Androgen Metabolism and Prostate Cancer: Establishing a Model of Genetic Susceptibility


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Abstract

The prostate is an androgen-regulated organ, which has led to long-standing interest in the role of androgens in prostate carcinogenesis. Although evidence of a hormonal etiology for prostate cancer is strong, it is almost entirely circumstantial. Much of the problem in proving a causal relationship relates to the continued difficulties in reliably measuring human tissue-specific exposure to endogenous steroid hormones.

The international and racial-ethnic variations in prostate cancer incidence, combined with the effects of migration on risk patterns, have suggested that whereas environmental factors are likely to be important, genetic factors might also play a central role in determining prostate cancer risk. We are developing a polygenic model of prostate carcinogenesis focused around a series of genes involved in androgen biosynthesis and androgen activation, transport, and metabolism in the prostate. In this developing model, we have initially targeted four genes based on three main criteria: (a) all encode products that play important roles in inducing androgen stimulation in the prostate; (b) all are polymorphic; and (c) all show substantial allelic variation in the polymorphic marker among the racial-ethnic groups of greatest interest in terms of prostate cancer risk. In addition to studying how the polymorphic markers of interest are related to prostate cancer development within and between racial-ethnic groups, we are concurrently evaluating whether genotypic variations correlate in the anticipated direction with biochemical parameters in vitro and in vivo.

We summarize the development of this model and the state of knowledge related to each of the genes comprising the current model. We discuss the extent to which the current model can explain demographic variation in prostate cancer risk as well as the potential for future expansion of the model to incorporate environmental risk factors as well as additional genes. The model, when fully developed, can potentially provide a basis for targeting populations for screening interventions and/or preventive strategies aimed at the multigene products or at the genes themselves.

Introduction

The scientific community has been interested in the possible role of steroid hormones in cancer development for over 50 years (1, 2). During that period, extraordinary amounts of indirect evidence have accumulated for a role of such hormones in the etiology of multiple human cancers (3). This evidence has come from animal models for such cancers as breast, endometrial, and prostate cancer and from epidemiological risk factor data. The well-established and highly reproducible hormonal risk factors for breast cancer (age at menarche, age at menopause, parity, age at first term pregnancy, lactation, physical exercise activities, and postmenopausal body mass; Ref. 4) and endometrial cancer (use of hormone replacement therapy, use of oral contraceptives, obesity, and parity; Ref. 5), in particular, come to mind. Nonetheless, despite this extensive circumstantial evidence, which in its entirety becomes compelling that hormones play a major role in the etiology of human cancer, proving a direct link between levels of steroid hormones and human cancer development has proven difficult. Much of the difficulty relates to the difficulties inherent in reliably assessing human exposure to endogenous steroids. We have summarized in detail the methodological challenges in human hormonal studies (6). Among many others, these include intra- and interbatch variability in the assays themselves, cyclical and sporadic variability in the circulating levels of the hormones, the requirement for prospective assessments to causally link hormone levels to the development of a specific disease, and the often imprecise relationship between the circulating levels of a hormone and the active intracellular concentrations of that hormone.

Although there is evidence that hormonal secretion and metabolism can be environmentally influenced (for example, through dietary change), the control of hormonal patterns is, either directly or indirectly, also genetically regulated. Based on the extensive evidence of the hormone dependency of human prostate cancer, we are developing a polygenic model of prostate cancer susceptibility. We describe below the rationale on which this prostate cancer model is being built, the current model, the preliminary data that we and others have accumulated in support of this model, and the future research directions designed to fine-tune the existing model and expand it to capture additional genetic and environmental risk factors. We begin with a brief discussion of the relevant prostate cancer epidemiology.

