Short Communication

Genetic Variants in the Vitamin D Receptor Gene and Prostate Cancer Risk

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Abstract

Vitamin D receptor (VDR), a member of the steroid/thyroid hormone nuclear receptor family, is bound by the steroid hormone 1,25-dihydroxyvitamin D3, which is thought to play a role in the etiology and progression of prostate cancer. Polymorphisms in the VDR gene have been associated with prostate cancer risk, although findings are inconclusive. The purpose of this study was to determine if VDR polymorphisms were associated with prostate cancer risk using a large, Australian population–based study of 812 cases and 713 controls frequency-matched by age. As the 3′ region polymorphisms are in strong linkage disequilibrium, for joint effects, we only evaluated the common g.60890G > A polymorphism with the unlinked g.27823C > T (5′ region) polymorphism. Allele frequencies were similar in cases and controls (g.27823C > T, 36% versus 36%; g.60890 G > A, 41% versus 43%). No genotypes were individually associated with prostate cancer risk (all P > 0.3). All nine possible genotype combinations were evident, and although the g.27823CT/g.60890GA combination was nominally more prevalent in controls (24%) than in cases (19%, P = 0.03), there was no difference in the combined genotype distribution between cases and controls (P = 0.2). The associations of risk with genotype were between 0.91 and 1.03, all with 95% confidence intervals within 0.81 to 1.15. In conclusion, VDR polymorphisms either alone or in combination do not seem to contribute appreciably to prostate cancer risk. (Cancer Epidemiol Biomarkers Prev 2005;14(4):997–9)

Introduction

The vitamin D receptor (VDR), a member of the steroid/thyroid hormone nuclear receptor family, is expressed in both normal and malignant prostate cells (1). The active form of vitamin D, 1,25-dihydroxyvitamin D3, binds VDR, which plays a role in maintaining calcium homeostasis and regulating cellular growth and differentiation of many cell types (2). A number of polymorphic markers of unknown functional consequence within the VDR gene have been studied for their potential role in prostate cancer risk, with inconsistent findings. These include a polyadenine (A) microsatellite repeat polymorphism located in the 3′-untranslated region, which is in linkage disequilibrium with three single nucleotide polymorphisms (SNP), two located in intron 8 (IVS8 +283G > A, also known as BsmI, and IVS8 -49G > T, also known as ApaI), and a synonymous variant in exon 9 (I349, ATT to ATG, also known as TaqI polymorphism). As the 3′-untranslated region polymorphisms have no effect on amino acids within the VDR protein, one can assume that they do not alter protein function, however, their effect on mRNA stability and VDR levels is undetermined. The C to T polymorphism, also known as FokI, located eight nucleotides upstream of the initiation start site creates an additional start codon (ACG to ATG) and thus a three–amino acid longer protein (reviewed in ref. 3). The FokI polymorphism is not linked to the 3′-untranslated region polymorphisms, and the C allele has been shown to be more transcriptionally active than the T allele (4).

The aim of this study was to re-address the hypothesis that polymorphisms in the VDR gene are associated with prostate cancer risk. We tested this hypothesis in a large case-control study of Caucasian men, where, in contrast to previous studies, age-matched controls were randomly sampled from the general population and the possible effect of the combination of the genotypes in relation to prostate cancer was assessed. As is becoming standard practice in the genetics literature, we refer to these common polymorphisms according to their genomic position (Genbank accession #AY342401), namely g.27823C > T for FokI and g.60890G > A for BsmI.

Materials and Methods

Study Population. A detailed description of the study has been published previously (5). In brief, random samples of 100%, 50%, and 25% of the cases diagnosed in the age groups younger than 60, 60 to 64 years, and 65 to 69 years, respectively, were asked to participate in this study conducted in Perth and Melbourne, Australia, from 1994 to 1997. Cases were identified from the cancer registries of Victoria or western Australia, presenting with a histopathologically confirmed adenocarcinoma of the prostate and a Gleason score of 5 or more. Controls were randomly selected from the State Electoral Rolls (registration to vote is compulsory in Australia) and frequency-matched to the expected age distribution of the cases in a ratio of one control per case. Response rates were 65% and 50% for cases and controls, respectively.

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A face-to-face interview was done using structured questionnaires to obtain information on potential risk factors including age, history of prostate cancer in first-degree relatives, country of birth, life-style (including diet), and other potential risk factors for prostate cancer. Tumor stage (stage I to IV) and grade (moderate, Gleason 5-7; high, Gleason 8-10) was recorded from histopathology reports. The great majority of subjects (98.5%) were born in Australia, the British Isles, or Western Europe and therefore of Caucasian descent. Informed consent was obtained from all study participants. Blood samples were collected from 862 cases (83%) and 745 controls (71%).

**Genotyping.** Genomic DNA was extracted from whole blood and genotyped in a blinded manner. Both the g.27823 and g.60890 SNPs were analyzed using denaturing gradient gel electrophoresis, which included the analysis of the entire coding region of exon 2, including intron-exon boundaries (VDR amplicon 2), and partial analysis of intron 8 (VDR amplicon 8). Primer sequences and conditions are available on request (V.M. Hayes). As the genotyping assay used in this study is capable of detecting all sequence variation within the amplicons, the g.27878 (N16, AAC to AAT) and g.27975 (IVS2 +8C > T) SNPs were also detected and assessed. Genotyping was successfully performed on 812 cases and 713 controls.

