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A CASE-CONTROL STUDY OF PROSTATE CANCER IN THE GREATER BALTIMORE AREA

AN EPIDEMIOLOGICAL STUDY OF GENETIC AND ENVIRONMENTAL RISK FACTORS FOR PROSTATE CANCER IN AFRICAN-AMERICAN AND CAUCASIAN MALES

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Multi-institutional Protocols

Our protocol is a multi-institutional protocol for which the NCI is the coordinating center. The University of Maryland Medical Center and the Baltimore Veterans Affairs Medical Center are participating institutions.

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PRECIS

We are conducting an epidemiological prostate cancer case-control study in Baltimore, Maryland. Participants will be African-American and Caucasian males who reside in Baltimore city and surrounding areas. The study is ongoing and will recruit 1000 prostate cancer cases and 1000 population-based controls. The cases are recruited at two Baltimore hospitals, the Veterans Affairs Medical Center and the University of Maryland Medical Center. Cases will have pathologically confirmed prostate cancer. The population-based controls are identified through the Maryland Department of Motor Vehicle database, and are frequency-matched by age and race to cases. Enrollment of controls started concurrently with case accrual. The first 12 months of the study constituted a pilot study, during which we evaluated the recruitment procedures. The study involves the administration of a survey and collection of blood from all study subjects. The survey evaluates family cancer history, tobacco use, medication, occupational history, socioeconomic status, and risk factors for prostate cancer. Fresh-frozen tumor specimens will be obtained from cancer patients if available. The study is supported by an epidemiological infrastructure that has been developed by our resource contractor at the University of Maryland for a lung cancer case-control study. This lung cancer study is ongoing, and the controls that are recruited for the prostate cancer study are joint controls with the lung cancer study. Hence, population-based male controls recruited by our contractor have double eligibility for the concurrent lung and prostate cancer studies. To achieve an age and race matching of cases and controls in the prostate study, we will over-sample male controls in the lung study.

We will test the primary hypothesis that genes and environmental exposures, including infections and medical history, and interactions between these factors modify prostate cancer susceptibility and contribute to the excessive disease risk among African-American men. As the secondary goal, we will study gene expression profiles of prostate tumors to identify changes in tumor biology that are associated with exposure to genetic and environmental risk factors. It is a focus of this research to identify mechanisms in tumor biology that may cause the aggressive nature of prostate cancer in African-American men.

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1 INTRODUCTION

Prostate cancer (PCa) is a leading cause of cancer death among men in Western countries (1,2). About 220,000 new cases of PCa were projected for the United States (US) in 2004, and one in six men will develop PCa during his life (1). The disease incidence is still increasing, and disease rates have risen during recent decades in virtually every population-based registry around the world (2). PCa is usually a latent disease, but 25-30% of the tumors behave aggressively, resulting in almost 30,000 deaths among US males per year. Large racial and geographic differences have been observed with a 40-fold difference in incidence between low-risk and high-risk populations. The highest incidence rates were found among African-American men in the US with 137 reported cancers per age-adjusted 100,000 man-years for 1988-1992, versus only 2.3 for men from Shanghai, China (2). Although Caucasians have a significantly lower incidence and mortality rate than African-Americans in the US, the rates are still relatively high when compared with some European countries.

The reasons for the large racial and geographic differences are poorly understood but both environmental and genetic risk factors are thought to be involved. Migrant studies support the involvement of environmental factors (2-4). The adoption of a Western-type diet has been found to strongly increase the risk for PCa (5,6). High meat and animal fat intake, and excessive alcohol consumption, correlate positively with PCa risk (7-9), while a diet that is either high in phytoestrogens (10), such as a soy diet, or in allium vegetables (11) appears to be protective. Although smoking does not appear to be associated with the incidence of PCa (12,13), an association between current smoking and high grade or metastatic PCa has been reported (14-17). It suggests that smoking promotes the progression to a more advanced disease. Other observations point to the role of genetic susceptibility factors in human PCa. The lifetime risk of PCa increases 1.5 to 4-fold in men with one or two first-degree relatives with PCa (18). A Scandinavian study of twins estimated that 42% (95% CI: 29% - 50%) of the observed PCa susceptibility was associated with inherited genetic risk factors (19). Genetic risk factors are particularly significant at a younger age, and the attributable risk of inherited susceptibility is thought to be as high as 30%-40% among men diagnosed with PCa at age 55 years or younger (18). The importance of genetic susceptibility is further suggested by the finding that only humans and dogs, but not other mammals develop PCa (20). Recent reports indicate that PCa biology is different in African-Americans when compared with Caucasians. African-American men develop higher stage carcinomas earlier in life than Caucasian men (21). It appears that both an earlier onset and a more rapid progression of the disease in African-Americans contribute to the difference in mortality between the two ethnic groups (22). Such observations are supported by additional data. The Prostate Cancer Outcomes Study concluded that traditional socioeconomic, clinical, and pathologic factors, could not explain the increased risk of advanced-stage PCa in African-American men (23). Autopsy examination of prostate specimens revealed that the frequency of small latent carcinomas is similar in low-risk and high-risk populations (4,24). In contrast, larger and higher-grade carcinomas are more common in high-risk than in low-risk populations, and the frequency correlates with the incidence of clinical PCa. The data show that genetic and/or environmental factors modulate the progression from a small, microscopic carcinoma into a large carcinoma (4,25), and that tumor progression is the critical step that defines the strong ethnic differences in PCa risk and mortality.

Most prostate tumors are adenocarcinomas. Several benign and malignant lesions have been identified that precede clinical PCa. Focal prostatic glandular atrophy and post-atrophic hyperplasia are potential precursors of prostatic intraepithelial neoplasia (PIN) and adenocarcinoma, and they occur in close association with chronic inflammation (26,27). PIN is physically and temporally associated with concurrent malignancies. It frequently arises in inflamed areas (28). Many consider PIN as the most likely precursor of invasive adenocarcinoma. Acute and chronic inflammation is commonly observed in prostate biopsies (29) and causes atrophic acini and hyperplasia. It has been proposed that the inflammation-to-carcinoma sequence is of great importance in prostate carcinogenesis (28,30). The finding that two food supplements with antioxidant properties, selenium and vitamin E, protect against PCa (31,32) supports this notion.

Endogenous Mediators of Prostate Cancer Progression

Androgens are essential for the growth of the prostate gland, and are believed to be significant in the etiology of PCa (33). Large doses of androgen can induce PCa in rodents, and eunuchs rarely develop PCa. Androgen ablation, or anti-androgen therapy, frequently causes the regression of prostate tumors, at least temporarily (34). Men with serum testosterone in the upper quartile of the population have an approximately two-fold higher risk for developing PCa, as indicated by a meta-analysis after adjusting for age and body mass index (35). However, many studies that examined the relationship between circulating sex hormones and PCa risk failed to show an association (33). It is possible that blood hormone concentrations may not reflect intraprostatic androgen activity.

Non-androgenic mediators of prostatic growth have been described. A high blood concentration of insulin-like growth factor-1 (IGF-1) has been associated with an increased risk of advanced PCa (36-39). Insulin resistance, and chronically high insulin blood concentrations, are also predictors of increased cancer risk (40,41). The increased secretion of IGF-1 and insulin appears to be linked to a Western-style diet, a known risk factor for PCa. Other data suggest that several endothelial-derived factors and mediators of inflammation, such as interleukin-6 and -8, endothelin-1, vascular endothelial growth factor (VEGF), eicosanoids and nitric oxide, contribute to PCa progression (42-48). Mediators of inflammation, such as eicosanoids and nitric oxide, have also pro-angiogenic properties and induce functional changes in the vasculature that will lead to vessel remodeling, stimulation of endothelial cell mitosis and vessel growth. Overexpression of cyclooxygenase-2 and nitric oxide synthase-2 in human PCa has been reported (48-51). Chronic inflammation of the prostate may, therefore, promote cancer progression through the angiogenesis pathway.

1.1 STUDY OBJECTIVES

PRIMARY OBJECTIVE

We will examine the association of allele variant genes with PCa risk. The key aim is to examine pathways in relation to PCa, e.g., host defense, inflammation pathways. Within cases, we will examine the relation of genetic variants to molecular, clinical, and prognostic endpoints. The objective will be accomplished with 1) genotype analysis of blood DNA from 1000 cases and 1000 controls and 2) collection of data from questionnaires, medical and pathology records, and the National Death Index. *We are testing the hypothesis that susceptibility to nonhereditary PCa is modified by allele variant genes that modulate host defense, inflammation, hormone-stimulated survival and proliferation of mutant cells, the DNA repair capacity, or tumor angiogenesis. The susceptibility may vary by race and lifestyle (gene-environment interaction).*

SECONDARY OBJECTIVES

- We will develop gene expression profiles of 60-100 tumors, and will compare gene expression in low-stage and high-stage tumors of African-Americans with gene expression in same stage tumors of Caucasians. The objective is to find gene expression profiles of cancerous and non-cancerous prostate tissue that are characteristic for the disease in African-American men. *We are testing the hypothesis that the molecular signature of primary prostate tumors is different between African-American and Caucasian men. The gene expression profile of African-Americans is associated with an early onset of the disease, a more rapid cancer progression and poor outcome.*

- We will examine the gene expression profiles of tumors from current (n = 10-15) and never smokers (n =10-15). *We are testing the hypothesis that current smoking induces a gene signature of tumor progression that reveals the mechanism by which tobacco smoke promotes prostate cancer progression.*

- We will determine IGF-1 serum concentrations in our study population and compare gene expression profiles of about 10-15 low-stage tumors from patients with blood IGF-1 levels in the extreme quartiles, respectively. *We are testing the hypothesis that a high IGF-1 blood concentration modulates gene expression in primary tumors. The molecular profile is associated with disease progression and poor outcome.*

- We will correlate IGF-1 serum concentrations with tissue markers such as phoshorylated Akt, reduced apoptosis, increased VEGF expression, and with the phosphorylation of genes that are regulated by Akt. *We are testing the hypothesis that IGF-1 promotes cancer progression by activating the Akt/protein kinase B pathway in the prostate.*

- We will compare gene expression profiles of about 10-15 low-stage tumors from patients with a vegetable consumption (tomato, allium) in the extreme quartiles, respectively. The dietary intake of these vegetables is assessed by our questionnaire. *We are testing the hypothesis that a diet high in these vegetables modulates gene expression in primary tumors. The molecular profile correlates with good outcome.*

1.2 BACKGROUND AND RATIONALE

1.2.1 RATIONALE FOR CONDUCTING THE STUDY IN THE GREATER BALTIMORE AREA

Our goal is to identify and compare genetic risk factors in two ethnic groups, African-Americans and Caucasians. The two hospitals that we chose to recruit PCa patients are located in Baltimore City, Maryland. Baltimore has a large African-American population, and we estimate that about half of the PCa patients at the two hospitals will be of African-American descent. The study will be supported by an existing epidemiologic infrastructure with our contractor at the University of Maryland and an established relationship between our group and the Departments of Pathology at the two hospitals. The Laboratory of Human Carcinogenesis is currently conducting a lung cancer case-control study at the same hospitals and is recruiting population-based controls to match the cases. The controls recruited for the lung study meet the eligibility criteria of the proposed PCa study. The mean and median age of male cases in the lung cancer study is 65-66 years. If the age distribution of the PCa patients does not change in the next 5 years, we expect about the same mean and median age for the PCa cases, as indicated by hospital records and cancer registry data (Tables 1 and 2). Such an age distribution will allow the matching of PCa cases with population-based controls using the established resources. The recruitment will be accomplished by over-sampling of male controls under the current protocol for the lung study. PCa cases will be identified primarily by PSA screening. Needle biopsies are taken to confirm the presence of cancer in screen-positive patients. We expect to recruit up to 25 patients per year, who present with cancer in a needle biopsy and undergo a radical prostatectomy prior to chemotherapy, radiation, or hormone therapy. The remaining patients will have chemotherapy, radiation, or hormone therapy, where only blood samples but no tumor specimens will be collected.

