in African American cannot be measured using pigmentation-based index. Skin color is a compromise between latitude, extent of exposure and conflicting requirements of photoprotection against UVR-induced photolysis of key chemicals such as folate and adequate synthesis of vitamin D [160]. Linking outcome in prostate cancer with UV is a challenge because of the assessment of lifetime exposure must include intensity, duration

and timing of exposure. Such data can only be collected retrospectively, usually many decades after exposure events.

Association between Vitamin D and Prostate Cancer Risk

The third aim of the study was to assess the association between serum vitamin D levels and risk of prostate cancer in African Americans. It was hypothesized that a significant inverse relationship between serum vitamin D levels and the risk of prostate cancer was expected. The mean values of all vitamin D variables including serum vitamin D levels, food vitamin D, and supplemental vitamin D; were found to be higher in the control group when they were compared to case group, although the difference was not significant. After applying a binary logistic regression model for association analysis, the findings suggested that serum vitamin D concentrations, food vitamin D, and supplemental vitamin D also had a degree of association with the increasing risk of prostate cancer, but were not significant. Similar results were found using Point-biserial correlation coefficient which demonstrating negative correlation coefficient. This indicated slight inverse, yet not achieving the level of significance, for the association between serum vitamin D levels, food vitamin D, and supplemental vitamin D; with the risk of prostate cancer. The findings from this study show that the risk of prostate cancer did not vary significantly by serum concentration of 25(OH)D. Despite the widespread notion that vitamin D insufficiency is an important risk factor for prostate cancer [161, 162], this theory has not been verified by results from the majority of published

prospective studies [98, 99, 117, 153, 155, 163-165]. One major factor that may contribute to these inconsistent findings is that most studies did not specifically examine aggressive prostate cancer, the etiology of which appears to differ from that of indolent disease [98, 153, 164, 165]. Also consistent with our findings are the results from two studies that showed support for lower concentrations of 25(OH)D and increased risk of prostate cancer. Ahonen et al. [70] demonstrated a greater risk of prostate cancer for Nordic men with a 25(OH)D concentration of ≤40 nmol/L in comparison to men with concentrations of >40 nmol/L. They also showed a second Nordic study reporting an U-shaped relation with a higher risk for men with low (≤19 nmol/L) and high (≥80 nmol/L) concentrations of 25(OH)D in comparison to men with moderate concentrations [166]. Some studies have suggested that the inconsistent results regarding an association between vitamin D and risk of prostate cancer may be due to the variation in vitamin D concentrations between populations. The median levels of 25(OH)D for men of these studies were around or below 20 ng/ml so that at least half of the study participants were vitamin D deficient. For instance, the proportion of men with low 25(OH)D concentrations (<50 nmol/L) was higher among the Nordic populations [70, 166, 167] than in most of the US cohorts, where sun exposure is likely to be greater [155, 163, 164], and/or study populations were drawn from health conscious populations [117, 165], whose intake of vitamin D may be higher. Several studies have evaluated the risk of prostate cancer associated with concentrations of 1,25(OH)2D; where in experimental studies it has been shown to reduce the degree of cell proliferation in the

prostate [79, 100, 168]. In two studies, the investigators reported a non-significant decreased risk for men with high concentrations of both vitamin D metabolites [99, 155], whereas several others have reported no association [98, 117, 164, 165, 169]. In this study the association between prostate cancer risk and serum 25(OH)D is assessed and not for 1,25(OH)2D, because circulating 25(OH)D (from diet, supplementation, and sun exposure) is probably a better marker of an individual's with vitamin D exposure, than circulating concentrations of 1,25(OH)2D, which are homeostatically controlled, have a half life of 4 hours, and most probably reflect the production of 1,25(OH)2D in the kidneys [170]. However, a potential limitation of the current study and all previous epidemiologic studies is that it is not clear to what extent circulating 25(OH)D reflects intraprostatic vitamin D concentrations because 1,25(OH)2D is produced locally in the prostate by cells expressing the enzyme 25(OH)-1a-hydroxylase [103]. Prostate cancer tissue expresses lower level of 25(OH)-1a-hydroxylase as compared to normal prostate tissue [171, 172]. The development of prostate cancer may therefore be enhanced not only by reduced circulating levels of 1,25(OH)2D, but also by decreased local production of 1,25(OH)2D [173]. Men who are deficient in 25-hydroxy vitamin D but not deficient in 1,25-dihydroxy vitamin D may represent a subpopulation that is likely to have a compensatory increase in plasma 1,25-dihydroxyvitamin D due to 25-hydroxy vitamin D deficiency. With more extreme 25-hydroxy vitamin D deficiency, 1,25-dihydroxy vitamin D levels drop due to inadequate substrate. Because plasma 25-hydroxy vitamin D has a much longer half-life than plasma 1, 25-dihydroxy

vitamin D, a single measure of low 25-hydroxy vitamin D may better reflect men who are likely to have a compensatory increase in 1,25-dihydroxy vitamin D, which could be protective. Thus, if circulating 1,25-dihydroxy vitamin D levels are relevant, men with normal or high 1,25-dihydroxy vitamin D and low 25-hydroxy vitamin D may be those most likely to have clinically high 1,25-dihydroxy vitamin D. Whether circulating 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D are relevant to intraprostatic levels needs to be determined [108, 174, 175].