**Statistical Analysis.** Estimates and comparisons of allele frequencies and tests of deviation from Hardy-Weinberg equilibrium were carried out using exact methods. Linkage disequilibrium was measured using Lewontin’s D’ and tested with methods based on asymptotic likelihood theory. Unconditional logistic regression adjusted for age was used to estimate associations between the dimorphic SNPs and prostate cancer, and tests of significance were based on asymptotic likelihood theory. Influence of potential confounding variables was assessed by including them in the models and assessing any subsequent change in estimates of genotype effects and their statistical significance. Two-sided Fisher’s exact test was used to test for independence between the SNPs and categorized risk factors; namely age (<60, 60-69), country of birth (Australia, others), family history of prostate cancer (affected first-degree relatives, no affected relatives), baldness (no balding, frontal, others), family history of prostate cancer (affected first-degree relatives, no affected relatives), baldness (no balding, frontal, vertex and frontal baldness combined, 51% versus 46% of controls), to be born in Australia (75% versus 70% of controls), Europe and therefore of Caucasian descent. Informed consent was obtained from all study participants. Blood samples were collected from 862 cases (83%) and 745 controls (71%).

**Discussion and Conclusion**

We have found no evidence for associations between genotypes defined by polymorphisms of the VDR gene and prostate cancer susceptibility using a large Australian population–based case-control study. As the common polymorphisms in the 3’ end of the VDR gene are in linkage disequilibrium (6), we focused on the g.60890 SNP located in this region of linkage disequilibrium because its rare variant was more common than for the other two linked polymorphisms in that region. The g.27823 SNP that creates an extended VDR, represented the 5’ region of the 5’-untranslated region. The allele frequencies for the two common polymorphisms were similar to those reported for European-based populations (7-9).
A recent study to determine the impact of UV radiation exposure on prostate cancer, and it has therefore been proposed that lower total free serum 1,25-dihydroxyvitamin D3 levels, the association with prostate cancer risk, although in men with lower total free serum 1,25-dihydroxyvitamin D3 levels, the association with prostate cancer risk may be attributed to inconsistencies or bias in both case and/or control selection, or to there being no real or detectable effect. In our study, we addressed the former issues by avoiding selection for family history in our cases and by recruiting the controls from the general population (via State Electoral rolls according to age-matching of cases) and not from a prostate cancer screening population. Use of prostate-specific antigen testing was already common in Australia at the time of the study. To reduce the possible impact of indolent, slow-growing tumors, we excluded well-differentiated tumors (Gleason < 5) and tumors in elderly men (age 70 or more). In agreement with most recent publications, we found a lack of association with prostate cancer risk between the g.27823 and g.60890 SNPs, as well as the previously unassessed rare g.27878 and g.27975 SNPs. Neither the meta-analysis, nor the large U.S.-based study, performed analyses combining the genotypes of different SNPs. This latter type of analysis in our study did not reveal any significant contribution of the g.27823 and g.60890 SNPs combined with prostate cancer risk, and the confidence intervals were small. We therefore conclude that genetic markers of VDR alone and in combination do not seem to contribute appreciably to risk of prostate cancer.

### Acknowledgments

We thank the study participants, and the many urologists, nurses, and histopathologists who kindly facilitated in the recruitment and collection of patient information and pathology reports.

### References


### Table 2. VDR genotype combinations (g.27823 and g.60890) and prostate cancer risk

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls, % (n = 713)*</th>
<th>Cases, % (n = 811)*</th>
<th>Odds ratios*</th>
<th>*Likelihood ratio test for the inclusion of the variable in the model.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC/GG</td>
<td>98 (14)</td>
<td>121 (15)</td>
<td>Reference</td>
<td>0.2</td>
</tr>
<tr>
<td>CC/GA</td>
<td>135 (19)</td>
<td>167 (21)</td>
<td>1.00 (0.92-1.09)</td>
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</tr>
<tr>
<td>AA/GG</td>
<td>60 (8)</td>
<td>52 (6)</td>
<td>0.91 (0.82-1.02)</td>
<td></td>
</tr>
<tr>
<td>CT/GG</td>
<td>101 (14)</td>
<td>134 (17)</td>
<td>1.02 (0.93-1.13)</td>
<td></td>
</tr>
<tr>
<td>CT/GA</td>
<td>170 (24)</td>
<td>153 (19)</td>
<td>0.92 (0.85-1.00)</td>
<td></td>
</tr>
<tr>
<td>CT/AA</td>
<td>51 (7)</td>
<td>72 (9)</td>
<td>1.03 (0.92-1.15)</td>
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<tr>
<td>TT/GG</td>
<td>33 (5)</td>
<td>39 (5)</td>
<td>0.99 (0.86-1.13)</td>
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</tr>
<tr>
<td>TT/GA</td>
<td>46 (7)</td>
<td>53 (7)</td>
<td>0.98 (0.87-1.10)</td>
<td></td>
</tr>
<tr>
<td>TT/AA</td>
<td>19 (3)</td>
<td>20 (3)</td>
<td>0.96 (0.81-1.14)</td>
<td></td>
</tr>
</tbody>
</table>

*Number of subjects with both variants measured.

Reference: 

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