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# Vitamin D receptor genotype and breast cancer in Latinas (United States)

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## Abstract

*Objective*: Polymorphism in the vitamin D receptor (VDR) gene has been associated with variation in bone mineral density and with prostate cancer risk. The purpose of this study was to determine whether polymorphism in the VDR gene may also influence breast cancer risk.
*Methods*: Polymorphisms in the 5′ and 3′ ends of the VDR gene were genotyped for 143 Latina women with breast cancer and 300 cohort controls.
*Results*: Both the BsmI and poly-A polymorphisms in the 3′ end of the VDR gene were associated with breast cancer risk, with a trend for increasing risk with increasing number of BsmI B alleles or short (S) poly-A alleles. Compared to subjects having two long poly-A alleles (genotype LL), odds ratios (and 95% confidence intervals) were 1.5 (1.0–2.3) and 3.2 (1.5–6.9) for subjects having genotypes SL and SS, respectively. Compared to BsmI genotype bb, odds ratios (and 95% confidence intervals) were 1.6 (1.1–2.5) and 2.2 (1.0–4.7) for genotypes Bb and BB respectively. The start codon polymorphism, FokI, was not associated with breast cancer risk.
*Conclusion*: These results suggest that polymorphic variation in or near the 3′ end of the VDR gene influences breast cancer risk in Latina women.

## Introduction

Vitamin D plays an important role in modulating transcription of genes involved both in calcium and phosphorus homeostasis and in cellular differentiation and proliferation (reviewed in refs. 1–3). An obligatory mediator of these effects is the vitamin D receptor (VDR). Both the 5′ and 3′ ends of the VDR gene are polymorphic. Polymorphism in the first of two possible translation start codons [4] produces receptor variants differing in size and activity [5]. Allelic variation in the 3′ end of the VDR gene, although less clearly related to function, appears to have phenotypic consequences for calcium metabolism [6, 7], vitamin D metabolism [8, 9], bone density (reviewed in ref. 10) and prostate cancer risk [9, 11–13]. The observation that normal human breast epithelial cells [14] and most breast cancers [15, 16] express VDR raise the possibility that polymorphism in the VDR gene may also influence breast cancer risk. To test this hypothesis we genotyped polymorphisms in the 5′ and 3′ ends of the VDR gene for 143 Latina women with breast cancer and 300 cohort controls.

## Materials and methods

### *Subjects*

The Hawaii–Los Angeles Multiethnic Cohort Study is an ongoing epidemiological study of Japanese-Americans and whites residing in Hawaii, and African-Americans and Latinos residing in California. For a study of vitamin D receptor genotypes and breast cancer we obtained DNA samples on female subjects from the

two Los Angeles-based (*i.e.*, African-American and Latino) sub-cohorts. In this first report we focus on Latinas, a population with strong linkage disequilibrium in the genomic region of interest, which allows BsmI and poly-A genotypes to be interpreted as markers of the VDR 3′UTR allelotypes [17]. African-Americans have not been included in this report because characterization of allelotypes and validation of markers in the African-American population is still in progress.

The Latina sub-cohort includes more than 12,000 Latina women, age 45–75 at recruitment, residing in Los Angeles County, who were recruited by sampling Los Angeles County residents from drivers' license files as previously described [18, 19]. Newly diagnosed cases of breast cancer among Latinas were ascertained through linkage of the cohort to the Los Angeles County Surveillance, Epidemiology, and End Results (SEER) cancer registry, which is estimated to be 99% complete. Blood samples were obtained from incident breast cancer cases and from approximately a 1% random sample of the cohort members to serve as a control group. The participation rate for sample collection was in excess of 70% among both cases and controls. All subjects signed informed consents and the study was approved by the University of Southern California Institutional Review Board, which oversees studies involving human subjects.

### *Genotyping*

An 825 bp region of genomic DNA containing the BsmI polymorphic site in intron 8 was amplified and analyzed as previously described [12]. The existence of the cut allele, b, is indicated by the formation of a 625 base-pair product.

A region surrounding the poly-A polymorphism was amplified as previously described [13]. Products were separated on polyacrylamide sequencing gels and autoradiographed. Alleles were sized and categorized as short (S) or long (L) as previously described [12].

A region of approximately 280 bp of genomic DNA containing the FokI polymorphic site was amplified and analyzed as previously described [20]. The presence of the cut allele, f, is indicated by a band at approximately 200 bp.

### *Statistical methods*

FokI, BsmI and poly-A odds ratios were estimated by fitting standard unconditional logistic regression models [21], using two indicator variables to encode the three FokI, BsmI or poly-A genotypes, and two indicator variables to adjust for tertiles of age. Tests for trend were performed using a likelihood ratio test for significance of a genotype variable coded as 0, 1, or 2.

Expected genotype frequencies under Hardy–Weinberg equilibrium and under the hypothesis of no linkage disequilibrium were calculated from observed genotype frequencies among controls, using standard methods [22]. Testing for departure from the hypothesis of no linkage disequilibrium was performed by comparing observed and expected joint genotypic distributions using a chi-square test.

