Short Communication

Vitamin D Receptor Genotypes/Haplotypes and Prostate Cancer Risk

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Abstract

The vitamin D receptor (VDR) gene has been associated with prostate cancer, although previous results are somewhat equivocal. To further study this, we did a family-based case-control study (N = 918) of the association between prostate cancer and six common VDR variants: Cdx2, FokI, BsmI, ApaI, TaqI, and the poly-A microsatellite. Looking at each variant alone, only FokI and ApaI were associated with disease. The FokI FF genotype was inversely associated with prostate cancer among men with less advanced disease (i.e., Gleason score <7 and tumor stage <T2a), where the odds ratio OR was 0.56 [95% confidence interval (95% CI), 0.31-1.01; P = 0.05]. ApaI, carrying one or two copies of the A allele, exhibited a weak inverse association with disease (OR, 0.64; 95% CI, 0.39-1.03; P = 0.06); this association was strengthened in Caucasian men with more advanced disease (OR, 0.44; 95% CI, 0.21-0.93; P = 0.03). We observed inverse associations between disease and the four-locus FBA haplotype (OR, 0.48; 95% CI, 0.30-0.76; P = 0.002) and the FbaT haplotype (OR, 0.60; 95% CI, 0.38-0.95; P = 0.03; i.e., in comparison with the FbaT haplotype). These were stronger among men with more advanced disease: for FBA, the OR was 0.31 (95% CI, 0.16-0.61; P = 0.0008); for FbaT, the OR was 0.32 (95% CI, 0.16-0.64; P = 0.001). These observations support a role for VDR variants in prostate cancer risk but suggest that any potential causal variant(s) may reside on the haplotypes reported here. This would help explain the somewhat equivocal results for VDR genotype-level associations with prostate cancer. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2549-52)

Introduction

Epidemiologic evidence supports an inverse association between vitamin D and risk of prostate cancer (1, 2). Vitamin D is mainly synthesized in the skin in response to UV radiation, may also be obtained from diet and supplements, and exerts its activities by binding to the vitamin D receptor (VDR). The active form of vitamin D, 1,25-dihydroxyvitamin D, is mediated through the VDR, which is expressed in both normal (3) and prostate cancer cells (4). Furthermore, 1,25-dihydroxyvitamin D3 inhibits prostate cell proliferation (4), invasion (5), and metastasis (6). Studies in animal tumor models (7) and human phase II clinical trials (8) support the role of 1,25-dihydroxyvitamin D3 in decreasing the incidence and slowing the progression of advanced prostate cancer.

The gene encoding VDR [MIM 601769] maps to chromosomal region 12q13 and contains several previously reported common variants that are hypothesized to influence the expression and/or function of the VDR protein. These include variants located in the promoter (Cdtx2), exon 2 (FokI), intron 8 (ApaI and BsmI), exon 9 (TaqI), and a mononucleotide repeat (poly-A) in the 3’ untranslated region. The TaqI, ApaI, and BsmI variants are in linkage disequilibrium, resulting in two common haplotypes, BAT and bAT (9, 10).

Materials and Methods

Study Subjects. The study design and population have been described elsewhere (23). Briefly, siblings (N = 918; 439 cases and 479 controls from 413 families) were recruited from the major medical institutions in the greater Cleveland, Ohio area and from the Henry Ford Health System, Detroit, Michigan. Ninety-one percent of the subjects were Caucasian, 8% were African American, and 1% were Asian or Latino. Institutional Review Board approval was obtained from the participating institutions and all study participants gave informed consent.Sibling sets consisted of men with prostate cancer diagnosed at age 73 years or younger and at least one brother without prostate cancer. The cases’ clinical characteristics at diagnosis (e.g., Gleason score, tumor stage) were obtained from medical records and their disease status was confirmed histologically. All controls were no more than 8 years younger than their brothers at diagnosis. The disease status of unaffected brothers was further confirmed through testing of prostate-specific
antigen levels whenever possible (93% of controls). If the prostate-specific antigen level of a control was elevated (>4 ng/mL), they were referred for further evaluation by our collaborating urologists. These individuals were retained in the study as controls unless a subsequent diagnosis of prostate cancer was made, at which time they were reclassified as cases. These criteria were selected in an attempt to minimize potential for misclassification of prostate cancer status among controls. We chose to use this sibling-based study design to ensure that our control group was selected from the same genetic source population as the cases, and is thus not subject to population stratification from factors such as race.

**Genotype Analysis.** Standard venipuncture was used to collect blood samples from all study participants in tubes with EDTA as an anticoagulant. Genomic DNA was extracted from buffy coats using the QIAamp DNA Blood Kit (Qiagen, Inc., Valencia, CA). All purified DNA samples were diluted to a constant DNA concentration of 5 ng/µL in 10 mmol/L Tris, 5 mmol/L EDTA buffer (pH 8).

