[CANCER RESEARCH 60, 305–308, January 15, 2000

Association of *Vitamin D Receptor* Gene Polymorphism with Prostate Cancer and Benign Prostatic Hyperplasia in a Japanese Population[1]

Tomonori Habuchi,[2] Takehiro Suzuki,[2] Ryusei Sasaki, Lizhong Wang, Kazunari Sato, Shigeru Satoh, Toshiya Akao, Norihiko Tsuchiya, Naotake Shimoda, Yasuhiko Wada, Akio Koizumi, Junichi Chihara, Osamu Ogawa, and Tetsuro Kato[3]

Departments of Urology [T. H., T. S., R. S., L. W., K. S., S. S., T. A., N. T., N. S., T. K.], Hygiene [Y. W., A. K.], Clinical and Laboratory Medicine [J. C.], Akita University School of Medicine, Akita 010-8543, and Department of Urology, Kyoto University Graduate School of Medicine, Shogoin, Sakyo-ku, Kyoto 606-8507 [O. O.], Japan

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 *Bsm*I, *Apa*I and *Taq*I. In the *Bsm*I polymorphism, heterozygosity or homozygosity for the absence of the *Bsm*I 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 *Taq*I and *Apa*I polymorphisms did not show any significant association with either prostate cancer or BPH. The results indicate that the *Bsm*I polymorphism in the *VDR* gene plays a significant role in protection against prostate cancer and BPH. Because of the racial difference 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 D_3, 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.

VDRs[4] mediate the action of their cognate ligand 1,25-dihydroxyvitamin D_3 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 *Bsm*I, *Apa*I, and *Taq*I 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, osteomalasia, insulin-dependent diabetes mellitus, and osteoarthritis (14–16).

Recent molecular epidemiological studies have shown that inherited polymorphisms including the *Bsm*I, *Apa*I, and *Taq*I 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 *Taq*I 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 *Bsm*I, *Apa*I, and *Taq*I 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.

Received 6/24/99; accepted 11/12/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Grants-in-Aid B10470331, B10470330, B09557129, B10470336 from the Ministry of Education, Science, Sports and Culture of Japan and by the Seventh Annual Grant-in-Aid from the Japanese Urological Association.

[2] These authors contributed equally to this work.

[3] To whom requests for reprints should be addressed, at Department of Urology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan. Fax: 81-18-836-2619; E-mail: tkato@hos.akita-u.ac.jp.

[4] 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 prostatic 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 *Bsm*I polymorphic site in intron 8 was amplified using primers and PCR conditions described by Morrison *et al.* (13). When the *Bsm*I site was present, the PCR fragment was divided into 650 and 175 bp by *Bsm*I endonuclease digestion. The 490-bp fragment encompassing *Apa*I and *Taq*I was amplified using specific primers 5′-cag agc atg gac agg gag caa-3′ in intron 8, and 5′-cac ttc gag cac aag 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 *Apa*I digestion or into 290 and 200 bp by *Taq*I digestion. The PCR products were digested overnight with *Bsm*I (65°C), *Apa*I (25°C), or *Taq*I (65°C) and were electrophoresed on 2.0% agarose gels. The genotypes were designated as "*A*," "*B* " or "*T* " when the *Apa*I, *Bsm*I, or *Taq*I 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 χ^2 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 χ^2 test. A probability of less than 0.05 was required for statistical significance.

RESULTS

Frequencies of *Apa*I, *Bsm*I, and *Taq*I 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 *Bsm*I 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 *Apa*I genotype was observed between prostate cancer patients and the female controls ($P < 0.05$). No significant difference was found in the *Taq*I genotype. In allelic frequencies, significant differences in the *Bsm*I 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 *Apa*I and *Taq*I allelic prevalence was found among these four groups.

Because of the significant differences in the *Bsm*I genotype and allelic frequencies involving the male controls, the *Bsm*I 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 *Bsm*I *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 *Bsm*I 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 *Bsm*I *B* and *b* alleles are in disequilibrium with the *Taq*I *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 *Ta* haplotypes was also statistically significant because the agreement was 56% ($P < 0.0001$) and 58%

Table 1 *Frequencies (%) of VDR polymorphisms in patients with prostate cancer, patients with BPH, male controls, and female controls*

	VDR genotype (%)								
	*Bsm*I[a]			*Apa*I[b]			*Taq*I		
	BB	*Bb*	*bb*	*AA*	*Aa*	*aa*	*TT*	*Tt*	*tt*
Prostate cancer, $n = 222$	8 (3.6)	42 (18.9)	172 (77.5)	32 (14.4)	85 (38.3)	105 (47.3)	176 (79.3)	44 (19.8)	2 (0.9)
BPH $n = 209$	10 (4.8)	54 (25.8)	145 (69.4)	25 (12.0)	93 (44.5)	91 (43.5)	157 (75.1)	51 (24.4)	1 (0.5)
Male control $n = 128$	5 (3.9)	59 (46.1)	64 (50.0)	11 (8.6)	55 (43.0)	62 (48.4)	96 (75.0)	30 (23.4)	2 (1.6)
Female control $n = 198$	3 (1.5)	42 (21.2)	153 (77.3)	17 (8.6)	100 (50.5)	81 (40.9)	155 (78.3)	43 (21.7)	0 (0)