## Results

Of the 300 control women, 55 were included in our previous report describing linkage disequilibrium in the 3′UTR of the VDR gene [17]. Among 55 female and 43 male Latino cohort members, we had observed 81% agreement between BsmI and poly-A genotypes. With 300 control women now available from the same cohort, agreement between the BsmI and poly-A genotypes is now estimated to be 88% (Table 1).

*Table 1.* Joint distribution of 3′VDR marker genotypes (BsmI and Poly-A) among Latina controls

| | Poly-A | | | |
|---|---|---|---|---|
| | LL | SL | SS | Total |
| BsmI | | | | |
| bb | 12 | 7 | 0 | 19 |
| Bb | 4 | 94 | 14 | 112 |
| BB | 0 | 12 | 157 | 169 |
| Total | 16 | 113 | 171 | 300 |

Agreement = (12 + 94 + 157)/300 = 88%.

*Table 2.* Age-adjusted odds ratios and 95% confidence intervals for breast cancer by 3′VDR genotype in Latinas

| | Controls *n* (%) | Cases *n* (%) | OR (95% confidence interval) |
|---|---|---|---|
| BsmI | | | |
| bb | 169 (56.4) | 61 (42.7) | 1.0 |
| Bb | 112 (37.3) | 68 (47.5) | 1.6 (1.1–2.5) |
| BB | 19 (6.3) | 14 (9.8) | 2.2 (1.0–4.7) |
| | | | *p*-trend = 0.01 |
| Poly-A | | | |
| LL | 171 (57.0) | 62 (43.4) | 1.0 |
| SL | 113 (37.7) | 65 (45.4) | 1.5 (1.0–2.3) |
| SS | 16 (5.3) | 16 (11.2) | 3.2 (1.5–6.9) |
| | | | *p*-trend < 0.01 |
| Total | 300 (100) | 143 (100) | |

Both BsmI and poly-A genotype frequencies were in Hardy–Weinberg equilibrium among the cohort controls, with observed genotype frequencies (Table 2) being nearly identical to the expected Hardy–Weinberg frequencies of 57%, 37%, and 6% for genotypes bb or LL, Bb or SL, and BB or SS, respectively. Cases, on the other hand, had a lower than expected frequency of bb or LL and higher than expected frequencies of Bb or SL and BB or SS genotypes. Thus 3′VDR genotype, as measured by either the BsmI or the poly-A marker, was associated with breast cancer risk (Table 2). Compared to subjects having the BsmI bb genotype, risk was increased by approximately 60% in heterozygotes (Bb) and more than two-fold in BB homozygotes. Similar results were obtained using poly-A as a marker of 3′VDR genotype.

The start codon polymorphism, FokI, was not in linkage disequilibrium with the BsmI and poly-A polymorphisms in the 3′ end of the gene. The observed joint distribution of FokI and BsmI genotypes did not differ significantly from the expected distribution under the hypothesis of no linkage disequilibrium (Table 3), indicating that the two ends of the VDR gene segregate independently, or nearly so in this population. Moreover, the FokI polymorphism was not associated with breast cancer risk (Table 4). Genotype frequencies were similar for cases and controls, and odds ratios comparing genotypes Ff and ff to FF were not significantly different from the null hypothesis value of 1.

*Table 3*. Joint distribution of 5′VDR (FokI) and 3′VDR (BsmI) genotypes among Latina controls: observed frequencies (and expected frequencies under the assumption of no linkage disequilibrium)

| | BsmI | | |
|---|---|---|---|
| | bb | Bb | BB |
| FokI | | | |
| FF | 51 (61) | 45 (40) | 11 (7) |
| Ff | 89 (81) | 50 (54) | 8 (9) |
| ff | 29 (27) | 17 (18) | 0 (3) |

Test for departure from hypothesis of no linkage disequilibrium: $p = 0.21$.

*Table 4*. Age-adjusted odds ratios and 95% confidence intervals for breast cancer by 5′VDR genotype in Latinas

| | Controls *n* (%) | Cases *n* (%) | OR (95% confidence interval) |
|---|---|---|---|
| FokI | | | |
| FF | 107 (35.7) | 53 (37.1) | 1.0 |
| Ff | 147 (49.0) | 65 (45.5) | 0.9 (0.6–1.4) |
| ff | 46 (15.3) | 25 (17.5) | 1.1 (0.6–2.9) |
| Total | 300 (100) | 143 (100) | |

Of the 143 cases, 22 (15%) were diagnosed with *in-situ* disease, 86 (60%) with stage 1 disease, and 35 (25%) with higher-stage disease. Results did not appear to differ by stage; however, power to detect heterogeneity was low. The average age was 65.5 (s.d. = 8.3) years for cases and 62.7 (s.d. = 7.9) years for controls.

