ABSTRACT

Recent studies have suggested that vitamin D is an important determinant of prostate cancer risk and inherited polymorphisms in the 3′-untranslated region (3′UTR) of the vitamin D receptor (VDR) gene are associated with the risk and progression of prostate cancer. This study was conducted to explore the association of VDR gene polymorphisms with prostate cancer risk in Japanese men who are considered to be much less influenced by environmental risk factors for prostate cancer. We studied 222 prostate cancer patients, 209 benign prostatic hyperplasia (BPH) patients, 128 male controls who were over 60 years old and without any evidence of prostate cancer or BPH, and 198 female controls. A PCR-RFLP method was used to determine three VDR gene polymorphisms in the 3′UTR characterized by restriction enzymes BsmI, ApaI, and TaqI. In the BsmI polymorphism, heterozygosity or homozygosity for the absence of the BsmI restriction site was associated with one-third the risk of prostate cancer (P < 0.0001; odds ratio, 3.31; 95% confidence interval, 2.05–5.32) and with one-half the risk of BPH (P < 0.005; odds ratio, 2.07; 95% confidence interval, 1.33–3.22) compared with the male controls. The TaqI and ApaI polymorphisms did not show any significant association with either prostate cancer or BPH. The results indicate that the BsmI polymorphism in the VDR gene plays a significant role in protection against prostate cancer and BPH. Because of the racial differences in the strength of the linkage disequilibrium between the three polymorphisms, additional studies are required to apply the present results to other racial-ethnic groups.

INTRODUCTION

There are striking differences in the age-adjusted incidence of prostate cancer between different racial groups and between different geographic regions of the world. American blacks have the highest incidence of about 149 per 100,000 person-years and American whites have an intermediate incidence of 107 per 100,000 person-years (1). The incidence of 39 per 100,000 person-years in Japanese men is among the lowest in the world (2). On the other hand, Japanese immigrants in the United States have experienced a marked increase in prostate cancer incidence, although the rates in Japanese men in Los Angeles and the San Francisco Bay Area are still less than one-half of those in whites (1, 2). These epidemiological data emphasize that the incidence of prostate cancer is influenced by both genetic and environmental factors.

There is accumulating evidence that vitamin D may be an important determinant of occurrence and progression of prostate cancer. Because the prostate cancer mortality rate increases significantly as the availability of UV radiation exposure decreases, and the synthesis of vitamin D depends on UV radiation, it was hypothesized that vitamin D deficiency is a risk factor for prostate cancer (3). Laboratory studies revealed that vitamin D and vitamin D analogues have antiproliferative and differentiation effects on human prostatic cancer cells in vitro (4–9). Clinically, it was claimed that oral administration of 1,25-dihydroxyvitamin D3, an active form of vitamin D, may delay the recurrence of prostate cancer after primary therapy (10). These documents suggested that vitamin D had a protective effect on prostate cancer.

VDRs4 mediate the action of their cognate ligand 1,25-dihydroxyvitamin D3 by controlling the expression of hormone-sensitive genes (11, 12). The VDR gene consists of nine exons and has several polymorphisms in intron 8 and exon 9, which are in linkage disequilibrium with each other (13). Among these polymorphisms, the two most common haplotypes defined by BsmI, ApaI, and TaqI restriction site polymorphisms in the 3′UTR of the VDR gene may be associated with substantial differences in VDR expression (13). Molecular epidemiological studies have shown that certain VDR gene alleles could be associated with bone mineral density, hyperparathyroidism, osteomalacia, insulin-dependent diabetes mellitus, and osteoarthritis (14–16).

Recent molecular epidemiological studies have shown that inherited polymorphisms including the BsmI, ApaI, and TaqI polymorphisms in the 3′UTR may be linked with prostate cancer risk and/or its aggressive phenotype (17, 18). Taylor et al. (17) reported that the TaqI polymorphism in the 3′UTR of the VDR gene was associated with prostate cancer in American whites. Ingles et al. (18) reported that a novel VDR polymorphism defined by the length of polyadenosine residues in the 3′UTR was associated with the risk of prostate cancer. However, these investigations were performed mainly on white and black men in the United States who might be greatly influenced by environmental risk factors for prostate cancer and they included men with BPH in control groups (17, 18). BPH has an inheritable genetic component (19, 20), and vitamin D may play an important role in the growth and differentiation of stromal and epithelial cells of the prostate (21). Therefore, an analysis that includes men with BPH in a control group may mask the role of VDR polymorphisms in prostate cancer.

The evaluation of inheritable genetic risk factors in the Japanese population may be more valid than in western countries because native Japanese men are considered to be much less influenced by environmental risk factors for prostate cancer (2). The present study was conducted to explore the association of the BsmI, ApaI, and TaqI polymorphisms of the VDR gene with prostate cancer risk in Japanese men. In addition, we set up a group of men with apparent BPH and a group of older men without any evidence of prostate cancer or BPH.