Six VDR variants were examined: the Cdx2 single-nucleotide polymorphism (SNP) in the 5' promoter region of the VDR gene (rs11568820), the FokI polymorphism in exon 2 (rs10735810), two SNPs in intron 8 (BsmI, rs1455510 and Apal, rs7975232), TaqI in exon 9 (rs731236), and a mononucleotide (poly-A) microsatellite repeat in the 3' untranslated region.

The four polymorphisms, FokI, BsmI, Apal, and TaqI, were detected by PCR amplification followed by restriction enzyme digestion. The Cdx2 polymorphism was detected using a TaqMan assay (Applied Biosystems, Foster City, CA) using an ABI 7900HT Detection System. The poly-A fragment length polymorphism was detected using a CEQ 8000 genetic analysis system (Beckman Coulter, Fullerton, CA), and at least one sample of each poly-A fragment length observed was confirmed by DNA sequencing.

Five percent of samples were randomly selected and genotyped by a second investigator and 1% of samples were confirmed by DNA sequencing using a 377 ABI automated sequencer (all results were concordant). Consistent with the literature, genotypes for the four VDR variants, FokI, BsmI, Apal, and TaqI, are reported according to the standard nomenclature in which lowercase and uppercase letters represent the presence or absence of a restriction site, respectively. The FokI T and C alleles are represented by t and F, the BsmI G and A alleles by b and B, the Apal T and G alleles by A and a, and the TaqI T and C alleles by T and t, respectively.

**Statistical Analysis.** A χ² goodness of fit test was used to assess deviation from Hardy-Weinberg equilibrium for genotype frequencies of each variant. Linkage disequilibrium was calculated using HAPLOVIEW4 in Caucasian or African American controls (randomly selecting one control from each family). Next, haplotypes were predicted within major ethnic groups for three-locus (BsmI, Apal, and TaqI), four-locus (FokI, BsmI, Apal, and TaqI), and five-locus (Cdx2, FokI, BsmI, Apal, and TaqI) haplotypes, stratified by case-control status via the program tagSNPs (24).

Conditional logistic regression (using family as the match-variable) was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) for the relationship between genotypes/haplotypes and prostate cancer. Co-dominant and the most parsimonious mode of inheritance were used for single SNP analyses, whereas a log-additive model was used for SNP haplotype analyses. We grouped all of the less common haplotypes (<2.5% frequency) into a single category in the regression model.

To investigate the potential effect of genotypes/haplotypes on disease aggressiveness, the analyses were stratified by the clinical characteristics of cases at diagnosis. Disease was defined as “less advanced” if a case’s Gleason score was <7 and their tumor category was <T2c, and “more advanced” if their Gleason score was ≥7 and/or their tumor category was ≥T2c. All the effect estimates were adjusted for potential confounding by age. By using a family-based design and analysis, our results are controlled for population stratification. In addition, the analyses were stratified by ethnic group. All P values are from two-sided tests and all analyses were undertaken with SAS software (version 8.2, SAS Institute, Cary, NC).

**Results**

The mean age of cases and controls was 61 and 63 years, respectively. Approximately half of the cases were diagnosed with more advanced prostate cancer: 191 (45%) had Gleason scores ≥7 and 55 (13%) had tumor stage ≥T2c. Among Caucasian controls, the variants in VDR were in Hardy-Weinberg equilibrium, and the four variants (Apal, BsmI, TagI, and poly-A) in the 3' region of the gene were in strong linkage disequilibrium (D' > 0.96). FokI and Cdx2 exhibited weaker linkage disequilibrium with the 3' variants and with each other (D' < 0.20).

Table 1 provides results for the genotype-level associations between prostate cancer and the six vitamin D variants (Cdx2, FokI, BsmI, Apal, TagI, and poly-A). The FokI FF genotype was associated with decreased risk among men with less advanced disease (OR, 0.56; 95% CI, 0.31-1.01; P = 0.05). This result was strengthened among Caucasian men (OR, 0.48; 95% CI, 0.26-0.89; P = 0.02). A weak inverse association was also observed for the Apa AA and Aa genotypes when compared with the aa genotype (OR, 0.64; 95% CI, 0.39-1.03; P = 0.06), which was strengthened among Caucasian men (OR, 0.51; 95% CI, 0.31-0.86; P = 0.01), especially those with more advanced disease (OR, 0.44; 95% CI, 0.21-0.95; P = 0.03). We did not observe any noteworthy associations between the other vitamin D variants and prostate cancer (Table 1).

When looking at the three-locus haplotypes (BsmI, Apal, and TagI), there were no apparent associations with prostate cancer (Table 2). However, the four-locus haplotypes (+FokI) were associated with disease: in comparison with the reference haplotype (F-b-a-T), the F-B-A-t haplotype was inversely associated (OR, 0.48; 95% CI, 0.30-0.76; P = 0.002), as was the f-b-a-T haplotype (OR, 0.60; 95% CI, 0.38-0.95; P = 0.03; global P = 0.003). These associations were stronger among men with more advanced disease (Table 2).P In particular, the associations for the F-B-A-t and f-b-a-T haplotypes were now OR, 0.31 (95% CI, 0.16-0.61; P = 0.0008) and OR, 0.32 (95% CI, 0.16-0.64; P = 0.001), respectively (global P = 0.008). These findings were slightly weakened when adding the Cdx2 or Poly-A variants to the four-locus haplotypes (not shown).

