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Prostate cancer is the most common cancer in American men. It is also characterized by a substantial racial/ethnic variation in risk: highest in African-American men, lowest in Asian men and intermediate in Caucasian and Latino men. We propose to investigate genetic variants of genes involved in the regulation of prostatic growth and particularly in androgen metabolism, particularly the HSD3B2 gene which encodes the type II b-hydoxysteroid dehydrogenase. Our current progress is highlighted by the following three findings. First, our data indicate that the locus under investigation is polymorphic in constitutional DNA and mutated somatically in tumor tissue. Furthermore, our population-based investigations suggest that at least one polymorphism in the HSD3B2 gene is relatively common and in Hardy-Weinberg equilibrium in our population. Finally, our initial biochemical results point to an interesting regulation of the activity of this bifunctional enzyme.

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Table of Contents

PROGRESS REPORT

Juergen K. V. Reichardt, PhD

Introduction/Overview

This proposal is part of a research program aimed at identifying genes involved in the predisposition to and progression of prostate cancer among various racial/ethnic groups in the US. Prostate cancer will be diagnosed in 180,400 men in the US in the year 2002 alone. Some 31,900 individuals die of this disease annually. Prostate cancer is characterized by substantial racial/ethnic variation in risk: highest in African-American men, lowest in Asian men and intermediate in Caucasian and Latino men. We propose to investigate as our central hypothesis that genetic variants of genes involved in the regulation of prostatic growth and particularly in androgen metabolism by themselves and in combination significantly contribute to prostate cancer risk and progression. Specifically, we propose to examine the hypothesis that DNA sequence variations in the type II 3β-hydoxysteroid dehydrogenase (HSD3B2) gene contribute substantially to the risk of prostate cancer particularly across racial/ethnic lines. The "candidate gene", HSD3B2, was chosen because the reaction substrate [i.e. dihydrotestosterone (DHT)] of the enzyme encoded by this gene modulates directly cell division in the prostate. Epidemiologic evidence suggests that variation in DHT levels play an important role in risk of prostate cancer. Thus, 3β-hydoxysteroid dehydrogenase activity encoded by HSD3B2 variant alleles may be important in regulating intraprostatic DHT steady state levels by controlling its degradation. This candidate gene encodes the enzyme that initiates the irreversible inactivation of DHT.

Specifically, in this project we propose to test, using a case-control study approach within a multi-ethnic cohort study design, the association between prostate cancer risk and its progression and HSD3B2 allelic variants among four major racial-ethnic groups. Our three interrelated specific aims are:

- To identify all allelic variants in the HSD3B2 locus by sequencing 200 men from four racial/ethnic groups (African-American, Japanese-American, Latino and Americans of European ancestry (Caucasian) men).
- To determine the relationship between the HSD3B2 gene and prostate cancer by genotyping polymorphic DNA markers in the HSD3B2 gene in up to 800 men with prostate cancer and controls from four racial/ethnic groups who are at very different risks of prostate cancer.
- To determine the *in vitro* biochemical properties of HSD3B2 variants identified in specific aims 1 and 2.

Progress Report

Pursuant to specific aim 1, we have begun sequencing constitutional ("germline") DNA 120 men with the following make-up: 30 from each of the four racial/ethnic groups (African-American, Asian-(Japanese-)American, Caucasian and Latino. Half will are prostate cancer cases, half are controls. These samples are in addition to the 69 reported under "Preliminary Data" of the grant. Our total sample, therefore, will be 189. Our

preliminary sequencing has already identified two additional polymorphisms that change amino acids: E94Q where the normal glutamate is replaced by glutamine and T366N (threonine to aspartate). This is in addition to two polymorphisms (D74N and L236S) which we found previously (Fig. 1).