Prostate Cancer Epidemiology

By far, the two most important risk factors for prostate cancer are age and race-ethnicity. Prostate cancer is extremely rare before age 40, but the rate of increase with aging is greater than for any other cancer; rates increase at approximately the 9th-10th power of age (7), whereas comparable figures for other common epithelial cancers such as lung cancer and colon cancer are between 5 and 6. For prostate cancer, the rate of increase is steepest early in the age-specific curve and then gradually dissipates with age. This contrasts with some other epithelial cancers, such as colon cancer, for which the relationship between age and incidence is strictly linear in log-log units (Fig. 1).

Other hormone-related cancers, including endometrial cancer and female breast cancer, also demonstrate a nonlinear relationship between age and incidence, with the exception of grade 3 prostate cancer. Despite the increasing rate of increase, the relationship between age and prostate cancer risk is almost entirely circumstantial. Much of the problem in proving a causal relationship relates to the difficulties inherent in reliably assessing human exposure to endogenous steroids. We have summarized in detail the methodological challenges in human hormonal studies (6). Among many others, these include intra- and interbatch variability in the assays themselves, cyclical and sporadic variability in the circulating levels of the hormones, the requirement for prospective assessments to causally link hormone levels to the development of a specific disease, and the often imprecise relationship between the circulating levels of a hormone and the active intracellular concentrations of that hormone.
SHBG, sex hormone-binding globulin.

Chinese men (and probably other Asian populations such as Koreans) have the lowest known prostate cancer rates in the world. Historically, prostate cancer incidence is equally interesting. Native Japanese and can explain even a small fraction of the excess risk in this population. African-Americans have rates somewhat above those of men in their respective homelands (approximately 40–50% higher), even after attempting to adjust for these diagnostic biases (9). However, their prostate cancer incidence rates never approach those of Caucasian-Americans, much less those of African-Americans, although the Japanese, in particular, represent a highly acculturated immigrant population in the United States (10). Latino-Americans, a large and growing segment of the United States population, are another racial-ethnic group of interest epidemiologically, because their prostate cancer rates are more like those of Asian-Americans than those of their white non-Latino counterparts.

Androgens and Prostate Cancer

Over the past two decades, we have published several detailed articles on the role of steroid hormones in carcinogenesis (3, 11). Steroid hormones play a critical role in controlling cell proliferation in their primary target organs, and increased cell proliferation seems to be a common denominator in the pathogenesis of much human cancer (12). For a cell to undergo full transformation from a normal to a malignant phenotype requires a series of genetic changes resulting in the activation of proto-oncogenes and the inactivation of tumor suppressor genes. These occur through such mechanisms as somatic mutation, translocation, or amplification, but all require that cells be dividing. Genetic or environmental factors that alter the hormonal environment and thus alter the underlying rates of cell division in these target organs would be expected to alter the underlying rate of malignant transformation in these target tissues as well (11).

The prostate is a hormonally regulated organ. Testosterone diffuses freely into prostate cells, where it is rapidly and irreversibly converted to its reduced and metabolically more active form, dihydrotestosterone, through the activity of a single enzyme, steroid 5α-reductase type II. Dihydrotestosterone and, to a lesser extent, testosterone bind to the androgen receptor; this receptor/ligand complex translocates to the nucleus for DNA binding and transactivation of genes with androgen-responsive elements, including those that control cell division (13, 14).

Among mammalian species with nonrudimentary prostate glands, only men and dogs have a high naturally occurring incidence of prostate cancer (13). Moreover, prostate cancer that histologically mimics the human disease has proven difficult to produce experimentally. This lack of both experimental and naturally occurring models of the disease has dampened progress in understanding the pathogenesis of prostate cancer. The first experimental model of prostate adenocarcinoma (the human histological equivalent) was produced by Noble in 1977 (15). This rat model used testosterone propionate administered s.c. as the sole induction agent. Subsequently, additional experimental rodent models of prostate cancer have been developed, nearly all of which have an androgen requirement for tumor induction. A notable exception is the recently developed transgenic adenocarcinoma mouse prostate model (16). Although this model has histopathological characteristics in common with the human disease, it remains unclear how this model relates to human prostate carcinogenesis.