Table 1: Age Distribution of PCa Patients at the Two Participating Hospitals

Calendar Year 2001	
Age (yrs)	**Number of Patients**
40-49	10
50-59	67
60-69	126
70-79	93
80+	17
Total	313

Table 2: Age Distribution by Race/Ethnic Background

University of Maryland Medical Center Calendar Year 2001			
Age (yrs)	**African-American**	**Caucasian**	**Total**
40-49	2	5	7
50-59	21	27	48
60-69	22	42	64
70-79	18	19	37
80+	2	2	4
Total	65	95	160

1.2.2 RATIONALE FOR SELECTION OF SUBJECTS BY RACE

PCa has the largest health disparity for any cancer site in the US (52). The causes of this health disparity are multifactorial and may include tumor biology. African-American men have the highest incidence and mortality rates. The disease accounts for about 39% of all cancers diagnosed in African-American males (53). The age-adjusted African-American to Caucasian cancer incidence ratio is about 1.6 but the mortality rate is 2 to 3-fold higher for African-Americans than Caucasians (22). African-American men develop PCa at a younger age, and the disease progression is faster than in Caucasians (22). It is hypothesized that the tumor biology of PCa is different in the two ethnic groups.

1.2.3 RATIONALE TO STUDY ALLELE VARIANT GENES AS RISK FACTORS IN PROSTATE CANCER

Allele variant genes belong to the category of low penetrance susceptibility genes (54). A mutation in a low penetrance gene does not cause cancer but contributes to cancer risk, and affects large segments of the population. The investigation of allele variant genes will provide clues to the role of genetic risk factors in nonhereditary cases. Further, the identification of low penetrance susceptibility genes can have important public health implications.

The association between allele variant genes and cancer risk has been studied extensively (55-57). Only a small number of disease-associated allele variants have been identified at this point. There are various reports on PCa susceptibility as it relates to low penetrance susceptibility genes (58,59). Polymorphisms in cell cycle genes, such as cyclin D1, CDKN1A, and CDKN1B, have been studied and an association between some allele variants and an increased PCa risk has been reported (60,61). Most of the epidemiological studies focused on genes in the androgen pathway. CYP17 is a key enzyme in the synthesis of sex hormones. A polymorphism in this gene is associated with an increased cancer risk in African-American men, but not in Caucasians, as suggested by several reports and confirmed by a recent meta-analysis (62). Testosterone is irreversibly converted to dihydrotestosterone (DHT) by the enzyme steroid 5α-reductase

type II (SRD5A2). The conversion to DHT is a major modulator of androgenic activity. Thus, SRD5A2 polymorphisms that modulate the bioavailability of DHT have been studied as risk factors in PCa. A SRD5A2 polymorphism, Ala49Thr, has been found to be associated with markers of poor prognosis or cancer risk (63,64), and a frequency gradient of another polymorphism, Val89Leu, in the SRD5A2 gene exists among racial/ethnic groups, which parallels PCa risk (65). However, a more recent meta-analysis of the published literature did not support an association between SRD5A2 genotypes and PCa risk (66). A variant polyglutamine tract in the androgen receptor gene (67,68) has also been studied, but the association with cancer risk is rather inconsistent. Recently, a joint effect of two polymorphisms in the 3β-hydroxysteroid dehydrogenases I and II has been observed. Men with the two variant genotypes had a significantly higher risk to develop PCa. A relative risk of 2.6 to 3.1 for hereditary and sporadic PCa was observed among carriers with the two mutant alleles (39).

Another essential pathway in prostate carcinogenesis is tumor angiogenesis. Recent publications point to the potentially great importance of allele variant genes in the angiogenesis pathway for PCa progression (Table 3). A single nucleotide polymorphism in the angiogenesis inhibitor, endostatin, has been found to predispose to PCa (69). The Asp1437Asn polymorphism is located in a conserved region and, presumably, inhibits protein function. Furthermore, two genetic variants that reduce the promoter activity of two pro-angiogenic factors, interleukin-8 and VEGF, were found to be significantly less frequent in PCa patients than in a control population (70). Lastly, a polymorphism in the endothelial nitric oxide synthase, Glu298Asp, was found to be significantly more common among cancer patients with an advanced disease than in the control population (71). The data indicate that inherited PCa susceptibility may be partly explained by genetic traits in genes of the angiogenesis pathway.

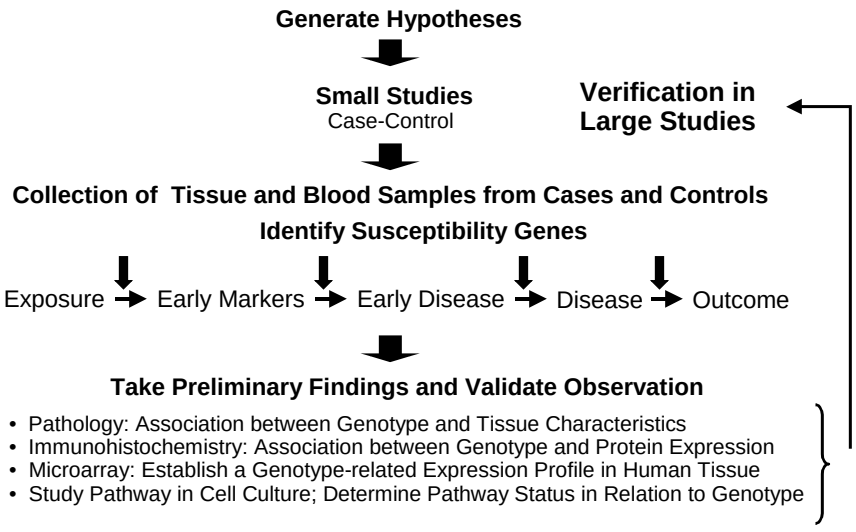
Table 3: Allele Variant Genes of the Angiogenesis Pathway as Risk Factors for Prostate Cancer

Gene	Variant Allele	Rare or Mutant Allele Frequency (%)	Reported Risk Association *(mut versus wt)*
Interleukin-8	A(-251)T	50	OR 0.66 95% CI (0.44 - 0.99)
VEGF	G(-1154)A	30	OR 0.45 95% CI (0.24 - 0.86)
eNOS	Glu298Asp	40	OR 2.1 95% CI (1.12 - 4)
Endostatin	Asp1437Asn	5-10	OR 2.4 95% CI (1.4 - 4.16)

Our goal is to study the modulation of PCa risk by allele variant genes that regulate five distinct pathways. We will explore genetic susceptibility that is driven by 1) host defense, 2) chronic inflammation, 3) hormone-stimulated survival and proliferation

of mutant cells, 4) an impaired DNA repair capacity, and 5) tumor angiogenesis. We will analyze genotype-risk associations in the context of race and the environment because dominant gene effects without environmental involvement are unlikely to occur. We will also determine associations between genotypes and poor prognosis markers. An allele variant that is associated with cancer risk will be further validated to establish biological plausibility and mechanisms of causality (Table 4). The additional verification steps, as shown in the table, are important to minimize the possibility of a false positive relationship between a genotype and cancer risk. These experiments will provide important mechanistic information. Disease-associated genotypes will subsequently be validated in larger studies.

Table 4: Strategy to Identify and Validate Genetic Risk Factors in Human Cancer



1.2.4 RATIONALE TO STUDY MOLECULAR PROFILES OF PROSTATE CANCER

Current therapeutic approaches to treat advanced stages of human PCa are palliative rather than curative. In the majority of cases, an aggressive cancer ultimately becomes androgen-refractory resulting in the death of the patient. There is an immediate need to prevent the progression of the clinically localized disease by increasing the understanding of the basic biology of PCa. The propensity for disease progression is different for African-Americans and Caucasians and may define the strong ethnic differences in PCa mortality (53). A critical challenge is to develop means that distinguish indolent cancers from those that are potentially lethal. The application of gene expression profiling to study PCa has resulted in significant successes, including the identification of novel clinical markers that improve tumor classification and the prediction of outcome (72).

The genetics of PCa are poorly understood (73). Oncogenically activated RAS is common in some types of cancer, but the underlying mutations that lead to the activation

are rarely found in prostate tumors (74). Mutations in the p53 tumor suppressor gene are more common, and approximately one third of advanced prostate carcinomas have either a mutated p53 or over-express a functionally inactivated p53 protein (75). Germline mutations in the ribonuclease L (RNASEL) gene have been associated with hereditary PCa (76), and the common Arg462Gln allele variant may have a major role in the development of some PCa (77). Other putative tumor suppressor genes are located on chromosomes 8p, 10q, 13q, and 17p (73). Two candidate susceptibility genes at chromosome 8p and 17p have been described (78,79). The genes, macrophage scavenger receptor 1 (MSR1) and ELAC2, were found to be associated with PCa in high-risk families and two allele variants in the MSR1 gene were found to be weakly associated with PCa risk in African-American men (80). However, a molecular mechanism that could explain the aggressive nature of PCa in African-Americans has not been established.