Another potential effect modifier of the association between vitamin D and prostate cancer risk is calcium. A high intake of calcium coupled with low vitamin D status may increase the risk of prostate cancer by reducing the amount of 1,25(OH)2D synthesized [176]. High levels of calcium may suppress the release of parathyroid hormone, and the action of this hormone tightly regulates conversion of 25(OH)D to 1,25(OH)2D in renal cells [177]. Nevertheless, others showed no evidence that the association between concentrations of 25(OH)D and the risk of prostate cancer varied according to calcium intake, [117, 163, 165, 178], or status [155, 179]. The majority of recent studies have reported no substantial difference in the relation between vitamin D and prostate cancer risk by age [117, 153, 155, 164, 165], with the exception of two studies [70, 99]. Ahonen et al. [70] demonstrated an inverse association between 25(OH)D concentrations and prostate cancer risk for men aged than age 52 years. Corder et al. [99] reported a similar inverse association but only for men older than age 57 years. Reduced enzyme activity of 1a-hydroxylase due to

aging [180] or other factors, especially under low 25(OH)D status, could predispose a man to a higher risk of prostate cancer. Not withstanding the possibility of a differential effect by age, both of these subgroup analyses were based on a small number of cases (n =67, and n =91 men, respectively), and, because a number of comparisons were made in these analyses, the role of chance cannot be excluded. Due to the varying serum 25(OH)D concentrations according to the month in which the blood sample was collected, there is the risk of confounding by season of blood collection. The mean preclinical duration of prostate cancer has been estimated to be 10 years [181]. Another confounding may be the frequency of vitamin D measurement. The measurement of serum 25(OH)D concentrations reflects internal dose and status, which encompasses cutaneous production of the vitamin and is considered superior to vitamin D intake alone or predictors of vitamin D status. A single measurement of 25(OH)D in adulthood may not reflect long-term vitamin D status. In a steady-state context, it represents the past several weeks to several months of exposure [182]. Others have also demonstrated that the influence of low concentrations of 25(OH)D on the risk of prostate cancer differed according to several polymorphisms located on the vitamin D receptor gene, including Cdx2, Fok1,and Bsm1 [91, 117]. Moreover, evidence also suggests that polymorphisms in the vitamin D binding protein affect circulating concentrations of 25(OH)D [183].

Given that the genotype of vitamin D binding protein was not determined for the men in this study, our results do not rule out the possibility that low levels of circulating 25(OH)D may be associated

with a greater risk of prostate cancer for certain individuals with a specific genotype or haplotype. Evaluating our results in addition to results from other prospective studies we can not rule out that vitamin D concentration may play a critical role in the etiology of prostate cancer.

Association between Vitamin D Receptor Polymorphism, UV Exposure and Prostate Cancer Risk

In this study fourth aim was to assess the association between VDR polymorphisms and risk of prostate cancer in African American. It is hypnotized that there would be a significant relationship between VDR polymorphism and the risk of prostate cancer. VDR polymorphisms have been evaluated as markers of prostate cancer risk; however, their impact remains unclear especially in African American. In this study, we have assessed seven polymorphisms in VDR gene which had significant association with the risk of prostate cancer. Two of these SNPs, c.907+75C>T and c.1025-56A>G were not reported in dpSNPs data base. Thus, we postulate that these two SNPs are novel VDR polymorphisms associated with the risk of prostate cancer.

This study showed that two frequently reported VDR polymorphisms, TaqI (rs731236) and ApaI (rs7975232) had association with the risk of prostate cancer. TaqI was associated with the decrease risk of prostate cancer, although it was not significant. Also this study determined a strong association between ApaI and increased risk of prostate cancer.

In this study it is determined that VDR polymorphisms rs11168266 (c.278-96>A), c.1025-56A>G were directly associated with the risk of prostate cancer, whereas rs61614728 (c.755+25G>A), rs51574114 (c.1025-95G>A), and c.907+75C>T showed association with the decreased risk of prostate cancer. This study is the first to report these polymorphisms as determinants of prostate cancer risk. There is no report of frequency of these SNPs in African American. We indicated that frequency of altered allele in rs11168266, rs11574114, rs731236, and rs7975232 (0.32, 0.01, 0.27, and 0.20 respectively) were lower than their European Caucasian (CEU) counterparts (0.605, 0.027, 0.44, and 0.578 respectively) when compared with Hapmap database.

