Clinical and Pathological Significance of Vitamin D Receptor Gene Polymorphism for Prostate Cancer which Is Associated with a Higher Mortality in Japanese

TAKASHI HAMASAKI, HISATO INATOMI, TAKAHIKO KATOH*, TOSHIRO IKUYAMA AND TETSURO MATSUMOTO

Abstract. The purpose of this study was to investigate the TaqI vitamin D receptor (VDR) polymorphism in both Japanese prostate cancer patients and Japanese noncancer controls in order to determine if an association exists between VDR genotype with clinical and pathological risk of prostate cancer patients. This study involved 115 patients with prostate cancer and 133 male age-matched noncancer controls genotyped for a previously described TaqI restriction fragment length polymorphism (RFLP) at codon 352 in exon 9 of the VDR gene. Products were digested into T allele or t allele according to the absence or presence of TaqI restriction site with individuals being classified as TT, Tt, or tt. The genotype tt was higher among the control group (6.0%) compared to the patients with prostate cancer (1.8%), but not so (OR=0.28; 95% CI, 0.06-1.33; p=0.081). In addition, the genotype TT was statistically higher among patients with locally advanced or metastatic disease (T3/T4/N1/M1) compared to controls (OR =2.52; 95% CI, 1.21-5.27; p=0.009). Lastly, the genotype TT was statistically higher among patients with poorly differentiated adenocarcinoma compared to controls (OR=5.38; 95% CI, 1.57-18.50; p=0.002). These data demonstrate that VDR genotype plays an important role in determining the risk of more clinically advanced and pathologically aggressive prostate cancer which is associated with a higher mortality rate in Japanese men.

Key words: Vitamin D receptor, Gene polymorphism, Prostate cancer, Japanese

Several factors are associated with an increased risk for prostate cancer including ethnicity, environmental exposure, age, family history and lifestyle. African-American men have the highest rate of prostate cancer in the world (incidence approximately 149 per 100,000 person-year). Caucasians living in the USA have an intermediate incidence (107 per 100,000 person-year) whilst Japanese men have the lowest incidence (39 per 100,000 person-year) [1, 2]. However, the incidence of prostate cancer in Japanese immigrants to the USA has markedly increased, although the rate on the west coast of the USA is still significantly less than that among Caucasians [1, 2]. Furthermore, recent reports have demonstrated a significant north-south trend of prostate cancer mortality rates in the USA with significantly lower mortality rates being found in the southern USA [3, 4]. Apart from emphasizing that the development of prostate cancer is influenced by both genetic and environmental factors, these epidemiological studies suggested that exposure to UV radiation may be protective against prostate cancer. A possible mechanism for the proposed protective effect of UV radia-
tion has been suggested by recent studies, which have shown an association between vitamin D receptor (VDR) polymorphisms and the development of prostate cancer [5-7]. However, recent studies included men with benign prostate hyperplasia (BPH) in the control groups. BPH has an inherent genetic component [8, 9], and since vitamin D may play a role in the growth and differentiation of prostatic stromal and epithelial cells, it is possible that VDRs may be associated with the development of BPH [10, 11]. If this is the case then analysis of data from studies that include BPH patients in the control group may mask the association of VDR polymorphism and prostate cancer. In this study, we excluded BPH patients from control group.

The VDR is known to have polymorphisms that may result in individual variations including variations in the circulating levels of active vitamin D. The alleles can be assayed using restriction fragment length polymorphisms (TagI, BsmI, ApaI) that lie in the region from exon 8 to the 3' untranslated region (3'UTR). The two most frequent vitamin D receptor alleles are BAt and baT. BAt allele means BsmI and ApaI absent but TaqI present, whereas baT allele means BsmI and ApaI present but TaqI absent. These alleles are in linkage disequilibrium and occur together in 97% of the cases [12]. Therefore, we have developed an assay for one of the candidate sequences, the TaqI polymorphism. In this study, we tested the hypothesis that the genotype tt, which is associated with higher circulating levels of active vitamin D [12], would be associated with a diminished risk of prostate cancer risk in Japanese men compared to the TT and Tt genotype. We also examined the possible associations between the VDR polymorphisms and the clinical stage and pathological finding of prostate cancer.