Recent progress in whole genome expression profiling has generated prognosis-related molecular profiles for many cancer types (81-83). A four-gene expression model has been described that discriminated between seven PCa patients with relapse and seven PCa patients without relapse, independently of stage and grade (84). The gene expression profiles were obtained from clinically localized tumors that were removed by radical prostatectomy. Another research group studied a group of 23 PCa patients, who were treated with adjuvant androgen suppression therapy before surgery (85). The surgical specimens were investigated for correlations between a patient's gene expression profile and disease recurrence with a 5-year follow-up. An eight-gene signature was identified that classified patients into a PCa recurrence group and a non-recurrence group with an overall correct prediction of 83%. A combination of gene profile and clinical/pathological parameters yielded a correct prediction of 96%.

Although these investigations have shown that gene expression profiles can predict PCa recurrence, there is no such study in the literature that has attempted to identify the genes and pathways with relevance to the etiology and aggressive behavior of PCa in African-Americans. Our objective is to find molecular signatures of low-stage and high-stage tumors in African-Americans, and to identify genes that are relevant to metastasis and patient outcome. The research is also aimed at determining molecular profiles in the context of lifestyle and diet if a strong association between a lifestyle/dietary component and cancer risk has been reported or can be established in our study population. We expect to collect up to 100 fresh-frozen tumor specimens that 1) are primarily identified by PSA screening, 2) are positive for cancer by needle biopsy, and 3) are surgically removed by radical prostatectomy. Of those, we expect that about 25% of the patients will have an advanced pathological stage ($\geq$ pT3) or a Gleason score ≥ 7 (86). Of the 52 patients, who underwent radical prostatectomy at the 2 participating hospitals in 2001, 49 were informative for staging and 24.5% of these had an advanced pathological stage (Table 5).

We will investigate gene expression patterns in both tumor tissue and the surrounding non-tumor tissue. We will develop gene expression profiles of 60-100 microdissected tumors (Table 6). We hypothesize that gene products of both tumor and stromal cells drive tumor progression and the invasion of normal surrounding tissue. We will use laser-captured microdissection to separate tumor from normal tissue, and to determine the molecular profiles that separate 1) PCa in African-Americans from PCa in Caucasians, 2) high pathological stage from low pathological stage tumors, and 3) poor

from good outcome. Candidate marker genes for tumor progression and outcome will be validated by quantitative PCR, and will be further studied.

Table 5: Stage Distribution of 49 PCa Patients Undergoing Radical Prostatectomy at the Two Participating Hospitals

Calendar Year 2001	
Pathological Stage	**Number of Patients**
Stage I + II	37
Stage III	11
Stage IV	1

Table 6: Tumors for Expression Profiling Stratified by Race and Pathological Stage

Race	Pathological stage	Number of specimens
African-American	Low (pT2)	20-30
	High (pT3)	10-20
Caucasian	Low (pT2)	20-30
	High (pT3)	10-20

1.2.5 RATIONALE TO STUDY ASSOCIATIONS BETWEEN IGF-1 AND TUMOR MARKERS

Westernization is a major risk factor for PCa. A Western-style diet increases IGF-1 blood concentrations and PCa risk (6,87). Deregulated expression of IGF-1 in the prostate epithelium leads to neoplasia in transgenic mice (88). More recent data have shown that IGF-1 induces the activation of the Akt pathway, inhibits apoptosis, and up regulates VEGF expression (89). Akt is a tumor oncogene that is activated by phosphorylation (90). It is anti-apoptotic and induces a survival response in cells (91,92). We will test the hypothesis that a high IGF-1 bioavailability promotes PCa by activating the Akt pathway in prostate tissue. We will measure plasma IGF-1 and IGFBP3 protein concentrations and correlate IGF-1 bioavailability with tissue markers of Akt activation, such as phosphorylated Akt, phosphorylated target genes of Akt (e.g., eNOS, caspases, Bad, IKK), apoptotic index, expression of VEGF and COX2, and tumor microvessel density. We will also explore whether a diet high in allium vegetables, which has been shown to reduce PCa risk (11), is an inhibitor of the IGF-1 pathway.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

The verification of eligibility and enrollment of subjects will follow the procedures that are outlined below and summarized in the two flow diagrams at the end of the section (Figures 1 and 2). A nurse will assess the eligibility of PCa patients, and an interviewer determines the eligibility of population-based controls. Eligible candidates will be offered an incentive with a monetary value of up to $75 to participate in the study.

ENROLLMENT OF CASES

Cases will be identified through resources including, but not limited to, daily afternoon visits, or phone calls, to the pathology departments to identify all cases diagnosed that day with PCa. We will also review the weekly lists of scheduled surgeries. We have established active collaborations with the Departments of Pathology and Urology at the University of Maryland and the Baltimore Veterans Affairs Medical Center to maximize our ability for recruitment of new PCa cases.

Case recruitment will follow the new HIPAA regulations ("HIPAA Privacy Rule"). However, to better assess eligibility, we will review medical and pathology records, medical examiner reports, cancer center registries and hospital databases. Our contractor has been granted a HIPAA waiver to perform the aforementioned study activities for our protocol.

We have obtained an agreement with the Departments of Urology and the treating physicians that they will put a note into the medical record of severely ill PCa patients. The note will indicate that we should not contact this patient because of health concerns. If no such note is found in the medical record, we will proceed and contact eligible patients. If a note is found, the interviewer will record the reason for refusal on the eligibility record, in addition to age, race, and family history. Other physicians who see prostate cancer patients will contact us through their staff.

After the eligibility has been confirmed, a nurse will contact patients to get both informed consent and authorization to obtain, use and disclose protected health information for research. A trained interviewer and phlebotomist will administer the questionnaire to those who consented. After the interview, blood will be collected. The investigators will make every effort to obtain informed consent and administer the questionnaire well in advance of either a scheduled surgery or an alternative treatment such as radiation or hormone therapy. Most of the patients will not be rushed to surgery. The disease is detected by needle biopsy and patients may seek a second or third opinion before they decide on a treatment option. Thus, we will have the opportunity of recruiting most patients weeks or months before either a surgery is scheduled or an alternative treatment will start. It is always at the discretion of the patient to give informed consent on one day but to have the interview or the blood collection on another day that is convenient for him. In the situation where we cannot get access to an eligible patient well in advance of surgery, we will approach the patient up to one day before surgery to seek consent. We will offer to administer the questionnaire and obtain blood after the surgery. However, if the patient wishes to be interviewed and have the blood drawn at the same day when he gives informed consent, we will do so. If we would limit our access to surgery patients because of a longer waiting period where we do not approach eligible cases prior to surgery, it could seriously compromise our ability of collecting the number of fresh-frozen tumor specimens that are needed for our research study.

PROCEDURES

1. Interviewer obtains patient's name, location and physician's name
2. Interviewer screens medical record for eligibility. If eligible, record age, race and family history. Record reasons for ineligibility. Also record histology of tumor
3. Interviewer obtains a non-objection statement from the treating physician to contact the patient. If physician refuses, interviewer records reason for refusal on eligibility record, in addition to age, race, and family history of PCa
4. Interviewer contacts case and obtains informed consent and authorization to obtain, use and disclose protected health information for research
5. If patient refuses (either right away or repeatedly delaying consent [failure to make commitment in response to 3 requests]), then record brief information from medical record about family history, age, and race, on eligibility record. If subject requests that no such information will be kept on file, we will not record this information and will delete any information that already exists on file. However, we will keep the medical record number to avoid re-contacting those subjects that refused to participate in our study
6. Perform interview and complete questionnaire for those consented. The interviewer will give a copy of the questionnaire to the patient before the interview begins. The patient will have the opportunity to read a question while being interviewed. The last section of the questionnaire which is examining sexual history is self-administered. Provide incentive of $25
7. Collect 45 cc of blood in one 15 cc red top tube and three 10 cc green top tubes. Place tubes in a thermos for short-term storage and transport
8. Collect urine (50 ml)
9. Provide incentive of $25
10. Blood (separation of serum and blood clot; buffy coat; plasma and red blood cells) and urine to be processed within 8 hours at the University of Maryland Department of Pathology
11. Review pathology report to confirm diagnosis and abstract pathological data
12. Request confirmation of the pathology for the specimens that are collected from radical prostatectomy.
13. Request part of tumor block and frozen tumor, if available, for storage
14. If subject is re-contacted at a later time for additional phlebotomy, then an additional $25 incentive is given. If subject is contacted for additional questionnaire information, then no additional award is given

ENROLLMENT OF POPULATION-BASED CONTROLS

Identify subjects through Department of Motor Vehicles database, frequency-matched on age and race. Search the Internet for the home phone number. We will contact the person by phone to verify eligibility and the willingness to participate in the study. We will obtain both informed consent and authorization to obtain, use and disclose protected health information for research at a location that is convenient to the subject. A trained interviewer and phlebotomist will contact the person and administer the

questionnaire to those who consented. Blood and urine will be collected either at the interview or at a separate appointment.

PROCEDURES

1. An introductory letter is sent to a prospective subject to notify him of the study
2. The subject is contacted by phone to explain the study and how he was selected, and to request participation. The permission to interview the person is obtained. If person refuses to participate (either right away or repeatedly delaying consent [failure to make commitment for 3 requests]), then record brief information about age, race, and family history of lung and prostate cancer. If subject refuses to give the information and also requests that no such information will be kept on file, we will not record this information and will delete any information that already exists on file. However, we will keep a DMV ID number to avoid re-contacting those subjects that refused to participate in our study
3. Obtain informed consent and authorization to obtain, use and disclose protected health information for research. This is done either at the study office, or other locations such as person's home or person's work location. If person refuses (failure to make commitment for 3 requests), then record brief information about age, race, and family history of lung and prostate cancer. If subject refuses to give the information and also requests that no such information will be kept on file, we will not record this information and will delete any information that already exists on file. However, we will keep a DMV ID number to avoid re-contacting those subjects that refused to participate in our study
4. Perform interview and complete questionnaire. This is done either at the study office, or other locations such as person's home or person's work location. The interviewer will give a copy of the questionnaire to the subjects before the interview begins. Subjects will have the opportunity to read a question while being interviewed. The last section of the questionnaire which is examining sexual history is self-administered. Provide incentive of $25
5. Collect about 55 cc of blood. One 15 cc red top tube, three 10 cc green top tubes and one 10 cc red-green top tube. Place tubes in a thermos for short-term storage and transport
6. Collect urine (50 ml)
7. Provide incentive of $25
8. Blood (separation of serum and blood clot; buffy coat; plasma and red blood cells) and urine to be processed within 8 hours at the University of Maryland, Department of Pathology. Four 2 cc aliquots of heparinized blood will be preserved. The red-green top tube will be sent to LHC within 24 hours for lymphocyte culture
9. If subject is re-contacted at a later time for additional phlebotomy, then an additional $25 incentive is given. If subject is contacted for additional questionnaire information, then no incentive is given

RE-CONTACTING SUBJECTS FOR ADDITIONAL INFORMATION OR BLOOD

The interviewer who administered the questionnaire will be the person who will re-contact the subject. This re-contact will not involve a script because of the large number of possible circumstances. Subjects will be re-contacted only under certain conditions. The conditions are as follows:

1. If there is missing information in the questionnaire
2. If the provided information is illegible
3. If there is a discrepancy between questionnaire and medical record information
4. If there is an obvious inconsistency in the answers to two similar questions in the questionnaire
5. If an insufficient volume of blood has been collected
6. If there has been mishandling of a blood sample in the laboratory
7. If there has been an accident leading to the loss of a blood sample
8. If the analysis of a blood sample returns a highly unusual result

We will not contact subjects, who refused to answer questions during the interview process, for responses to those questions they refused. Missing information due to refusal will be marked as such in the questionnaire. We will also not contact a person who failed to complete the sexual history part of the questionnaire.