[a] Prostate cancer *versus* male control, $P < 0.0001$; BPH *versus* male control, $P < 0.001$; Female control *versus* male control, $P < 0.0001$.
[b] Prostate cancer *versus* female control, $P < 0.05$.

Table 2 *OR by BsmI genotype*

	Allele frequency (%)			Non-age-adjusted		Age-adjusted	
	bb	*Bb + BB*		OR (95% CI)	*P*	OR (95% CI)	*P*
Prostate cancer $n = 222$	172 (77.5)	50 (22.5)	Prostate cancer *vs.* Male	3.44 (2.15–5.49)	<0.0001	3.31 (2.05–5.32)	<0.0001
BPH $n = 209$	145 (69.4)	64 (30.6)	Prostate cancer *vs.* BPH	1.52 (0.99–2.34)	0.0567	1.67 (1.07–2.61)	<0.05
Male control $n = 128$	64 (50.0)	64 (50.0)	BPH *vs.* Male	2.27 (1.44–3.57)	<0.0005	2.07 (1.33–3.22)	<0.005
Female control $n = 198$	153 (77.3)	45 (22.7)					

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

DISCUSSION

The present study on native Japanese men and women unexpectedly revealed significant differences in the prevalence of *Bsm*I alleles and genotypes in the "male controls" compared with that of the female controls or BPH patients 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 others (26), we considered that our female control group represented the general Japanese female population. In addition, the difference in *VDR* allelic and genotype frequencies between males and females has not been reported. Therefore, it is most likely that the *Bsm*I allelic and genotype frequencies in the male controls in this study significantly deviated from those of general Japanese men and women. On the basis of the absence of a significant difference in the *Bsm*I allelic and genotype frequencies between the female controls and prostate cancer patients, and a marginal significant difference between the female controls and BPH patients, we reasoned that the presence of at least one "*B* " allele played 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 *Bsm*I 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 *Bsm*I 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 *Bsm*I *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 *Bsm*I *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 histopathological 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 *Bsm*I 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 inheritable 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 Japanese men compared with men in Minnesota or Scotland have been reported (20). On the other hand, it has been noted that normal prostatic epithelial cells express VDRs, and 1,25-dihydroxyvitamin D_3 exerts an antiproliferative effect on both normal and malignant prostatic 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 *Taq*I and *Bsm*I polymorphisms and the estimated frequency of as high as 97% for the *Bt* and *bT* haplotypes in Caucasians (13, 25), the findings by Taylor *et al.* could be translated as reflecting a protective effect of the *B* allele against prostate cancer. Although we could not find any correlation between the *Taq*I polymorphism and prostate cancer, this may have come from the weaker linkage disequilibrium between the *Taq*I and

Table 3 *Linkage disequilibrium between three VDR polymorphisms in female controls*[a]

	*Taq*I					*Taq*I					*Apa*I			
	TT	*Tt*	*tt*	total		*TT*	*Tt*	*tt*	total		*AA*	*A*	*aa*	total
*Bsm*I					*Apa*I					*Bsm*I				
BB	0	3	0	3	*AA*	6	11	0	17	*BB*	3	0	0	3
Bb	3	39	0	42	*Aa*	69	31	0	100	*Bb*	8	32	2	42
bb	152	1	0	153	*aa*	80	1	0	81	*bb*	6	68	79	153
	155	43	0	198		155	43	0	198		17	100	81	198
Agreement				0.96					0.56					0.58
Expected agreement				0.66[b]					0.44[b]					0.44[b]
P				< 0.0001					< 0.0001					< 0.0001

[a] All of the *P*s are calculated with χ^2 test between agreement and expected agreement.
[b] Expected agreement under the assumption of no linkage disequilibrium.

*Bsm*I polymorphisms in Japanese than that in Caucasians (25, 26). By studying the *poly*(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 *poly*(*A*) alleles compared with men heterozygous or homozygous for the long *poly*(*A*) alleles (18). Because the short *poly*(*A*) alleles have been shown to be closely linked with the *Bsm*I *B* allele (31), their results may partly support our conclusion. Although Ma *et al.* claimed no significant association of the *Bsm*I or the *Taq*I polymorphism with prostate cancer risk, they found a 57% reduction in risk for prostate cancer with the *Bsm*I *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 *Bsm*I polymorphism in the *VDR* gene may influence the risks for prostate cancer and BPH is unclear because the *Bsm*I and the other two *VDR* polymorphisms do not alter the amino acid sequence of the VDR protein (13). The *Bsm*I, *Apa*I, and *Taq*I polymorphisms located in intron 8, intron 8, and exon 9 near the 3′UTR, respectively, are in strong linkage disequilibrium with *poly*(*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 mutation(s) that alters VDR function.

