

NOTE

Vitamin D Receptor Gene Polymorphism in Japanese Patients with Prostate Cancer

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Abstract. Vitamin D receptor gene polymorphism was determined in 66 and 60 Japanese patients with prostate cancer and non cancer controls, respectively. In contrast to previous reports showing an association between vitamin D receptor polymorphism of a TaqI restriction fragment length polymorphism at codon 352 (genotype *tt*) and prostate cancer in an American population, the frequency of genotype *tt* is less than one percent in the Japanese population. There was no difference between the patients and the controls in the vitamin D receptor TaqI genotype. In patients with metastatic prostate cancer, genotype *TT* had a tendency to shorter progression free survival compared to genotype *Tt*, but the number of patients was limited.

Key Words: Prostate cancer, Vitamin D receptor, Polymorphism

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THE incidence of prostate cancer is increasing worldwide. In the United States, it is the most diagnosed malignant tumor and the second most common cause of cancer death in men [1]. In Japan, the number of prostate cancers is smaller than that in western countries, but it is increasing [2]. Recently vitamin D deficiency has been hypothesized to be a risk factor for prostate cancer. The risk of prostate cancer decreases with higher levels of serum 1,25-hydroxyvitamin D3 [3]. Vitamin D may act as a tumor inhibitor, retarding the progression of clinical prostate cancer [4]. In cell culture studies, vitamin D analogue inhibited the growth of prostate cancer cells via apoptosis [5]. A total of 13 polymorphisms have been identified in the vitamin D receptor locus [6]. Although the exact sequence elements responsible for functional variation are not known, the alleles can be assayed by restriction fragment length polymor-

phism. It was presumed that the variations are responsible for differences in translation efficiency or in RNA stability [6].

It was recently reported that vitamin D receptor genotype is related to the risk of prostate cancer [7]. Taylor *et al.* examined the presence of TaqI restriction fragment length polymorphism at codon 352 and a less active vitamin D receptor allele (TaqI site absent) is associated with an increased risk of prostate cancer [7]. In the present study, we examined vitamin D receptor polymorphism in Japanese patients with prostate cancer in order to determine if an association exists between vitamin D receptor genotype and the incidence and/ or progression of prostate cancer.

Patients and Methods

A total of 66 prostate cancer patients and 60 control patients were enrolled in this study. All patients gave informed consent to participate in the study. Non cancer controls were urology patients without evidence of prostate cancer on PSA tests, digital

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rectal examination, and/or prostate needle biopsy. DNA was extracted from the peripheral blood with a DNA extraction kit (Takara, Kyoto). Vitamin D receptor TaqI genotype was determined as described by Riggs *et al.* [8]. C for T synonymous changes at the third position of codon 352 coding for isoleucine in exon 9 alters a TaqI restriction site. A 740-bp fragment was generated by polymerase chain reaction (PCR) with primers located within intron 8 and exon 9. The primers used were 5'-cag agc atg gac agg gag caa (forward), and 5'-gca act cct cat ggc tga ggt ctc (reverse). The PCR protocol consisted of 35 cycles of denaturation (92°C, 60 s), annealing (60°C, 60 s), and extension (72°C, 90 s) in a Thermal Sequencer (Iwaki, Chiba). The product was subjected to TaqI digestion and separated on 3% agarose gels. Three possible genotypes were present: *TT*, *Tt* and *tt*, where *T* designates TaqI site absent (495 and 245 bp fragments) and *t* designates TaqI site present (290, 245, and 205 bp fragments).

Staging of the tumor was evaluated according to the classification of the General Rule for Clinical and Pathological Studies on Prostatic Cancer [9]. Progression following endocrine therapy in metastatic prostate cancer was defined as the appearance of at least one of the following: new or worsened bone metastases, more than 25% increase in soft tissue disease and/ or in the prostate. Statistical significance was estimated by a one-way analysis of variance and the Cox-Mantel method [10]. Progression-free survivals were calculated by the Kaplan-Meier method [11].

Results

Age distribution in the cases and the controls ranged from 57 to 84 and 47 to 79 years, with a mean \pm SD of 68.5 ± 7.0 and 67.7 ± 6.9 , respectively. The clinical stages of prostate cancer were stage A in 4, stage B in 9, stage C in 33 and stage D in 20 patients. None of 66 prostate cancer patients and only one of 60 controls (1.7%) had genotype *tt*. Because of the very low frequency of the *tt* genotype, we could not estimate the odds ratio of *TT/Tt* vs *tt* groups, which was reported previously [7, 12]. Therefore we divided the subjects into two groups according to the presence or absence of the *t* allele, that is the *TT* group and *Tt/tt* group.

No differences were found between prostate cancer cases and the controls in the frequencies of the *T* or *t* alleles (Table 1). In prostate cancer patients, there were no differences between the two groups in clinical stages. When survival in the *TT* and *Tt/tt* groups in patients with metastatic prostate cancer was compared, the *Tt/tt* group had a tendency to better progression free survival than the *TT* group, although only twenty patients could be evaluated (Fig. 1).

