Vitamin D Receptor Start Codon Polymorphism (FokI) and Prostate Cancer Progression[1]

Yue Xu, Atsuko Shibata, John E. McNeal, Thomas A. Stamey, David Feldman, and Donna M. Peehl[2]

Departments of Urology [Y. X., J. E. M., T. A. S., D. M. P.], Health Research and Policy [A. S.], and Medicine [D. F.], Stanford University School of Medicine, Stanford, California 94305

Abstract

Vitamin D plays an important role in cell growth and differentiation and is proposed to protect against cancer initiation and/or progression. The vitamin D receptor (VDR) has a thymine/cytosine (T/C) polymorphism located in the first of two potential start (ATG) codons that can be detected by a RFLP using the endonuclease FokI. The C variant, which lacks the first ATG, results in a shorter VDR and is referred to as the *F* allele. The T variant (*f* allele) initiates at the first ATG. We examined the association of the VDR FokI genotype with histopathological characteristics and prognosis of prostate cancer among 191 mostly Caucasian subjects who had undergone radical prostatectomy between 1984 and 1992. The frequencies of the *FF*, *Ff*, and *ff* genotypes were 41%, 38%, and 21%, respectively. Subjects with the *ff* genotype had a lower mean percentage of Gleason grade 4/5 cancer (30.3%) than subjects with the *FF* or *Ff* genotypes (42.8% and 43.8%, respectively; $P = 0.015$ by *t* test for *ff* versus *FF* + *Ff*). The data suggest that the presence of an *F* allele increased the risk of being diagnosed with more aggressive cancer because higher percentage of Gleason grade 4/5 is associated with worse prognosis. The age-adjusted risk of prostate-specific antigen failure was lower for the *ff* genotype than for the *FF* genotype by Cox proportional hazards analysis but did not achieve statistical significance (hazard ratio = 0.76; 95% confidence interval, 0.44–1.32). This risk reduction disappeared after further adjustment for percentage of Gleason grade 4/5, cancer volume, and preoperative serum prostate-specific antigen level (hazard ratio = 1.03; 95% confidence interval, 0.58–1.85). In conclusion, the *ff* genotype was associated with less aggressive histopathological findings than *Ff* or *FF* genotypes. Additional studies with a larger sample size and investigation of the functional significance of the FokI polymorphism in prostate cancer cells are warranted.

Introduction

Prostate cancer is the most commonly diagnosed noncutaneous cancer in men in the United States. Incidence rates of prostate cancer increased markedly from the late 1980s to 1992, due largely to the increased testing of serum PSA[3] for cancer screening, followed by a decrease to a level still higher than that of the pre-PSA era (1). In contrast, mortality rates of prostate cancer have shown less remarkable time trends (1, 2). The majority of prostate tumors never become metastatic or life-threatening. Thus, the identification of biomarkers that predict progression to advanced disease carries major public health significance (2–4).

Vitamin D maintains calcium homeostasis and regulates growth and differentiation of many types of cells (5). The active form of vitamin D, $1,25(OH)_2D_3$, exerts its activity through the intracellular VDR[3] (6). The VDR, a member of the steroid/thyroid hormone nuclear receptor family, is expressed in both normal and malignant prostate cells (7, 8). $1,25(OH)_2D_3$ binds to the VDR, which forms a heterodimer with the retinoid X receptor. The heterodimer in turn binds to vitamin D response elements in the promoter regions of target genes and regulates transcription of those genes (5).

Several polymorphisms have been identified in the *VDR* gene, and their functional significance and potential effects on disease susceptibility have been investigated (9). One of the known DNA sequence variants is a thymine/cytosine (T/C) polymorphism in the first of two potential start (ATG) codons separated by 3 codons. This polymorphism results in two alleles that can be distinguished by a RFLP[3] using the endonuclease FokI (10). The *F* allele lacks the first ATG, and translation starts at the second ATG, with the resulting VDR being shorter by three amino acids than that of the *f* allele, which starts at the first ATG. Findings regarding functional significance of the FokI polymorphism have been inconsistent (11–13). However, most data indicate that the *F* allele is more effective than the *f* allele in transactivation of the $1,25(OH)_2D_3$ signal (12, 13). In recent studies, FokI genotypes have been associated with risk of malignant melanoma (14) and colon adenomas (15). To our knowledge, the association between FokI genotypes and pros-

[1] Supported by the Cancer Research Fund, under Interagency Agreement 97-12013 (University of California, Davis Contract 98-00924V) with the Department of Health Services, Cancer Research Section./. Mention of trade name, proprietary product or specific equipment does not constitute a guaranty or warranty by the Department of Health Services, nor does it imply approval to the exclusion of other products. The views expressed herein represent those of the authors and do not necessarily represent the position of the State of California, Department of Health Services.

