Vitamin D Receptor Gene Polymorphisms, Insulin-like Growth Factors, and Prostate Cancer Risk: A Population-based Case-Control Study in China

Anand P. Chokkalingam,1 Katherine A. McGlynn, Yu-Tang Gao, Michael Pollak, Jie Deng, Isabell A. Sesterhenn, F. K. Mostofi, Joseph F. Fraumeni, Jr., and Ann W. Hsing

Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, Maryland 21201 [A. P. C.]; Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland 20892 [A. P. C., K. A. M., J. F. F., A. W. H.]; Shanghai Cancer Institute, Shanghai 200032, China [Y.-T. G., J. D.]; Cancer Prevention Research Unit, Jewish General Hospital and Department of Oncology, McGill University, Montreal, Ontario H3T 1E2, Canada [M. P.]; and Armed Forces Institute of Pathology, Washington, DC 20306 [I. A. S, F. K. M.]

Abstract

Operating through the vitamin D receptor (VDR), vitamin D inhibits prostate cancer growth and increases insulin-like growth factor binding protein (IGFBP) expression, suggesting that the vitamin D and insulin-like growth factor (IGF) regulatory systems may operate together to affect prostate cancer. Among 191 newly diagnosed prostate cancer cases and 304 randomly selected population controls in Shanghai, China, we found no significant association between the BsmI or FokI VDR gene polymorphisms and prostate cancer risk. However, we found that among men with the ff FokI genotype, those in the highest tertile of plasma IGFBP-3 had a decreased risk versus those in the lowest tertile (odds ratio, 0.14; 95% confidence interval, 0.04–0.56; 95% confidence interval, 0.07–0.85). No such FokI genotype-specific effects were observed for IGF-I or IGF-II. Our findings in a low-risk population suggest that the IGF and vitamin D regulatory systems may interact to affect prostate cancer risk. Larger studies are needed to confirm these findings and clarify the underlying mechanisms.

Introduction

Laboratory studies demonstrate a strong and consistent prodifferentiative and growth-inhibitory effect of the steroid hormone 1,25(OH)2D3 and its analogues on prostate cancer both in vitro and in vivo (1). Because the effects of 1,25(OH)2D3 are mediated by the VDR (2) and because both normal and malignant prostate cells express active VDR (2), it has been suggested that polymorphic markers within the VDR gene may be related to prostate cancer risk. To date, a number of such markers have been identified, including the FokI and the 5′ BsmI markers; the latter have been shown to have functional effects. However, epidemiological studies of these two VDR polymorphisms have been inconsistent, with both positive (3) and null (4–6) results. In recent laboratory studies, the inhibition of prostate cancer cell growth by 1,25(OH)2D3 and its analogues has been associated with increased expression of IGFBPs and decreased expression of IGF-II (7–9). Because IGFBPs inhibit the mitogenic actions of IGFs on cancer cells (10, 11) and because we and others have found previously that systemic levels of IGFBP-3 and IGFBP-1 are inversely associated with prostate cancer risk (12, 13), it has been suggested that the growth inhibition of prostate cancer cells induced by vitamin D through the VDR occurs via a pathway involving the IGF axis (9). To date, no epidemiological studies have addressed the potential combined effects of the IGF axis and vitamin D regulatory pathway. In the current population-based investigation, we examined the a priori hypothesis that VDR gene polymorphisms affect prostate cancer risk, both independently and in conjunction with plasma levels of IGFBP-1, IGFBP-3, IGF-I, and IGF-II, among newly diagnosed prostate cancer cases and healthy controls randomly selected from the population in Shanghai, China.

Materials and Methods

Study Population. Details of this study have been described previously (14, 15). Briefly, histologically confirmed cases of primary prostate cancer newly diagnosed in urban Shanghai between 1993 and 1995 were identified through a rapid reporting system established by the Shanghai Cancer Registry. On the basis of a regional registry of all persons >16 years in urban Shanghai, male population controls were selected at random from the 6.5 million permanent residents and frequency-matched to the expected age distribution (by 5-year age category) of the cases. Using a structured questionnaire, trained interviewers elicited information on epidemiological risk factors from cases and controls within 30 days after selection. Anthropometric measures were taken during the interview. Of the 268 cases who were permanent residents of Shanghai (95% of cases diagnosed in Shanghai during the study period), 243 (91%) were interviewed. Of the 495 selected controls, 472 (95%) were interviewed. This study was approved by the Office of Human Subjects Research, NIH, and the Institutional Review Board, Shanghai Cancer Institute, Shanghai, China.