Prostate cancer is itself androgen dependent, and various methods of androgen ablation or blockade have been a mainstay of prostate cancer treatment, especially for early metastatic disease, for several decades (8). Men with highly underdeveloped prostates, such as eunuchs or men with constitutional 5α-reductase deficiency, have...
never been reported to develop prostate cancer, although there seems to be little systematic epidemiological research on this issue (8).

Whereas studies of hormone levels and prostate cancer risk are hampered by the difficulties listed above (inter- and intra-assay variability, cyclical and sporadic variations in circulating levels, the requirement for large prospective studies, and the difficulties in determining tissue-specific exposures), one well-conducted prospective study recently found that prediagnostic circulating testosterone levels were strongly predictive for prostate cancer development (17). In that study, when SHBG levels, which were strongly correlated with testosterone levels, were taken into account, a particularly strong trend of increasing prostate cancer risk with increasing levels of circulating free testosterone was observed [risks by quartile were 1.0 (low), 1.4, 2.0, and 2.6 (high); \( P = 0.004 \) for trend].

Because racial-ethnic variation in incidence is such a powerful feature of prostate cancer epidemiology, we have explored over the past decade whether underlying differences exist in the androgen milieu of populations in the middle and at the extremes of prostate cancer incidence. These studies were conceptually simple but logistically complex for reasons we have described in detail elsewhere (6). Two of these studies were particularly pertinent in providing baseline phenotype data for our polygenic model.

After establishing that hormonal differences among racial-ethnic groups might be present even in the in utero period (18), we compared testosterone levels in healthy young adult white and African-American men (19). Although prostate cancer is primarily a disease of elderly men, we focused on the young adult period for several reasons: (a) we wanted to avoid possible confounding due to the effects of other chronic diseases associated with aging; and (b) we recognized that the racial-ethnic differences in prostate cancer incidence are maximal at the earliest ages at which prostate cancer appears, suggesting that the responsible hormonal milieu must occur or first occur relatively early in life. In this study of 100 African-American and white men in Los Angeles (average age, 21 years), we found that the African-American men had testosterone levels that averaged some 19% higher than their white counterparts and were 15% higher even after adjusting for known correlates of testosterone (including body mass index, smoking and alcohol use, and time of sampling). We have argued that differences of this magnitude, sustained over a lifetime, are sufficiently large to explain the substantial differences in prostate cancer incidence (19). The bases for this argument are as follows: (a) as noted above, prostate cancer incidence rates increase as a power function of age; (b) tissue age is a function of the rate of cell division in the target tissue; and (c) a direct relationship exists between hormonal changes and prostate tissue age (20).

We later extended this study of hormone patterns in African-American and white men to include young Japanese men born and raised in rural Japan, a group of men with a low expected lifetime risk of prostate cancer compared to their previously contrasted white and African-American men (21). Contrary to our expectations, these young Japanese men had testosterone levels that were intermediate between those of whites and African-Americans, and that did not differ significantly from those of either African-Americans or whites. However, in this extended study, we also measured circulating levels of androstanediol glucuronide, a reliable index of in vivo 5α-reductase activity, in all three groups of men. The Japanese men had levels of this hormone that were 25–35% lower than the African-American men and white men (21). Chinese men in Hong Kong have been shown to have similarly low levels of androstanediol glucuronide (22). Although we were initially unable to demonstrate a difference in this hormone between African-Americans and whites, in a later study of healthy men in their 60s, we confirmed the deficit of androstanediol glucuronide in Asian men and also found elevated levels in the African-Americans compared to whites.4

We concluded from this body of work that the differences in prostate cancer incidence among African-Americans, white Americans, and Asians (Chinese and Japanese men) could be explained by differences in testosterone biosynthesis, on the one hand, and testosterone metabolism, on the other. These testosterone differences could be due to environmental influences, genetic control, or an interplay of genetic and environmental factors. The international and racial-ethnic patterns in prostate cancer incidence described above have led us to focus on genetic control.