Received 6/13/02; revised 10/18/02; accepted 10/26/02.

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.

[2] To whom requests for reprints should be addressed, at Department of Urology, Stanford Medical Center, Stanford, CA 94305-5118. Phone: (650) 725-5531; Fax: (650) 723-0765; E-mail: dpeehl@stanford.edu.

[3] The abbreviations used are: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; PSA, prostate-specific antigen; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; CI, confidence interval; HR, hazard ratio; VDR, vitamin D receptor.

tate cancer risk has thus far only been investigated in men in the United Kingdom (16) and in China (17). Other VDR polymorphisms located at the 3′ end of the gene have been associated with risk of prostate cancer in some studies (17–21) but not in others (22–26).

Because of the important role of vitamin D and its receptor in prostate cell growth and differentiation (27–30), we hypothesized that the FokI polymorphism in the *VDR* gene might be associated with progression of prostate cancer. The goal of this study was to examine the association of the FokI polymorphism with histopathological features and prognosis of prostate cancer among men who had undergone radical prostatectomy. Because the percentage of Gleason grade 4/5 has been found to be a strong predictor of progression of prostate cancer after radical prostatectomy (31, 32), we focused on the relationship of the VDR FokI genotype and this variable.

Materials and Methods

A total of 227 subjects who had undergone radical prostatectomy at Stanford Medical Center between 1984 and 1992 were selected for the study (31). These subjects were those who were found to have at least 5% of Gleason grade 4/5 in their cancers. Thirty-six of the selected subjects were excluded because: (*a*) DNA from archived tissue failed to yield detectable PCR products in the genotyping assay for the VDR polymorphism (35 subjects); or (*b*) cancer was in the transition zone rather than in the peripheral zone (1 subject). These exclusions resulted in a sample size of 191 subjects. None of the subjects had received hormonal therapy before surgery. Although race was not recorded for individual patients, the source population historically had been predominantly (>95%) Caucasian.

Surgically removed prostates were routinely subjected to a comprehensive histopathological review by a single pathologist according to the method described previously (33). When two or more distinct foci existed in a single prostate, the volume of the largest (index) cancer was used in statistical analysis. The Stanford modified Gleason scale was used to estimate the proportion of each cancer that was poorly differentiated in each prostatectomy specimen (34). From the Gleason scale of 5 grades (35), grades 4 and 5 (poorly differentiated) constitute a variety of histological patterns having the common feature that they do not form intact glands. For each patient, an estimate was made of the percentage of the total cancer represented by Gleason grade 4 or 5 (percentage of Gleason grade 4/5). This measurement of tumor grade was shown to be the strongest predictor of PSA failure in a study of 379 men who had undergone surgery at the Stanford Medical Center between 1983 and 1992 (31). Information on the subject's age at surgery and serum PSA level before surgery was abstracted from an existing database. The study protocol was approved by the Panel on Medical Human Subjects at Stanford University.

DNA was extracted from formalin-fixed, paraffin-embedded prostate tissue as described previously (36). Because we were interested in DNA sequence variation of constitutional (*i.e.*, germ-line) DNA, rather than somatic changes in malignant cells, we selected tissue blocks that contained no tumor according to histological review. The primers and PCR[3] conditions for amplifying exon 2 (containing the FokI site) of the *VDR* gene were described previously (37). Five μl of DNA were used for each PCR reaction. The resulting 260-bp PCR products were digested with FokI endonuclease (New England Biolabs, Beverly, MA) at 37°C for 3 h and then electrophoresed through a 3% agarose gel containing Tris-acetate-EDTA buffer and ethidium bromide. PCR products with an undigested large band were scored as *FF* homozygotes, those with a smaller digested band were scored as *ff* homozygotes, and those with a large and small band were scored as *Ff* heterozygotes.

