Vitamin D Receptor Polymorphisms, Circulating Vitamin D Metabolites,
and Risk of Prostate Cancer in United States Physicians

Jing Ma, Meir J. Stampfer, Peter H. Gann, 
Heather L. Hough, Edward Giovannucci, Karl T. Kelsey, 
Charles H. Hennekens, and David J. Hunter

Channing Laboratory, Department of Medicine [J. M., M. J. S., H. L. H., E. G., 
K. T. K., D. J. H.], and Division of Preventive Medicine, Department of 
Medicine and Department of Ambulatory Care and Prevention [C. H. H.], 
Brigham and Women’s Hospital and Harvard Medical School, and 
Departments of Nutrition [M. J. S., E. G.], Epidemiology [M. J. S., C. H. H., 
D. J. H.], Environmental Health [K. T. K.], and Cancer and Cell Biology 
[K. T. K.], and Harvard Center for Cancer Prevention [D. J. H.], Harvard 
School of Public Health, Boston, Massachusetts 02115; and Department of 
Preventive Medicine, Northwestern University Medical School, Chicago, 
Illinois 60611 [P. H. G.]

Abstract
Prostatic cells express vitamin D receptor (VDR), which 
mediates the functions of 1,25-dihydroxyvitamin D. Two 
recent case-control studies suggested strong inverse 
associations between two VDR polymorphisms, TaqI and 
and risk of prostate cancer. These two and a 
third polymorphism, BsmI, are closely linked. In a case-
control study nested in the Physicians’ Health Study, a 
randomized double-blind trial of aspirin and β-carotene 
among 22,071 United States male physicians, we 
examined the associations between BsmI and TaqI and 
prostate cancer risk and whether the associations varied 
according to age and vitamin D metabolite levels among 
372 incident cases and 591 controls. Among controls, the 
BB genotype was significantly associated with higher 
1,25-dihydroxyvitamin D (median = 36.2 pg/ml for the 
BB versus 33.9 pg/ml for the bb genotype; \( P = 0.02 \)), 
suggesting an association of the VDR polymorphisms 
with VDR function. Overall, we observed no significant 
associations of these VDR polymorphisms with prostate 
cancer risk: relative risk (RR) = 0.86 [95% confidence 
interval (CI) = 0.57–1.29] for the BB genotype and \( \text{RR} = 0.92 \) [95% \( \text{CI} = 0.69–1.22 \)] for the Bb genotype, 
compared with the \( \text{bb} \) genotype (results were similar for 
the \( \text{TaqI} \) polymorphism). Stratification by age (≤61 and 
>61 years) and tumor aggressiveness showed no 
significant associations. However, in an analysis restricted 
to men with plasma 25-hydroxyvitamin D below the 
median, we observed a 57% reduction (RR = 0.43, 95% 
\( \text{CI} = 0.19–0.98 \)) in risk for those with the \( \text{BB} \) versus the \( \text{bb} \) genotype; the risk reduction was particularly marked 
among older men (RR = 0.18, 95% \( \text{CI} = 0.05–0.68 \)). We 
did not observe this inverse association among men with 
25-hydroxyvitamin D levels above the median, nor did we 
observe it among younger men.

Introduction
Vitamin D maintains calcium homeostasis and regulates many 
aspects of bone metabolism. The activated form of vitamin D, 
1,25-D, inhibits proliferation and induces differentiation 
in human prostate cancer cell lines (1–4). A vitamin D analogue 
reduced prostate cancer incidence in an experimental rat model 
(5). These laboratory data are supported by ecological obser-
vations showing that certain populations at high risk for pros-
state cancer, including the elderly, African-Americans, and resi-
dents of northern latitudes, tend to have reduced levels of 
circulating vitamin D (6, 7). However, inconsistent results have 
been reported from several prospective epidemiological studies 
of prostate cancer that assessed vitamin D metabolite levels in 
stored blood samples (8–10).

1,25-D exerts its activity through the intracellular VDR 
(11, 12); normal and malignant prostatic cells express VDR (13, 
14). Polymorphisms in the 3′-UTR of the VDR gene alter 
transcriptional activity and mRNA stability in minigene re-
porter constructs (15). An inherited haplotype near this region 
comprises several RFLPs in strong linkage disequilibrium. 
These polymorphisms, located in intron B (BsmI and Apal) 
and exon 9 (TaqI), form two common haplotypes in Caucasians, 
\( \text{BAI} \) and \( \text{baT} \); a fourth polymorphism, a poly(A) microsatellite, 
is in the 3′-UTR itself (16). In a study of non-Hispanic whites, 
the S (short) and L (long) alleles of the poly(A) microsatellite 
were 97% concordant with the BsmI alleles, with \( \text{B} \) correspon-
ding to \( \text{S} \) and \( \text{L} \) corresponding to \( \text{L} \) (17). Two recent small 

case-control studies [96 cases (18) and 57 cases (16)] reported 
strong and significant associations of the \( \text{TaqI} \) \( \text{tt} \) genotype 
and the poly(A) \( \text{SS} \) genotype with reduced risk of prostate cancer.