Genes and Cancer

There are two types of germ-line (constitutional DNA) genetic alterations that predispose to cancer (23). Some germ-line genetic loci predispose directly to the disease. These single inherited genetic traits are uncommon in the population; hence, they carry with them low population attributable risks, although individuals with these genetic traits may have a very high absolute risk of developing the disease. A number of such genes, e.g., \( BRCA1, BRCA2, APC, \) and \( p53 \) (Li-Fraumeni), have been identified.

Much more common are susceptibility genes with low absolute risk but possibly high population-attributable risk, because the high-risk genotype may be quite common in the population. These types of genes, such as those involved in androgen metabolism and therefore in cell proliferation in the prostate as discussed below, do not directly cause cancer but affect the risk of cancer indirectly. They probably influence cancer risk in conjunction with one another, creating a polygenic etiology of cancer, thereby allowing the identification of individuals with high- or low-risk polygenic profiles (23).

Establishing a Genetic Model for Prostate Cancer

In developing a polygenic model of prostate carcinogenesis, genes involved in androgen biosynthesis, androgen activation and inactivation, and androgen transport are all of interest. We have initially targeted four genes: (a) all four encode products that potentially play an important role in inducing androgen stimulation of the prostate; (b) all are polymorphic; and (c) those that have been sufficiently evaluated show substantial allelic variation among the racial-ethnic groups of greatest interest in terms of prostate cancer risk. We list these genes in Table 1 along with the major activity of their product. The initially targeted polymorphic markers in these genes were chosen because there was a priori evidence that each was associated with functional change in the protein product, and/or that the polymorphic marker shows substantial interracial allelic variation among the populations at the extremes of prostate cancer risk. The major metabolic androgen pathways pertaining to the prostate and the relevant sites of activity of the gene products of current interest as they pertain to the prostate are illustrated in Fig. 2. The four genes are as follows: (a) the cytochrome \( p450c17a (CYP17) \) gene, which encodes the enzyme that regulates...

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critical steps in testosterone biosynthesis; (b) the steroid 5α-reductase type II (SRD5A2) gene, which encodes the enzyme responsible for the conversion of testosterone to the metabolically more active (in terms of androgen receptor affinity) dihydrotestosterone; (c) the androgen receptor (AR) gene, which encodes the androgen receptor allowing for androgen binding and transport, DNA binding, and transactivation of genes with androgen response elements; and (d) the 3β-hydroxysteroid dehydrogenase type II (HSD3B2) gene, which encodes one of the two β-hydroxysteroid dehydrogenase enzymes involved in the metabolism of dihydrotestosterone in the prostate and also catalyzes a critical reaction in testosterone biosynthesis (possibly by a different isozyme). Because of this dual role of HSD3B2 in the pathway of interest, it is difficult to predict precisely how functional variants of this gene might relate to prostate cancer risk.

Our primary initial goals for each of these genes are to demonstrate: (a) that the polymorphic marker of interest is related to prostate cancer risk; (b) that the prevalence of high-risk genotypes correlates with the racial-ethnic variation in prostate cancer incidence; and (c) when appropriate, that the genotypic variation correlates in the anticipated direction with biochemical variation in vitro and in vivo. Although we have begun initial exploration of all four genes, we have focused initially on two genes involved in androgen activation and transport within prostatic epithelial cells (SRD5A2 and AR). We discuss these two genes in some detail followed by brief discussions of the other two genes (CYP17 and HSD3B2).

Steroid 5α-reductase Type II (SRD5A2) Gene

There are two distinct steroid 5α-reductase enzymes encoded by two different genes. There is only a 50% homology in amino acid sequence between the two enzymes, and there is a clear distinction in optimal pH. The type II enzyme encoded by the SRD5A2 gene is of interest here, because it is active primarily in the prostate and in genital skin (24). The SRD5A2 gene contains five exons and is located on chromosome 2p. Germ-line mutations of SRD5A2 have been identified in a highly inbred kindred in the Dominican Republic (25). Boys with this mutated gene are phenotypically female and have a persistent vaginal pouch until puberty, at which time there is some phallic enlargement and development of some secondary sex characteristics, but the prostate remains highly underdeveloped. The germ-line mutations in this kindred have not been identified in the general population.