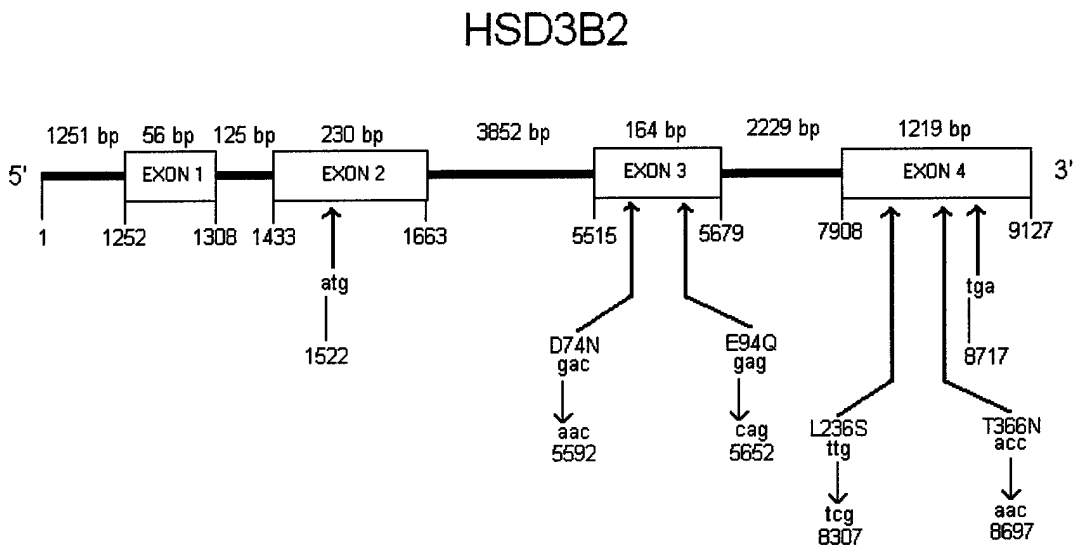

Figure 1: Newly discovered constitutional ("germline") SNPs in the HSD3B2 gene.

We have also sequenced 30 tumors and found a large number of somatic mutations which are not present in matched constitutional DNA (Fig. 2).

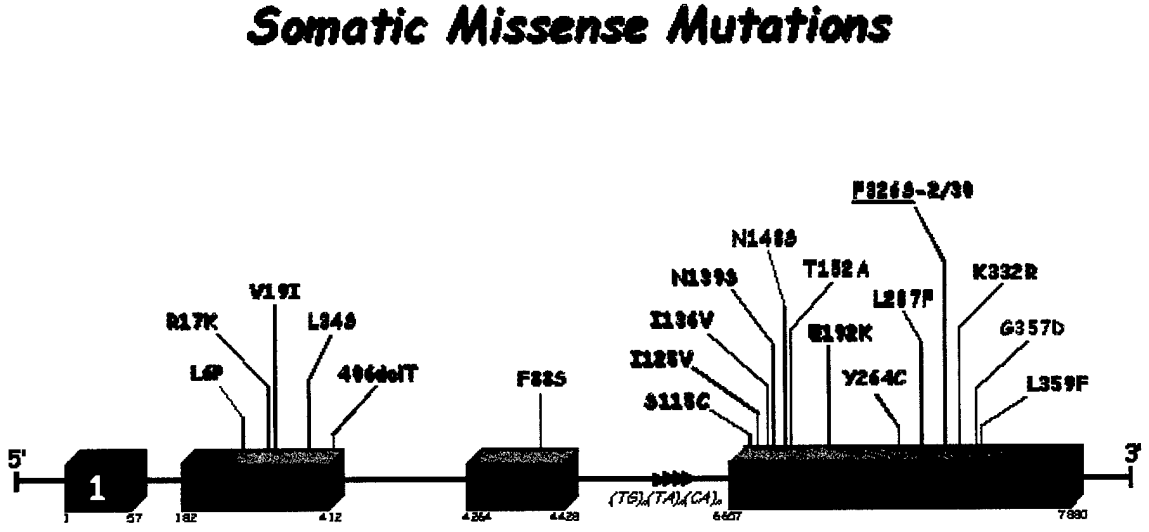

Figure 2: Somatic mutations in the HSDS3B2 gene.

The P326S somatic mutation (proline-326 replaced by serine) is a recurrent somatic mutation since it is found twice in the 30 tumors we examined.

In order to advance specific aim 2 we have genotyped 588 men for the L236S constitutional DNA polymorphism (cf. Fig. 1). The polymorphism was found 10 times in the heterozygous state and was in Hardy-Weinberg equilibrium. There was, however, no significant association with disease. We, therefore, plan to genotype up to 1,000 men with prostate cancer and cohort controls for this and other polymorphisms (cf. Fig. 1). We are contemplating increasing the sample size because of the data reported above that we obtained for the L236S polymorphism.

We have made progress in specific aim 3 on the *in vitro* biochemistry on several fronts. We have established optimal pHs for the two activities, isomerase/dehydrogenase and reductase, which differ by about 2 units (or a 100-fold difference in H^+ ion concentration), time course etc. We have reconstructed all four missense SNPs in constitutional DNA (cf. Fig. 1) and plan to assay them soon. We have reconstructed roughly half of the somatic mutations that change amino acids (cf. Fig. 2). We expect to complete the mutagenesis soon and will then assay all mutants.