We previously studied circulating vitamin D metabolites and 
subsequent risk of prostate cancer in a case-control study 
nested in the Physicians’ Health Study, a randomized double-
blind trial of aspirin and β-carotene among 22,071 United 
States male physicians and found no overall significant asso-
ciations (10); however, a nonsignificant inverse association for 
1,25-D was present for older men with low 25-D levels. We 
now report the associations between two VDR polymorphisms 
(BsmI and TaqI) and prostate cancer risk. We also assessed 
the combined effects of these VDR polymorphisms and plasma

\[ \text{CI} = 0.19 – 0.98 \]

The abbreviations used are: 1,25-D, 1,25-dihydroxyvitamin D; VDR, vitamin D 
receptor; UTR, untranslated region; 25-D, 25-hydroxyvitamin D; DBP, vitamin 
D-binding protein; OR, odds ratio; CI, confidence interval; RR, relative risk.
levels of the vitamin D metabolites in predicting the occurrence of prostate cancer and whether these effects varied by age.

Subjects and Methods

Subjects. This was a prospective case-control study nested in the Physicians' Health Study, a randomized, double-blind, placebo-controlled trial of \( \beta \)-carotene among 22,071 healthy United States male physicians, aged 40–84 years, in 1982 (19). The participants were predominantly Caucasians (over 95%). Men were excluded at baseline if they had a history of myocardial infarction, stroke, or transient ischemic attack; cancer (except nonmelanoma skin cancer), current renal or liver disease, peptic ulcer, or gout; and current use of a vitamin A or \( \beta \)-carotene supplement. Study participants completed two mailed questionnaires before randomization, additional questionnaires at 6 and 12 months, and annual questionnaires thereafter. Written consent was obtained from each participant, and the project has received ongoing approval from the Institutional Review Board at Brigham and Women's Hospital in accord with federal requirements. Blood samples were collected at baseline, in 1982, as described previously (10). We received specimens from 14,916 (68%) of the randomized physicians.

When participants reported a diagnosis of cancer, we requested medical records (including pathology reports), which were reviewed by study physicians from the Study End Points Committee. By March 1992, we had confirmed 372 prostate cancer diagnoses among those who provided blood samples at baseline. For each case, two controls were selected who had provided blood, had not had a prostatectomy, and had not reported a diagnosis of prostate cancer at the time the diagnosis was reported by the case. Controls were also matched for age (±1 year) and smoking status (never, past, or current). Because we could identify a second control for only 219 cases, a total of 591 men formed the control group.

Assays for VDR Polymorphisms and Vitamin D Metabolites. DNA from these cases and controls was extracted from baseline blood specimens, and \( B sml \) and \( T a q l \) genotypes were analyzed for all cases and controls in the Molecular Epidemiology Laboratory of the Channing Laboratory, with the laboratory personnel blinded to case-control status. The VDR-RFLP genotypes were determined by PCR amplification, followed by restriction enzyme digestion, as described previously (15, 18).

Plasma 25-D and 1,25-D were assayed in the laboratory of Dr. Bruce Hollis (Medical University of South Carolina, Charleston, SC). Plasma 25-D was measured by a modified technique of a direct RIA using an antibody to the 23,24,25,26,27-petanor-C(22)-carboxylic acid of vitamin D (20). Plasma 1,25-D was determined by a radioceptor assay with calf thymus receptor, after extraction and purification of the supernatant of the acetonitrile-treated plasma sample on a nonpolar octadecyl-silanol cartridge (21). DBP was measured by radial immunodiffusion assays in the laboratory of Dr. John G. Haddad (University of Pennsylvania Medical Center, Philadelphia, PA; Ref. 22). The mean intra-assay coefficients of variation for 25-D, 1,25-D, and DBP, from our blinded quality control data, were 8.1, 7.9, and 8.7%, respectively. We used the ratios of 1,25-D:DBP and 25-D:DBP as indices of the free hormone concentrations (10). We used the ratio of 25-D:1,25-D as an index of the conversion of 25-D to the active metabolite. Because of inadequate plasma volume, vitamin D metabolites were measured in only 232 cases and 414 controls. One case-control pair was missing \( B sml \) and \( T a q l \) genotypes, and three controls who later became cases were excluded from the control group, leaving 231 cases and 410 controls in these analyses.