Discussion

A number of risk factors for patients with prostate cancer have been reported. Recently vitamin D deficiency has been hypothesized to be a risk factor [3]. Lack of exposure to ultraviolet radiation is a

Table 1. Frequency of vitamin D receptor alleles

	No. (%)		
	<i>TT</i>	<i>Tt</i>	<i>tt</i>
Controls (60 pts) ^a	41 (68.3)	18 (30.0)	1 (1.7)
Cases (66 pts) ^a	41 (62.1)	25 (37.9)	0
stage A	3	1	0
stage B	5	4	0
stage C	21	12	0
stage D	12	8	0

^aOdds ratio = 1.32 (95% CI 0.62–2.83) *TT* vs *Tt/tt*

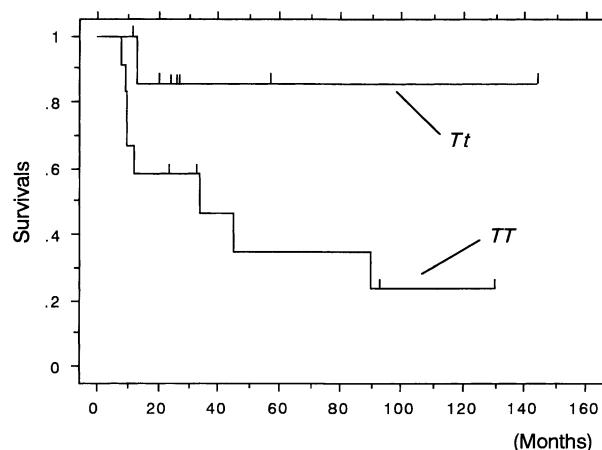


Fig. 1. Progression-free survivals according to the vitamin D receptor genotype in patients with metastatic prostate cancer. $p=0.072$

potential risk factor for prostate cancer [13]. A low serum level of vitamin D3 has been linked to increased risk of clinically significant prostate cancer [3]. As the serum level of vitamin D3 rises, the risk of clinically significant prostate cancer decreases in Americans [3]. Recent evidence has suggested the potential effects of 1,25-dihydroxyvitamin D3, the active analogue of vitamin D3, in inducing apoptosis of a variety of cancer cell lines [14, 15]. Vitamin D is active in binding the vitamin D receptor [4]. Therefore the function of vitamin D receptor might be associated with the incidence and/or progression of prostate cancer. Mutation in the vitamin D receptor results in vitamin D-resistant rickets [16] but there is only limited information on the potential contribution of natural allelic variation in receptor genes to the diversity of response to vitamin D in disease. Recently a series of common polymorphisms in the vitamin D receptor gene were reported to be associated with bone density and the risk of osteoporosis [8]. Significantly higher serum levels of 1,25-dihydroxyvitamin D3 have been reported in those who are homozygous for the *t* allele relative to those who are heterozygous or homozygous for the *T* allele in healthy females [6].

Taylor *et al.* reported that 8 % of prostate cancer patients and 22% of controls were genotype *tt* [7]. They concluded that homozygosity for the *tt* (the more active allele) may protect individuals from prostate cancer. Ingles *et al.* reported 37 patients with poly A microsatellite repeat in the 3'-untranslated region [17]. Because shorter alleles play a protective role, patients with prostate cancer have less homozygous alleles for the shorter repeat. Vitamin D receptor genotype with at least one long allele was more closely associated with advanced disease than with localized disease. TaqI restriction fragment length polymorphism and the poly-A microsatellite are in strong linkage disequilibrium. Kibel *et al.* suggested that TaqI polymorphism and poly-A microsatellite caused the same phenomenon [12]. They also concluded that there is no association between the vitamin D receptor genotype alone and lethal metastatic prostate cancer. In the present

study, we did not show a difference between prostate cancer patients and the controls in the vitamin D receptor genotype. Genotype *tt* is rare in Japanese patients. Therefore we could not show the effect of *tt* genotype in protecting against prostate cancer in Japanese. Suzuki *et al.* reported that genotype *tt* is also limited in Japanese prostate cancer patients as well as Japanese controls [18]. The present results show that in patients with metastatic prostate cancer, genotype *Tt* might protect against disease progression, but too few metastatic patients were available for study. Reports concerning vitamin D receptor genotype and progression of advanced prostate cancer are very limited in number [12]. Details of the mechanism of vitamin D receptor polymorphism in the progression of prostate cancer has been obscure up to now. Another polymorphism in the translation initiation site of the vitamin D receptor gene, which results in receptor proteins that differ in length by three amino acids, has been reported in association with bone mineral density [19, 20]. There appears to be no link between this polymorphism and the TaqI polymorphism [21]. Analysis of the polymorphism in the translation initiation site will be needed in patients with prostate cancer. Alternatively it is possible that the vitamin D receptor polymorphisms may be linked to another nearby gene and that these associations may not be related to the vitamin D receptor itself [22].

In conclusion, there are racial differences in vitamin D receptor gene polymorphism in prostate cancer patients, and this polymorphism might be associated with the progression of prostate cancer, especially in patients with advanced prostate cancer, although further study is needed.

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