When we began our work on the SRD5A2 gene, the only polymorphic marker that had been identified was a dinucleotide (TA)n repeat in the 3' untranslated region of the gene. Moreover, this marker seemed unlikely to be highly informative because other investigators had reported that one allele [the (TA)₉ allele] accounted for over 96% of all alleles (26). Our initial assessment demonstrated that the prevalence of variant alleles using this polymorphic marker was substantially greater than initially reported, and that novel alleles existed for this polymorphism among both Asians and African-Americans (27). Although our preliminary work suggested that a series of alleles with a relatively high number of repeats [(TA)₇ or greater] was not only unique to African-Americans but was also somewhat more common in African-American men with prostate cancer than in healthy African-American control men, we had no other evidence of functional relevance per se for this dinucleotide repeat (28). Moreover, expanded data in African-Americans and other racial-ethnic groups did not provide any strong support for an association with prostate cancer. Therefore, we proceeded to sequence the SRD5A2 gene in a sample of men with either high or low circulating levels of androstenediol glucuronide (the biochemical serological correlate of prostatic 5α-reductase activity in vivo) using single-strand conformational polymorphism followed by sequencing of any aberrant single-strand con-
formational polymorphism patterns identified. We identified seven missense mutations that resulted in an altered amino acid (Fig. 3) and six nucleotide substitutions that were either silent (no amino acid change) or intronic. Among the missense mutations, only one mutation, the valine to leucine substitution at codon 89 (V89L substitution), appeared to be sufficiently common to possibly explain a substantial proportion of prostate cancer incidence (29). We examined the distribution of this polymorphic marker in 286 control men from the racial-ethnic groups of greatest interest. The valine 89 homozygote genotype (VV) was the most common genotype in both African-Americans (59%) and whites (57%) but was much less common in Chinese and Japanese men (29%). On the other hand, the leucine 89 homozygote genotype (LL) was most common in Chinese and Japanese men (22%) but accounted for the genotype of only 4% of whites and 3% of African-Americans [the differences between Asians and the other two groups were highly statistically significant (P < 0.001; Ref. 29)]. Among Asians, there was a strong correlation between the V89L genotype and the circulating levels of androstenediol glucuronide, with VV homozygotes having a median level of 4.7 ng/ml (n = 30), VL heterozygotes having a median level of 4.0 ng/ml (n = 50), and LL homozygotes having a median level of 3.4 ng/ml (n = 22; P = 0.04).

A second missense mutation resulting in an alanine to threonine substitution at codon 49 (A49T) was uncommon in healthy men, so we did not initially evaluate it further in terms of prostate cancer risk. We then discovered that although the allele frequency of this A49T mutation was only 0.5% in healthy high-risk African-American men and 1.8% in healthy low-risk Latino men, it was considerably more common in men with prostate cancer, especially those presenting with advanced (i.e., clinically significant) disease. Among African-Americans, the prevalence was 0.9% for men with early-stage low-grade prostate cancer and 6.1% for men with advanced disease, resulting in relative risk estimates of 1.9 and 10.6 (P = 0.001), respectively, for presence of the T allele; for Latinos, the comparable figures were 4.6 and 7.0%, respectively, with resultant relative risks of 4.6 and 7.0% (P = 0.04). We concluded that this single mutation, although very uncommon in healthy men, is associated with nearly 10% of all advanced prostate cancers in these two populations.

We have provided evidence that the V89L and A49T substitution mutations in SRD5A2 may have functional consequences by studying the in vitro kinetic properties of both the A49T and the V89L mutations using a site-directed mutagenesis approach.6 The A49T enzyme has a 5-fold higher V_max for testosterone conversion than the normal enzyme, whereas the V89L enzyme shows reduced activity (~33%) compared to that of the wild type, as predicted by the in vivo data on circulating androstenediol glucuronide levels (29).


