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<b>13. ABSTRACT (Maximum 200 Words)</b>  The goals of this project are to (1) provide a formal evaluation of variation at three candidate gene regions for prostate cancer (the androgen receptor gene, 5 $\alpha$ -reductase type 2 gene, and the chromosome X region Xq27-28) in 2,000 clinically evaluated unrelated men, and (2) exploit the evolutionary history of haplotypes in order to determine if haplotypic differences account for phenotypic variation in prostate cancer, prostate specific antigen (PSA) levels and disease progression. To date we have collected blood and clinical data from about 1700 unrelated men with and without prostate cancer. Molecular genotyping of three polymorphisms within the androgen receptor has been performed in 1700 individuals and three polymorphism within the 5 $\alpha$ -reductase type 2 gene in about 1100 of the subjects. In addition, five microsatellites have been typed within the candidate gene region in and around Xq27-28 for all subjects. Preliminary analyses reveal significant linkage disequilibrium between the androgen receptor markers in African Americans and suggest a strong correlation between specific androgen receptor haplotypes and prostate cancer. The other two gene regions have yet to be analysed for association with prostate cancer.			
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## **INTRODUCTION**

For unknown reasons, prostate cancer incidence and mortality rates for African American males are among the highest in the world. Very few hereditary prostate cancer studies have included African Americans. This is unfortunate, especially since the population history of African Americans is quite different than other populations. Thus, genetic predisposition to a common disease like prostate cancer may also be different. The identification of susceptibility genes will provide insight into critical rate limiting steps in the carcinogenic pathway of both inherited and sporadic cases of the disease. The specific goals of this project are as follows: (1) Extraction of genomic DNA from blood collected from 2000 unrelated men (1500 African Americans and 500 European Americans) from Columbia, South Carolina; Chicago, Illinois; and Washington, D.C. (2) The genotyping of microsatellite (STRs) loci and single nucleotide polymorphisms (SNPs) in order to construct compound haplotypes from three candidate genomic regions. (3) Analyze the effects of differences between haplotypes on the vulnerability to prostate cancer and related PSA levels using cladistic association analysis (Templeton et al., 1987). Our expectations for this project are to determine if any of the candidate gene regions from a large sample of clinically evaluated and unrelated African American males are significantly associated to prostate cancer and related physiological biomarkers.

## **BODY**

The specific aims for the first 12 months as listed in the Statement of Work are as follows:

Task 1. Start-up phase and subject recruitment (Months 1-5).

- Recruit and hire a research associate.
- Identify and recruit subjects into study.
- Evaluate clinical status of subjects.

Task 2. Data collection (Months 3-20).

- Extraction of genomic DNA from blood samples.
- Genotyping of DNA samples.
- Collection of Epidemiological data.

Task 3. Interim analyses (Months 10-22).

- Infer haplotypes from genotypic data.
- Enter genetic data and epidemiological data into database.
- Perform preliminary data analysis.

## **TASK 1:**

Dr. Kittles has established individual collaborations with Srinivasan Vijayakumar, M.D., a radiation oncologist, from the University of Chicago and Michael Reese Hospital in

Chicago; Sally Weinrich, Ph.D. at University of South Carolina, Columbia, SC; and Chiledum A. Ahaghotu, MD., a urologist at Howard University Hospital. Over the past year, the collaborators have been quite successful in recruiting a cohort of cases and controls from the African American and European American communities in Columbia, SC, Chicago and Washington, DC. The table below details the numbers recruited thus far.

<b>Population</b>	<b>Prostate cancer patients</b>	<b>Age matched controls</b>	<b>Total</b>
African Americans	510	705	1,215
European Americans	200	300	500
<b>TOTAL</b>	<b>710</b>	<b>1,005</b>	<b>1,715</b>

### **TASKS 2 and 3:**

Genomic DNA has been extracted from all blood specimens collected using a slight variation of the Puregene DNA extraction protocol. We have started genotyping the androgen receptor gene trinucleotide repeat polymorphisms. The androgen receptor (AR) interacts with androgens to promote cell division (normal and malignant) in the prostate gland. The AR binds dihydrotestosterone and stimulates the transcription of a cascade of androgen responsive genes. Because of this relationship, it has been proposed by many that the AR may be one genetic predictor of susceptibility to prostate cancer. There are two polymorphic regions in the N-terminal protein domain of the AR, which are encoded in the first exon of the AR gene. These are the polyglutamine repeat region (CAG)<sub>n</sub> and the polyglycine repeat region (GGC)<sub>n</sub> (Stanford et al., 1997; Irvine et al., 1995; Giovannucci et al., 1997; Edwards et al., 1992). We genotyped the CAG and GGC loci for approximately 950 individuals (1063 chromosomes) using florescent-dye labeled PCR primers and the ABI 377 DNA sequencer. Control populations we have examined thus far for the CAG and GGC markers include African Americans (N=520), Gold Coast Africans (Nigeria and Ghana, N=85), Sierra Leoneans (N=210), European Americans (N=85), Amerindians (N=103), and Asians (Chinese, N=60).