PSA failure (or biochemical failure) was used as the outcome event for longitudinal analysis. Typically, follow-up PSA test results were available approximately every 6 months, although the length of follow-up and intervals between PSA tests varied among subjects. PSA failure was defined as two consecutive PSA values above a cutoff point of 0.07 ng/ml for PSA measured by the sensitive Tosoh method and 0.2 ng/ml for measurements by less sensitive methods. When a patient had experienced PSA failure, time to failure was calculated as the number of months between the date of surgery and the date of the first of the two consecutive PSA values that exceeded the cutoff point.

Twenty-nine subjects whose serum PSA levels never dropped to zero (or undetectable level) after surgery were classified as experiencing PSA failure at time 0 (*i.e.*, at the time of surgery). Three subjects had a serum PSA level above the cutoff point only in the last follow-up PSA test available, and thus we could not confirm their failure status definitively in the absence of a subsequent PSA test also exceeding the cutoff point. We performed longitudinal analysis for PSA failure in two ways, classifying these three subjects with indeterminate failure status as (*a*) experiencing PSA failure or (*b*) not experiencing PSA failure.

All statistical analyses were performed using the SAS statistical package, release 8.1 (SAS Institute, Inc., Cary, NC). Means of age, percentage of Gleason grade 4/5, and cancer volume were compared among the three VDR FokI genotypes (*FF*, *Ff*, and *ff*) by ANOVA, using the GLM procedure (38). For a comparison between the two groups (*e.g.*, *FF* + *Ff versus ff*), the *t* test was used (39).

The relationship of the VDR FokI genotype to the risk of PSA failure was examined by Cox proportional hazards analysis, using the PHREG procedure with the "exact" option for handling ties (40). The Cox model included the VDR FokI genotype (*FF*, *Ff*, or *ff*), age at surgery (<60, 60–64, 65–69, and ≥70 years), percentage of Gleason grade 4/5, cancer volume, and preoperative PSA level (quartiles based on the distribution in the study sample for the last three variables).

Results

Table 1 summarizes the characteristics of 191 study subjects, their tumors, and their VDR FokI genotype. In this population with prostate cancer, the frequencies of the *F* allele and the *f* allele were 60% and 40%, respectively. The mean percentage of Gleason grade 4/5 cancer in subjects with the *ff* genotype was 30.3%, which was lower than that in subjects with the *FF* and *Ff* genotypes (42.8% and 43.8%, respectively; $P = 0.015$ by *t* test for *ff versus FF* + *Ff*). No statistically significant differences among the VDR FokI genotypes were observed for age at surgery, index cancer volume, or preoperative serum PSA level.

Among the 191 subjects, the duration of follow-up ranged from 0 to 175.1 months (median, 54.7 months). Serum PSA levels never dropped to zero or to an undetectable level after surgery in 29 subjects, who were considered to experience PSA failure at time 0 (*i.e.*, at the time of surgery). For three subjects, PSA failure status was not definitive because their last available PSA values were above the cutoff for the failure definition without subsequent elevated PSA levels to confirm the failure status. When these three subjects were regarded as experiencing failure, the total number of PSA failures was 102; otherwise

Table 1 Mean ± SD of baseline characteristics of the 191 study subjects by the VDR FokI genotype

Variable	VDR FokI genotype			P^a
	FF	*Ff*	*ff*	
No. of subjects (%)	78 (41%)	73 (38%)	40 (21%)	
Age at surgery (yrs)	64.8 ± 6.8	65.2 ± 6.5	63.2 ± 6.2	0.28
% Gleason grade 4/5	42.8 ± 30.0	43.8 ± 30.2	30.3 ± 28.6	0.05[b]
Index cancer volume (cc)	5.2 ± 5.3	5.3 ± 5.7	5.7 ± 6.0	0.88
Preoperative serum PSA (ng/ml)	24.5 ± 39.5	19.7 ± 23.2	18.5 ± 14.1	0.49

[a] By ANOVA.
[b] $P = 0.015$ by t test when the *FF* and *Ff* genotypes were combined and compared with the *ff* genotype.

