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 D_3, 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 D_3, 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 *Bsm*I, and IVS8 -49G > T, also known as *Apa*I), and a synonymous variant in exon 9 (I349, ATT to ATC, also known as *Taq*I 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 *Fok*I, 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 *Fok*I 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 *Fok*I and g.60890G > A for *Bsm*I.

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 i8), 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 changes 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, vertex, frontal and vertex combined), history of benign prostatic hyperplasia (yes, no) and tumor stage (stage I to IV), and grade (moderate and high). The likelihood ratio test was used to test for an overall difference in genotype distribution between cases and controls. All statistical analyses were done using the R statistical language (www.r-project.org).

Results

Cases were slightly younger than controls (18% aged <55 versus 14% in controls), were more likely to have a history of prostate cancer in first-degree relatives (18% of cases versus 6% of controls), to be bald (proportion of cases with vertex or vertex and frontal baldness combined, 51% versus 46% of controls), to be born in Australia (75% versus 70% of controls), and report a personal history of benign prostatic hyperplasia (20% versus 16% in controls). In cases, 27% presented with high-grade tumors (Gleason 8 or higher), and 69% with tumors confined within the prostate (T1-T2), whereas only 5% were metastatic (T4 or N1 or M1).

Table 1 shows that there was no evidence that any of the polymorphisms were not in Hardy-Weinberg equilibrium for cases, controls, or cases and controls combined (all $P \geq 0.2$). The prevalence of the g.27823 T allele was 36% in both the cases and controls, whereas the prevalence of the g.60890 A allele was 41% in the cases and 43% in the controls. The prevalences of the g.27878C > T and g.27975C > T SNPs were 2% and 4% in both cases and controls, respectively. Table 1 also shows that none of the four loci were associated with prostate cancer risk (all $P \geq 0.3$). None were associated with any of the known measured prostate cancer risk factors including age, family history of prostate cancer, country of birth, and androgenetic alopecia (baldness), or with a personal history of benign prostatic hyperplasia (all $P > 0.1$). Genotype distribution did not vary by tumor grade (all $P > 0.2$). Although the genotype distribution was not associated with tumor stage for any of the SNPs, the proportion of homozygotes for the T (g.27823) and A allele (g.60890) was lowest in metastatic cases (9% versus 14% in stage I-III tumors for g.27823, $P = 0.6$ and 9% versus 18% for g.60890). Both rare polymorphisms were in linkage disequilibrium with g.27823, with the T alleles of g.27878 and g.27975 in linkage disequilibrium with the g.27823 T allele ($D' = 0.96$, $P < 0.001$) and C allele ($D' = 0.66$, $P < 0.001$), respectively. Neither was in linkage disequilibrium with g.60890 (both $P \geq 0.1$).

Table 2 shows that all nine possible genotype combinations among the unlinked dimorphic markers g.27823 and g.60890 were observed. Overall, there was no evidence of a difference in the distribution of the genotypes of the two SNPs combined between cases and controls ($P = 0.2$) with all odds ratios relative to male homozygotes for both common alleles (CC/GG) between 0.91 and 1.03 and all 95% confidence intervals within 0.81 to 1.15. The only odds ratio of marginal statistical significance was the one relative to heterozygotes for both markers (CT/GA) who were more prevalent in controls (24%) than in cases (19%; odds ratio, 0.92; 95% confidence interval, 0.85-1.00). The associations between genotypes and prostate cancer risk were not modified by the inclusion in the models of family history, country of birth, baldness, and personal history of benign prostatic hyperplasia.

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. The allele frequencies for the two common polymorphisms were similar to those reported for European-based populations (7-9).

Table 1. *VDR* gene polymorphisms in controls and prostate cancer cases

	Controls, % (n = 713)*	Cases, % (n = 812)*	Odds ratios† (95% confidence interval)	P‡
g.27823	$P_{HWE} = 0.5$§	$P_{HWE} = 0.3$		>0.9
CC	293 (41)	340 (42)	Reference	
CT	322 (45)	359 (44)	0.97 (0.78-1.20)	
TT	98 (14)	112 (14)	0.99 (0.72-1.35)	
g.27878	$P_{HWE} = 0.1$	$P_{HWE} = 0.4$		0.6
CC	688 (96)	777 (96)	Reference	
Any T	25 (4)	33 (4)	1.17 (0.69-1.99)	
g.27975	$P_{HWE} = 0.9$	$P_{HWE} = 0.3$		0.7
CC	664 (93)	752 (93)	Reference	
Any T	49 (7)	59 (7)	1.07 (0.72-1.58)	
g.60890	$P_{HWE} = 0.9$	$P_{HWE} = 0.2$		0.3
GG	232 (33)	295 (36)	Reference	
GA	351 (49)	373 (46)	0.84 (0.67-1.05)	
AA	130 (18)	144 (18)	0.87 (0.65-1.17)	