In conclusion, the present results indicate that the *Bsm*I 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.

ACKNOWLEDGMENTS

We are indebted to many physicians and urologists of the Akita University Medical Center and other community hospitals for providing samples and clinical information.

REFERENCES

1. Taylor, J. D., Holmes, T. M., and Swanson, G. M. Descriptive epidemiology of prostate cancer in metropolitan Detroit. Cancer (Phila.), *73:* 1704–1707, 1994.
2. Oishi, K., Yoshida, O., and Schroeder, F. H. The geography of prostate cancer and its treatment in Japan. Cancer Surv., *23:* 267–280, 1995.
3. Schwarz, G. G., and Hulka, B. S. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Res., *10:* 1307–1311, 1990.
4. Miller, G. J., Stapleton, G. E., Ferrara, J. A., Lucia, M. S., Pfister, S., Hedlund, T. E., and Upadhya, P. The human prostatic carcinoma cell line LNCaP expresses biologically active, specific receptors for 1α, 25-dihydroxyvitamin D_3. Cancer Res., *52:* 515–520, 1992.
5. Peel, D. M., Skowronski, R. J., Leung, G. K., Wong, S. T., Stamey, T. A., and Feldman, D. Antiproliferative effects of 1,25-dihydroxyvitamin D_3 on primary cultures of human prostatic cells. Cancer Res., *54:* 805–810, 1994.
6. Skowronski, R. J., Peehl, D. M., and Feldman, D. Actions of vitamin D_3 analogs on human prostate cancer cell lines: comparison with 1,25-dihydroxyvitamin D_3. Endocrinology, *136:* 20–26, 1995.
7. Wang, X., Chen, X., Akhter, J., and Morris, D. L. The *in vitro* effect of vitamin D_3 analogue EB-1089 on a human prostate cancer cell line (PC-3). Br. J. Urol., *80:* 260–262, 1997.
8. Blutt, S. E., Allegretto, E. A, Pike, J. W., and Weigel, N. L. 1,25-dihydroxyvitamin D_3 and 9-*cis*-retinoic acid act synergistically to inhibit the growth of LNCaP prostate cells and cause accumulation of cells in G_1. Endocrinology, *138:* 1491–1497, 1997.
9. Zhuang, S. H., and Burnstein, K. L. Antiproliferative effect of 1α,25-dihydroxyvitamin D_3 in human prostate cancer cell line LNCaP involves reduction of cyclin-dependent kinase 2 activity and persistent G_1 accumulation. Endocrinology, *139:* 1197–1207, 1998.
10. Gross, C., Stamey, T., Hancock, S., and Feldman, D. Treatment of early recurrent prostate cancer with 1,25-dihydroxyvitamin D_3 (carcitriol). J. Urol., *159:* 2035–2040, 1998.
11. McDonnell, D. P., Mangelsdorf, D. J., Pike, J. W., Haussler, M. R., and O'Malley, B. W. Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. Science (Washington DC), *235:* 1214–1217, 1987.
12. Baker, A. R., McDonnell, D. P., Hughes, M., Crisp, T. M., Mangelsdorf, D, J, Haussler, M. R., Pike, J. W., Shine, J., and O'Malley, B. W. Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc. Natl. Acad. Sci. USA, *85:* 3294–3298, 1988.
13. Morrison, N. A., Qi, J. C., Tokita, A., Kelly, P. J., Nguyen, T. V., Sambrook, P. N., and Eisman, J. A. Prediction of bone density from vitamin D receptor alleles. Nature (Lond.), *367:* 284–287, 1994.
14. Carling, T., Kindmark, A., Hellman, P., Holmberg, L., Akerstrom, G., and Rastad, J. Vitamin D receptor alleles *b*, *a*, and *T*: risk factors for sporadic primary hyperparathyroidism (HPT) but not HPT of uremia or MEN 1. Biochem. Biophys. Res. Commun., *231:* 329–332, 1997.
15. McDermott, M. F., Ramachandran, A., Ogunkolade, B. W., Aganna, E., Curtis, D., Boucher, B. J., Snehalatha, C., and Hitman, G. A. Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. Diabetologia, *40:* 971–975, 1997.
16. Uitterlinden, A. G., Burger, H., Huang, Q., Odding, E., Duijn, C. M., Hofman, A., Birkenhager, J. C., and van Leeuwen, J. P. Vitamin D receptor genotype is associated with radiographic osteoarthritis at the knee. J. Clin. Invest., *100:* 259–263, 1997.