Table 1. Frequencies of VDR polymorphisms and RR* of prostate cancer according to \( B sml \) and \( T a q l \) genotypes among cases and controls (Physicians' Health Study)

<table>
<thead>
<tr>
<th>VDR polymorphisms</th>
<th>Cases</th>
<th>Controls</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B sml )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( bb )</td>
<td>135</td>
<td>201</td>
<td>34</td>
<td>1.00</td>
</tr>
<tr>
<td>( Bb )</td>
<td>185</td>
<td>300</td>
<td>51</td>
<td>0.92</td>
</tr>
<tr>
<td>( BB )</td>
<td>52</td>
<td>90</td>
<td>15</td>
<td>0.86</td>
</tr>
<tr>
<td>Total</td>
<td>372</td>
<td>591</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>( Taq1 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( TT )</td>
<td>134</td>
<td>204</td>
<td>35</td>
<td>1.00</td>
</tr>
<tr>
<td>( Tr )</td>
<td>186</td>
<td>299</td>
<td>51</td>
<td>0.95</td>
</tr>
<tr>
<td>( t )</td>
<td>52</td>
<td>86</td>
<td>15</td>
<td>0.92</td>
</tr>
<tr>
<td>Total*</td>
<td>372</td>
<td>589</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for age and smoking status (never, past, or current).

The Tumor Characteristics. Tumor aggressiveness was determined on basis of pathology reports (10). Cases without pathologic staging were classified as indeterminate stage unless there was clinical evidence of distant metastases. "Aggressive" cases were defined as those diagnosed at stage C or D (extra-prostatic) and those diagnosed at stage A or B or indeterminate stage with either poor histologic differentiation or a Gleason score of ≥7.

Statistical Analysis. We quantified the linkage disequilibrium between the \( B sml \) and \( T a q l \) alleles with a maximum likelihood approach using the linkage utility program EH (23). We used contingency tables and calculated ORs and 95% CIs to assess whether the \( B sml \) and \( T a q l \) polymorphisms were related to prostate cancer risk. Because cases and controls were matched by age and cigarette smoking status (never, past, or current), we used age- and smoking-adjusted ORs to estimate the RR, with unconditional logistic regression analysis. The results were virtually identical with or without smoking status in the model. To be consistent with our previous analyses, we also assessed risks according to two age categories (≤61 years and >61 years), dichotomized at the median age, and according to tumor aggressiveness. To assess whether the vitamin D metabolite levels differed by age and the \( B sml \) and \( T a q l \) genotypes, we computed Wilcoxon rank sum statistics using untransformed values. Finally, we assessed the risks for the joint effect of \( B sml \) and \( T a q l \) polymorphisms and vitamin D metabolite status (low versus high according to the median levels of the control distribution) using an indicator variable for each category in logistic regression models. The median cutoff points were 34 pg/ml for 1,25-D and 0.108 for free 1,25-D index. Levels of 25-D within individuals vary by season, with higher levels observed in the summer and fall (from June to November). We, therefore, used separate median cutoff points for 25-D, 25-D:1,25-D ratio, and free 25-D index according to two seasonal groups [December/January–May (winter/spring) and June–November (summer/fall)]. These median cutoff points were: 25-D, 24.8 ng/ml and 29.8 ng/ml; 25-D:1,25-D ratio, 0.72 and 0.90; and free 25-D index, 0.074 and 0.099, for winter/spring and summer/fall, respectively. We compared the log likelihood statistics of the main effect model with the joint effect model to assess interaction. All P s are two-sided; all analyses were performed with SAS (24).
Table 2  RR* of prostate cancer according to BsmI and TaqI genotypes among 185 cases with aggressive tumors* and 225 matched controls overall and by two age groups (Physicians’ Health Study)