6 N. M. Makridakis and J. K. V. Reichardt, unpublished data.

The Androgen Receptor (AR) Gene

The AR gene, located on the long arm of the X-chromosome, codes for a transcription factor within the superfamily of steroid receptors (Fig. 4). The large exon 1 encodes the transactivation domain that mediates target gene transcriptional activation. Exon 1 also encodes two polymorphic polynucleo acid tracts, a poly-Q (CAG)_n and a poly-G (GGC)_n (30). Because the role of the AR is well-established during all phases of prostate cancer progression, genetic variation at this locus might contribute significantly to prostate cancer risk.

We have focused our attention on the (CAG)_n sequence because an expansion of this repeat is the cause of a rare, X-linked, adult-onset, motor neuron disease, spinal and bulbar muscular atrophy, or Kennedy’s disease (31), which is associated with low virilization, reduced sperm production, testicular atrophy, and reduced fertility (32, 33). More than double the average number of CAG repeats are found in the AR of such patients. Among otherwise healthy men, impaired sperm production and male infertility are associated with CAG repeat sizes in the long end of the “normal” size distribution (34). Thus, AR activity seems to be negatively correlated with CAG size. Consistent with this notion, we hypothesized (35) and subsequently provided evidence (30, 36) that shorter alleles of the (CAG)_n would predispose men to prostate cancer. Two independent epidemiological studies confirmed our original observations (37, 38), and an additional two studies (39, 40) identified phenotypic subgroups among prostate cancer cases on the basis of CAG repeats.

A possible direct causal link between CAG repeat variation and AR transactivation was first suggested by transfection assays using AR genes cloned from patients with Kennedy’s disease. Receptors containing an expanded CAG repeat from these patients have blunted transactivation activity (about 50% of normal activity) but normal androgen-binding activity (41), and there is a negative relationship between CAG length and transactivation within and beyond the normal range. The link between CAG size and AR transactivation was further reinforced by studies in which the elimination of the CAG repeat in both human and rat AR caused a marked elevation of transcriptional activation in vitro (42, 43).

We (44) and others (45) have observed an excess risk of prostate cancer in men with prostate cancer-affectected fathers compared to those with prostate cancer-affectected fathers. We have interpreted this to indicate either an X-linked or a recessive model of prostate cancer risk inheritance. Although the AR gene would be a strong candidate to explain such an inheritance pattern, a group in Montreal (46) found no strong evidence of AR-linked inheritance of prostate cancer risk among a group of 47 white multiplex families. One potential locus for hereditary prostate cancer that is neither X-linked nor recessive has been identified on chromosome 1q (47). Nonetheless, we have continued to pursue the issue of hereditary prostate cancer linked to the
AR gene among African-American men. In this analysis, three polymorphic markers at the AR locus were assessed. In addition to the two trinucleotide microsatellites referred to above, we analyzed a third marker, a Stul single-nucleotide polymorphism (Ref. 48; silent) at codon 211 (G1733A), roughly halfway between the two satellites. Among healthy African-American men (n = 208), the Stul polymorphism (Stul alleles are defined as S1 and S2, uncut and cut by the restriction enzyme, respectively) was in disequilibrium with both satellites, but the two satellites were equilibrated with respect to each other. The S1 allele was associated with a statistically significant nearly 3-fold increased risk of prostate cancer among men under the age of 65 years. An excess proportion of this allele (12 of 14, 86%) was also found among prostate cancer cases with an affected brother; the corresponding proportion among control men was 58% (118 of 204). The Stul polymorphism did not seem to simply reflect shorter CAG repeats as a function of linkage disequilibrium (30). These preliminary data indicate that in African-American men, non-(CAG)n genetic variation(s) at the AR locus might contribute to hereditary prostate cancer among the brothers of men with this disease. These early provocative results suggest multiple important research pathways for further understanding the contribution of the AR to prostate cancer risk.