Table 2 Results of Cox proportional hazards model analysis for PSA failure

Covariates adjusted for[a]	VDR FokI genotype[b]			
	Ff		*ff*	
	HR	95% CI	HR	95% CI
Age	1.02	0.66–1.58	0.76	0.44–1.32
Age, Gleason grade, cancer, volume, and preoperative serum PSA	1.07	0.68–1.69	1.03	0.58–1.85

[a] The following categories were used: age at surgery in years (<60, 60–64, 65–69, 70+); % Gleason grade 4/5 (<15%, 15–34%, 35–69%, ≥70%); cancer volume in cc (<2.00, 2.00–3.25, 3.26–6.68, ≥6.69); and preoperative serum PSA level in ng/ml (<9.1, 9.1–14.8, 14.9–24.9, ≥25.0).
[b] The *FF* genotype was considered as a reference category.

there were 99 PSA failures. For the 102 PSA failures, time to failure ranged from 0 to 149.2 months (median, 16.6 months).

Table 2 shows the HR[3] estimates from the Cox proportional hazards analysis for the association between the FokI genotype and risk of PSA failure. One analysis was performed with adjustment for age at surgery alone. In another analysis, HR estimates were further adjusted for percentage of Gleason grade 4/5, cancer volume, and preoperative serum PSA level. Risk of PSA failure in subjects with the *Ff* genotype showed little difference from that in subjects with the *FF* genotype, regardless of whether the HR estimate was adjusted for age alone or for age and additional covariates. In contrast, the risk of PSA failure for subjects with the *ff* genotype was lower than that for men with the *FF* genotype, although the association was not statistically significant when the HR estimate was adjusted for age alone (HR = 0.76; 95% CI,[3] 0.44–1.32). This decrease in PSA failure risk for the *ff* genotype disappeared when the HR was further adjusted for Gleason grade, cancer volume, and preoperative serum PSA level. HR estimates were essentially the same as those shown in Table 2 when the three subjects with indeterminate failure status were reclassified as not experiencing PSA failure (data not shown).

Discussion

It is plausible to search for potential prognostic markers of prostate cancer among the genes and gene products related to the pathophysiology of prostate cells. The active form of vitamin D, $1,25(OH)_2D_3$, has growth-suppressive and differentiating effects on prostate cells (27–30). These effects are mediated by the nuclear receptor, VDR, in cancer cell lines as well as in nonmalignant and malignant primary cultures of prostatic epithelial cells. However, the cellular response to vitamin D does not necessarily correlate with the receptor content, suggesting that additional factors are involved in vitamin D-mediated growth regulation (5, 41). Small changes in the VDR structure, including changes in protein composition caused by DNA sequence variants, might have subtle influences on the cellular response to $1,25(OH)_2D_3$. Naturally occurring DNA polymorphisms in the *VDR* gene are of interest as candidate prognostic markers.

The goal of our study was to determine whether the VDR FokI genotype affected outcome after radical prostatectomy to cure prostate cancer. To carry out this study, we chose men who had undergone radical prostatectomy at the Stanford Medical Center between 1984 and 1992. These men have a relatively long follow-up (median, 54.7 months). We observed a lower percentage of Gleason grade 4/5 cancer in subjects with the *ff* genotype than in those with the *FF* or *Ff* genotype. Percentage of Gleason grade 4/5 cancer is the primary independent predictor of biochemical (PSA) failure in men undergoing radical prostatectomy. The cure rate for men with no Gleason grade 4/5 was 93% in the Stanford series reported in 1999 (31). The critical biological change that occurs when cancers become grade 4/5 is shown by the fact that every 10% increase in the percentage of grade 4/5 cancer in radical prostatectomy specimens correlates with an almost proportionate increase in biochemical failure (31). Therefore, an association of the *ff* genotype with a lower percentage of Gleason 4/5 cancer suggests that this VDR variant slows the progression of prostate cancer from the less aggressive and curable grade 3 to the more aggressive grade 4/5. To our knowledge, this is the first report of an association between the VDR FokI genotype and histopathological Gleason grade of prostate cancer. Luscombe *et al.* (42) reported that the VDR FokI genotype was not associated with histopathology of prostate cancers in men in the United Kingdom, but cancers were classified only as moderately or poorly differentiated.

Longitudinal analysis of our cases suggested that age-adjusted risk of PSA failure was lower in subjects with the *ff* genotype, although the association was not statistically significant. This decrease in risk disappeared when the risk estimate was further adjusted for Gleason grade, cancer volume, and preoperative serum PSA level, which was not surprising because the *ff* genotype was associated with lower Gleason grade.

However, in the study in the United Kingdom, the VDR *ff* genotype was associated with increased risk of bone metastases (42). Several years are required from first evidence of biochemical PSA failure after radical prostatectomy to positive bone scans (43, 44), therefore it is not possible to directly compare our findings with those of the British study.