<table>
<thead>
<tr>
<th></th>
<th>BsmI</th>
<th>TaqI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bb</td>
<td>Bb</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>Tt</td>
</tr>
<tr>
<td>All ages'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.00</td>
<td>0.82</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.54-1.25)</td>
<td>(0.53-1.78)</td>
</tr>
<tr>
<td>No.</td>
<td>78/86</td>
<td>89/108</td>
</tr>
<tr>
<td>Age ≤ 61 yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.00</td>
<td>0.73</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.41-1.32)</td>
<td>(0.69-3.65)</td>
</tr>
<tr>
<td>No.</td>
<td>42/50</td>
<td>35/58</td>
</tr>
<tr>
<td>Age &gt; 61 yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.00</td>
<td>0.94</td>
</tr>
<tr>
<td>95% CI</td>
<td>(0.51-1.76)</td>
<td>(0.23-1.41)</td>
</tr>
<tr>
<td>No.</td>
<td>36/36</td>
<td>45/50</td>
</tr>
</tbody>
</table>

* Adjusted for age and smoking status (never, past, or current).
* An aggressive tumor was defined as stage C/D or stage A/B/indeterminate stage with either poor histological differentiation or a Gleason score of ≥7.
* Age at blood collection.
* Number of cases/number of controls.

Results

Linkage Disequilibrium between BsmI and TaqI. The frequencies of the BsmI B allele (41%) and TaqI T allele (40%) among our control group are consistent with previous reports (15, 17, 18). We estimated BsmI/TaqI haplotype frequencies and compared the observed frequencies of two haplotypes, Bt and bT, with expected frequencies. Linkage disequilibrium was nearly complete among both cases and controls, as indicated by estimated Bt and bT haplotype frequencies, totaling 97% in each group.

BsmI and TaqI Polymorphisms and Prostate Cancer. Overall, the frequencies of BB or tt genotypes of VDR were nearly the same in cases (14%) as controls (15%; Table 1). The overall prostate cancer risks were not materially different for men with the homozygous variants (BB and tt) or those with the heterozygous (Bb and Bt) variants, compared with men with the bb and TT genotypes, and none of the RRs were close to statistical significance. To assess whether the association was modified by age, we further stratified the analyses by two age groups (≤61 and >61 years). Among older men, the RR for the BB genotype versus the bb genotype was 0.73 (95% CI = 0.42–1.27), and the RR for the TT genotype versus the bb genotype was 0.84 (95% CI = 0.48–1.47); among younger men, these RRs were close to 1. Table 2 shows the gene frequencies and RRs in a subgroup of cases with aggressive tumors and their matched controls. Although there were no overall associations between the BB or tt genotype and risk of developing aggressive tumors, the BB and tt genotypes had divergent trends by age, with increased risk among younger men but lower risk among older men. None of these RRs were close to statistical significance. We found similar results by restricting these analyses to men with data on plasma vitamin D metabolites.

VDR Polymorphisms and Circulating Vitamin D Metabolites. The BB genotype was related to higher 1,25-D levels in a previous study (15). In the control group of our study, men with the BB genotype also had significantly higher levels of 1,25-D than did men with the Bb or bb genotypes (Table 3). The associations between the BsmI genotype and vitamin D metabolites varied by age. Significantly higher 1,25-D levels and lower free 25-D indices and 25-D:1,25-D ratios for the BB genotype were observed among older but not younger men (Table 3). We observed similar patterns for the tt versus TT genotype (data not shown).

Effect Modification by Plasma Vitamin D Metabolites and Age. Because plasma or serum 25-D is the most commonly used index of vitamin D status (25, 26) and VDR mediates the functions of 1,25-D, we assessed whether the associations between the VDR polymorphisms and prostate cancer risk may differ by levels of vitamin D metabolites. Table 4 shows the interactions between the BsmI genotype and plasma 25-D and 1,25-D, the 25-D:1,25-D ratio, and the free 25-D and 1,25-D indices. We observed a significant 57% reduction in risk (RR = 0.43, 95% CI = 0.19–0.98) for the BB versus the bb genotype among men with low 25-D levels. No apparent association between the BsmI genotype and cancer risk was observed among men with plasma 25-D above the median. This pattern was slightly stronger for the free 25-D index, with a significant 62% reduction in risk (RR = 0.38, 95% CI: 0.16–0.89). Results were similar in analyses that did not use the season-specific cutoff points for 25-D, 25-D:1,25-D ratio, and the free 25-D index but instead used the overall median values. We also observed nonsignificant reductions in risk for the BB genotype versus the bb genotype among men with higher levels of 1,25-D or the free 1,25-D index (Table 4). Similar associations were found for the TaqI alleles (data not shown). Because the associations between the VDR genotypes and levels of vitamin D metabolites among older men were different from those among younger men, we further stratified these analyses by age. Among men over age 61 years with low 25-D, free 25-D index, and 25-D:1,25-D ratio, we observed much stronger and more significant inverse associations between the BB genotype and prostate cancer risk (Table 5); similar inverse associations were also observed for the tt genotype (data not shown). No significant associations or interactions were observed among younger men (data not shown).