A total of 27 CAG (range 5-31 repeats) and 23 GGC alleles (range: 2-24 repeats) were observed. Not surprisingly, African Americans had the most alleles. The European and Asian populations possessed the least number of alleles at the two loci. Populations of African descent possessed significantly shorter repeats than non-African populations (paired t-test,  $p < 0.00001$ ). The entire range of CAG repeat variation was observed among populations of African descent. Interestingly, the purported high-risk CAG repeat lengths  $< 20$ , were most prevalent among populations of African descent. The non-random association of CAG and GGC alleles, linkage disequilibrium (LD), was assessed for each study population. Significant evidence for linkage disequilibrium between the two markers was observed only among African Americans cases and controls ( $p = 0.00001$ ) and Amerindians ( $p = 0.009$ ). The LD observed in Amerindians was consistent with their population history of recent population bottlenecks. The high level of linkage

disequilibrium among African Americans is likely due to admixture. This assessment of linkage disequilibrium in the African American population is quite significant for several reasons. First, the high level of stratification in the African American population may be a confounder in disease association studies if the substructure is not controlled for. Secondly, the identification of high-risk haplotypes is potentially more powerful in disease studies than single locus analyses. A preliminary analysis of androgen receptor haplotype risk and prostate cancer has revealed an association of closely related haplotypes with high-grade cancer. We intend to increase the resolution in identification of these possible high-risk haplotypes by typing single nucleotide polymorphisms (SNPs) within the gene.

Another gene we have studied is the human steroid  $5\alpha$ -reductase type 2 gene (SRD5A2) located on chromosome 2. SRD5A2 encodes the isoenzyme  $5\alpha$ -reductase, which is responsible for the intracellular conversion of testosterone to its reduced form, dihydrotestosterone (DHT). DHT promotes prostate cell division and may be involved in benign and neoplastic growth of the prostate in elderly men (Labrie et al., 1993). It has also been suggested that differences in androgen synthesis and metabolism may be responsible for ethnic variation in prostate cancer risk (Ross et al., 1992). Thus genetic variability of the SRD5A2 gene and subsequent enzyme activity may be important risk factors in prostate cancer. A dinucleotide repeat (TA) marker has been observed in exon 5 of the gene. Preliminary studies have shown that, like the androgen receptor CAG and GGC repeat loci, allelic distributions of this polymorphic marker vary considerably between high-risk and low-risk populations (Reichardt et al., 1995). Similarly to the androgen receptor, the TA-repeat and a SNP which creates the loss of an RsaI restriction site within the SRD5A2 gene has been typed for all the samples collected thus far. In addition we have started screening the entire gene for SNPs using a core set of DNA samples from our cohort. Exon 1 has been screened and sequenced for about 60 samples of men with prostate cancer, 10 African control samples, and 25 Asians. We are currently characterizing a SNP in this exon, which contributes to a loss of a BstUI site. We plan to type this SNP using florescent labeled primers and the ABI 377 sequencer and include it with the two other markers, the (TA) repeat located in exon five, and an RsaI RFLP in order to create haplotypes.

Another genetic region that has been shown to play a role in hereditary forms of prostate cancer is on chromosome X. Evidence for a prostate cancer susceptibility locus on the X chromosome has been observed using linkage analysis on certain families by NHGRI investigators (Xu et al., 1998). The region implicated, Xq27-28 is not near the androgen receptor. In fact more than 50cM separates the suspected locus from the androgen receptor. Five microsatellites on chromosome X near the Xq27-28 region were genotyped using florescent-labeled primers and the ABI 377 sequencer. The microsatellites included DXS8106, DXS984, DXS1193, DXS1205, and DXS1227.

## KEY RESEARCH ACCOMPLISHMENTS

- Recruitment of 1800 clinically evaluated prostate cancer patients and healthy volunteers.
- Collected over 5,400 genotypes from the androgen receptor gene.
- Collected over 5,200 genotypes from the steroid 5 $\alpha$ -reductase type 2 gene.
- Genotyped 5 microsatellites within and around the chromosome Xq27-28 region.

## REPORTABLE OUTCOMES

### Manuscript submission:

Kittles R, et al. (submitted to Human Genetics) Extent of linkage disequilibrium between the Androgen Receptor gene CAG and GGC repeats in human populations: Implications for prostate cancer risk assessment.

### Published Abstracts:

Kittles et al., (1999) Natural variation of the androgen receptor gene CAG repeat in human populations. *American Journal of Human Genetics*. 65(4): A207, Supplement.

### Presentations:

American Society of Human Genetics, 50<sup>th</sup> Annual Meeting, Philadelphia, PA. October, 2000. "Extent of linkage disequilibrium between the Androgen Receptor gene CAG and GGC repeats in human populations."

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## **CONCLUSIONS**

The African and African American populations are ideal study populations to examine interactions between genes and environments. The African American population possesses unique genetic features due to its history of antiquity and admixture. When a disease such as prostate cancer manifests variation in incidence and mortality between populations, admixed populations provide a population based approach to evaluate the relative importance of genetic factors. The genetic resources generated by our project will be directed towards this end and will enable us to utilize genomic technologies to characterize the functional implications of DNA variation in these populations. Since this study takes advantage of the genetics of unrelated men from diverse ethnic populations, the results may be generalizable to the larger American population. The research design is quite significant mainly because interactions between genotype and environmental influences on the disease can be evaluated and the ability to uncover genetic influences on prostate cancer is greatly enhanced. More importantly, since exposure to many environmental factors that influence genetic risk can be modified, the discovery of these interactions may have major public health implications. Finally, the assessment and publication of genetic variation within the candidate genes for prostate cancer is quite significant because it will (1) provide accessibility to our data and allow others to compare their data on other populations for the same markers; (2) encourage others to study the same markers in other populations so that their populations can be placed into a global framework; and (3) stimulate researchers to develop new models and methods to analyze the data.



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