GEN GEN: THE GENOMIC GENETIC ANALYSIS OF ANDROGEN-METABOLIC GENES AND PROSTATE CANCER AS A PARADIGM FOR THE DISSECTION OF COMPLEX PHENOTYPES

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1. ABSTRACT

Prostate cancer will be diagnosed in about 179,300 men in the US in 1999 alone. Some 37,000 individuals die of this disease annually. Prostate cancer is 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 set out to investigate as our central hypothesis that genetic variants of genes involved in androgen metabolism by themselves and in combination significantly contribute to prostate cancer progression and its racial/ethnic variation. Specifically, we examined the hypothesis that DNA sequence (allelic) variations in the type II (or prostatic) steroid 5alpha-reductase (SRD5A2) gene contribute substantially to the risk and progression of prostate cancer particularly across racial/ethnic lines. The "candidate gene", SRD5A2, was chosen because the reaction product [i.e. dihydrotestosterone (DHT)] of the enzyme encoded by this gene modulates directly cell division in the prostate. DHT binds to the androgen receptor (AR) and the DHT-AR complex leads to the transactivation of a variety of genes which ultimately modulates cell division in the prostate. Epidemiologic evidence suggests that variation in DHT levels play an important role in risk of prostate cancer. Thus, steroid 5alpha-reductase activity encoded by SRD5A2 variant alleles may be important in regulating intraprostatic DHT steady state levels by controlling its biosynthesis. A second candidate gene, the type II 3beta-hydoxysteroid dehydrogenase (HSD3B2) gene, encodes the enzyme that initiates the metabolic inactivation of testosterone (T) to DHT. We have identified allelic variants in this gene as well.

Here I review our strategy for identifying candidate genes for prostate cancer, a multifactorial disease. I summarize the significant findings, particularly of allelic variants in the HSD3B2 and SRD5A2 genes and discuss how they by themselves, in combination and through interactions with the environment may play a role in prostate cancer predisposition and its progression. Our approach, a multidisciplinary genomic genetic (GEN GEN) attack on the problem, may be useful in the analysis of other complex phenotypes as well.

2. INTRODUCTION

In the US some 179,300 men will be diagnosed with prostate cancer in 1999 alone and 37,000 men will die of the disease this year (1). In fact, prostate cancer is the most commonly diagnosed malignancy among men in this country (1).

Prostate cancer is rare before the age of 40, but the rate of increase thereafter is greater than for any other cancer (2). There is a large variation in prostate cancer rates between racial/ethnic groups in the US. In Los Angeles, African-Americans, who have by far the highest prostate cancer rates in the world, have a prostate cancer rate that is 70% higher than that of Caucasians (non-Latino Whites), who have a substantially higher rate than Latinos (L-Whites), while Chinese- and Japanese-Americans (Asians or Asian-Americans) have still lower rates, roughly one-half those of Caucasians (3). A most striking feature of prostate cancer epidemiology has been the great international variation in reported rates. China and Japan, have among the lowest prostate cancer rates, 1/8th to 1/20th the rates in the U.S. (3). These epidemiologic data strongly suggested a substantial genetic component to prostate cancer risk, which is differentially expressed among various racial/ethnic groups. Finally, there is substantial evidence of more aggressive disease and a less favorable outcome (e.g. survival) for African-American prostate cancer patients than their Caucasian counterparts (4).

There is also a significant familial component to risk of prostate cancer. First-degree relatives of men with prostate cancer have roughly three times the risk of prostate cancer of men with no such history. In young men, the familial form is most consistent with an autosomal dominant mode of inheritance (5). The contribution of this familial susceptibility locus to all prostate cancer is estimated to be about 9% (5). A candidate locus, HPC1 (hereditary prostate cancer 1), was reported about three years ago on the long arm of chromosome 1 (located on 1q24-25) (6). Linkage to this locus in familial prostate cancer was not confirmed by some groups (e.g. 7). Significant linkage (LOD=4.6) to a locus on the X-chromosome (mapping to Xq27-28; 8) and more modest linkage (LOD=2.7) to a second locus on the long arm of chromosome 1 (which maps to 1q42.2-43; 9) were recently reported (9).

