Association of Prostate Cancer with Vitamin D Receptor Haplotypes in African-Americans

Sue Ann Ingles, Gerhard A. Coetzee, Ronald K. Ross, Brian E. Henderson, Lawrence N. Kolonel, Laura Crockett, Wei Wang, and Robert W. Haile


Abstract

In previous studies, allelic variation in the 3' end of the vitamin D receptor gene was associated with increased risk of prostate cancer in white men. Several polymorphisms, including a BsmI restriction site and a poly(A) microsatellite, can be used interchangeably to mark the unidentified locus in whites. In African-Americans, these markers are not interchangeable, due to weaker linkage disequilibrium in this genomic region in this population. Here, we genotyped both the BsmI and poly(A) markers for 151 African-American prostate cancer cases (102 localized and 49 advanced) and 174 African-American male controls from a large epidemiological cohort. A direct haplotyping procedure was devised to determine BsmI/poly(A) haplotypes for double heterozygotes so that haplotypes could be used as allelic markers in standard logistic regression analyses. Using BsmI alone, b alleles were associated with a 2-fold increase in risk of advanced prostate cancer. The association was, however, confined to haplotypes carrying a long (L) allele of the poly(A) microsatellite. BL and BL haplotypes were associated with increased and decreased risk, respectively, whereas neither BS nor bS haplotypes were associated with prostate cancer risk. An allelic variant that confers increased risk of advanced prostate cancer appears to be associated with the BsmI/poly(A) BL haplotype in African-Americans.

Introduction

Prostate cancer is an increasingly common disease. More than 200,000 cases are newly diagnosed annually in the United States. Mortality rates, however, have increased more slowly (35,000 deaths in 1994, up just 7% from 1990 levels; Ref. 1). Although approximately 50% of men between 70 and 80 years of age have latent, histologically localized prostate cancers (2), the majority of these tumors never become metastatic or life threatening. Thus, the identification of biomarkers that predict progression to advanced disease carries tremendous public health significance.

We previously reported that allelic variation in the VDR gene was associated with increased risk of prostate cancer, particularly for advanced disease. For non-Hispanic white males, risk increased approximately 2-fold for localized prostate cancer and 4-fold for all prostate cancers combined (localized and advanced) among men carrying one or more long alleles of the poly(A) microsatellite. BL and BL haplotypes were associated with increased and decreased risk, respectively, whereas neither BS nor bS haplotypes were associated with prostate cancer risk. An allelic variant that confers increased risk of advanced prostate cancer appears to be associated with the BsmI/poly(A) BL haplotype in African-Americans.

Genotyping. An 825-bp region of genomic DNA containing the BsmI polymorphic site in intron 8 was amplified and analyzed as described previously (3). The existence of the cut allele, b, is indicated by the formation of a 625-bp product.

A region of approximately 411 bp surrounding the poly(A) polymorphism was amplified using primers VDRPAF1 and VDRPAR1 (Fig. 1), as described previously (3). This PCR product was diluted 1:150, and a nested amplification was performed using primers VDRPAF2 (5'-GAGACCAACCTGACCA-3') and VDRPAR2 (5'-CCTCAGCCTCGTAGT-3'). The forward primer was first end-labeled using [γ-32P]ATP (1 μCi/μmol). Products were separated on polyacrylamide sequencing gels and autoradiographed. Alleles were sized as described previously (3).

Haplotyping. BsmI/poly(A) haplotypes can be inferred from BsmI and poly(A) genotypes for all subjects, except for double heterozygotes (BS, SE). For these double heterozygotes, we developed the following direct haplotyping procedure. A region of more than 3 kb, spanning the BsmI and poly(A) loci, was first amplified using the Perkin-Elmer GeneAmp XL PCR kit with a hot start (using the Perkin-Elmer AmpliWax kit), according to manufacturer's instructions. Primers used were CZ-53 (5'-CAACCAAGACTACAAGTACCGGA-3').

