Association of Prostate Cancer with Vitamin D Receptor Gene Polymorphism

Jack A. Taylor, Ari Hirvonen, Mary Watson, Gary Pittman, James L. Mohler, and Douglas A. Bell

Epidemiology Branch [J. A. T.], Laboratory of Biochemical Risk Analysis [A. H., M. W., D. A. B.], National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, and University of North Carolina, Chapel Hill, North Carolina 27599 [G. P., J. L. M.]

Abstract

The incidence of prostate cancer in the United States is second only to skin cancers, and the disease kills almost the same number of men as breast cancer does women. Relatively few risk factors are known for prostate cancer, although several lines of evidence suggest that vitamin D may be an important determinant of prostate cancer risk. A series of common polymorphisms in the vitamin D receptor gene were recently reported to be associated with bone density and risk of osteoporosis (Morrison et al., Nature (Lond.), 367: 284-287, 1994). These genetic variants have been correlated with both circulating levels of active vitamin D hormone and in vitro measures of gene expression (Morrison et al., Nature (Lond.), 367: 284-287, 1994). We tested the hypothesis that vitamin D receptor gene polymorphisms are associated with prostate cancer risk using a case-control study of 108 men undergoing radical prostatectomy and 170 male urology clinic controls with no history of cancer. Among the white control group, 22% were homozygous for the presence of a TaqI RFLP at codon 352 (genotype t), but only 8% of cases had this genotype (P < 0.01). A similar trend was seen among the small number of blacks in this study (13% for controls, 8% for cases), although the difference was not statistically significant. Race-adjusted combined analysis suggests that men who are homozygous for the t allele (shown to correlate with higher serum levels of the active form of vitamin D) have one-third the risk of developing prostate cancer requiring prostatectomy compared to men who are heterozygotes or homozygous for the T allele (odds ratio = 0.34; 95% confidence interval, 0.16-0.76; P < 0.01). These results support recent ecological, population, and in vitro studies suggesting that vitamin D is an important determinant of prostate cancer risk and, if confirmed, suggest strategies for chemoprevention of this common cancer.

Introduction

Prostate cancer is a common malignancy worldwide and a dominating one in western countries (1). In the United States, it is the most commonly diagnosed tumor in men and the second most common cause of cancer death in men; the disease strikes and kills almost the same number of men as breast cancer does women (2). Until recently, however, few epidemiological studies have been conducted, and there are scant clues as to etiology and pathogenesis. There are striking differences in prostate cancer incidence rates among racial and ethnic groups, with African-American men displaying the highest incidence of prostate cancer in the world, whereas Japanese and Chinese men have the lowest rates (1).

In the United States, prostate cancer mortality rates exhibit a marked north-south gradient, with higher rates observed in the north (3, 4). This gradient correlates well with ambient levels of UV radiation, giving rise to the hypothesis that low UV exposure may be a risk factor for prostate cancer (3, 4). Most of the body's supply of vitamin D is synthesized in the skin in response to UV radiation. Vitamin D has potent antitumor properties, and studies have suggested that vitamin D metabolites and analogues may be modifiers of the growth of various cancers (5-11). A recent report suggested a strong relationship between higher serum levels of 1,25-D (2), the active hormonal form of vitamin D, and decreased risk of developing prostate cancer (12). 1,25-D exerts its activities by binding the VDR (5), a nuclear hormone receptor.

Inherited polymorphisms in the 3' UTR of the VDR gene correlate with transcriptional activity and mRNA stability in minigene reporter constructs (13). The 3' UTR polymorphisms (Fig. 1) are in strong linkage disequilibrium with RFLPs located in intron 8 (BsmI and Apal) and exon 9 (TaqI) and result in two common haplotypes, BaT and baT (small letters denote a restriction site is present). In a study of 117 subjects, 1,25-D levels were significantly higher among individuals who were homozygous for the BaT haplotype (134 ± 42 pm) compared to individuals who were heterozygous or homozygous for baT haplotype (104 ± 30 pm and 99 ± 40 pm, respectively; P = 0.0008; Ref. 13).

We hypothesized that individuals who are homozygous for the BaT haplotype would be at decreased risk for prostate cancer. In the present study, we determined VDR TaqI genotypes in prostate cancer cases and controls, detecting significantly decreased risk associated with the t allele.

Materials and Methods

A total of 108 consecutive prostatectomy cases (96 white and 12 black) collected at University of North Carolina hospitals were used as the prostate case group. A total of 170 noncancer patients (162 white and 8 black) were enrolled from the Urology Clinics at Duke University Medical Center and the University of North Carolina hospitals. Noncancer control patients were male urology clinic patients, the majority of whom presented with benign prostatic hypertrophy or impotence, who had no history of any cancer other than non-melanoma skin cancer. Blood samples were obtained from both cases and controls, and DNA was extracted using standard methods.

VDR TaqI genotype was determined by a PCR-based method described by Riggs et al. (14). Briefly, a 740-bp fragment was generated using PCR primers (5'-cag agc atg gac agg gag can and 5' gca act ccl cat ggc tga ggt ctc) located within intron 8 and exon 9. The PCR fragment was subjected to TaqI digestion and then separated on 3% NuSieve 3:1 agarose gels (FMC Bioproducts, Rockland, ME; Fig. 2).