**Discussion**

Our study of the VDR gene and prostate cancer detected weak inverse associations for FokI and Apal genotypes, but stronger associations when looking at four-locus haplotypes composed of FokI-BsmI-Apal-TagI variants. These results were also generally strengthened when restricting analyses to men with more advanced prostate cancer (as defined by high grade or stage). Further stratifying these analyses by whether the cases had high grade or stage indicated that the inverse associations for men with advanced cancer are driven by high grade (data not shown). Our observation of limited genotype-level associations agrees with some (1, 14, 18) but not other genetic epidemiologic studies (21, 25).
The *FokI* polymorphism is functional, and the short 424-amino-acid VDR protein variant (C allele or F allele) is more active than the long 427-amino-acid variant (T allele or f allele) in terms of its transactivation capacity (26-28). Our finding that the *FokI* variant was most strongly associated with reduced risk within a common haplotype suggests that other variants within this haplotype may also have some functional relevance. In support of this, Whitfield et al. (28) concluded that the *FokI* and poly-A variants in combination affect VDR transcriptional activity and suggested that there exist one or more additional polymorphisms, possibly in the 5′ region of the VDR gene, which may also affect VDR activity.

For example, the VDR 5′ regulatory region polymorphism in *Cdx2* has been shown to affect transcriptional activity of VDR, with the G allele activity being 70% that of the A allele. A recently published study found that the high-activity *Cdx2* A allele was associated with reduced risk (15). In contrast, men with high UV exposure were at higher risk when they carried the *Cdx2* AA genotype (29). Our findings do not support the involvement of *Cdx2* with prostate cancer, either alone or on a haplotype, although we did not assess UV exposure.

In a similar fashion, results for the other VDR variants have been extremely mixed to date. Among Japanese populations, *ApaI* was not statistically significantly associated with either familial prostate cancer (30) or sporadic prostate cancer and benign prostatic hyperplasia (13). The *Taql* tt genotype has been inversely associated with prostate cancer (25), although most studies did not detect any such a results (12, 16, 17).

### Table 1. Association between VDR genotypes and prostate cancer risk and aggressiveness

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Entire sample</th>
<th>Less advanced</th>
<th>More advanced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/controls</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td><em>Cdx2</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>257/269</td>
<td>1.00</td>
<td></td>
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<tr>
<td>GA</td>
<td>146/167</td>
<td>0.92 (0.63-1.34)</td>
<td>0.66</td>
</tr>
<tr>
<td>AA</td>
<td>36/43</td>
<td>0.63 (0.30-0.31)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

### Table 2. Association between VDR haplotypes and prostate cancer risk and aggressiveness

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Entire sample</th>
<th>Less advanced</th>
<th>More advanced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases/controls</td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>(BsmI b/B)-(ApaI A/a)-(Taql T/t)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b-A-T</td>
<td>0.47/0.45</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>b-A-T</td>
<td>0.13/0.13</td>
<td>0.97 (0.62-1.51)</td>
<td>0.89</td>
</tr>
<tr>
<td>B-A-T</td>
<td>0.35/0.38</td>
<td>0.83 (0.61-1.13)</td>
<td>0.24</td>
</tr>
<tr>
<td>Global P</td>
<td>0.02/0.03</td>
<td>0.79 (0.35-1.81)</td>
<td>0.58</td>
</tr>
<tr>
<td>(FokI F/f)-(BsmI b/B)-(ApaI A/a)-(Taql T/t)</td>
<td>0.08</td>
<td>0.50 (0.35-1.57)</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Includes cases with Gleason score <7 and tumor stage <T2c and their brothers.
*Includes cases with Gleason score ≥7 and tumor stage ≥T2c and their brothers.
*Adjusted for age.
The findings for the poly-A are also conflicting; however, our results are consistent with those that found no association between prostate cancer and the poly-A polymorphism (11, 16, 20, 31). Of the variants studied here, only FokI and Cdx2 are known to be functional. These variants, and others studied here, may be in linkage disequilibrium with another functional variant, which would explain the strong haplotype-level results we observed.

Our family-based case-control study had several strengths, including the sample size, control for potential population stratification by studying sibships, and stratifying the data by whether man had nonadvanced or advanced disease. A potential limitation of this study is our lack of information on vitamin D status, which could modify the effects observed here. Nevertheless, our findings provide additional information about the main effect of VDR variants that are not susceptible to confounding by population stratification, which are pertinent to men with advanced prostate cancer.

In summary, our findings reveal a strong inverse association between VDR variants and prostate cancer that emerged primarily when VDR haplotypes were considered. The results may not apply to men diagnosed at later ages. This may help explain why several previous reports considering single VDR variants have show limited, if any, associations with disease. A recently developed dense SNP and linkage disequilibrium map of the VDR gene (32) should help advance future studies and our understanding of the potential functional role of variants residing on the VDR haplotypes associated with prostate cancer.

References

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