Blood Collection and Laboratory Assays. Two hundred cases and 330 controls (82 and 70% of those interviewed, respectively) provided 20 ml of fasting blood for the study. Samples were processed within 3 h of collection at a central laboratory in Shanghai. DNA extracted from the buffy coat fractions was used for VDR genotyping, whereas IGFs and IGFBPs were quantified from the plasma samples. All laboratory personnel were masked to case-control status, and samples from cases and controls were physically arranged and assayed in an alternating fashion to minimize bias attributable to day-to-day laboratory variation.

Plasma levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3 were determined using ELISA kits from Diagnostic Systems Laboratories (Webster, TX). Analytical sensitivities of the assays are 0.03, 2.4, 0.04, and 0.04 ng/ml, respectively. For all four analytes, each sample was assayed twice, and the mean of the two determinations was used for data analysis.

We genotyped the study subjects for both the 5′ FokI and the 3′ BsmI VDR markers; the latter were in linkage disequilibrium with other 3′ markers, including TaqI and poly(A) (2). For each VDR gene marker, 50 ng of genomic DNA were amplified by PCR in a total volume of 10 μl using the primers described by Morrison et al. (16) for the BsmI marker and by Gross et al. (17) for the FokI marker. After sequence-specific digestion with either 2 units of BsmI or 0.8 unit of FokI restriction enzyme (New England Biolabs), the samples were electrophoresed through a 2% agarose gel containing ethidium bromide and scored for genotypes of the BsmI (bb, Bb, or BB) and FokI (FF, FF, Ff, ff).
Results

One hundred ninety-one prostate cancer cases and 304 controls were assayed for the BsmI and FokI markers. Compared with controls, cases were more likely to have an education level higher than middle school, less likely to be married, and less likely to smoke or consume alcohol.

The BsmI and FokI genotyping results are shown in Table 1. Among the population controls, the prevalences of each of the bb, Bb, and BB BsmI genotypes were 87.2, 10.4, and 2.4%, respectively, yielding only 7 controls with the BB genotype. For the FokI marker, the prevalences of the FF, Ff, and ff FokI genotypes were 28.8, 50.7, and 20.5%, respectively. Both markers were in Hardy-Weinberg equilibrium. We found no significant association of either marker with total prostate cancer risk or stage-specific cancer (clinical staging: early versus late). However, despite the fact that early-stage cancers made up only one-third of the total case group, all four cases with the BB BsmI genotype had early-stage disease.

To assess whether systemic levels of IGFBPs and IGFs are associated with VDR genotype, we compared age-adjusted means of IGFBP-1, IGFBP-3, IGF-I, and IGF-II in each genotype of the FokI marker among the 304 population controls (Table 2). Because of the rarity of the B allele of the BsmI marker in this study population, we did not assess this marker in relation to IGF/IGFBP levels or to their combined effects on prostate cancer risk. Those with the ff genotype of FokI had higher IGFBP-1 levels (P = 0.07) and significantly lower IGF-II levels (P = 0.03) than those with the FF and Ff genotypes.

Among those with the FF and Ff FokI genotypes, IGFBP-1 was not significantly associated with prostate cancer risk after adjusting for age and IGF-I (Table 3). In contrast, among those with the ff genotype, those in the highest tertile of IGFBP-1 had a 75% decreased risk compared with those in the lowest tertile, with a significant trend (OR, 0.04–0.56; P trend < 0.01). However, no differences were observed in the association of either IGF-I or IGF-II with prostate cancer across strata of FokI genotypes.