17. Taylor, J. A., Hirvonen, A., Watson, M., Pittman, G., Mohler, J. L., and Bell, D. A. Association of prostate cancer with vitamin D receptor gene polymorphism. Cancer Res., *56:* 4108–4110, 1996.
18. Ingles, S. A., Ross, R. K., Yu, M. C., Irvine, R. A., La Pera, G., Haile, R. W., and Coetzee, G. A. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. J. Natl. Cancer. Inst., *89:* 166–170, 1997.
19. Sanda, M. A., Beaty, T. H., Stutzman, R. E., Childs, B., and Walsh, P. C. Genetic susceptibility of benign prostatic hyperplasia. J. Urol., *152:* 115–119, 1994.
20. Tsukamoto, T., Kumamoto, Y., Masumori, N., Miyake, H., Rhodes, T., Girman, D. J., Guess, H. A., Jacobson, S. J., and Lieber, M. M. Prevalence of prostatism in Japanese men in a community-based study with comparison to a similar American study. J. Urol., *154:* 391–395, 1995.
21. Konety, B. R., Schwartz, G. G., Acinerno, J. S., Becich, M. J., and Getzenberg, R. H. The role of vitamin D in normal prostate growth and differentiation. Cell Growth Differ., *7:* 1563–1570, 1996.
22. Kuriyama, M., Akimoto, S., Akaza, H., Arai, Y., Usami, M., Imai, K., Tanaka, Y., Yamazaki, H., Kawada, Y., Koiso, K., Yoshida, O., Kotake, T., Yamanaka, H., Machida, T., Aso, Y., and Shimazaki, J. Comparison of various assay systems for prostate-specific antigen standardization. Jpn. J. Clin. Oncol., *22:* 393–399, 1992.
23. Kiel, D. P., Myers, R. H., Cupples, L. A., Kong, X. F., Zhu, X. H., Ordovas, J., Schaefer, E. J., Felson, D. T., Rush, D., Wilson, P. W., Eisman, J. A., and Holick, M. F. The *Bsm*I vitamin D receptor restriction fragment length polymorphism (bb) influences the effect of calcium intake on bone mineral density. J. Bone Miner. Res., *12:* 1049–1057, 1997.
24. Sainz, J., Van Tornout, J. M., Load, M. L., Sayre, J., Roe, T. F., and Gilsanz, V. Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent. N. Engl. J. Med., *337:* 77–82, 1997.
25. Morrison, N. A., Yeoman, R., Kelly, P. J., and Eisman, J. A. Contribution of *trans*-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. Proc. Natl. Acad. Sci. USA, *89:* 6665–6669, 1992.
26. Tokita, A., Matsumoto, H., Morrison, N. A., Tawa, T., Miura, Y., Fukamauchi, K., Mitsuhashi, N., Irimoto, M., Ymamamori, S., Miura, M., Watanabe, T., Kuwabara, Y., Yabuta, K., and Eisman, J. A. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. J. Bone Miner. Res., *11:* 1003–1009, 1996.
27. Guess, H. A., Arrighi, H. M., Metter, E. J., Fozard, J. L. Cumulative prevalence of prostatism matches the autopsy prevalence of benign prostatic hyperplasia. Prostate, *17:* 241–246, 1990.
28. Kato, T. Histological study on hyperplasia of the prostate with special reference to histogenesis of nodule. Nippon Hinyokika Gakkai Zasshi, *58:* 469–483, 1967.
29. Yanagisawa. J., Yanagi, Y., Masuhiro, Y., Suzawa, M., Watanabe, M., Kashiwagi, K., Toriyabe, T., Kawabata, M., Miyazono, K., and Kato, S. Convergence of transforming growth factor-β and vitamin D signaling pathways on SMAD transcriptional coactivators. Science (Washington DC), *283:* 1317–1321, 1999.
30. Ma, J., Stampfer, M. J., Gann, P. H., Hough, H. L., Giovannucci, E., Kelsey, K. T., Hennekens, C. H., and Hunter, D. J. Vitamin D receptor polymorphisms, circulating vitamin D metabolites and risk of prostate cancer in United States physicians. Cancer Epidemiol. Biomark. Prev., *7:* 385–390, 1998.
31. Ingles, S. A., Haile, R. W., Henderson, B. E., Kolonel, L. N., Nakaichi, G., Shi, C. Y., Yu, M. C., Ross, R. K., and Coetzee, G. A. Strength of linkage disequilibrium between two vitamin D receptor markers in five ethnic groups: implications for association studies. Cancer Epidemiol. Biomark. Prev., *6:* 93–98, 1997.