Material and Methods

A total of 115 patients with prostate cancer and 133 age-matched controls were enrolled from our urology clinic. All patients gave their informed consent to participate in this study. The diagnosis of prostate cancer was confirmed histologically in the 115 patients of the study group. The 133 control patients consisted of male urology clinic patients with benign retroperitoneal and genitourinary diseases. Physical, serological (prostate specific antigen: PSA), and radiological examinations were performed in all controls to exclude the possibility of prostate cancer, BPH or other malignant disease. The control patients had no BPH-related symptoms, a normal serum PSA level (<4.0 ng/ml by the Tandem-R assay) and no clinical signs of prostate hyperplasia or cancer by rectal digital examination. Clinical BPH patients were excluded from control group in this study.

Blood samples were obtained between March 1997 and February 2001 from both patient groups and patient data were obtained from the medical records of patients. Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol/chloroform extraction. VDR TaqI genotype was determined by a polymerase chain reaction (PCR) method described by Riggs et al. [13]. A 740 base pairs (bp) fragment was generated by PCR with primers located in exon 9. The primer sequences were 5’ cag agc atg gac agg gag caa 3’ (forward) and 5’ gca act cct cat ggc tga ggt ctc 3’ (reverse). The 35 cycles were performed using Taq polymerase (Perkin Elmer Co., Ltd, New Jersey, USA) at denaturation (94°C, 1 minute), annealing (57°C, 1 minute) and extension (72°C, 1 minute). Following PCR, 10μL PCR products were removed and subjected to restriction digestion with TaqI at 65°C for 3 hours (restriction enzyme from Takara Shuzou Co., Ltd, Kyoto, Japan) and run on 3% Nusieve agarose gels. The presence of C > T change at the third position of codon 352 in exon 9, which is the code for isoleucine, is associated with the loss of a TaqI restriction site. The resulting alleles are designated T (TaqI site absent; 2 fragments of 495 bp and 245 bp) or t (TaqI site present; 3 fragments of 290 bp, 245 bp and 205 bp). Individuals were classified as TT, Tt, or tt (Fig. 1). Clinical staging of patients with prostate cancer at diagnosis was evaluated on the basis of the TNM classification edited by UICC in 1997 [14] whilst the histological study of cases was performed according to the General Rule for Clinical and Pathological Studies on Prostate Cancer in 2001 [15].

We attempted to determine whether the tt genotype decreased the risk of prostate cancer compared to the TT and Tt genotype. We also examined the associations between the VDR genotypes and clinical stage and pathological finding of prostate cancer patients. The relative associations between prostate cancer patients and controls were assessed by cal-
Calculating the odds ratios (OR) from contingency tables. The OR and 95% confidence intervals (CI) were calculated by multiple regression analysis using the JMP program package (Version 3, SAS Institute Inc., NC, USA). The difference in age distribution between prostate cancer patients and controls was analyzed with the unpaired t test. Data are presented as mean ± SD. Statistical significance was defined as a p value of less than 0.05.

Results

There was no statistical difference in the age at diagnosis between the groups (70.3 ± 5.4 vs 67.7 ± 7.7, prostate cancer patients vs controls, p = 0.214). The first major finding of this study was that there was no significant difference in the occurrence of the genotype tt between the control and prostate cancer groups, although there was a trend to a slightly higher incidence in the control group (6.0% vs 1.8%, control group vs prostate cancer group; OR = 0.28; 95% CI, 0.06–1.33; p = 0.081, tt vs TT /Tt) (Table 1). We therefore divided patients into two groups according to the absence or presence of the t allele (i.e. TT and Tt/tt groups) in order to analyze potential risk factors for more clinical advanced and pathological aggressive disease in prostate cancer patients. The genotype TT is associated with lower circulating levels of active vitamin D compared to the tt and Tt genotype [12].

For analysis of the risk factor associated with advanced and lethal prostate cancer, we divided the clinical stages into two groups; the organ-confined group (intra-prostate disease: T1a-c /T2a-b, N0, M0: 44 patients) and the locally advanced or metastatic

Table 1. Frequency of the vitamin D receptor alleles using the TaqI restriction fragment length polymorphism in prostate cancer patients and controls.