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The abbreviations used are: VDR, vitamin D receptor; 3′UTR, 3′-untranslated region; BPH, benign prostatic hyperplasia; OR, odds ratio; CI, confidence interval; PSA, prostate-specific antigen.
MATERIALS AND METHODS

Subjects. A total of 757 subjects, consisting of 222 prostate cancer patients, 209 BPH patients, 128 male controls, and 198 female controls treated at Akita University Medical Center and related community hospitals were enrolled in this study. All of the prostate cancer patients were diagnosed histologically with specimens obtained from transrectal needle biopsy or transurethral resection of the prostate for voiding symptoms. All of the BPH patients had various degrees of lower urinary tract symptoms and an apparent prostatic enlargement by digital rectal examination. The serum PSA levels were measured in all of the BPH patients, and men with elevated PSA levels (4.0 ng/ml or more by the Tandem-R assay; Hybritech Inc., San Diego, CA) were proved not to have prostate cancer by transrectal sextant biopsies. The male control group comprised 128 volunteers over 60 years old without any voiding symptoms. They were mainly selected from the patients admitted because of different nonurological diseases in several community hospitals. They all had normal serum PSA levels (<4.0 ng/ml by the Tandem-R assay) and showed no signs of prostate cancer and no prostatic enlargement by digital rectal examination. Serum PSA was measured using the Tandem-R assay in most cases. When serum PSA was measured by kits other than the Tandem-R, the measured PSA level was adjusted to that of the Tandem-R assay using a formula published elsewhere (22). The female controls, who required complete blood count evaluation for other medical reasons, were selected randomly from our outpatient clinic. The mean ages of prostate cancer patients, BPH patients, male controls, and female controls were 72.1 ± 8.7 (± SD), 70.4 ± 9.4, 73.5 ± 7.1, and 48.6 ± 21.8 years, respectively. There was a statistically significant difference in the mean age between the female controls and the other three groups by unpaired two-tailed t test.

Genotyping of Three VDR Polymorphisms. DNA was extracted from blood samples collected from each patient using a QIAamp Blood Kit (QIAGEN, Germany) or by the standard method with proteinase K digestion followed by phenol/chloroform extraction. The 825-bp fragment encompassing the BsmI polymorphic site in intron 8 was amplified using primers and PCR conditions described by Morrison et al. (13). When the BsmI site was present, the PCR fragment was divided into 650 and 175 bp by BsmI endonuclease digestion. The 490-bp fragment encompassing Apal and TaqI was amplified using specific primers 5′-cag atgc gag gag cca-3′ in intron 8, and 5′-cacttc gac cag ggg cgt tag c-3′ in exon 9 (14, 23, 24). Thirty cycles of PCR were performed with each cycle, consisting of 94°C for 30 s, 94°C for 30 s, and 72°C for 30 s. The 490-bp fragment was divided into 280 and 210 bp with Apal digestion or into 290 and 200 bp by TaqI digestion. The PCR products were digested overnight with BsmI (65°C), Apal (25°C), or TaqI (65°C) and were electrophoresed on 2.0% agarose gels. The genotypes were designated as “A,” “B,” or “T” when the Apal, BsmI, or TaqI restriction site is absent, and as “a,” “b,” or “t” when each restriction site is present, respectively.

Statistical Methods. Associations between diseases and genotypes were assessed by calculating ORs and 95% CIs. VDR genotype distribution in BsmI, Apal (65°C), and TaqI (96°C) polymorphic site in intron 8 was amplified using specific primers 5′-cag atgc gag gag cca-3′ in intron 8, and 5′-cacttc gac cag ggg cgt tag c-3′ in exon 9 (14, 23, 24). Thirty cycles of PCR were performed with each cycle, consisting of 94°C for 30 s, 94°C for 30 s, and 72°C for 30 s. The 490-bp fragment was divided into 650 and 175 bp by BsmI endonuclease digestion. The 490-bp fragment encompassing Apal and TaqI was amplified using specific primers 5′-cag atgc gag gag cca-3′ in intron 8, and 5′-cacttc gac cag ggg cgt tag c-3′ in exon 9 (14, 23, 24). Thirty cycles of PCR were performed with each cycle, consisting of 94°C for 30 s, 94°C for 30 s, and 72°C for 30 s. The 490-bp fragment was divided into 280 and 210 bp with Apal digestion or into 290 and 200 bp by TaqI digestion. The PCR products were digested overnight with BsmI (65°C), Apal (25°C), or TaqI (65°C) and were electrophoresed on 2.0% agarose gels. The genotypes were designated as “A,” “B,” or “T” when the Apal, BsmI, or TaqI restriction site is absent, and as “a,” “b,” or “t” when each restriction site is present, respectively.