PROCEDURES

1. Subject is contacted by phone, or by letter, notifying him of the need for additional information or blood. If notified by letter, we will include a postage paid envelope and form to be returned if the subject does not want to be contacted
2. If only additional information is requested, we will ask the subject by phone for the information. This will be done at the first phone contact, or two weeks after the notification letter was sent out
3. If subject agrees, then additional phlebotomy can be done at the home or in the hospital, whichever is convenient

Figure 1: Flow Diagram for the Accrual of Cases

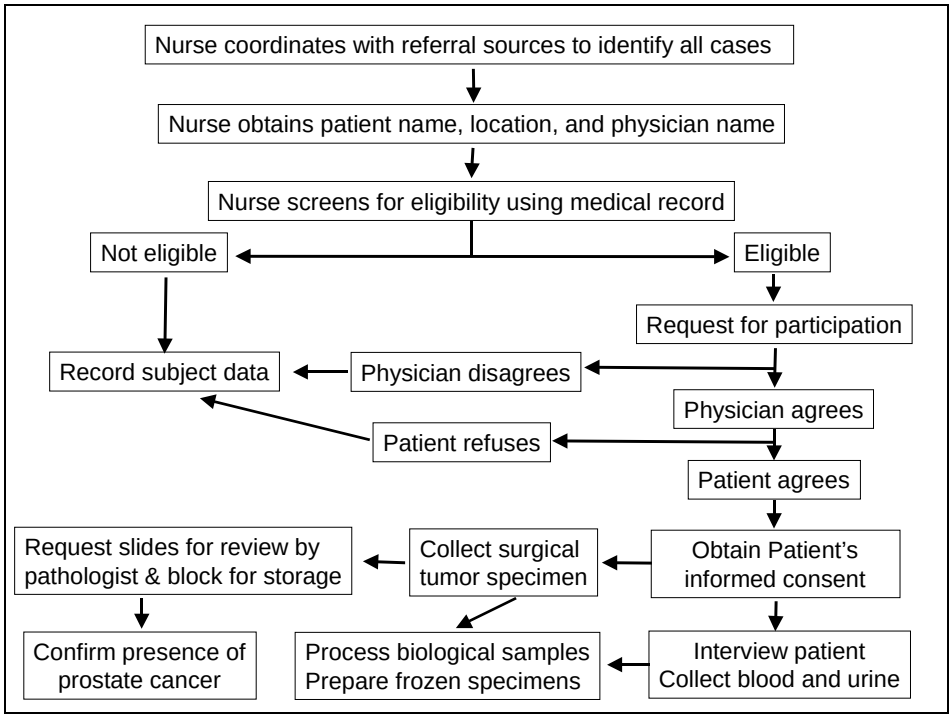
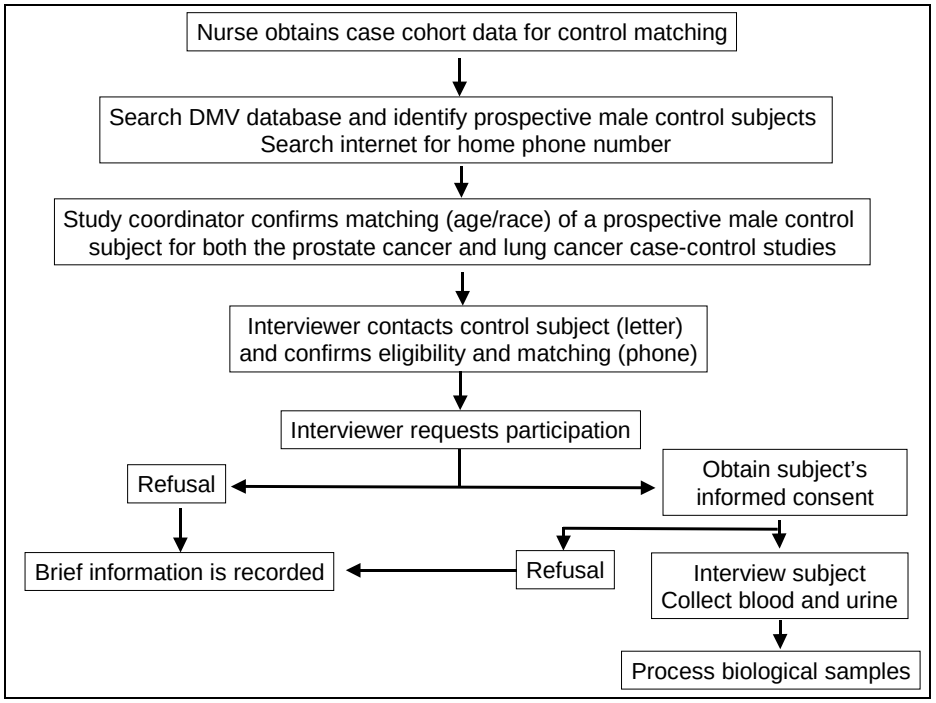


Figure 2: Flow Diagram for the Accrual of Controls



2.1 ELIGIBILITY CRITERIA

CASE SUBJECT SELECTION

We will recruit incident cases of pathologically confirmed PCa at all stages of the disease that are age 40 to 90 years. Treatment can be surgery or therapy. The following check list will be used to verify eligibility of a case subject.

ELIGIBILITY CHECK LIST - PROSTATE CANCER CASES

NAME_____ DATE_____

DATE OF BIRTH_____

ID# _____

Yes	No	Criteria (ALL MUST BE CHECKED)
		Has been diagnosed with prostate cancer within the last two years
		Pathological diagnosis of prostate cancer made at the local hospital pathology department
		Resides in Baltimore City or contiguous metropolitan counties, Anne Arundel and Prince George's counties, Eastern Shore, western and northern Maryland, or adjacent counties in Pennsylvania, Delaware, Virginia, or the Washington Metropolitan area
		Has a residential working phone within his home
		Born in the United States
		Of African-American or Caucasian descent, and age 40 to 90 years
		A non-objection statement by the physician from the hospital where the patient was identified, or listed as the treating physician by the tumor registry or surgical pathology report, to contact the patient
		Is not currently residing in an institution, such as a prison, nursing home, or shelter
		Is not a severely ill patient in the intensive care unit
		Speaks English well enough to be interviewed
		Is able to give informed consent
		Is physically and mentally capable of performing the interview
		Has never been interviewed as a control for this study
		Subject provides informed consent and signs form. _____Unwilling _____Unavailable

SELECTION OF POPULATION-BASED CONTROLS

Population-based controls will be identified through the Department of Motor Vehicles (DMV), and matched on age (5-year intervals), gender and race to cases. Recruitment of controls will start concurrently with case accrual, using the age and race frequency distribution of cancer patients in previous years, but recruitment objectives can be re-assessed to ensure a frequency-matched study. We will exclude controls that do not have a listed home phone number. The following check list will be used to verify eligibility of a control subject.

ELIGIBILITY CHECK LIST - POPULATION-BASED CONTROLS

NAME_____ DATE_____

DATE OF BIRTH_____

ID# _____

Yes	No	Criteria (ALL MUST BE CHECKED)
		Resides in Baltimore City or contiguous metropolitan counties, Anne Arundel and Prince George's counties, Eastern Shore, western and northern Maryland, or adjacent counties in Pennsylvania, Delaware, Virginia, or the Washington Metropolitan area
		Has a residential working phone within his home
		Born in the United States
		Of African-American or Caucasian descent, and age 40 to 90 years
		Does not have a personal history of cancer other than basal and non-melanomic skin cancer
		Have never had radiation therapy or chemotherapy
		Is not currently residing in an institution, such as a prison, nursing home, or shelter
		Speaks English well enough to be interviewed
		Is able to give informed consent
		Is physically and mentally capable of performing the interview
		Has never been interviewed as a control for the study
		Subject provides informed consent and signs form. _____Unwilling _____Unavailable

2.1.1 INCLUSION CRITERIA

CASES

1. Has been diagnosed with PCa within the last two years
2. Pathological diagnosis of PCa made
3. Must reside in Baltimore City or contiguous metropolitan counties, Anne Arundel and Prince George's counties, Eastern Shore, western and northern Maryland, or adjacent counties in Pennsylvania, Delaware, Virginia, or Washington DC area
4. Has a residential working phone within his home
5. Was born in the United States
6. Of African-American or Caucasian descent, and age 40 to 90 years
7. Speaks English well enough to be interviewed
8. Is physically and mentally capable of performing the interview
9. Never has been interviewed as a control for the study
10. A non-objection statement to contact the patient has been obtained from the treating physician at the hospital where the patient was identified, or from the physician who is listed as the treating physician

CONTROLS

1. Stratified to match cases by age (5-year intervals), gender and race
2. Must reside in Baltimore City or contiguous metropolitan counties, Anne Arundel and Prince George's counties, Eastern Shore, western and northern Maryland, or adjacent counties in Pennsylvania, Delaware, Virginia, or Washington DC area
3. Has a residential working phone within his house
4. Was born in the United States
5. Speaks English well enough to be interviewed
6. Is physically and mentally capable of performing the interview
7. Never has been interviewed as a control for the study (with our tracking database)

2.1.2 EXCLUSION CRITERIA

CASES

1. Severely ill subjects in the intensive care unit (they can be reconsidered after discharge from ICU)
2. Currently residing in an institution, such as a prison, nursing home, or shelter
3. Patient is unable to give informed consent

CONTROLS

1. Personal history of cancer other than basal and non-melanomic skin cancer
2. History of radiation therapy or chemotherapy
3. Currently residing in an institution such as a prison, nursing home or shelter
4. Subject is unable to give informed consent

2.2 RESEARCH ELIGIBILITY EVALUATION

The research eligibility for cases is determined by the inclusion and exclusion criteria (see 2.1.1 and 2.1.2) and a non-objection statement by the physician from the hospital where the patient was identified, or listed as the treating physician by the tumor registry or surgical pathology report. Severely ill subjects are not eligible but can be reconsidered after recovery. Under the protocol, no invasive procedure will be performed. Cases will be asked to participate in an interview session and to donate blood and urine, and if applicable, to donate a surgically removed tumor specimen. We will not apply specific tests to evaluate eligibility. Information regarding the pathological diagnosis of PCa will be obtained from pathology and medical records.