<table>
<thead>
<tr>
<th>Genotype (%)</th>
<th>OR* (95% CI*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tt vs (TT + Tt)</td>
</tr>
<tr>
<td>Controls (n=133)</td>
<td>91 (68.4)</td>
</tr>
<tr>
<td>Prostate Cancer (n=115)</td>
<td>91 (79.1)</td>
</tr>
</tbody>
</table>

*OR; Odds ratio, *95% CI; 95% confidence interval
Table 2. Frequency of the vitamin D receptor alleles of prostate cancer patients between clinical stages.

<table>
<thead>
<tr>
<th>Genotype (%)</th>
<th>ORa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>Tt</td>
</tr>
<tr>
<td>Controls (n=133)</td>
<td>91 (68.4)</td>
</tr>
<tr>
<td>Prostate Cancer (n=115)</td>
<td></td>
</tr>
<tr>
<td>&lt;T1a-c/T2a-b N0 M0 (n=44)</td>
<td>31 (70.5)</td>
</tr>
<tr>
<td>≧T3a-b/T4/N1/M1a-c (n=71)</td>
<td>60 (84.5)</td>
</tr>
</tbody>
</table>

aOR; Odds ratio, b95% CI; 95% confidence interval, cTNM classification edited by UICC in 1997. T: tumor extension (T1–T4), N: lymph node metastasis (N0/N1), M: distant metastasis (M0/M1). dT1a-c/T2a-b N0 M0: organ-confined group (intra-prostate disease). dT3a-b/T4/N1/M1a-c: locally advanced or metastatic group (extra-prostate disease).

Table 3. Frequency of the vitamin D receptor alleles of prostate cancer patients between pathological grades.

<table>
<thead>
<tr>
<th>Genotype (%)</th>
<th>ORa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>Tt</td>
</tr>
<tr>
<td>Controls (n=133)</td>
<td>91 (68.4)</td>
</tr>
<tr>
<td>Prostate Cancer (n=115)</td>
<td></td>
</tr>
<tr>
<td>&lt;Wel-Mod (n=77)</td>
<td>56 (72.7)</td>
</tr>
<tr>
<td>≧Por (n=38)</td>
<td>35 (92.1)</td>
</tr>
</tbody>
</table>

aOR; Odds ratio, b95% CI; 95% confidence interval, Pathological finding is defined as low and intermediate grade group: well and moderately differentiated adenocarcinoma (Wel and Mod), high grade group: poorly differentiated adenocarcinoma (Por).

In patients with prostate cancer, the genotype TT was significantly higher among the locally advanced and metastatic group (84.5%) compared to controls (68.4%) (OR = 2.52; 95% CI, 1.21–5.27; p = 0.009, TT vs Tt/tt). However, there was no statistically significant difference in the distribution of genotype TT between the organ-confined group (70.5%) and controls (OR = 1.10; 95% CI, 0.52–2.32; p = 0.479, TT vs Tt/tt) (Table 2).

The pathological findings of prostate cancer cases were well differentiated adenocarcinoma (Wel) in 29 (25.2%), moderately differentiated adenocarcinoma (Mod) in 48 (41.7%), and poorly differentiated adenocarcinoma (Por) in 38 patients (33.1%). We also divided the pathological findings into two groups; the low and intermediate grade group (Well and Mod) and the high grade group (Por). The genotype TT was significantly higher among the high grade group (92.1%) compared to controls (68.4%) (OR = 5.38; 95% CI, 1.57–18.50; p = 0.002, TT vs Tt/tt), but there was no statistical difference in the distribution of genotype TT between the low and intermediate grade group (72.7%) and controls (OR = 1.23; 95% CI, 0.66–2.29; p = 0.310, TT vs Tt/tt) (Table 3).

Therefore, the second major finding of this study is that the genotype TT is associated with a more advanced clinical stage and a pathological grade of prostate cancer which is associated with a higher mortality rate.
Discussion

Recent studies of associations between VDR gene polymorphisms and prostate cancer are problematical since these studies included men with BPH in the control groups. Recent evidence has suggested that VDR gene polymorphisms may be associated with the development of BPH [10, 11]. If this is the case then analysis of data from studies that include BPH patients in the control group may mask the association of VDR polymorphism and prostate cancer. BPH is benign disease, however, hence BPH patients were excluded from control group in this study.