Statistical Methods. Associations between diseases and genotypes were assessed by calculating ORs and 95% CIs. VDR genotype distribution in prostate cancer and BPH was compared with male and female controls using a χ2 test (two-sided). In addition, multivariate logistic regression analyses were performed for each polymorphism with inclusion of age using a computer software SPSS. The linkage disequilibrium between the three polymorphisms was evaluated by a χ2 test. A probability of less than 0.05 was required for statistical significance.

RESULTS

Frequencies of Apal, BsmI, and TaqI genotypes and allele types in the four groups (prostate cancer, BPH, male control, and female control) are shown in Tables 1 and 2. Statistical analyses of the genotype prevalence showed that significant differences in the BsmI genotype were observed between prostate cancer patients and the male controls (P < 0.0001), between BPH patients and the male controls (P < 0.001), and between the male controls and the female controls (P < 0.0001; Table 1). A marginal significant difference in the Apal genotype was observed between prostate cancer patients and the female controls (P < 0.05). No significant difference was found in the TaqI genotype. In allelic frequencies, significant differences in the BsmI allelic prevalence were observed between prostate cancer patients and the male controls (P < 0.0001), between BPH patients and the male controls (P < 0.005), between BPH patients and the female controls (P < 0.05), and between the male controls and the female controls (P < 0.0001). No significant difference in the Apal and TaqI allelic prevalence was found among these four groups.

Because of the significant differences in the BsmI genotype and allelic frequencies involving the male controls, the BsmI genotype was further analyzed by dividing it into two groups, i.e., the bb genotype or the Bb+BB genotypes, to clarify the influence of a B allele on the male controls (Table 2). Significant differences were observed between prostate cancer patients and the male controls (P < 0.0001), between BPH patients and the male controls (P < 0.0005), and between the male controls and the female controls (P < 0.0001). Consequently, the frequency of at least one B allele being present was significantly higher in the male controls (50%) compared with prostate cancer patients (22.5%; OR, 3.44; 95% CI, 2.15–5.49), BPH patients (30.6%; OR, 2.27; 95% CI, 1.44–3.57) and the female controls (22.7%; Table 2). The BsmI B allele was associated with about one-third the risk for prostate cancer and about one-half the risk for BPH compared with the male controls (Table 2). To adjust for age, multivariate logistic regression analyses were performed for each polymorphism with inclusion of age. The multivariate analyses provided almost the identical significant differences in BsmI polymorphism alone (Table 2). In addition, there was a significant result in age-adjusted OR between BPH patients and prostate cancer patients (P < 0.05).

Because a series of the three polymorphisms have been shown to be in strong linkage disequilibrium with one another in Western countries (13, 14, 25), we analyzed the presence of the linkage disequilibrium in the female controls (Table 3). Assuming that BsmI B and b alleles are in disequilibrium with the TaqI t and T alleles, respectively (i.e., an excess of Bt and Bt haplotypes), the observed agreement was 96%. This was significantly higher than the expected agreement (66%; P < 0.0001). Linkage disequilibrium as indicated by an excess of BA and ba haplotypes or Ta and Tt haplotypes was also statistically significant because the agreement was 56% (P < 0.0001) and 58%
50% of men ages 51–60 and 90% of men over age 80 have his-
trectal examination, and a normal PSA level. It has been reported that
associated with survival to old age.

However, the male controls were on average much older than the female controls. I B allele may be associated with survival to old age because the
Bsm allele might be

expected to arise from the weaker linkage disequilibrium between the
TaqI and BsmI alleles. Considering the strong linkage disequilibrium between the TaqI and BsmI polymorphisms and the estimated frequency of as high as 97% for the

TT and the strong linkage disequilibrium between the TaqI and BsmI alleles. Considering the strong linkage disequilibrium between the TaqI and BsmI polymorphisms and the estimated frequency of as high as 97% for the B allele, our T allele, and the B allele would have been enriched for the

P<0.0001, respectively, compared with the expected agreement of 44% in both haplotype groups (Table 3).