In addition, the risk of disease associated with the ff FokI genotype relative to the FF and Ff genotypes differed by tertiles of systemic IGFBP-1 and IGFBP-3. Among those in the lowest IGFBP-1 tertile, the ff genotype was associated with increased risk relative to the FF and Ff genotypes (OR, 2.08; 95% CI, 0.86–5.01), whereas among those in the highest tertile of IGFBP-1, the ff genotype was associated with a reduced risk (OR, 0.39; 95% CI, 0.14–1.11). Similarly, among those in the lowest tertile of IGFBP-3 levels, the ff genotype was associated with increased risk of prostate cancer relative to the FF and Ff genotypes, whereas among those with the highest IGFBP-3 levels (OR, 2.20; 95% CI, 0.94–5.11), the ff genotype was associated with a

<table>
<thead>
<tr>
<th>VDR marker</th>
<th>N1/N2*</th>
<th>OR (95% CI)</th>
<th>N1/N2*</th>
<th>OR (95% CI)</th>
<th>N1/N2*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BsmI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>140/259</td>
<td>1.00 (ref)</td>
<td>48/259</td>
<td>1.00 (ref)</td>
<td>91/259</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Bb</td>
<td>17/1</td>
<td>1.01 (0.54–1.90)</td>
<td>3/31</td>
<td>0.52 (0.15–1.78)</td>
<td>14/1</td>
<td>1.29 (0.65–2.52)</td>
</tr>
<tr>
<td>BB</td>
<td>4/7</td>
<td>1.06 (0.30–3.67)</td>
<td>4/7</td>
<td>3.08 (0.87–10.94)</td>
<td>0/7</td>
<td>0.07 (0.05–0.96)</td>
</tr>
<tr>
<td>(BB + Bb) vs. bb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Ff + Ff) vs. FF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>51/87</td>
<td>1.00 (ref)</td>
<td>17/87</td>
<td>1.00 (ref)</td>
<td>34/87</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Ff</td>
<td>95/153</td>
<td>1.06 (0.69–1.63)</td>
<td>35/153</td>
<td>1.17 (0.62–2.21)</td>
<td>59/153</td>
<td>0.99 (0.60–1.62)</td>
</tr>
<tr>
<td>ff</td>
<td>41/62</td>
<td>1.13 (0.67–1.91)</td>
<td>17/62</td>
<td>1.40 (0.66–2.96)</td>
<td>24/62</td>
<td>0.99 (0.54–1.83)</td>
</tr>
</tbody>
</table>

* N1, number of cases; N2, number of controls.

Table 2 Age-adjusted mean levels of plasma IGFs and IGFBPs by VDR FokI genotype among controls

<table>
<thead>
<tr>
<th>IGF-I</th>
<th>IGFBP-1</th>
<th>IGFBP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td>(FF + Ff)</td>
<td>125.3</td>
<td>(120.0–130.6)</td>
</tr>
<tr>
<td>FF</td>
<td>125.5</td>
<td>(116.2–134.8)</td>
</tr>
<tr>
<td>Ff</td>
<td>125.2</td>
<td>(118.7–131.8)</td>
</tr>
<tr>
<td>ff</td>
<td>117.2</td>
<td>(106.5–127.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pdiff*</th>
<th>Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF vs. Ff</td>
<td>0.96</td>
<td>0.67</td>
</tr>
<tr>
<td>Ff vs. ff</td>
<td>0.20</td>
<td>0.03</td>
</tr>
<tr>
<td>(Ff + Ff) vs. ff</td>
<td>0.18</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Pairwise t test P for difference between means.
reduced risk (OR, 0.39; 95% CI, 0.12–1.25). In contrast, the effect of the \textit{ff} genotype relative to the \textit{FF} and \textit{Ff} genotypes did not differ markedly by tertiles of IGF-I or IGFBP-II.

**Discussion**

In this low-risk Chinese population, there was no evidence linking either the \textit{BsmI} or \textit{FokI} polymorphisms of the VDR gene with prostate cancer risk, although small to moderate effects of \textit{BsmI} marker cannot be ruled out because of the low prevalence of its \(B\) allele. We have reported previously that systemic levels of IGF-I and IGFBP-3 in this population were inversely associated with prostate cancer risk, whereas plasma levels of IGF-I showed a positive relation to risk (12). In the current study, we found that the inverse associations of IGFBP-3 and IGFBP-1 according to \textit{BsmI} genotype effects by analytic tertile\(d\) were mainly limited to subjects with the \textit{FF} genotype of the \textit{FokI} marker, whereas the effects of IGF-I did not differ across strata of \textit{FokI} genotypes.