The research eligibility of population-based controls is defined by the inclusion and exclusion criteria (see 2.1.1 and 2.1.2). Eligible subjects must be physically and mentally capable of performing the interview. Under the protocol, no invasive procedures will be performed. Subjects will be asked to participate in an interview session and to donate blood and urine.

2.3 PATIENT REGISTRATION AND TREATMENT RANDOMIZATION

The proposed study is not a clinical investigation but an epidemiological case-control study. Subjects will not receive any type of therapy under the protocol. The collection of blood is the only medical procedure that is performed for this protocol. Patient care is not modified by the protocol. Cancer patients will receive their usual care and are asked to donate tumor specimens that are surgically removed as part of their routine treatment.

3 STUDY IMPLEMENTATION

The study will be implemented in two phases. The first phase is conducted as a pilot study to evaluate recruitment procedures. The second phase is the main study that will be closed when the projected 1000 cases and 1000 controls have been enrolled. Participants in the pilot study will count toward the total accrual.

TIME SCHEDULE OF THE STUDY

Month 1	Begin pilot study
Month 13	Review and revise protocol if needed Resubmit to IRB if needed
Month 14	Begin accrual for the study at the projected rate of 80 cases* and 80 population-based controls* per year. Cases with prostatectomy and/or therapy will be eligible
Month 62*	Close enrollment if target accruals are met

* have been increased to 120 cases and 120 controls per year; now projected for 2014/15

PILOT STUDY (was completed in 2006)

During the 12-month pilot phase, we will recruit about 80 patients who are either African-Americans or Caucasians, and have had a radical prostatectomy and/or therapy. We will also start recruiting controls that match the cases on age (5-year intervals) and race. We will recruit the cases at the 2 participating hospitals.

We will assess the availability of PCa patients, who 1) are willing to participate in the study, 2) are of African-American descent or 3) provide tumor specimens. In this period, we expect to recruit 25 patients who will have a radical prostatectomy as the initial treatment. Approximately half of the patients should be of African-American descent. We will also assess the availability of controls that are eligible for both the lung cancer and the PCa case-control study and frequency-match the PCa cases by age and race.

The criteria that are used to determine if the pilot is successful are 1) the accrual of 80 cases and 80 matched controls, 2) the accrual of about 40 cases who are of African-American descent, and 3) the collection of 25 fresh-frozen tumor specimens. If major modifications are required to improve accrual rates or other aspects of the study, then the pilot study will be extended to address these issues. Otherwise, we will start recruiting for the main study at the projected rate of 80 cases and 80 controls per year.

PERSONNEL

The Principal Investigator, Dr. Stefan Ambs, and the NCI Project Officer, Dr. Glenwood Trivers, both at the Laboratory of Human Carcinogenesis, NCI, will be responsible for the overall monitoring of the study. The associate investigators of the study, who are at Baltimore Veterans Affairs Medical Center and the University of Maryland, support the study with their expertise in pathology and clinical aspects of PCa. The study will be facilitated through a service contract to our laboratory. The contractor is the University of Maryland Pathology Department. The contract supports an established epidemiological infrastructure at the 2 participating hospitals. Contract personnel include a PI for the contract and a case-control supervisor, a nurse and interviewers, staff to collect and process biological specimens, data entry personnel, and a statistician and epidemiologist for data analysis and quality control assessment. Leoni Leondaridis, a software consultant to the Laboratory of Human Carcinogenesis, will assist with database development and management.

PREVIOUS EXPERIENCE OF THE UNIVERSITY OF MARYLAND CONTRACTOR WITH EPIDEMIOLOGICAL CASE-CONTROL STUDIES

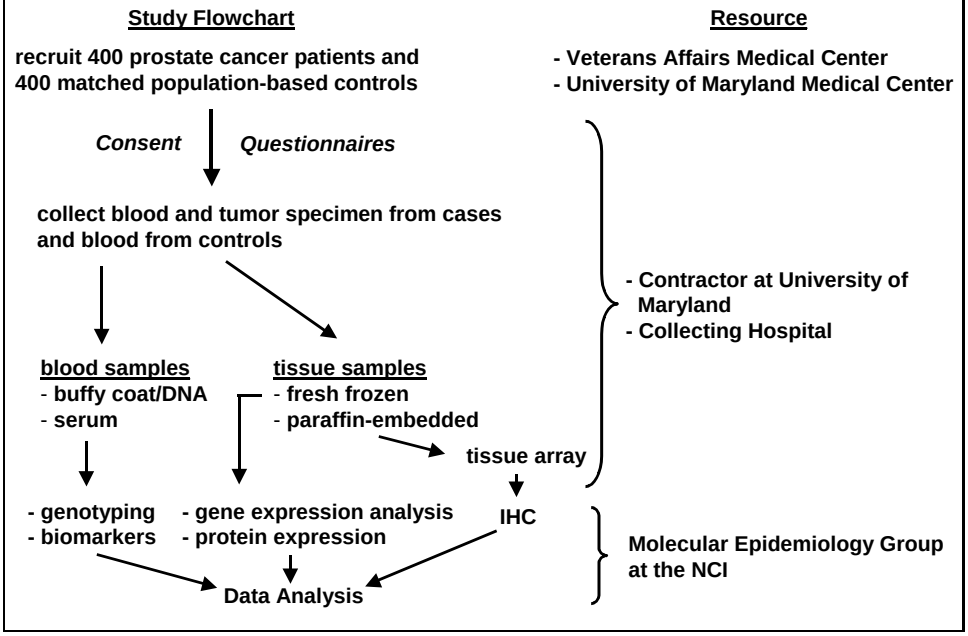
The first case-control study conducted by the University of Maryland at Baltimore contractor began in 1986, using the University of Maryland Medical Center and the Baltimore Veterans Affairs Medical Center to recruit cases and hospital-based controls. It was a lung cancer study to investigate the association between genotypes and lung cancer risk. A second, follow-up study was completed in 1998, which was designed to replicate the first study with some minor study modifications. A third study begun in 1999. This lung cancer case-control study had a target accrual of 1,200 subjects, separated into 400

cases, 400 frequency-matched hospital controls, and 400 frequency-matched population-based controls. Target recruitment in the lung study has now been changed to 1500 cases and 1500 population-based controls. The aim of this study is to investigate lung cancer risk with respect to gender, race, and inherited susceptibility as it relates to mutagen sensitivity, p53 mutation load, and allele variant genes. Mutagen sensitivity is assayed in blood lymphocytes, and compared between cases and controls. Preliminary results confirm the hypothesis that lung cancer patients are more sensitive to mutagen-induced DNA damage. This study (NCI protocol OH98-C-N027) is scheduled to continue for several more years, as the request for recruitment above target accrual has been approved.

3.1 STUDY DESIGN

We are proposing an epidemiological case-control study in which we collect 1) questionnaire information and 2) blood and urine samples from all subjects, and 3) tumor specimens from cases if available. Additional information for cases will be abstracted from medical and pathology records. The duration of the recruitment phase is 5 years. Research laboratory studies will begin at the end of the recruitment phase. The design of the study is illustrated in Figure 3.

Figure 3: Design Of Prostate Cancer Case-Control Study



3.1.1 QUESTIONNAIRE

Trained interviewers will administer the questionnaire. The interview will last approximately one hour. The questionnaire assesses prior medical and cancer history, tobacco use, dietary risk factors, current medications, occupational history, medical

history and family medical history, socioeconomic status, anthropometry, and sexual history. The sexual history section is self-administered. This questionnaire is shared with the lung study and contains question relevant to both prostate and lung cancer.

INTERVIEWER TRAINING

The interviewers will receive a procedure manual. They will be trained in how to identify eligible subjects, how to provide informed consent, how to administer and properly complete the questionnaire, how to perform phlebotomy, and how to properly process blood and urine samples. The field supervisor will provide the training. Newly hired interviewers will practice the administration of the questionnaire to office volunteers. Interviewers will then administer the questionnaire and draw blood from subjects under the supervision of the field supervisor, who will provide feedback after the interview.

STEPS TO BE TAKEN IF A PATIENT BECOMES EMOTIONALLY UPSET DURING THE INTERVIEW PROCESS

The University of Maryland contractor will train the interviewers for the event that a subject will become emotionally upset during the interview. Part of this training will focus on the scientific background of the study, i.e., the rationale asking these specific questions. We have found that well-informed interviewers are often able to reassure subjects that the information being sought in the interview is needed for the scientific hypotheses that are under study. The interviewer will then be well prepared to answer questions that relate to the sexual history part on pages 18-20 of the supplemental questionnaire. If a subject continues to show signs of distress during the interview, the interviewer will explain that the subject has the right not to answer a question or section of the questionnaire if the subject does not feel comfortably to do so. Indeed, the questionnaire has a special code to denote that the respondent declined a particular question. The interviewers are trained to handle this with neutrality. If the interview is conducted on the premises of the University of Maryland and the Veteran Affairs Hospital, the interviewer will be able to call for help in the event that the above steps have failed to deal with a distressed subject. We have established a list of contacts. Dr. Plaut, Ms. Perlmutter, and Dr. Jones, can be called for immediate help. Dr. Smith will accept quick referrals and, if requested, will follow up on the incident in a special appointment with the subject. Drs. Plaut and Smith are psychologists at the University of Maryland School of Medicine. Dr. Plaut is a counselor for patients who have a sexual dysfunction. Dr. Smith is a counselor for cancer patients. In the event that an interview takes place off site, e.g., at a subject's home, and the subject has become distressed, the same contact people will be available by telephone.

The list of contacts is as follows:

1) Dr. S. Michael Plaut (Psychologist), 410-706-7476 or 410-303-4136 (pager)

2) Dr. Julie Smith (Psychologist), 410-328-6553

3) Donna Perlmutter (Field Supervisor), 410-706-0720

4) Dean L. Mann, M.D. (Project Director), 410-328-5512

3.1.2 COLLECTION AND PROCESSING OF BIOLOGICAL SAMPLES

We will collect blood, urine, and tumor specimens. The collected blood will be used for the analysis of genotypes and the measurement of hormone, cytokine, and eicosanoid concentrations in serum and plasma. Urine samples are collected to study the association between inflammation of the prostate and the progression of the disease. The collected urine will be analyzed for markers of inflammation and oxidative stress such 8-hydroxydeoxyguanosine, malondialdehyde, nitrate/nitrite, and metabolites of the eicosanoid pathway. We are also planning to measure nicotine metabolites in urinary samples to study the association between nicotine metabolism, genetic polymorphisms, and the risk of advanced prostate cancer. The tissue specimens are collected for whole-genome gene expression analysis and the preparation of tissue arrays for immunohistochemical analysis of protein expression.