**CYP17 Gene**

The CYP17 gene encodes the enzyme cytochrome p450c17, which catalyzes steroid 17–20 lyase activities at key points in testosterone biosynthesis in both the gonads and the adrenals (49). The CYP17 gene, on chromosome 10, is divided into eight exons. The 5' untranslated region of CYP17 contains a single-bp (a C to T transition) polymorphism that creates a Sp1 type (CCACC box) promoter site 34 bp upstream from the initiation of translation but downstream from the transcription start site. This change creates a recognition site for the MspA1 restriction enzyme. MspA1 digestion of a PCR fragment has been used to designate two alleles, A1 and A2. Because this bp change creates a CCACC box, and it is thought that the number of 5' promoter elements correlates with promoter activity, the A2 allele has been hypothesized to result in an increased rate of transcription (50). Carey et al. (49) have reported that carriers of the A2 allele are at increased risk of male pattern baldness in men and polycystic ovarian cancer in women, both of which are related to androgen biosynthesis or metabolism (2). We have shown that this marker predicts circulating estrogen levels in young women, supporting a functional relevance for this marker (51). We are now in the process of evaluating whether this genetic marker predicts circulating testosterone levels and the risk of prostate cancer across racial-ethnic groups and determining the prevalence of this marker among these groups with varying underlying prostate cancer risk.

**3ß-Hydroxysteroid Dehydrogenase Type II (HSD3B2) Gene**

The HSD3B2 gene encodes type II 3ß-hydroxysteroid dehydrogenase, one of two enzymes that initiate the inactivation of dihydrotestosterone in the prostate; this enzyme also catalyzes the conversion of androstenedione to testosterone in the biosynthetic pathway in the testes (52). At least two isozyme forms of 3ß-hydroxysteroid dehydrogenase are known to exist; the type I enzyme is encoded by the HSD3B1 gene that is expressed mostly in the breast, placenta, and skin. The type II enzyme, on the other hand, is expressed primarily in the adrenals, testis, ovary, and prostate (53). The HSD3B1 and HSD3B2 genes are closely linked on the short arm of chromosome 1. Both genes have been cloned and are structurally very similar. A number of mutations of the HSD3B2 gene have been found to cause a rare human disorder, congenital adrenal hyperplasia, but are apparently not involved in prostatic disease etiology (54).

A complex dinucleotide repeat polymorphism [(TG)n(TA)n(CA)m] has been described in intron 3 of the HSD3B2 gene (55). Although in the original description only eight alleles were reported, in our initial analysis of 312 healthy individuals from multiple racial-ethnic groups, we identified 25 alleles, with substantial heterogeneity in allele frequency among the racial-ethnic groups (56).

Although substantial differences exist among these racial-ethnic groups with underlying differences in prostate cancer risk, we have not yet established that this polymorphism correlates with biochemical phenotype, i.e., results in differences in enzyme activity and in differential rates of testosterone biosynthesis or degradation rates of dihydrotestosterone in the prostate. However, certain of these alleles were found to be modestly associated with prostate cancer risk in the small epidemiological study of prostate cancer in whites in which we have preliminarily evaluated other genetic markers (36).

**Does the Model Fit the Epidemiology of Prostate Cancer?**

If a fully developed model correctly describes prostate cancer pathogenesis and explains a substantial fraction of the population incidence of prostate cancer, then it would also be expected to possibly explain a substantial part of the demographic variation in prostate cancer incidence. We described above that important hormonal phenotypic differences exist among the racial-ethnic groups showing the greatest variability in prostate cancer incidence. We know of no data, however, that have demonstrated that these phenotypic variations result in differences among African-Americans, whites, and Asians in cell turnover rates in prostatic epithelium or potential correlates of this such as total prostatic volume or the glandular proportion of total prostatic volume.