Codon 352 in exon 9 is polymorphic, existing as either ATC or ATT, both of which code for isoleucine, and the C>T change is associated with the loss of a TaqI restriction site. The resulting alleles are designated t (TaqI site present) or T (TaqI site absent), and three possible genotypes result: TT, Tt, and tt. Three banding patterns are observed after digestion of the 740-bp amplification fragment, depending upon genotype (Fig. 2): (a) homozygous absence of the TaqI polymorphism (TT) results in two fragments of 495 bp and 245 bp; (b) homozygous presence (tt) of the TaqI polymorphism results in three
fragments of 290 bp, 245 bp, and 205 bp; and (c) heterozygotes have all four fragments 495 bp, 290 bp, 245 bp, and 205 bp. The 245-bp fragment is constant among all genotypes, having been created by a nonpolymorphic TaqI site within the amplification fragment, and acts as an internal control for digestion.

The VDR BsmI genotyping method of Morrison et al. (13) was also used, and this method produced qualitatively similar results (these restriction sites are separated by a few hundred bp and are in linkage disequilibrium). However, for approximately 8% of samples we observed technical problems with the assay, either unequal amplification of VDR BsmI alleles or incomplete digestion, making the VDR BsmI genotyping less reliable; thus BsmI results are not reported.

Associations between disease and genotype were assessed by calculating the OR and 95% CI. The OR is the odds of having a genotype in one group divided by the odds of having that same genotype in the referent (control) group. Because genotype frequency can differ by race, blacks and whites were analyzed separately. A combined OR was calculated on the basis of Mantel-Haenszel’s formula (15) to give a weighted average of the race-specific ORs. @ statistics can be used to test whether the ORs and combined ORs differ from their null value of unity. The OR closely approximates the RR of disease. The RR is the ratio of the probability of disease given a specific genotype to the probability of disease given alternative genotypes. ORs and RRs < 1 imply a protective effect of the specific genotype on disease risk relative to alternative genotypes.

Results and Discussion

A series of polymorphisms exist in intron 8, exon 9, and the 3’ UTR of the VDR gene that are in strong linkage disequilibrium with one another (13, 16). We measured one of these polymorphisms, a C for T synonymous change at the third position of codon 352 coding for isoleucine, which alters a TaqI restriction site in exon 9. Among whites, the occurrence of the t genotype was significantly lower among the prostate cancer patients (8%) compared to noncancer controls (22%; OR = 0.32; 95% CI, 0.15–0.75; P < 0.01; Table 1). Among the small number of blacks in this study, the t genotype was present in 8% of cases and 15% of controls, although this difference was not statistically significant (OR = 0.64; 95% CI, 0.03–12). Overall, men who were homozygous for the absence of this TaqI RFLP (genotype tt) appear to have one-third of the risk of developing prostate cancer requiring surgical resection as men who are heterozygous (Tt) or homozygous (TT) for the absence of the RFLP (Mantel-Haenszel combined race-adjusted ORMH = 0.34; 95% CI, 0.16–0.76; P < 0.01). Among cases, VDR genotype did not correlate with grade, stage, or age at diagnosis (data not shown).

Although neither the TaqI RFLP in exon 9 nor the linked BsmI and ApaI RFLPs in intron 8 are likely to have functional consequences themselves, these sequence polymorphisms have been shown to be associated with varying levels of the circulating VDR ligand 1,25-D. Significantly higher serum levels of 1,25-D have been reported in people who are homozygous for the haplotype with the t allele relative to those who are heterozygous or homozygous for the T allele (13).

Minigene reporter constructs of the two VDR 3’ UTRs, which encompass changes at more than 12 different sites in a 3.2-kb region, suggest that the haplotype corresponding to the t allele has about 140% of the transcriptional activity and mRNA stability of that corresponding to the T allele (13). Despite these findings, it is unclear how higher VDR expression is causally related to circulating ligand (1,25-D) levels, although there may be feedback pathways that link the expression of the receptor to the formation of the active hormone 1,25-D from the inactive precursor. Which of the multiple polymorphisms is actually responsible for altering functional activity has yet to be described. This is particularly important because the linkage disequilibrium between the RFLPs (in intron 8 and exon 9) and the multiple, and presumably functional, changes in the UTR may not exist in all ethnic groups. We had too few blacks in our sample to effectively test this association.

Although too few black prostate cancer patients (n = 12) and noncancer clinic controls were available to allow meaningful analysis of risk, the lower frequency of the tt VDR genotype reported among blacks (17, 18) is consistent with higher prostate cancer rates among blacks compared with whites (1). If the risk associated with VDR genotypes is similar in blacks and whites and the same linkage disequilibrium exists, then VDR genotype frequency differences may explain part of the difference in prostate cancer incidence observed between these groups.

Combined with the published data linking prostate cancer risk with low sunlight exposure (3, 4) and low 1,25-D levels (12), observations that 1,25-D inhibits proliferation and promotes differentiation in prostate cells in culture (8), and recent data that a vitamin D analogue can prevent prostate cancer in an experimental rat model (11), our findings support the hypothesis that vitamin D plays an important role in
prostate cancer. If these findings can be verified, VDR genotype represents an important determinant of prostate cancer risk. Although direct chemoprevention with 1,25-D may not be practical because of adverse effects on calcium homeostasis, other vitamin D congeners may prove useful for primary prevention of this common cancer.

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References

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