Previous studies of the \textit{BsmI} polymorphism among African-Americans (4) and Caucasian-Americans (5) have also shown no association with prostate cancer risk, although a study among Japanese men (also a low-risk population) found a significant reduction in risk associated with the \(B\) allele (3). The higher prevalence of the \(B\) allele in the Japanese study (3) relative to the current investigation (27% versus 8% among controls, respectively) is unlikely to explain the difference in results, because both United States studies had even higher \(B\) allele prevalences (32–41%; Refs. 4 and 5).

In contrast to the \textit{BsmI} marker and other \(3’\) polymorphisms of the VDR gene [including \textit{Apol}, \textit{Taql}, and poly(A)], polymorphism of the \(5’\) \textit{FokI} site alters the VDR amino acid sequence; the \textit{F} and \textit{f} alleles of the \textit{FokI} marker encode VDR proteins of 424 and 427 amino acids, respectively (19). Recent data suggest that the VDR coded by the \(F\) allele of the \textit{FokI} polymorphism is more responsive to vitamin D than that coded by the \(f\) allele (19). However, we found no significant association of the \textit{FokI} polymorphism with prostate cancer risk, despite high prevalence of the \(f\) allele (46%). Similarly, the only other published study of \textit{FokI} and prostate cancer, conducted in a European population with an \(f\) allele prevalence of 34%, also showed no association (6).

In cross-sectional analyses, we found that controls with the \textit{ff} genotype of the \textit{FokI} marker had significantly higher mean IGFBP-1 levels and significantly lower mean IGF-II levels than those with the \textit{FF} or \textit{Ff} genotypes. Indeed, systemic IGFBP-1 levels were elevated in parallel with increasing copies of the \(f\) \textit{FokI} allele. Given that we previously found that IGFBP-1 levels were inversely related to prostate cancer risk in this population (12), our results suggest that the reduced risk associated with the \textit{ff} genotype might be mediated through elevated levels of IGFBP-1. However, the overall null results for \textit{FokI} polymorphism genotypes on prostate cancer risk seem inconsistent with this notion, calling for further studies to elucidate the relationships observed. Furthermore, because systemic IGF-II levels have not been found to be associated with prostate cancer risk in our study and in others (11, 12), the implication of the significant difference in systemic IGF-II levels between the \textit{FF} and \textit{ff} genotypes is unclear.

In the current study, we found that levels of IGFBP-1 and IGFBP-3 were associated with reduced prostate cancer risk only among men with the \textit{ff} genotype of the \textit{FokI} polymorphism, but neither of the IGFBPs was related to prostate cancer risk among those with the \textit{FF} and \textit{Ff} genotypes. Given that the shorter VDR encoded by the \(F\) allele may be more effective at exerting vitamin D effects than that coded by the \(f\) allele (19), our results suggest that systemic levels of IGFBP-3 and IGFBP-1 are associated with risk reduction only among men with presumably lower VDR function. This issue should be investigated further, particularly because increases in IGFBP levels after vitamin D administration in laboratory studies occur at the local rather than systemic level (7–9), and it is as yet unclear how systemic and local levels of IGFBPs and IGFBs are related.

The association of the \textit{ff} \textit{FokI} genotype with prostate cancer relative to the \textit{FF} and \textit{Ff} genotypes differed by systemic IGFBP-3 and IGFBP-1 levels. Indeed, the \textit{ff} genotype was associated with increased risk of prostate cancer in the lowest IGFBP-1 and IGFBP-3 tertiles and decreased risk in the highest tertiles. Reasons for these findings are unclear.