The collection of blood, urine, and tissue specimens, will follow procedures that have been established for our protocol titled "Resource for the collection and evaluation of human tissues and cells from donors with an epidemiology profile", University of Maryland at Baltimore IRB #0298229. The same procedures are used in the lung cancer case-control study. For surgical specimens, each late afternoon one of the lab personnel checks the schedule for possible tissues. A note that reminds the operating room (OR) personnel to send the resected specimen to the pathology fresh, not fixed, is given to the OR. When the resection of a tumor is done, the OR will call the University of Maryland Department of Pathology. A designated person will transport the tissue to the pathology, where the tissue is procured as soon as possible. All biological samples will be stored at the pathology until further notice. The procedures are as follows.

PROCEDURES FOR COLLECTION OF BLOOD AND URINE

1. Observe universal precautions for prevention of transmission of blood-borne pathogens
2. Clean skin with alcohol wipe and wait to dry
3. Obtain blood: one 15 cc red top tube, two or three 10 cc green top tubes and one 10 cc red-green top tube. Apply pressure and band-aid
4. Place blood samples on ice
5. Request urine sample in plain sterile container (50 ml)

PROCEDURES FOR PROCESSING OF BLOOD AND URINE AT THE UNIVERSITY OF MARYLAND DEPARTMENT OF PATHOLOGY

1. Storage of urine at -70° C in 10 ml aliquots
2. Cryopreserve four 2 ml of whole heparinized blood
3. Send seven cc of heparinized whole blood to LHC within 24 hours for lymphocyte cultures by messenger
4. Separation of buffy coat from red cells and plasma. Wash red blood cells according to protocol. Storage of all three at -70° C
5. Separation of lymphocytes from red blood cells and plasma. Wash red blood cells according to protocol. Cryopreservation of two 0.5 ml tubes of lymphocytes. Storage of all tubes at -70° C

PROCEDURES FOR TISSUE COLLECTION

1. Tissues are collected at time of prostatectomy
2. Notify surgeon and the pathology department of tissue collection
3. A designated person will take tumor specimen as fast as possible, but within 10min of resection, to the pathology room
4. A pathology technician will immediately process the sample.
5. Within 20min of receipt, place half of the specimen in a pre-labeled container. Immediately flash-freeze and store the container at -70° C
6. Within 20min of receipt, place about a quarter to a third of the specimen in a pre-labeled container and cover with OCT. Immediately flash-freeze the OCT-embedded tissue and store the container at -70° C. The specimen will be processed at a later time point for laser-captured microdissection. If a specimen does not contain tumor, a flash-frozen tissue piece from step 5 can be used to replace the sample
7. Prepare a paraffin block of remaining tissue section
8. Prepare a H/E slide for diagnosis by pathologist

3.1.3 ASSESSMENT OF DISEASE STAGE AND OUTCOME

Information on disease stage, metastasis and outcome (relapse and survival) will be obtained from pathology and medical records, Veteran Administration databases, State of Maryland records, and from the National Death Index.

3.1.4 RESEARCH LABORATORY STUDIES

Genotyping at the NCI

- Isolate DNA from whole blood or buffy coat using a previously tested DNA isolation kit
- Determine DNA quality
- Perform genotype analysis with quality-controlled assays. Each assay will contain negative and positive controls, and 10% blinded duplicates

Gene expression analysis at the NCI

- Cut fresh-frozen tissue into serial 8μ section
- Microdissect tissue into tumor and non-tumor tissue, using laser-captured microdissection (LCM). A pathologist will help with the procedure. The equipment for LCM is provided by the NCI core facility
- Isolate, amplify and label RNA, using existing protocols. Currently, our laboratory uses a QIAGEN protocol for the isolation of total RNA from microdissected tissue and the small sample labeling protocol by Affymetrix to produce biotin-labeled cRNA
- Hybridize cRNA onto the Affymetrix GeneChip arrays
- Data analysis will be performed in collaboration with a biostatistician. We have established such collaboration for gene expression analysis of breast cancer

3.1.5 LIMITATIONS OF THE CASE-CONTROL DESIGN

A frequent limitation of case-control studies is the heterogeneity of the population as it relates to race, age, and catchment area. If not addressed, the study may yield differences in genetic frequencies that are unrelated to the case status. To correct for the problem, we have restricted the study to a narrow geographic area, and will match for race and age by frequency. We have conducted a survey to determine the expected age range for cases. We anticipate a mean and median age of about 66 years for the prospective cases, which will permit an age matching with population-based controls using the established resources. Most patients will be in moderately good health, as they are identified at a relatively young age through PSA screening. Cases could be rendered more similar to controls with a selection criterion for controls that would achieve matching of cases based on census tract. This alternative would not eliminate a possible selection bias but would make the study substantially more difficult to perform. Also, our experience in Baltimore is that population control accrual is not skewed to one part of Baltimore. A 2002 survey for the ongoing lung cancer case-control study indicated that the recruited cases and population-based controls have a similar residential history. Thirty-eight percent of the cases were from Baltimore City versus 54% of the controls, and 62% of the cases lived in Baltimore suburbs or Eastern Shore counties versus 46% of the controls. We do not expect a selection bias based on genotype that would be confounded by behavior or education, as the genotype is unknown to the subjects and there is no plausible relation to either behavior or other factors that might affect the selection. A limitation common to other case-control studies is the possibility of differences between cases and the control population that are caused by a confounding disease, and not the disease under investigation (case identification among a hospitalized cohort, hospital-based controls). We are limiting the bias by matching cases with population-based, but not hospital-based, controls. The relatively young age of our cases will also limit this problem.

Another limitation relates to problems associated with rapid subject accrual. We expect to identify all cases through hospital pathology and oncology departments. This will be verified through tumor registry records at 6-months intervals. Based on our experience in the ongoing lung cancer case-control study, we expect to contact most of the subjects prior to treatment or surgery, but will confirm this during our pilot study. We will allow for accrual of subjects before and after surgery, which will give us more flexibility for the accrual process.

3.2 DRUG ADMINISTRATION

Not applicable for our study.

3.3 TREATMENT MODIFICATIONS

Not applicable for our study.

3.4 PHARMACOKINETIC STUDIES

Not applicable for our study.

3.5 PROTOCOL EVALUATION

Not applicable for our study.

3.6 CONCURRENT THERAPIES

Not applicable for our study.

3.7 SURICAL GUIDELINES

Not applicable for our study.

3.8 RADIATION THERAPY GUIDELINES

Not applicable for our study.

3.9 RADIATION THERAPY GUIDELINES

Not applicable for our study.

3.10 POST STUDY EVALUATION

Not applicable for our study.

4 SUPPORTIVE CARE

Not applicable for our study.

5 DATA COLLECTION AND EVALUATION

The University of Maryland contractor is responsible for the collection and evaluation of the study data.

5.1 DATA COLLECTION

All data will be entered and stored in a centralized case-control study database at the University of Maryland. Leoni Leondaridis, a software consultant to the Laboratory of Human Carcinogenesis, is assisting the contractor with database maintenance and upgrades. The centralized database consists of three databases, a tissue, a survey, and a tracking database, that are linked through unique identifiers. The tracking database contains the personal information of each study participants. Access to this database is strictly controlled, and the database is not accessible to NCI researchers. The tissue database is a tracking and inventory database for fixed and fresh-frozen tissues, and for blood samples. Updated files of the database are available to the NCI researcher. The survey database contains the demographic and epidemiological information of the study participants. Access to information in this database has to be requested. Information from the tissue and survey databases cannot be linked to any personal identifiers.

5.1.1 SURVEY DATA

Initially, all forms including the eligibility screener forms, consents, HIPAA authorization forms, interview forms, specimen collection forms, pathology and medical reports, etc., will be assigned a study ID number, which is common only to all forms and specimens that belong to one particular participant. All forms are then entered into the centralized case-control study database for tracking and storage. The forms are filed in locked file cabinets in the secured study office.

5.1.2 QUESTIONNAIRE DATA

The questionnaire data are entered into the survey database twice with a different data entry person each time. If a discrepancy occurs, a supervisor will verify the correct entry. Prior to entry, all questionnaires are coded and edited using a standardized code for occupation, illness, and medication. When appropriate, we utilize the ICD-9 codes (or further updated codes as they become available in the future). A coder will highlight all sections in a questionnaire that contain either an illogical response or missing data. The highlighted sections will be reviewed by a supervisor, or the Research Study Coordinator where needed. A patient may be re-contacted to clarify an answer or to complete a missing response. The supervisor will also examine the coding of about ten percent of the questionnaires. The questionnaires are then entered into the database and verified by the Epi Info software. A contractor to the University of Maryland developed the Epi Info software. The software is programmed to include range checks, skip patterns, and codes for missing data and don't know answers. If errors are detected, a supervisor will assist with the correction. Monthly reports on coding and editing, data entry and the verification status are prepared and presented at monthly staff meetings and site visits.

During the study, quality control will consist of data comparisons among interviewers to determine the quantity and quality of information that they have gathered, by evaluating characteristics such as interview duration, number of interview problems reported, number of refusals, distribution of subject answers, and number of missing and incomplete answers. In addition, a small random sample of subjects will be re-contacted to inquire about the interview experience, to note any problems, and to confirm that the interview actually took place. Quality assessment will follow procedures that are already in place for the lung cancer study.

5.1.3 TISSUE DATA AND TRACKING OF BIOLOGICAL SPECIMENS

Tissues and blood samples are entered into the database under the patient's unique study ID number. The information on tissue and blood samples includes date procured, type of tissue, type of preservation, serum, plasma, buffy coat, amount, number of vials, date shipped. Different tissue preservation methods, e.g., formaldehyde-fixed, OCT-fixed, flash-frozen, will generate different records. Frozen tissues are intermediately stored at the University of Maryland and shipped in batches, on dry ice, to the NCI. Paraffin-embedded blocks are kept at the University of Maryland Department of Pathology. The tissues and blood samples that are received at the NCI will be entered into an inventory database at the Laboratory of Human Carcinogenesis and will be stored long-term at a contracting facility in Frederick, MD.

5.1.4 DATABASE SETUP

The current system is designed using Access 97. It is housed on a secure Windows Server NT with Windows authentication and only authorized users have access to the shared folder. In addition, the database itself has another layer of security that lets only registered users log on to the application. The database was specifically designed to be a tracking system for all data from hospital cases and the controls recruited through the DMV database.