Results of *in vitro* studies have not conclusively demonstrated whether one or the other of the FokI alleles (*F* versus *f*) leads to more efficient responses of cells to vitamin D. Gross *et al.* (11) did not detect allelic differences in transactivation activity of the VDR alleles. Jurutka *et al.* (12) found that the *F* variant of VDR had more potent transactivation activity than the *f* variant in transient transfection assays in COS-7 cells. Recent studies demonstrated that the *F* allele, as well as the *L* allelic form of a singlet (A) repeat in the 3′-untranslated region, displayed higher transcriptional activity induced by $1,25(OH)_2D_3$ in human fibroblast cell lines (13). However, only when genotypes at both sites were considered simultaneously did statistically significant differences emerge.

Several studies found that the *ff* genotype was associated with lower bone mineral density in postmenopausal women (10, 37). In contrast, the *F* allele was associated with an increased risk of developing rickets in Nigerian children (45). If indeed the *F* allele creates a better-functioning VDR variant, then this finding, like our finding of a protective effect of the *f* allele on prostate cancer progression, is counterintuitive. However, the FokI genotype has not been found, at least in females, to correlate with serum levels of 25-hydroxyvitamin D_3, $1,25(OH)_2D_3$, parathyroid hormone, or osteocalcin (10, 37). Because these factors are all regulated by VDR, it might be expected that their levels would depend on the level of activity of the VDR genotype. Overall, it appears that additional studies are required to comprehensively determine whether VDRs translated from *F* versus *f* alleles have differential activity. It will be important to examine this in appropriate model systems because activity of VDR variants may be cell type specific.

Some limitations of the study design in the present investigation are worth noting. First, the statistical power of the study was limited for longitudinal analysis. Therefore, confirmation of the present findings in larger study samples is needed. Second, subjects in the present study were selected because they underwent radical prostatectomy. Caution is due in extrapolating the results of this study to prostate cancer patients who choose other forms of therapy or watchful waiting. However, our present finding shows that the *ff* genotype is associated with lower percentage of Gleason grade 4/5 than the *FF* and *Ff* genotypes, suggesting a protective effect of the *ff* genotype on prostate cancer progression. The mechanistic basis of this finding is under investigation.

References

1. Shibata, A., and Whittemore, A. S. Re: Prostate cancer incidence and mortality in the United States and the United Kingdom. J. Natl. Cancer Inst. (Bethesda), *93:* 1109–1110, 2001.

2. Abbas, F., and Scardino, P. T. The natural history of clinical prostate carcinoma. Cancer (Phila.), *80:* 827–833, 1997.

3. Boccon-Gibod, L. Significant *versus* insignificant prostate cancer: can we identify the tigers from the pussy cats? J. Urol., *156:* 1069–1070, 1996.

4. Montie, J. E., and Meyers, S. E. Defining the ideal tumor marker for prostate cancer. Urol. Clin. N. Am., *24:* 247–252, 1997.

5. Feldman, D., Malloy, P. J., and Gross, C. Vitamin D: biology, action and clinical implications. *In:* R. Marcus, D. Feldman, and J. Kelsey (eds.), Osteoporosis 1, pp. 257–304. San Diego, CA: Academic Press, 2001.

6. Malloy, P. J., Pike, J. W., and Feldman, D. The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. Endocr. Rev., *20:* 156–188, 1999.

7. Peehl, 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.

8. Skowronski, R. J., Peehl, D. M., and Feldman, D. Vitamin D and prostate cancer: 1,25-dihydroxyvitamin D_3 receptors and actions in human prostate cancer cell lines. Endocrinology, *132:* 1952–1960, 1993.

9. Zmuda, J. M., Cauley, J. A., and Ferrell, R. E. Molecular epidemiology of vitamin D receptor gene variants. Epidemiol. Rev., *22:* 203–217, 2000.

10. Gross, C., Eccleshall, T. R., Malloy, P. J., Villa, M. L., Marcus, R., and Feldman, D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J. Bone Miner. Res., *11:* 1850–1855, 1996.

11. Gross, C., Krishnan, A. V., Malloy, P. J., Eccleshall, T. R., Zhao, X. Y., and Feldman, D. The vitamin D receptor gene start codon polymorphism: a functional analysis of FokI variants. J. Bone Miner. Res., *13:* 1691–1699, 1998.