Classic cytogenetic investigations have identified several commonly rearranged chromosomal locations in prostate cancer. These include very common translocations, deletions and inversions on the short arm of chromosome 2 (10). It is noteworthy in this context that the SRD5A2 gene is located precisely on that arm (in band 2p23; 11).

3. ANDROGEN ACTION

Androgens play a critical role in normal and abnormal prostate development. Studies of androgens and prostate cancer go back nearly 60 years (12).

Normal prostate development is induced by DHT which is formed from testosterone (T) by the enzyme steroid 5alpha-reductase (13). T is produced in large amounts primarily by the testes (13). T is then irreversibly metabolized intracellularly to DHT. DHT (or, less efficiently, T) is bound by an intracellular cytosolic receptor, the androgen receptor (AR). This complex is then translocated to the cell nucleus where it activates transcription of genes with androgen-responsive elements (ARE) in their promoters (13). DHT is known to promote DNA synthesis and cell replication in the prostate (13). DHT can be inactivated in the prostate by further reduction to 3alpha- or 3beta-androstanediol which circulate as glucuronide conjugates.

In summary, DHT steady state levels are determined a) by its biosynthesis (catalyzed by steroid 5alpha-reductase which is encoded by the prostate-specific SRD5A2 gene; 14) and b) by its degradation initiated through reductive inactivation by the 3alpha- and 3beta-hydroxysteroid dehydrogenase enzymes. In fact it has been reported that these two sets of reactions (catalyzed by steroid 5alpha-reductase and the two 3-hydroxysteroid dehydrogenase enzymes) are by far the most active in the human prostate (15).

4. STEROID 5ALPHA-REDUCTASE

Steroid 5alpha-reductase (testosterone 5alpha-reductase; EC 1.3.99.5) is a membrane-bound enzyme that catalyzes the irreversible conversion of T to DHT with NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) as a cofactor (13). Two isozymes exist: the type I enzyme with an alkaline pH optimum, encoded by the SRD5A1 gene, and the type II isozyme with an acidic pH optimum, encoded by the SRD5A2 gene (14). Thigpen *et al*. (14) reported immunologic studies that showed that the type I enzyme is expressed primarily in newborn scalp, and in skin and liver. The type II isozyme protein is expressed primarily in genital skin, liver and the prostate (14).

Cloning and characterization of the SRD5A2 gene has demonstrated that it is located on the short arm of human chromosome 2 (band 2p23) spanning over 40 kb of genomic DNA in five exons (14).

We have reported a number of investigations on genetic variation in the prostatic steroid 5alpha-reductase gene and its implications for prostate cancer risk (16, 17, 18). Highlights of these investigations include the first report of racial/ethnic variation at the SRD5A2 locus which was identified by examining a polymorphic $(TA)_n$ dinucleotide repeat marker (16). We have also reported the identification allelic variants that may reduce risk in Asians (17): the V89L substitution which replaces valine at codon 89 with leucine and reduces enzyme activity. Finally, we have identified a missense mutation, A49T (alanine-49 to threonine), that increases the risk of prostate cancer in African-American and Latino men significantly and increases steroid 5alpha-reductase activity five-fold (18).

We have also investigated the SRD5A2 gene and its possible contribution to tumor progression (19). We reported common somatic genetic alterations in the SRD5A2 gene in prostate tumors at the polymorphic $(TA)_n$ dinucleotide repeat in its 3' UTR (19).

5. 3BETA-HYDROXYSTEROID DEHYDROGENASE

DHT -as discussed above- is the most active intraprostatic androgen (13). It is synthesized from testosterone by the enzyme steroid 5alpha-reductase and DHT is inactivated through a reductive reaction catalyzed by 3alpha- or 3beta-hydroxysteroid dehydrogenase (13). Both reductions are reversible and use NAD(P)H (nicotinamide adenine dinucleotide (phosphate) in its reduced form) as a cofactor (13). Thus, the 3beta-dehydrogenase reaction initiates the irreversible inactivation of DHT in the prostate. Therefore, this enzyme is critical for the regulation of intraprostatic DHT steady state levels by controlling its degradation rate. Finally, it has been reported that activity of the two 3-hydroxysteroid dehydrogenase enzymes is significantly lower in abnormal prostatic tissue (15). Thus, DHT might accumulate because of slowed degradation.