A complementary approach to studying the role of vitamin D in prostate cancer is to examine genetic polymorphisms in vitamin D pathway genes, such as the VDR gene. Although two initial studies found 3 to 4 fold increased risks of prostate cancer associated with VDR polymorphisms in the 3’ end of the gene [31, 36], a recent meta-analysis involving 17 studies that assessed the TaqI, BsmI, and poly-A repeat polymorphisms as well as the FokI polymorphism in exon 2 concluded that none of these variants were likely to be a major determinant of prostate cancer risk [184]. There is some suggestion that VDR polymorphisms may be more strongly associated with advanced disease [31, 37, 42, 47, 152], most previous studies included a mix of cases with localized and advanced disease. If the effects are indeed stronger for advanced disease, the inclusion of localized cases would attenuate risk estimates, which may explain some of the inconsistent findings. The observed genotypic associations are consistent with

functional data. In exon 2, use of the second start codon, as occurs in the F polymorphic variant lacking the first start codon [185], results in a VDR protein with an activation domain shortened by three amino acids [186]. This protein is more efficient at transactivating a vitaminD regulated target gene [187]. In our study, no association with FokI was found. Previous studies in men from Spain [41] and U.S. Whites [160] as well as a study of advanced disease in Chinesemen [39] found no association with FokI genotype. In African Americans, FokI FF (versus ff or Ff) genotype was associated with a 2-fold increase in risk [160]. Because known polymorphisms in the 3′ region of the VDR gene do not alter the amino acid sequence of the VDR protein, the functional significance of these variants is unclear. 3′ untranslated region sequence variants may interact differently with other upstream sequences in the VDR gene to regulate transcription, translation, or RNA processing [188, 189]. Of those studies that found an association, reduced prostate cancer risk was always associated with the TaqI t allele or an allele in LD with TaqI t (BsmI B, ApaI A,or poly-A S). We found reduced risks associated with TaqI t allele and increased risks associated with ApaI t. Similarly, Ma et al. [37] reported reduced risk associated with the TaqI tt genotype but, conversely, only among men with low serum 25-OHD levels. Although our result for TaqI is not consistent with other null findings [184], none of the other studies considered the modifying effect of sun exposure.

We speculated that the level of UVR exposure is a surrogate for long-term serum vitamin D levels. Thus, stratifying cancer and control subjects into low and high exposure group based on cumulative UVR

exposure might cover any effect VDR variants have in men with different vitamin D levels [31]. VDR polymorphisms were associated with prostate cancer risk in men with UVR exposure levels above the median. We used the median value for cumulative exposure to stratify subjects as it allowed the maximum number in both groups. A test of effect modification showed that the association of VDR polymorphisms with prostate cancer risk was dependent on the level of exposure. These analyses are compatible with a recent data indicating that the pathogenesis of prostate cancer in men with low levels of exposure to UVR is different to that in men with higher levels [5, 11, 148, 190]. This study had higher median of cumulative UV exposure (20800 hr) than Bodiwala et al [190] (1100 hr) study. So the outcomes are slightly differences. We found that low and high UV exposure reduced the risk of prostate cancer in subjects who had SNPs associated with increased risk while they didn't alter the risk in subjects with SNPs which were associated with low risk. It is possible that levels of UVR exposure below the median value are associated with prostate cancers that develop because of relative vitamin D deficiency. If the functional differences between the VDR genotypes are small relative to the consequences of low vitamin levels, the impact of the polymorphisms may be masked. By contrast, men with exposure above the median would be expected to synthesize adequate amounts of vitamin D. Thus, the functional consequences of the polymorphisms may be sufficiently great in the presence of adequate levels of the vitamin to influence prostate cancer risk.

Strengths and Limitations

This population-based case-control study adds to the emerging epidemiologic evidence that vitamin D from sun exposure and VDR genotype play a role in the development of prostate cancer. Several strengths are noteworthy. This study is the first that studied the association of four risk factors; UV exposure, vitamin D, skin pigmentation, and VDR variants and the risk of prostate cancer in African American. None of the previous studies investigated these factors and their interactions together. Between numerous advantages of this study, determination of strong association between skin pigmentation and prostate cancer risk was an exceptional strength that could not be find in other similar research reports. Unlike most other studies, ours is one of the few to inspect variants in all exons of VDR gene. Thus, this study led to identify two novel VDR SNPs associated with the risk of prostate cancer in African American, which can be applied as a foundation for future studies.

There are multiple issues associated with serum measurements of vitamin D: treatment of sample during storage and method of analysis are the major ones. In this study serum were separated immediately after blood collection, kept in -80◦C, and 25(OH)D was measured by using one of the most reliable techniques, EIA.