*Number of subjects with at least one of the two variants measured (we were unsuccessful in genotyping 20 controls and 18 cases).
†From logistic regression analysis adjusted for age.
‡Likelihood ratio test for the inclusion of the genotype in the model.
§Test for Hardy-Weinberg equilibrium.

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

	Controls, % (n = 713)*	Cases, % (n = 811)*	Odds ratios† (95% confidence interval)	P‡
g.27823/g.60890				
CC/GG	98 (14)	121 (15)	Reference	0.2
CC/GA	135 (19)	167 (21)	1.00 (0.92-1.09)	
CC/AA	60 (8)	52 (6)	0.91 (0.82-1.02)	
CT/GG	101 (14)	134 (17)	1.02 (0.93-1.11)	
CT/GA	170 (24)	153 (19)	0.92 (0.85-1.00)	
CT/AA	51 (7)	72 (9)	1.03 (0.92-1.15)	
TT/GG	33 (5)	39 (5)	0.99 (0.86-1.13)	
TT/GA	46 (7)	53 (7)	0.98 (0.87-1.10)	
TT/AA	19 (3)	20 (3)	0.96 (0.81-1.14)	

*Number of subjects with both variants measured.
† From logistic regression analysis adjusted for age.
‡ Likelihood ratio test for the inclusion of the variable in the model.

Previous studies assessing the g.27823 polymorphism and prostate cancer risk in Caucasian populations (Europe) were limited by their sizes (132 and 210 cases, 105 and 155 controls, respectively; refs. 7, 9), whereas Xu et al. (U.S., ref. 10), although finding no association with prostate cancer risk, suggested a protective effect of the TT genotype on disease progression. The g.60890 polymorphism has only been assessed by one Caucasian-based (U.S.) case-control study (372 cases and 591 controls), which found no overall association with prostate cancer risk, although in men with lower total free serum 1,25-dihydroxyvitamin D_3 levels, the AA genotype was associated with an increased risk of prostate cancer compared with the GG genotype (8). These markers were included in a meta-analysis of three studies of g.27823 (514 cases and 545 controls) and five studies of g.60890 (987 cases and 1,504 controls), which showed no evidence of an overall association of prostate cancer risk on a population basis (11). More recently, Keitheri Cheteri et al. (12), who did, prior to our study, the largest reported Caucasian-based case-control study of the g.27823, g.60890, and poly(A) repeat polymorphisms (U.S., 559 cases and 523 controls), showed no association between these markers and prostate cancer risk. They did report, however, an association between the g.60890 AA genotype and a modest, marginally significant, increase in the risk of localized prostate cancer (P = 0.04). The latter observation was not confirmed by our study. Although clinicopathologic correlations between the *VDR* polymorphisms have been suggested (reviewed in ref. 11), these studies have not been replicated either in previous studies or in our own.

It has been suggested that UV radiation protects against prostate cancer, and it has therefore been proposed that protection is also afforded by increased vitamin D intake (13). As vitamin D exerts its effects in the prostate via the VDR, this has led to the hypothesis that polymorphisms within the *VDR* may influence prostate cancer risk in a way that is dependent on the individual extent of UV radiation exposure (14). A recent study to determine the impact of *VDR* polymorphisms with prostate cancer risk in northern European men stratified into low and high UV radiation exposure, found the TT genotype to be associated with increased prostate cancer risk in men with UV radiation exposure above 1,100 hours per year (15). As the Australian population is exposed to UV radiation well in excess of that experienced by their Caucasian ancestors, one cannot exclude the possibility that the null findings in this study is not due to there being a modest effect of the *VDR* polymorphisms on VDR production and/or function which may only manifest in populations with inadequate sunlight hours.

Inconclusive findings for the role of *VDR* polymorphisms and 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.

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