The database is backed up by the School of Medicine server at the University of Maryland, and also locally by the study data manager. The database has an incremental daily backup, and a regular full back up each weekend. This system is constantly updated.

The database is also used to perform the frequency matching of cases with population-based controls from the DMV database. The DMV data sets are imported into the database and can be searched for matching controls but the contract statistician performs the actual matching. The system has a built-in tool that loads the names and/or addresses into search engines like Yahoo, and if the home phone is found for the candidate control subject, a unique registration number is generated for the person. This study ID number is then used in all subsequent steps. Once a study ID number has been generated, the subjects can be contacted for study eligibility and consent.

5.2 RESPONSE CRITERIA

Not applicable for our study.

5.3 TOXICITY CRITERIA

Not applicable for our study.

5.4 STATISTICAL SECTION

5.4.1 SUMMARY OF STUDY DESIGN AND OBJECTIVES

As of 2010, our last amendment for the study, we are proposing an epidemiological prostate cancer case-control study that will include 1000 prostate cancer cases and a sample of 1000 population-based controls (Table 7). The aim of the study is to identify genetic susceptibility factors for nonhereditary PCa with a particular emphasis on genetic factors that might explain the comparatively aggressive nature of PCa in African-American men. The accrual time is projected to end in 2014/2015, averaging an annual accrual of 120 cases and 120 frequency-matched controls. The participants will be African-American and Caucasian males.

Women are excluded from the study because they do not develop PCa. Men, who are younger than 40 years and older than 90 years are excluded. Nonhereditary PCa is rare at an age younger than 40 years. PCa patients, who are older than 90 years, are excluded because of co-morbidity considerations. Subjects with a race/ethnic background other than African-American or Caucasian have been excluded. Less than 10% of the PCa patients at the 2 participating hospitals have a racial/ethnic background that is not African-American or Caucasian. The inclusion of such a small number of cases would not allow a risk analysis based on stratification by race.

The primary objectives of the study are to examine the association of 1. allele variant genes with PCa risk and outcome, and 2. exposures with gene expression profiles in prostate tumors. The objective will be accomplished with 1) genotype analysis of blood DNA from 1000 cases and 1000 controls, 2) collection of data from questionnaires, medical and pathology records, and the National Death Index, and 3) expression profiling of fresh-frozen tumor samples.

Genome-wide gene expression analysis of prostate carcinomas will compare expression profiles among groups (e.g., African-American versus Caucasian, high versus low IGF-1) that consist of 10-30 tumors per group. The minimum number of tumors needed to generate a molecular profile that differentiates two groups cannot be determined for the proposed experiments. A best estimate is 13 per group based on the following assumptions: A significance level of 0.001 and a power of 0.05, assuming the data is base 2log transformed and that the variance in expression for a gene of interest is 0.5, and that one wishes to be able to detect genes for which one of the four subgroups exhibits a two-fold change in expression at the specified power (using the nQuery software). However, some recent PCa gene expression studies used fewer tissues than we are proposing for our experiments and yielded expression signatures that predicted clinical behavior in response to treatment (84,85) or differentiated tumors according to

the Gleason score (93). Thus, the proposed sample size for our investigations appears be adequate and is still suitable to do laser-captured microdissection. Dr. Ming Yi at the Advanced Computing Center at the NCI-Frederick will conduct the analysis of the expression data.

Table 7: Target Accrual Numbers for the Proposed Study (as of 2010)

Target Accrual Numbers of Cases and Controls[1]			
	African-American Males	**Caucasian Males**	**Total**
Cases	500	500	1000
Population Controls	500	500	1000
Total	1000	1000	2000
[1] Race will be identified by subject self-report			

5.4.2 AVAILABILITY OF CASES AND JUSTIFICATION OF SAMPLE SIZE

A review of cancer registry data shows that the 2 participating hospitals treated 313 PCa cases in 2001 of which 150 cases were identified as being of African-American and 143 cases as being of Caucasian descent (Table 8). Twenty cases were either not informative for race or had a different race/ethnic background (e.g., Asian, Hispanic). Most of the cases had residency in the greater Baltimore area. There were 52 known available cases with radical prostatectomy in 2001, which is sufficient to meet our goal of recruiting at least 25 cases with radical prostatectomy per year.

We will use the case-control design to detect statistically significant differences in the frequency of genotypes between PCa patients and randomly selected population-based controls that are matched by age, gender and race to the cases. For the initial analysis, all cases will be grouped together. We will then examine the study population stratified by race and/or exposure. The study is designed to detect risk associations that have odds ratios of ≥ 1.5. Associations between PCa and genotypes of the angiogenesis and hormone pathways have been found to have odds ratios that are in the range of 1.5-2.5 (39,69-71). Examples are shown in Table 3. We are not aiming to detect weak risk associations that are reflected by odd ratios between 1 and 1.5. Such associations are prone to a selection bias and confounding, and would require a larger sample. Our proposed study is exploratory and designed to detect potentially novel genotype–cancer risk associations. The finding of a new disease-associated genotype can subsequently be validated in a larger study.

Table 8: Known Available Cases from Hospital Tumor Registries

Known Available Cases by Hospital Calendar Year 2001			
	African-American	**Caucasian**	**Total**
VA Hospital All Cases	85	48	133
UM Medical Center All Cases	65	95	160 [1]
Total	150	143	293
[1] >70% of patients are from the greater Baltimore area			

POWER CALCULATIONS

The power calculation assumes a study size of 400 cases and 400 controls which was the study size in the originally approved protocol. The study size was subsequently increased to 600/600 after approval of an amendment in 2006 to 1000/1000 after approval of an amendment in 2010. Power calculations were performed with the Power and Sample Size (PS) software, Version 2.1.30. William D. Dupont and Walton D. Plummer at the Vanderbilt University Medical Center have developed this software to conduct power calculations for clinical and epidemiological studies. The program is available on the Internet at http://www.mc.vanderbilt.edu/prevmed/ps/.

ANALYSIS OF ALLELE VARIANT GENES WITH ALL CASES GROUPED TOGETHER

We assume 1) a study population of 400 cases and 400 controls, and 2) two-sided alpha = 0.05, and 3) a prevalence of 5%, 20% or 40% for the disease-associated allele. We selected the candidate allele variants using the following criteria (and/or): 1) the allele variant gene is functionally important (literature), and 2) the polymorphism will likely affect protein expression/stability/activity or mRNA splicing/stability, and 3) the frequency of the rare allele is greater 5% in the general population. The power to detect an association between an allele variant and cancer risk at a given odds ratio is shown in Table 9.

Table 9

Odds ratio	5% Prevalence **Power**	20% Prevalence **Power**	40% Prevalence **Power**
1.5	0.55	0.93	0.98
1.8	0.86	0.999	1
2	0.95	1	1
2.5	0.997	1	1

ANALYSIS OF ALLELE VARIANT GENES AFTER STRATIFICATION BY RACE

We assume 1) a study population of 200 cases and 200 controls, and 2) two-sided alpha = 0.05, and 3) a prevalence of 5%, 20% or 40% for the disease-associated allele. The power to detect an association between an allele variant and cancer risk at a given odds ratio is shown in Table 10.

Table 10

Odds ratio	5% Prevalence **Power**	20% Prevalence **Power**	40% Prevalence **Power**
1.5	0.35	0.71	0.82
1.8	0.62	0.95	0.99
2	0.76	0.99	0.998
2.5	0.94	1	1

DETECTION OF A GENE-ENVIRONMENT INTERACTION - ANALYSIS OF ALLELE VARIANT GENES AFTER STRATIFICATION BY EXPOSURE

We assume 1) a study population of 400 cases and 400 controls, and 2) two-sided alpha = 0.05, and 3) a prevalence of 20% for the disease-associated allele, and 4) a prevalence of 25% for exposure. We model the control =0, and case=1, and specify the relationship to exposure with a logistic regression model. We categorize exposure into quartiles and assume a linear trend. We will calculate an odds ratio for 4th quartile (highest exposure) compared to first (lowest exposure or baseline). The power to detect an association between an allele variant and cancer risk after stratification for exposure is given in Table 11.

Table 11

Risk Factor	**Prevalence**	**Odds ratio**	**Power 400/400**
Genotype	0.2	1.5	0.93
		1.8	0.999
		2	1
		2.5	1
Genotype + Exposure[1]	0.05	1.5	0.55
		1.8	0.86
		2	0.95
		2.5	0.997

[1] 4th quartile compared to first

DETECTION OF A GENE-ENVIRONMENT INTERACTION - ANALYSIS OF ALLELE VARIANT GENES AFTER STRATIFICATION BY RACE AND EXPOSURE

We assume 1) a study population of 200 cases and 200 controls, and 2) two-sided alpha = 0.05, and 3) a prevalence of 20% for the disease-associated allele, and 4) a prevalence of 25% for exposure. We model the control =0, and case=1, and specify the relationship to exposure with a logistic regression model. We categorize exposure into quartiles and assume a linear trend. We will calculate an odds ratio for 4th quartile compared to first. The power to detect an association between an allele variant and cancer risk after stratification for exposure (highest versus lowest quartile) is given in Table 12.

Table 12

Risk Factor	Prevalence	Odds ratio	Power 200/200
Genotype	0.2	1.5	0.71
		1.8	0.95
		2	0.99
		2.5	1
Genotype + Exposure[1]	0.05	1.5	0.35
		1.8	0.62
		2	0.76
		2.5	0.94

[1] 4th quartile compared to first

5.4.3 DATA ANALYSIS PLAN

ANALYSIS OF GENOTYPES

We will analyze genotype-risk associations in the context of race or other known risk factors of PCa, and will investigate relationships between genotypes and tissue markers, disease progression and poor outcome. If we investigate the association using a priori hypotheses, we will use an unadjusted alpha = 0.05 as the cutoff for significance. If we do not use a priori hypotheses, we will adjust alpha appropriately. An a priori hypothesis would be based on existing knowledge that associates the genotype with cancer risk.