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allele-specific amplifications using Taq polymerase. The allele-specific forward primers are CZ174 for the B allele (5'-GGCCACAGACAGGGCCTCA-3') and CZ175 for the b allele (5'-GGCCACAGACAGGGCCTCG-3'). These nested reactions were carried out in 5% DMSO with VDRPAR1 as the downstream primer. The resulting allele-specific amplification is biased toward the BsmI allele of interest, with the poly(A) allele that was in cis with the specific BsmI allele being preferentially amplified. The two allele-specific products are then amplified as in the second PCR of the poly(A) assay described above (primers VDRPAR2 and VDRPAR3). These two products are then run on a sequencing gel, alongside the product from the original (non-allele-specific) poly(A) assay for reference, such that each subject has three genotype calls: BsmI (B or b), poly(A) (S or L), and BsmI/poly(A) (BB, Bb, bb, SS, SL, LL). Statistical Methods. BsmI and poly(A) odds ratios were estimated by including appropriate indicator variables in an unconditional logistic regression model. Haplotype odds ratios were similarly estimated after subjects were categorized as to whether they carried 0, 1, or 2 copies of each haplotype. All logistic models included indicator variables to adjust for tertiles of age. Tests for trend were performed using a likelihood ratio test for significance of a poly(A) or BsmI term in the model. The addition of BsmI genotype and haplotype number improved model fit (P < 0.05). Addition of BL haplotypes significantly improved model fit (P = 0.04), but bl, BS, and bS haplotypes did not (P = 0.09, 0.08, and 0.09, respectively).

Discussion

Allelic variation in the 3' end of the VDR gene has been associated with increased risk of prostate cancer in white men (3, 7). Several polymorphisms, including BsmI and TaqI sites and a poly(A) microsatellite, have been used to mark the 3' VDR locus (Fig. 1). In whites, only two BsmI/TaqI/poly(A) haplotypes, BS and bTL, have been commonly observed (4, 8). Both the poly(A) L (3) and the TaqI T (7) alleles have been associated with increased risk of prostate cancer, suggesting that an at-risk allelic variant is associated with the bTL haplotype in the white population.

In this study of African-American men, we found that poly(A) genotype was not associated with risk of localized or advanced prostate cancer (Table 1). BsmI b alleles were associated with a 2-fold decrease in risk of advanced prostate cancer in African-Americans, in contrast to a 4-fold increase in risk previously observed in Caucasians (3). On the surface, these results appear to be inconsistent; however, either or both markers might substantially misclassify the relevant VDR genotype in African-Americans due to weak linkage disequilibrium in this genomic region in this ethnic group (4).

Table 1. Age-adjusted odds ratios (95% confidence intervals) for prostate cancer by VDR genotype in African-Americans

<table>
<thead>
<tr>
<th></th>
<th>All cases</th>
<th>Advanced</th>
<th>Localized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>BsmI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>1.00</td>
<td>0.97 (0.47, 2.03)</td>
<td>0.47 (0.20, 1.11)</td>
</tr>
<tr>
<td>Bb</td>
<td>0.97 (0.47, 2.03)</td>
<td>0.47 (0.20, 1.11)</td>
<td>1.96 (0.71, 5.36)</td>
</tr>
<tr>
<td>bb</td>
<td>1.13 (0.54, 2.38)</td>
<td>0.39 (0.15, 0.96)</td>
<td>2.66 (0.97, 7.32)</td>
</tr>
<tr>
<td>Poly(A)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>1.00</td>
<td>0.71 (0.32, 1.59)</td>
<td>0.97 (0.32, 2.96)</td>
</tr>
<tr>
<td>SL</td>
<td>0.71 (0.32, 1.59)</td>
<td>0.97 (0.32, 2.96)</td>
<td>0.61 (0.24, 1.51)</td>
</tr>
<tr>
<td>LL</td>
<td>0.93 (0.42, 2.06)</td>
<td>0.79 (0.25, 2.46)</td>
<td>1.00 (0.41, 2.45)</td>
</tr>
</tbody>
</table>