3Beta-hydroxysteroid dehydrogenase in humans can act on a number of steroid substrates, including DHT (20). Enzyme activity is encoded by two closely linked yet distinct loci: the HSD3B1 and HSD3B2 genes which are both located in chromosome band 1p13 (21). The type I gene encodes the isoform present in placenta and peripheral tissue such as skin and mammary gland while expression of the type II enzyme is restricted to adrenals and gonads (20,

22). Thus, the type II enzyme encoded by the HSD3B2 gene would regulate DHT levels by initiating the inactivation of this potent androgen in the prostate.

Cloning and characterization of the human HSD3B2 gene has revealed that it spans about 7.8 kb of genomic DNA in four exons (23).

A complex dinucleotide repeat polymorphism has been reported in the third intron of the HSD3B2 gene (24). These authors discovered nine alleles of a $(TG)_n$ $(TA)_n$ $(CA)_n$ STR. We have subsequently identified substantial allelic differences, which include at least 25 different alleles, at the HSD3B2 locus among African-Americans, Asians and Caucasians (25).

6. PROSTATE CANCER PROGRESSION

Little is presently known about somatic mutations during prostate cancer progression. Classic cytogenetic investigations (reviewed in 10) revealed very common chromosomal abnormalities on the long arm of chromosome 10 and the short arm of chromosome 2. More recently investigators have focused on candidate genes for somatic mutations such as certain oncogenes or tumor suppressor genes. *De novo* mutations in the tumor suppressor gene p16 have been identified but are rare (26). Somatic mutations in the p53 tumor suppressor gene have also been described as infrequent in most series (e.g., 27, 28, 29). Mutations in the AR (androgen receptor) gene occur as somatic mutations during tumor progression (e.g. reviewed by 30; see also 31, 32). Examination of the PTEN protein phosphatase tumor suppressor gene on 10q23 have revealed important somatic mutations involved in tumor progression (e.g. 33, 34, 35, 36). There is growing evidence that prostate cancer may in fact be multifocal in origin characterized by different somatic mutations in different tumor foci of the same prostate (e.g. 29, 37). Microsatellite instability appears commonly during prostate cancer progression (e.g. 38, 39).

Molecular studies by Southern blotting of the type I 5 alpha-reductase gene did not uncover any abnormalities in prostate carcinomas (40), but we have molecular evidence that SRD5A2 abnormalities may be commonplace in tumor tissue (19).

7. FUTURE PROSPECTS

Future investigations will include the thorough examination of the regulatory elements in the SRD5A2 gene and mutations in the entire HSD3B2 gene. These investigations will examine both constitutional ("germline") and tumor DNA. Mutations will be characterized biochemically and epidemiologically. Their individual contributions and in concert ("gene-gene interaction") will be examined along with possible environmental contributors ("gene-environment interaction"). Diet, and in particular animal fat intake which contains cholesterol and steroids, are likely to be important factors.

8. GEN GEN

The genomic genetic analysis (GEN GEN) of multifactorial human phenotypes is likely to lead to significant insights into the etiology of complex diseases. One can envisage a strategy that takes advantage of the decades of epidemiologic investigations into human diseases as guidelines to identify likely pathogenetic pathways. All genes in these pathways will then be subjected to rapid molecular analyses to identify likely candidate genes. We have used dinucleotide repeat markers in our investigations, although they are likely to be replaced by SNPs (single nucleotide polymorphisms) in the future. Once candidate genes are identified, they are subjected to intense molecular searches for allelic variants. These allelic variants are then examined for their contributions to the phenotype by themselves, in concert with other allelic variants in the same of other genes and finally in conjunction with environmental exposure. These investigations are likely to involve epidemiologic, molecular and biochemical methods. This convergence of various disciplines is likely to yield a genomic genetic (GEN GEN) view of complex phenotypes which will be complemented by the analysis of environmental contributions.

9. ACKNOWLDEGMENTS

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