Several recent studies of VDR TaqI genotypes in Japanese men have found that the genotype tt is uncommon in Japanese men, occurring with a frequency of 0%-2.0% in prostate cancer patients and 1.6%-3.0% in controls [10, 16, 17]. These three studies reported that TaqI polymorphisms did not show a significant association with developing prostate cancer in Japanese men. In this study, our data is in agreement with these previous Japanese studies in reporting a lack of an association between TaqI tt genotype and the development of prostate cancer.

Currently, there is a lack of data regarding the actual association of VDR genotype with the clinical stage of disease and pathological finding of the tumor in prostate cancer patients. In this study, the genotype TT was significantly associated with a more advanced clinical stage of disease. Furthermore, the genotype TT was associated with a higher pathological grade of prostate cancer. Morrison et al. [12] demonstrated that the BAt allele had 140% greater receptor activity than baT allele. This difference was believed to be secondary to enhanced gene transcription or mRNA stability, resulting in individual variation in VDR expression [5, 12]. These evidences support our studies which suggest a clinical and pathological significance of vitamin D receptor gene polymorphism for prostate cancer.

Recently, Habuchi et al. [10] reported that the BsmI polymorphism in the VDR gene plays a significant role in protection against prostate cancer in Japanese. Much further work is required in this field including a large scale analysis of VDR gene polymorphisms in different parts of Japan as well as additional analysis of other VDR polymorphisms.

Clinically, vitamin D may act as a tumor inhibitor by retarding the progression of indolent prostate cancer to more active disease [3, 5]. Indeed, oral administration of 1,25-dihydroxyvitamin D3 (1,25 (OH)2D3) delays the recurrence of prostate cancer following primary therapy [18]. This evidence indicates that 1,25(OH)2D3 can be effective in slowing the progression of prostate cancer in patients. As to what might be the potential mechanism whereby VDR polymorphisms may modulate susceptibility to the development and progression of prostate cancer, it has been shown that the TaqI VDR polymorphism is associated with circulating levels of the active form of vitamin D [12] and there is now an impressive body of in vitro data indicating that vitamin D may exert important biological effects upon the behavior of prostate epithelial cells. For example, a recent study demonstrated that 1,25(OH)2D3 inhibits proliferation of both normal and malignant prostate epithelial cells [19]. Furthermore, 1,25(OH)2D3 and related compounds may also inhibit growth and promote the differentiation of prostate cancer cell lines [20-22]. For example, 1,25(OH)2D3 treatment of LNCaP cells may initiate differentiation by up-regulation of the expression of the androgen receptor (AR) and the secretion of prostate-specific antigen (PSA, a differentiation marker) [23, 24]. Lastly, reports have suggested that 1,25(OH)2D3 may induce apoptosis of human prostate cancer and other cancer cell lines [23, 25, 26] with cell lines that lack VDR being unresponsive [27].

Recent studies have shown an association between VDR genotype and the development of prostate cancer [5-7]. Taylor et al. [6] reported that 22% of Caucasian controls were positive for the presence of the genotype tt (TaqI RFLP) whereas only 8% of Caucasian prostate cancer patients had this genotype (p<0.01). TaqI RFLP and the poly-A microsatellite repeat of the VDR gene are in strong linkage and Kibel et al. [7] reported that TT, Tt, and tt genotypes matched with LL, LS, SS genotypes respectively in 94% of prostate cancer patients. Kibel et al. [7] found no evidence of an increased risk of advanced prostate cancer based upon VDR genotypes. However, Ingles et al. [5], who noted that 20% of controls were genotype SS compared to only 5% of prostate cancer cases, described that the VDR poly-A microsatellite repeat with genotype LL, which matched with the genotype TT, was more strongly associated with locally advanced and metastatic disease than organ-confined disease. The discrepancies
between these studies may be secondary to differences of sample size, ethnicity and staging of prostate cancers.

In conclusion, this study indicates that TaqI polymorphisms did not show a significant association with the risk of developing prostate cancer. However, TaqI polymorphisms do play an important role in determining the clinical and pathological risk factor of prostate cancer in Japanese.

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References