Data analysis will examine PCa occurrence, tumor stage, and outcome, in relation to allele variant genes and known risk factors of PCa. PCa cases will be grouped together and then examined separately by race. Allele variant genes will be categorized (homozygous wild-type, wild-type/mutant, homozygous mutant) for analysis. Other risk factors will also be examined as categorized variables, separated into subgroups of exposure (e.g., dichotomous, tertiles, quartiles). For an analysis by disease stage, we will stratify cases into two subgroups either by pathological stage (≤ pT2 versus ≥ pT3) or by Gleason score (≤ 6 versus ≥ 7). The outcome variables that are studied will be relapse and/or survival. The relationship between PCa and risk factors will be examined by the chi-square test, and by odds ratios with 95% confidence intervals, using the homozygous

wild-type genotype (major allele), or the lowest exposure subgroup, as the reference group. Odds ratios and 95% confidence intervals will be calculated by unconditional logistic regression. Regression analysis will be performed in univariate and multivariate models to assess associations between risk factors and cancer susceptibility, and to adjust for possible confounding factors. Case-case analyses, correlating tissue marker expression with tumor staging, hormone blood concentrations, diet or genotypes, will be done by ANOVA, with the student's t-test, or a nonparametric test, such as the Mann-Whitney U rank sum test. To look at trends, the Mantel-Haenszel test for trend will be used to assess the correlation between marker expression/frequency and categorized (e.g., low, medium, high) variables, such as tumor stage, intake of a dietary factor, or IGF-1 blood concentrations. The association between a genotype and outcome will be studied either in a logistic regression model after categorizing into a good and poor outcome group or with the Kaplan-Meier method and the log-rank test. All analyses will be performed with the STATA and SAS statistical software packages.

ANALYSIS OF GENE EXPRESSION PROFILES

The data from the Affymetrix HG-U133A array will be estimated using the RMA procedure from Bioconductor (www.bioconductor.org). Previous analysis on raw data from replicate samples with RMA indicates that the mean standard deviation of log2 intensities is 0.1 across all intensity levels. This corresponds to a typical technical error of 5%-10% (94).

The factorial study design suggests an analysis of variance. We will first examine the four groups for heterogeneity of variances by comparing the ratios of variances between groups to the F-distribution. If the distributions conform, we will follow standard ANOVA procedure. If there is significant departure from agreement, we will perform non-parametric ANOVA using the rank F-test and use the Welch t-test for two group comparisons. We will select differentially expressed genes for contrasts between racial groups at each level of tumor, and for the difference between tumor stages across racial groups. To address the multiple comparisons problem we will use a modified FDR procedure of Benjamini and Hochberg adapted to correlated data (95).

5.5 MULTI-INSTITUTIONAL GUIDELINES

Our protocol is a multi-institutional protocol for which the NCI is the coordinating center. The proposed study will be conducted through a service contract to our laboratory. The contractor is the University of Maryland Pathology Department. The University of Maryland Medical Center and the Baltimore Veterans Affairs Medical Center are participating hospitals. The MPA number of the 2 participating hospitals is M1174. Contact addresses at the participating hospitals are provided on page two of this protocol.

The PI of the study, Dr. Stefan Ambs, will seek Institutional Review Board (IRB) approval of the study from the NCI IRB, followed by IRBs at the University of Maryland and the Veterans Administration Office for Research. He will provide the NCI IRB with a copy of the participating institution's IRB approval prior to enrolling subjects. He will also provide the NCI IRB with a copy of the participating institution's approved yearly continuing review and with copies of all amendments, consents and approvals from each participating institution.

5.6 DATA AND SAFETY MONITORING PLAN

The resource contractor at University of Maryland will conduct the study and is responsible for data collection and storage. The collected data will be monitored as follows. The contractor is required to quality assess the questionnaire data, as described under 5.1. A small random sample of subjects will be re-contacted to inquire about their interview experience, to note any problems, and to confirm that the interview actually took place. The PI, Dr. Stefan Ambs, and the NCI Project Officer, Dr. Glenwood Trivers, together with the consulting epidemiologist under the contract, Dr. Christopher Loffredo, will monitor the study through weekly conference calls, regular meetings, and an annual site visit. The contractor will be required to provide a written monthly update, a six-month report, and a year-end report. The data that are particularly monitored are the accrual rates, participation rates, age and race distribution, the status of frequency matching, and the number and stage distribution of fresh-frozen tumor specimens. The PI, the associate investigators, and the consulting epidemiologists, will thoroughly review the study once per year at the annual site visit for the contract.

6 HUMAN SUBJECTS PROTECTIONS

The protection of study subjects from research risk will be achieved by several mechanisms. Prior to enrolling subjects, we will obtain approval of the study from the IRB at the NCI, followed by the IRBs at the University of Maryland and the Veterans Administration Office for Research. Written informed consent will be required from the study subjects for participation. The form will state that individual results will not be provided to the participants. Only overall study results and progress can be provided if requested by a subject. According to new HIPAA regulations ("HIPAA Privacy Rule"), a separate written authorization to obtain, use and disclose protected health information for research will be required from the study subjects for participation in the study.

Study subjects' confidentiality will be maintained at all times. Subjects will be assigned unique study ID numbers. These unique study numbers will be linked to the subject's identifier information in a database, and to the hard copy of the identifier sheet. This information will be secured at the University of Maryland at Baltimore. The database has two levels of password security, which will only allow authorized individuals to access the information. A log will automatically record who assessed the information, and what information was obtained. The identifier sheets from the questionnaire will be physically separated from the questionnaire, and stored at a secured location. The questionnaire will retain only the unique study number. Biological samples will be labeled with the unique study number but no other identifier information. No identifier information that can be linked to study results, or other data, will leave the University of Maryland at Baltimore premises. Thus, biological samples and results from the analyses of biological samples can neither be linked back to the participant nor be used in any way to identify the participants.

Identifier information for non-participants will also be recorded to avoid a re-contact. This information will be stored in a database with two levels of password security, which will allow only authorized individuals to access the information. A log will automatically record who assessed the information and what was assessed. Non-

participants will be assigned a unique study number. This number will be used for tracking of reasons for non-participation, and for available demographic information.

6.1 RATIONALE FOR SUBJECT SELECTION

We will recruit study subjects who are male and either of African-American or Caucasian descent. Eligible subjects have to be age 40 to 90 years. Women, children, and institutionalized patients, are excluded from the proposed study. PCa is mainly a disease of older men and does not develop in women and children. The nonhereditary disease rarely occurs at an age younger than 40 years. Very few PCa patients at the 2 participating hospitals are younger than 40 years of age. Males with a race/ethnic background other than being African-American or Caucasian are not recruited because of our specific study hypothesis that PCa biology in African-Americans and Caucasians is different. In addition, less than 10% of the PCa patients at the Baltimore Veterans Affairs Medical Center and the University of Maryland Medical Center have an ethnic background that is not African-American or Caucasian. The inclusion of such a small number of cases would not allow a risk analysis based on stratification by race. We will not recruit cases that are older than 90 years of age because of co-morbidity considerations, and the additional difficulty of matching those cases with population-based controls.

6.2 PARTICIPATION OF CHILDREN

Children are excluded from our study, as discussed under 6.1.

6.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Applicable for clinical trial protocols. Not applicable for our study.

6.4 RISKS/BENEFITS ANALYSIS

We are proposing an epidemiological study that is not a clinical investigation. Participating subjects will not receive treatment under the protocol. The only medical procedure is the collection of blood conducted by a nurse or trained phlebotomist. Thus, the treatment-related risk under the protocol is minimal. The main potential risk to participants is a loss of confidentiality about their disease or about their susceptibility to develop the disease. An interview may also cause distress for some of the participants. There will be no direct benefits for study participants. It is hoped that the research data will improve our understanding of PCa biology with respect to causes of the health disparity between African-Americans and Caucasians. Our research is also aimed to identify genetic markers that are useful prognostic markers for PCa.

STRATEGIES TO MINIMIZE RISK

- Study subjects' confidentiality will be maintained at all times. The guidelines are described in paragraph "Human Subject Protections". Our consent form will also state that individual results will not be provided to the participants. Only overall study results and progress can be provided if requested by a subject.
- All interviewers will be trained personnel. During the study, characteristics such as interview duration, number of interview problems reported, number of refusals, distribution of subject answers, and number of missing and incomplete answers will be evaluated. The reviews are aimed to improve the performance of the interviewers and to minimize the stress that is experienced by an interviewed person.

6.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

CONSENT PROCEDURE FOR CASES

A candidate participant is identified through hospital records and contacts with the Departments of Pathology and Urology. After a non-objection statement by the treating physician has been obtained, a nurse will contact the patient to get both informed consent and authorization to obtain, use and disclose protected health information for research. Severely ill patients in the intensive care unit will not be approached. If a patient is found to be unable to give informed consent, the consent procedure and the enrollment into the study will be stopped. The nurse will explain the purpose of the research study to assure that everything is understood. The patient will be given the option that either he reads the form by himself or that the nurse will read the form to him. The nurse will answer questions. The consent form describes the purpose of the study, procedures, risks and potential discomforts, benefits, and the independence of the quality of medical care from the decision to participate in the study. The form explains the confidentiality of the study, the right to withdraw from the study at anytime, and the protection of privacy as it relates to genetic testing. Consent for participation in the study has been obtained when the patient and the research study coordinator have both signed 2 identical consent forms. One form will be given to the patient and the other is put in the patient's study file. The proposed consent form for cases is attached to this protocol.

CONSENT PROCEDURE FOR CONTROLS

A candidate participant is identified through a DMV database. After a home phone number is confirmed, an interviewer will contact the subject to verify eligibility and the willingness to participate in the study. If the two criteria are fulfilled, the interviewer will schedule a meeting at a location that is convenient for the subject. If the subject is found to be unable to give informed consent, the consent procedure and the enrollment into the study will be stopped. The interviewer will explain the purpose of the research study to assure that everything is understood. The patient will be given the option that either he reads the form by himself or that the interviewer will read the form to him. The interviewer will answer questions. The consent form describes the purpose of the study, procedures, risks and potential discomforts, benefits, and the independence of

the quality of medical care from the decision to participate in the study. The form explains the confidentiality of the study, the right to withdraw at anytime, and the protection of privacy as it relates to genetic testing. Consent for participation in the study has been obtained when the patient and the research study coordinator have both signed 2 identical consent forms. One form will be given to the patient and the other is put in the subject's study file. The proposed consent form for population-based controls is attached to the protocol.

7 DATA REPORTING

The resource contractor will collect all data that pertain to the proposed study. The data will be stored in a central database at the University of Maryland, as described under 5.1. The contractor will provide data summaries in the semi-annual and annual reports. The database can be queried upon request from NCI investigators. The contractor will provide query results and statistical analysis. The contractor may also provide data in a file format to the NCI researcher for review and analysis at NCI.

8. PHARMACEUTICAL AND INVESTIGATIONAL DEVICE

Not applicable for our study.

9 ATTACHMENTS

1. MAIN QUESTIONNAIRE
2. SUPPLEMENTAL QUESTIONNAIRE
3. QUESTIONNAIRE APPROVAL LETTER
4. PRMC APPROVAL LETTER
5. PROPOSED CONSENT FORMS FOR CASES AND CONTROLS

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