12. Jurutka, P. W., Remus, L. S., Whitfield, G. K., Thompson, P. D., Hsieh, J. C., Zitzer, H., Tavakkoli, P., Galligan, M. A., Dang, H. T., Haussler, C. A., and Haussler, M. R. The polymorphic N terminus in human vitamin D receptor isoforms influences transcriptional activity by modulating interaction with transcription factor IIB. Mol. Endocrinol., *14:* 401–420, 2000.

13. Whitfield, G. K., Remus, L. S., Jurutka, P. W., Zitzer, H., Oza, A. K., Dang, H. T., Haussler, C. A., Galligan, M. A., Thatcher, M. L., Encinas Dominguez, C., and Haussler, M. R. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. Mol. Cell. Endocrinol., *177:* 145–159, 2001.

14. Hutchinson, P. E., Osborne, J. E., Lear, J. T., Smith, A. G., Bowers, P. W., Morris, P. N., Jones, P. W., York, C., Strange, R. C., and Fryer, A. A. Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. Clin. Cancer Res., *6:* 498–504, 2000.

15. Ingles, S. A., Wang, J., Coetzee, G. A., Lee, E. R., Frankl, H. D., and Haile, R. W. Vitamin D receptor polymorphisms and risk of colorectal adenomas (United States). Cancer Causes Control, *12:* 607–614, 2001.

16. Luscombe, C. J., French, M. E., Liu, S., Saxby, M. F., Jones, P. W., Fryer, A. A., and Strange, R. C. Prostate cancer risk: associations with ultraviolet radiation, tyrosinase and melanocortin-1 receptor genotypes. Br. J. Cancer, *85:* 1504–1509, 2001.

17. Chokkalingam, A. P., McGlynn, K. A., Gao, Y. T., Pollak, M., Deng, J., Sesterhenn, I. A., Mostofi, F. K., Fraumeni, J. F., Jr., and Hsing. A. W. Vitamin D receptor gene polymorphisms, insulin-like growth factors, and prostate cancer risk: a population-based case-control study in China. Cancer Res., *61:* 4333–4336, 2001.

18. Ingles, S. A., Coetzee, G. A., Ross, R. K., Henderson, B. E., Kolonel, L. N., Crocitto, L., Wang, W., and Haile, R. W. Association of prostate cancer with vitamin D receptor haplotypes in African-Americans. Cancer Res., *58:* 1620–1623, 1998.

19. 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.

20. 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. (Bethesda), *89:* 166–170, 1997.

21. Habuchi, T., Suzuki, T., Sasaki, R., Wang, L., Sato, K., Satoh, S., Akao, T., Tsuchiya, N., Shimoda, N., Wada, Y., Koizumi, A., Chihara, J., Ogawa, O., and Kato, T. Association of vitamin D receptor gene polymorphism with prostate cancer and benign prostatic hyperplasia in a Japanese population. Cancer Res., *60:* 305–308, 2000.

22. 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.

23. Kibel, A. S., Isaacs, S. D., Isaacs, W. B., and Bova, G. S. Vitamin D receptor polymorphisms and lethal prostate cancer. J. Urol., *160:* 1405–1409, 1998.

24. Watanabe, M., Fukutome, K., Murata, M., Uemura, H., Kubota, Y., Kawamura, J., and Yatani, R. Significance of vitamin D receptor gene polymorphism for prostate cancer risk in Japanese. Anticancer Res., *19:* 4511–4514, 1999.

25. Blazer, D. G., III, Umbach, D. M., Bostick, R. M., and Taylor, J. A. Vitamin D receptor polymorphisms and prostate cancer. Mol. Carcinog., *27:* 18–23, 2000.

26. Gsur, A., Madersbacher, S., Haidinger, G., Schatzl, G., Marberger, M., Vutuc, C., and Micksche, M. Vitamin D receptor gene polymorphism and prostate cancer risk. Prostate, *51:* 30–34, 2002.

27. Konety, B. R., Johnson, C. S., Trump, D. L., and Getzenberg, R. H. Vitamin D in the prevention and treatment of prostate cancer. Semin. Urol. Oncol., *17:* 77–84, 1999.

28. Blutt, S. E., and Weigel, N. L. Vitamin D and prostate cancer. Proc. Soc. Exp. Biol. Med., *221:* 89–98, 1999.

29. Miller, G. J. Vitamin D and prostate cancer: biologic interactions and clinical potentials. Cancer Metastisis Rev., *17:* 353–360, 1998.