The biological mechanism underlying the differential effects of IGFBP-3 and IGFBP-1 according to \textit{FokI} genotype, if confirmed in other studies of prostate cancer, is as yet unclear. One possible mechanism is that, if the VDR coded by the \(F\) \textit{FokI} allele (\(F\)-VDR) is
indeed more transcriptionally active than that coded by the \( f \) allele (19), men with the \( F \) allele (either the \( FF \) or \( Ff \) genotypes) may more easily activate the local, prostatic IGFBP expression that occurs in rats and in cultured human cells in response to vitamin D administration (7–9), thus inhibiting prostate cellular proliferation irrespective of systemic IGFBP levels (Fig. 1A). In contrast, men without the VDR coded by the \( F \) allele (those with the \( ff \) genotype) would have a lowered production of local IGFBPs so that inhibition of IGF-mediated cellular proliferation would be more dependent on systemic IGFBP levels (Fig. 1B). It should be noted, however, that it is as yet unclear whether systemic IGFBPs can influence local IGFBP levels. Other possible mechanisms exist as well; because IGFBP-3 can bind to the retinoid X receptor \( \alpha \) (an obligatory cofactor of the VDR) to induce apoptosis in prostate cancer cells (20), IGFBPs may also interact directly with the VDR, or even replace it under certain circumstances, to effect transcriptional change. Such mechanisms are speculative at this point but may provide directions for further research to explain the interactions observed in our study. It is also noteworthy that although the \( F0kI \) genotypes appeared to interact with systemic levels of both IGFBP-1 and IGFBP-3, the correlation of \( F0kI \) genotype with levels of IGFBP-1, but not IGFBP-3, suggest that different mechanisms may be involved for each observed interaction.

Because the plasma samples from the cancer cases in this study were collected after diagnosis, it is possible that the presence of disease among cases affected the results of this study. However, this potential disease effect, if present, is likely to be minimal, because the age-adjusted mean levels of IGFBP-3, IGF-I, and IGF-II did not differ appreciably between localized and advanced stage cancer cases. Levels of IGFBP-1 were slightly higher among localized relative to advanced stage cases (112.0 ng/ml, respectively), but not significantly so \((P = 0.35)\), and it is unclear whether this difference is biologically meaningful. In addition, the associations with prostate cancer of IGF-I and IGFBP-3 reported previously in this study population (12) are similar in direction and magnitude to those reported in a prospective study (13), which is not subject to disease effects. Finally, despite the fact that the combined effects of systemic IGFBP levels and \( F0kI \) genotypes were based on small numbers, it is noteworthy that no such effects were seen for the IGFs, a very different class of proteins.

In summary, the results of this study suggest that the \( BsmI VDR \) gene polymorphism is not related to prostate cancer (though a small effect cannot be ruled out), and that the \( f f \) genotype of the \( F0kI VDR \) gene polymorphism may combine with systemic levels of IGFBP-3 and IGFBP-1 to modulate disease risk. Indeed, it appears that the inverse association of both IGFBP-3 and IGFBP-1 with prostate cancer we observed previously in this population (12) is confined to men with the \( f f \) \( F0kI \) genotype. These results suggest that the vitamin D regulatory system and the IGF axis may influence prostate cancer risk. These findings should be explored in large prospective investigations of populations at varying risk of prostate cancer that include measurements of circulating vitamin D metabolites.

References

Fig. 1. Hypothesized mechanism for observed interaction between VDR \( F0kI \) polymorphism and IGFBP-1 and IGFBP-3 levels. A, men with at least one copy of the more transcriptionally active VDR encoded by the \( F \) allele (\( F-VDR \)) have increased VDR functionality, yielding higher local vitamin D-dependent IGFBP expression and therefore higher progesterone IGFBP levels, irrespective of systemic IGFBP levels. IGFBP-mediated cellular proliferation is thus inhibited at the local level, and the risk of prostate cancer is decreased. B, in contrast, in absence of the \( F-VDR \), men have reduced vitamin D-dependent prostatic IGFBP expression. In the presence of high systemic IGFBP levels, levels of prostatic IGFBPs could still be high enough to inhibit cellular proliferation, thereby reducing prostate cancer risk. However, in the presence of low systemic IGFBP levels, prostatic levels remain exclusively low and IGF-mediated cellular proliferation is not inhibited, resulting in elevated prostate cancer risk.
Vitamin D Receptor Gene Polymorphisms, Insulin-like Growth Factors, and Prostate Cancer Risk: A Population-based Case-Control Study in China


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/11/4333

Cited articles
This article cites 15 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/11/4333.full#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/11/4333.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/61/11/4333.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.