## Discussion

In this cohort of Latina women, the frequency of the BsmI b allele, at 75%, is higher than frequencies typically reported for non-Latino white populations (*e.g.*, 59% among 591 control subjects in the Physician's Health Study [9]; 57% among 169 non-Latino white control subjects residing in Los Angeles County [17]). However, the BsmI b allele frequency among our Latina control subjects is similar to the frequency of 71% observed among 103 Mexican-American women in an observational study of fracture risk in Northern California [23]. Although the Northern California study was not population-based, any bias due to selection of women at increased fracture risk would be expected to produce downward bias in the frequency of the BsmI b allele, which is associated with lower fracture risk. The BsmI b allele frequency among Latinos, at 71–75%, is lower than frequencies typically reported in Asian populations (*e.g.*, 88% among 488 healthy premenopausal Japanese women [24]; 95% among 96 members of a Singapore cohort [17]). The observation of BsmI allele frequencies in Latinos that are intermediate between non-Latino whites and Asians is consistent with the known ethnic make-up (part European, part native-American) of this population.

In this cohort of Latina women, breast cancer risk was associated with polymorphic variation in the 3′ end of the VDR gene. The VDR, a transcription factor which regulates a number of genes involved in cell proliferation and differentiation (reviewed in refs 1–3), was first observed in human breast cancer cells nearly 20 years ago [25]. Since that time, numerous studies have demonstrated that vitamin D and deltanoids (vitamin D analogues) inhibit proliferation of breast cancer cells both *in vitro* [25–33] and *in vivo* [15, 30–32, 34–36]. The anti-proliferative effect is confined to those cells possessing VDRs [28, 37], and is roughly proportional to VDR number [37].

The simplest hypothesis that might explain an association between breast cancer risk and 3′VDR genotype is that two allelic variants encode receptors differing in steady-state expression or functional activity. Tests of this hypothesis, however, have yielded conflicting results, with VDR gene expression having been reported

as: higher for BsmI B compared to b alleles [8, 38]; higher for b compared to B alleles [39]; and indistinguishable for B and b alleles [40, 41]. Contributing to these contradictory results may be the use of markers, such as BsmI, which do not lie in the 3′UTR itself, to classify a relatively small number of cell lines in all of these studies. Although BsmI can be used as a marker of 3′UTR polymorphisms in most populations [17], it cannot be presumed that the B allele, for example, is in *cis* with the functional allele of interest in any single cell line. Potentially functional polymorphisms in the 3′UTR itself have not yet been tested in *in vitro* systems. Based on *in vitro* studies reported to date, the VDR 3′UTR cannot be ruled out as a functional locus contributing to breast cancer risk.

At the 5′ end of the VDR gene, the start codon polymorphism, FokI, was not associated with breast cancer risk. Thus, the association between breast cancer and the 3′VDR genotype is not due to linkage disequilibrium between the 3′ end of the gene and the start codon polymorphism. Sequences upstream of the start codon are also unlikely candidates for the functional locus, since the start codon polymorphism lies outside the region of tight linkage disequilibrium surrounding the BsmI polymorphism. The region of disequilibrium extends at least 3 kb downstream from BsmI to the poly-A microsatellite approximately 1 kb from the end of the VDR 3′UTR [17], and may extend further downstream to include other genes. Thus, we cannot rule out the possibility that BsmI and poly-A are markers for a nearby downstream gene.

The association of 3′VDR genotype with variation in traits that are clearly dependent on vitamin D status, such as calcium metabolism and bone mineral density, lends support to the hypothesis that polymorphism within the VDR gene itself is functionally significant. The BsmI BB genotype, which we found to be associated with increased risk of breast cancer, has been associated with decreased bone density in most studies [42]. It can be hypothesized that variation in the VDR gene marked by the BsmI B allele, either by affecting VDR activity or VDR number, leads to decreased *trans*-activation of VDR target genes that influence calcium metabolism or cellular growth or proliferation. Likely target genes include genes for insulin-like growth factor (IGF) binding proteins, which in addition to regulating bone formation, may mediate growth inhibitory effects of vitamin D in breast cancer cell lines [43, 44]. Although breast cancer and osteoporosis have been found to be inversely correlated at the population level [45, 46], this may be explained by the role of estrogen both in maintaining bone mineral density and in driving cellular proliferation in the breast. The relative contributions of estrogen and vitamin D status to risk of breast cancer and osteoporosis, as well as possible interactions among estrogen, vitamin D, and the IGF system, need to be further studied.

Finally, we note that the BsmI b and poly-A L alleles that were associated with protection against breast cancer were previously found to be associated with increased risk of prostate cancer [9, 12]. While the reason for this finding is not yet clear, it is not surprising that a steroid hormone such as $1,25(OH)_2D_3$ may have different effects in different tissues. The VDR can act both as an activator and as a repressor of transcription, depending on the nature of the target gene promoter and on tissue-specific VDR interacting proteins [47]. The specific target genes and regulatory factors involved in breast- and prostate-specific VDR responses have not been identified; nevertheless, even in the absence of mechanistic explanations, the finding of an association between VDR genotype and breast cancer risk supports the hypothesis that vitamin D may influence breast cancer etiology. However, because the sequence variants defining the functional 3′VDR genotype have not yet been identified, it is especially important that this epidemiologic finding is replicated in other populations and in other ethnic groups. If confirmed, these findings suggest that vitamin D and/or vitamin D analogues may be useful for breast cancer prevention and/or treatment, and that assessment of VDR polymorphisms might someday be useful to identify individuals most at risk and/or most responsive to intervention.

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