* Underlining indicates positions of mismatch between primers or between primer and genomic sequence.
As a consequence of weak disequilibrium, all four possible BsmI/Poly(A) haplotypes, BS, BL, BL, and BS, are frequently observed in the African-American population (4). To increase genetic content information, VDR haplotypes can be treated as individual alleles (9), and given sufficient data, 9 odds ratios could be estimated to determine which of the 10 different allelotypes (BS/BS, BS/BL, BS/BS, BL/BL, BS/BL, BL/LS, BS/L, and BS/BS) are associated with prostate cancer risk. In our data set, however, statistical power for testing the significance of nine individual odds ratios was lacking, due to the relatively low frequency of BL and BS haplotypes (approximately 10% each). An alternative approach is to focus on a given haplotype, BL, for example, and to estimate only two odds ratios, comparing subjects carrying 0, 1, and 2 BL haplotypes. Because we had no a priori hypothesis as to which haplotype marks the relevant locus, each of the four haplotypes was examined in this way.

The excess of BL and deficit of BS haplotypes found among advanced prostate cancer cases (Table 2) can be most simply explained by association of either a high-risk allelic variant with BL or a protective variant with BL. Our data best support the former of these two hypotheses, that a high-risk variant is associated with the BL haplotype, BL, for example, and to estimate only two odds ratios, if one assumes that this hypothesis is true, then the protective odds ratios for BL can be explained by a predominance of high-risk BL-carrying subjects among the subjects who have no BS haplotypes.

Other explanations can be proposed for an excess of BL and a deficit of BS haplotypes among advanced cases. For example, the association of a high-risk variant with BL, a low-risk variant with BL, and “neutral” variants with BS and BS could explain the observed pattern of odds ratios. Alternatively, more than one crossing-over event may have occurred, so that the high-risk variant is not limited to a single haplotype. To distinguish among these possibilities and to identify the functional variants, comparison of the four haplotypes by functional assays and by sequence analysis is in progress in our laboratories.

Until VDR 3’ functional variants are identified, association studies using single VDR markers must be interpreted cautiously. The at-risk functional allele may be in linkage disequilibrium with different marker alleles in different ethnic groups, for example, BsmI b in whites and B in African-Americans, as we have observed. Moreover, if linkage disequilibrium between the functional at-risk allele and the marker allele is not strong, misclassification of the at-risk allele by the marker allele may result in attenuated odds ratios. For example, when only the BL (and not the BS haplotype) is associated with the at-risk allele, then BsmI alone misclassifies the causal locus, and odds ratios are expected to be attenuated. Indeed, we found that compared to the BsmI odds ratios (Table 1), the magnitudes of the BL and BS haplotype odds ratios (Table 2) were larger and were in closer agreement with the odds ratio magnitudes of 3 or 4 observed in white populations (3, 7).

Our finding that VDR markers are associated only with advanced and not with localized prostate cancer is consistent with our previous findings among whites (3) and is supported by experimental evidence that vitamin D and vitamin D analogues can inhibit invasiveness of human prostate cancer cells both in vitro (10) and in vivo (11). However, VDR genotype was equally associated with risk of localized and advanced prostate cancer in one previous study (7), and our data, although not statistically significant, show an approximate 2-fold increase in risk of localized cancer associated with BsmI b alleles (Table 1). After subdivision of b alleles into BS and BL haplotypes, odds ratios for localized prostate cancer were no longer elevated (Table 2), but a small nonsignificant excess of BL and a deficit of BS/BS subjects among localized cases compared to controls remained. Thus, until genetic variation in this genomic region is better understood, we cannot rule out the possibility that a second polymorphic site in the 3’ end of the VDR gene may be related to risk of localized prostate cancer.

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References


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