DISCUSSION

The present study on native Japanese men and women unexpect-
edly revealed significant differences in the prevalence of BsmI alleles and genotypes in the “male controls” compared with that of the female
controls or BPH and prostate cancer patients. Because the allelic and genotype frequencies of the three polymorphisms in our female controls were similar to Japanese healthy controls reported by

other studies are likely to be in line with ours. By testing mainly on whites,
Taylor et al. (17) claimed that men with the homozygous

TaqI genotype had a protective role against both BPH and prostate cancer. However, there may be some alternative explanations for the present results. Because the male controls were mostly chosen from

patients admitted to the hospitals for nonurological diseases, the hospitalized population might have been enriched for individuals carrying the BsmI B allele. We believe that this is unlikely because the male controls were selected from patients with different diseases in several community hospitals. Another possible explanation is that the

BsmI B allele may be associated with survival to old age because the male controls were on average much older than the female controls. However, the BsmI B allele was observed more frequently in the female controls younger than 50 than in those 50 or older (P, not significant). Therefore, it was unlikely that the BsmI B allele might be

associated with survival to old age.

In this study, we simply defined the male controls as men who had no voiding symptoms, no enlargement of the prostate gland by digital

rectal examination, and a normal PSA level. It has been reported that 50% of men ages 51–60 and 90% of men over age 80 have his-
topathological evidence of BPH (27, 28). Thus, the ‘male controls’ in this study may have belonged to a special aged-population having a normal prostate gland despite their high age of 73.5 on average. Because our criteria of male controls were based only on macroscopic

anatomical findings, and there is inevitable inaccuracy in measuring prostatic volume by digital examination, there may have been some overlap between BPH patients and the male controls. Irrespective of such problems, the large number of subjects manipulated in this study will warrant the conclusion that the BsmI genotype has a significant influence on the occurrence of both prostate cancer and BPH.

There are several documents describing BPH as a disease with an
inherent component. The presence of a familial form of BPH as well as the presence of a gene(s) contributing to the pathogenesis of
BPH (19), and a lower prevalence of prostatic enlargement in Japa-
nese men compared with men in Minnesota or Scotland have been reported (20). On the other hand, it has been noted that normal prostastic epithelial cells express VDRs, and 1,25-dihydroxyvitamin

D₃ exerts an antiproliferative effect on both normal and malignant prostastic epithelial cells (4–9, 21). More recently, cross-talk between the vitamin D signaling pathway and the transforming growth factor-β (TGF-β) signaling pathway which is involved in the formation of BPH was reported (29). These data also support our findings that the difference in the VDR genotype has a substantial influence on the occurrence of both prostate cancer and BPH.

Previous studies on VDR genotype in prostate cancer have analyzed a single polymorphism or two of the three polymorphisms or other VDR polymorphisms in each series (17, 18, 30). The results in these studies are likely to be in line with ours. By testing mainly on whites,
Taylor et al. (17) claimed that men with the homozygous

T allele had a one-third risk for developing prostate cancer compared with men who were heterozygotes or homozygotes for the

T allele. Considering the strong linkage disequilibrium between the TaqI and BsmI polymorphisms and the estimated frequency of as high as 97% for the

B allele, our TaqI and BsmI polymorphisms may have been enriched for the

T allele, and the B allele would have been enriched for the

T allele.
**BsmI polymorphisms in Japanese than that in Caucasians (25, 26). By studying the pol(A) length polymorphism in the 3'UTR of the VDR gene, Ingles et al. reported about a one-fifth risk of developing prostate cancer among men homozygous for the short pol(A) alleles compared with men heterozygous or homozygous for the long pol(A) alleles (18). Because the short pol(A) alleles have been shown to be closely linked with the BsmI B allele (31), their results may partly support our conclusion. Although Ma et al. claimed no significant association of the BsmI or the TaqI polymorphism with prostate cancer risk, they found a 57% reduction in risk for prostate cancer with the BsmI BB genotype compared with the bb genotype in men with low plasma 25-hydroxyvitamin D, the major circulating metabolite of vitamin D (30). Importantly, because none of the above-mentioned studies distinguished BPH patients from male controls (17, 18, 30), it would be interesting to know whether their results are further strengthened if the male controls were distinguished from men with BPH.**

**The mechanism through which the BsmI polymorphism in the VDR gene may influence the risks for prostate cancer and BPH is unclear because the BsmI and the other two VDR polymorphisms do not alter the amino acid sequence of the VDR protein (13). The BsmI, Apal, and TaqI polymorphisms located in intron 8, intron 8, and exon 9 near the 3'UTR, respectively, are in strong linkage disequilibrium with pol(A) length polymorphism in the 3'UTR (18, 31). Because the 3'UTR may be involved in the regulation of mRNA stability and degradation, these polymorphisms may alter the level of the VDR mRNA (13). Alternatively, it is possible that these polymorphisms are in linkage disequilibrium with other mutations that alters VDR function.**

**In conclusion, the present results indicate that the BsmI polymorphism in the VDR gene plays a significant role in protection against prostate cancer and BPH in Japanese men. Additional studies are warranted to verify the correlation among the age, the size of the prostate, and VDR polymorphisms in Japanese and other racial-ethnic groups.**

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