30. Feldman, D., Zhao, X. Y., and Krishnan, A. V. Vitamin D and prostate cancer. Endocrinology, *141:* 5–9, 2000.

31. Stamey, T. A., McNeal, J. E., Yemoto, C. M., Sigal, B. M., and Johnstone, I. M. Biological determinants of cancer progression in men with prostate cancer. J. Am. Med. Assoc., *281:* 1395–1400, 1999.

32. Stamey, T. A., Yemoto, C. M., McNeal, J. E., Sigal, B. M., and Johnstone, I. M. Prostate cancer is highly predictable: a prognostic equation based on all morphological variables in radical prostatectomy specimens. J. Urol., *163:* 1155–1160, 2000.

33. Stamey, T. A., McNeal, J. E., Freiha, F. S., and Redwine, E. Morphometric and clinical studies on 68 consecutive radical prostatectomies. J. Urol., *139:* 1235–1241, 1988.

34. McNeal, J. E., Villers, A. A., Redwine, E. A., Freiha, F. S., and Stamey, T. A. Histologic differentiation, cancer volume, and pelvic lymph node metastasis in adenocarcinoma of the prostate. Cancer (Phila.), *66:* 1225–1233, 1990.

35. Gleason, D. F., and Mellinger, G. T. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J. Urol., *111:* 58–64, 1974.

36. Shibata, A., Garcia, M. I., Cheng, I., Stamey, T. A., McNeal, J. E., Brooks, J. D., Henderson, S., Yemoto, C. E., and Peehl, D. M. Polymorphisms in the androgen receptor and type II 5 a-reductase genes and prostate cancer prognosis. Prostate, *52:* 269–278, 2002.

37. Harris, S. S., Eccleshall, T. R., Gross, C., Dawson-Hughes, B., and Feldman, D. The vitamin D receptor start codon polymorphism (FokI) and bone mineral density in premenopausal American black and white women. J. Bone Miner. Res., *12:* 1043–1048, 1997.

38. SAS Institute Inc. The GLM procedure. *In:* SAS/STAT User's Guide, pp. 549–640. Cary, NC: SAS Institute Inc., 1988.

39. Rosner, B. Fundamentals of Biostatistic. Boston: PWS-Kent Publishing Company, 1990.

40. SAS Institute Inc. The PHREG procedure. *In:* SAS/STAT Software: Changes and Enhancements through Release 6.12, pp. 873–948. Cary, NC: SAS Institute Inc., 1997.

41. Miller, G. J., Stapleton, G. E., Hedlund, T. E., and Moffatt, K. A. Vitamin D receptor expression, 24-hydroxylase activity, and inhibition of growth by 1α,25-dihydroxyvitamin D_3 in seven human prostatic carcinoma cell lines. Clin. Cancer Res., *1:* 997–1003, 1995.

42. Luscombe, C. J., French, M. E., Liu, S., Saxby, M. F., Jones, P. W., Fryer, A. A., and Strange, R. C. Outcome in prostate cancer associations with skin type and polymorphism in pigmentation-related genes. Carcinogenesis (Lond.), *22:* 1343–1347, 2001.

43. Carroll, P., Coley, C., McLeod, D., Schellhammer, P., Sweat, G., Wasson, J., Zietman, A., and Thompson, I. Prostate-specific antigen best practice policy. Part II: prostate cancer staging and post-treatment follow-up. Urology, *57:* 225–229, 2001.

44. Pound, C. R., Partin, A. W., Eisenberger, M. A., Chan, D. W., Pearson, J. D., and Walsh, P. C. Natural history of progression after PSA elevation following radical prostatectomy. J. Am. Med. Assoc., *281:* 1591–1597, 1999.

45. Fischer, P. R., Thacher, T. D., Pettifor, J. M., Jorde, L. B., Eccleshall, T. R., and Feldman, D. Vitamin D receptor polymorphisms and nutritional rickets in Nigerian children. J. Bone Miner. Res., *15:* 2206–2210, 2000.

Vitamin D Receptor Start Codon Polymorphism (FokI) and Prostate Cancer Progression

Yue Xu, Atsuko Shibata, John E. McNeal, et al.

Cancer Epidemiol Biomarkers Prev 2003;12:23-27.