This study suffers the lack of power because of the small size of samples; however, the findings can use as preliminary data for in detail planning of larger studies among African-American population. The major drawback to case-control studies is that they are retrospective and so the fact that the case group has recently been

diagnosed with cancer may affect the results, either through biased reporting or through the cancer itself affecting the biological sample. In general, the disadvantages of the case control studies are: it relies on subject's recall and/or completeness of excising records, it may be difficult or impossible to validate this information, there is incomplete allowance for extraneous factors, the selection of control group may be difficult, the mechanism of disease cannot be studied, and a proof of causation cannot be established. However, it is an excellent way to study rare diseases and diseases with long latency. In this study all exposure histories were based on self-report. Because the potential relation between sun exposure and prostate cancer risk is not widely recognized, it is unlikely that errors in reporting lifetime sun exposure histories differed by case-control status, thus potentially biasing the results toward the null. Confounding factors such as; physical activity and smoking are likely to affect the results, however, only few studies controlled these confounders. Questionnaire based data collection are subjects of variability in the answers subjects give and their reliability however, this fact dictates a larger number of subjects in order to avoid incorrect inferences. Observation of genetic variation is highly dependent on the underlying structure, that is, the racial/ethic composition, as well as the sample size.

Our work is extremely promising and also our data contributed to the current knowledge on DNA sequence variations. But more importantly, analysed of our populations allowed us to dissect the

role DNA sequence variations play in prostate carcinogenesis in high risk populations.

Conclusions

Our working hypothesis posed that increased incidence of prostate cancer in African Americans involves a dynamic interplay of environmental factors such as diet and UV exposure in addition to genetic factors, some which directly influence serum vitamin D levels. The results of our study and those by Bodiwala et al. [190] suggest the importance of considering both VDR genotype and sun exposure when assessing prostate cancer risk. Compared with men with low sun exposure and lacking protective genotypes, we found risk reductions in men with both high sun exposure and protective VDR genotypes. In this study, the findings and those by the others [5, 128, 129], suggest long term sun exposure may be important. A high percentage of African Americans have suboptimal blood levels of 25(OH)D and levels that are well below those of American whites. Poor vitamin D statues may increase the risk of African Americans as well as others for cancer and other serious conditions. Therefore, clinicians and educators should be encouraged to promote improved vitamin D statues among adult African Americans, as they have for infants and children. Despite the controversies between findings in respect to protective effect of vitamin D based on the studies that confirms anti- proliferate effect of vitamin D analogs on prostate cancer cell lines and established role of sun exposure in providing of the majority of vitamin D, we strongly support the idea that vitamin D has inverse association with the risk of prostate cancer especially in African American. The

absence of a link between vitamin D and prostate cancer risk, even if ultimately confirmed, should not be misinterpreted as evidence against other well documented health benefits of vitamin D. The weight of evidence does suggest that increased vitamin D levels from diet, supplementation, or sun exposure are likely to have a modest beneficial effect on the overall burden of chronic disease in the United States and other epidemiologically similar countries. Taken together, it is recommend at least 10 minute moderate sun exposure 2-3 days per week especially for people with darker skin/older who live in more northern latitudes, cover themselves with clothing, and having indoor life style. If sun exposure is not possible; a vitamin D supplement (vitamin D analogs) within the pharmacological non toxic dose (non- hypercalcemic), at least 400 IU per day, fortified food, and fish are highly recommended.

Future Perspectives

Further studies in large populations of African American are warranted to confirm the combined effects of sun exposure period that is important in influencing prostate cancer risk. Recruitment of participants in an organized manner along with blood collection coordinated with standardized season and time of diagnosis, stages of cancer, and follow up combined with complete pathological reports would provide more reliable and powerful results. This could allow studying the interactions of vitamin D related risk factors in stratified sub-groups. While the few clinical trials that have been conducted suggest that VDR agonists reduce prostate cancer progression, additional studies are required to define situations

where VDR agonist, either alone or in conjunction with other drugs, would serve as effective therapeutic agent for prostate cancer. It is planned to expand this study on positioning of polymorphisms or mutation of other genes in vitamin D pathway such as; vitamin D binding protein (DBP), CYP27B1, 1-α hydroxylase, and CYP24A1, that can reveal more molecular details of association between vitamin D and risk of prostate cancer. In this study, most of the detected VDR SNPs were located in non coding regions. Because microRNAs are noncoding small RNAs which regulate the expression of many genes, investigation of their possible presence in prostate cancer risk associated SNPs would be beneficial. Further, study on epigenetic effect of CYP27B1 among the larger samples would provide better understanding of the roles of theses alterations in the etiology of prostate cancer. We also recommended study of genes involved in vitamin D metabolism pathways in cancerous and normal tissues that gives an insight understanding of in vivo ongoing molecular events and could shed lights on many unknowns about prostate cancer.

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