As described above, we have evidence that at least some of the polymorphic genetic markers that we are studying result in detectable hormonal variation. For some of these genes, we also have evidence that the putative high-risk alleles are more common in high-risk populations and less common in low-risk populations, as defined by race-ethnicity. For example, the (CAG)n microsatellite in exon 1 of the AR gene shows the predicted length variation, with African-American men having shorter repeats, on average, and Asian men having longer repeats, on average, than whites. Two polymorphic markers relating to the SRD5A2 gene show the hypothesized racial-ethnic variation in allele frequency, as does the only available polymorphic marker in the CYP17 gene. The dinucleotide repeat marker on HSD3B2 shows substantial racial-ethnic variation, but the functional significance is unknown.

A troublesome aspect of the androgen hypothesis of prostate cancer etiology is the relationship between androgen levels and aging, on the one hand, and prostate cancer incidence rates and aging, on the other. Testosterone levels begin to decline in men at about age 40 [Ref. 57 (limited data suggest that this may even start earlier; Ref. 58)] and thereafter decrease roughly 10% per decade throughout the remainder of life. Prostate cancer is extremely rare before age 40 but increases at a more rapid rate with aging than any other cancer (7). The age-incidence curve for prostate cancer is very steep, but the slope declines slowly with age; this is consistent with the following two assumptions: (a) that there is a substantial pre-exposure and/or developmental time with a very high proportion of men “exposed” (i.e., at risk); and (b) that the intensity of exposure declines with age (7). Although both of these assumptions are compatible with an androgen-
related etiology, the slope of the age-incidence curve does not appear to start its decline until men approach age 60, well after the start of the testosterone decline. If the relevant hormone is free testosterone or non-SHBG-bound testosterone, then the discrepancy is exaggerated as SHBG increases with age (59). The discrepancy may possibly be explained by an up-regulation of AR due to an increase in the estrogen:testosterone ratio with age (59), but this remains to be thoroughly investigated.

The Future

The long-term goal of this approach to understanding prostate carcinogenesis is to definitively determine, first of all, the relationship between the polymorphic markers of each gene individually and collectively within each of the racial-ethnic groups contributing to the substantial variation in prostate cancer incidence. This will help us to establish: (a) the relationship between each gene and prostate cancer risk; (b) a polygenic etiology of prostate cancer; and (c) the extent that allelic variation in these genes individually and collectively explains the racial-ethnic variation in prostate cancer occurrence. When we began this work a few years ago, our goal was to identify a single marker in each gene of interest for which we could both demonstrate an association with prostate cancer risk and establish functional relevance. Over the course of our early investigations of both the 

17\(\beta\)-hydroxysteroid dehydrogenase type III gene that encodes the enzyme responsible for the conversion of androstenediol to testosterone in the biosynthetic pathway in the testis is also of great interest, and at least one polymorphic marker has been described (61). We have already begun to develop a similar model for breast cancer, and this concept can be readily extended to other hormone-related cancers such as ovarian, endometrial, and testicular cancers.

Concurrent with these epidemiological risk assessment studies, we will continue to engage in in vivo and in vitro studies to further establish the functional relevance of these genetic markers, with the goal of eventually definitively determining the relevant mutations responsible for prostate cancer risk. As epidemiologists seek to further expand research activities into the area of genetic polymorphisms and disease risk, it is critical that functional assessments be done in advance or concurrently with epidemiological risk assessments. Because correlations with hormonal phenotypes are fraught with difficulties, functional studies of genes involved in hormonal biosynthesis and metabolism will likely require careful and creative in vitro models, such as the site-directed mutagenesis assays to evaluate the kinetic properties of specific mutations as described above for the A49T and V89L mutations in SRD5A2.

Establishing a polygenic model of prostate cancer pathogenesis, in addition to the obvious importance of contributing valuable information regarding disease development, population risk assessment, and prostate biology and physiology, also has critical public health implications. Such a model can provide a basis for targeting populations for screening interventions and/or preventive strategies aimed at the multigene products or at the genes themselves.

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