Discussion

In this well-defined prospective cohort of United States physicians, we found no significant associations of two VDR polymorphisms, BsmI and TaqI, with prostate cancer risk overall, in younger or older men or in analyses limited to aggressive tumors. However, in analyses restricted to men whose circulating 25-D levels or free 25-D index were below the median levels, we observed a significant 57–62% reduction in risk for men with the BsmI BB genotype or the TaqI tt genotype.
compared with those with the bb or TT genotype; the risk was reduced by about 80–90% among older men (over 61 years).

The VDR polymorphisms, BsmI, TaqI, and poly(A), are closely linked among whites; concordance was 97% between the BsmI B allele and the TaqI T allele in our study and 97% between the BsmI B allele and the poly(A) S allele in the study of Ingles et al. (16). The overall frequencies of the BsmI B allele, the TaqI T allele, and the poly(A) S allele are similar (40–43%) among our study and other studies (15, 17, 18). However, our results on overall risk are inconsistent with previous findings from smaller studies (16, 18). In a hospital-based case-control study of the TaqI polymorphism (96 cases), Taylor et al. (18) reported that homozygotes for the T allele have one-third the risk (OR = 0.32, 95% CI = 0.15–0.75) of developing prostate cancer requiring prostatectomy of that of heterozygotes or homozygotes for the T allele. In a study of the poly(A) polymorphism (57 cases), Ingles et al. (16) reported an OR of 0.22 (95% CI = 0.06–0.75) for the risk of prostate cancer among men with the SS genotype compared with men with the SL or LL genotypes. Selection bias in the case-control studies is one possible explanation, but the prevalence of the polymorphisms in our control series was similar to that of the previous studies, suggesting that differences in control selection were probably not the reason for the divergent results. Cases in our study were incident cases arising in a defined cohort, whereas the two previous studies enrolled prevalent cases; if these VDR polymorphisms were associated with more aggressive prostate cancer and higher mortality, they may have been underrepresented in a series of prevalent cases due to a “survivor effect.” However, we observed no association with tumor aggressiveness, thus reducing the probability of this form of bias in the previous studies. We and others found previously that the inverse association between circulating 1,25-D levels and prostate cancer risk was much stronger among older men (8,10). In this analysis, we found evidence of effect modification of the relation of the VDR polymorphisms and prostate cancer risk by age (the association was more strongly inverse among older men). However, men in the study of Ingles et al. (16) were actually somewhat younger on average than the men in our study, which suggests that differences in age were not the reason for the divergent results. We also observed effect modification according to plasma 25-D levels (the association was more strongly inverse in the lower half of the 25-D distribution standardized by season). It is possible that men in the previous studies had lower 25-D levels; however, indices of vitamin D status were not reported in those studies.

The mechanism(s) through which these genetic variations in the VDR gene may influence prostate cancer risk are unclear because these specific VDR polymorphisms do not alter the amino acid sequence of the VDR protein. The BsmI and TaqI polymorphisms located in intron 8 and exon 9 near the 3'-UTR of the VDR gene are in strong linkage disequilibrium with the poly(A) polymorphism in the 3'-UTR region (15, 17). The 3'-UTR region is involved in the regulation of mRNA stability, and mutations at this site can alter mRNA degradation (15). It is also possible that these polymorphisms are in linkage disequilibrium with other mutations that alter VDR function. Consistent with the findings of Morrison et al. (15), our observation of significantly elevated circulating levels of 1,25-D and lower free 25-D index and 25-D:1,25-D ratio among older men with the BsmI homozygous variant BB strongly suggests a biological effect of this VDR haplotype on regulation of circulating vita-
The conversion of 25-D to the active hormone 1,25-D is under stringent control, so that the renal output of 1,25-D is genotype and the BB genotype have an attenuated decrease in serum parathyroid hormone or regulate the hydroxylation of 25-D to 1,25-D may modify the association of the VDR polymorphisms with prostate cancer risk, and these factors merit further study.

In conclusion, these prospective data among United States physicians indicate that, overall, the VDR polymorphisms BsmI and TaqI are not strong independent predictors of prostate cancer risk, as reported previously. However, the data suggest an inverse association of these VDR polymorphisms with prostate cancer risk among older men with low 25-D levels.

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References
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