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Vitamin D receptor gene polymorphisms in breast cancer

Nur Buyru[1], Ayda Tezol[1]
Elif Yosunkaya-Fenerci[1] and Nejat Dalay[2,3]

[1]Cerrahpasa Medical Faculty
Department of Medical Biology
[2]Oncology Institute, Istanbul University
Istanbul, Turkey
[3]Corresponding author: Tel, 90-212-5313100;
Fax, 90-212-5348078; E-mail, ndalay@yahoo.com

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Abbreviation: VDR, vitamin D receptor

Abstract

Breast cancer is the leading cause of cancer death among women around the world and its incidence is annually increasing. The vitamin D receptor (VDR) gene is a member of the nuclear receptor superfamily, which is expressed in breast tissue and known to modulate the rate of cell proliferation. Association between the VDR gene polymorphisms and cancer development has been suggested by several studies. However, the relationship between VDR polymorphisms and breast cancer is controversial and has not been confirmed by all studies. The purpose of this study was to investigate the genotype frequencies and association of the VDR *Bsm I* and *Taq I* polymorphisms with breast cancer in Turkish patients. In this study, 78 patients with breast cancer and 27 healthy individuals were enrolled. The prevalence of the VDR *Taq I* and *Bsm I* alleles and the genotype frequencies in patients with breast cancer was similar to that in the normal population. Our data indicate that no significant differences exist between the patients and control subjects.

Keywords: breast cancer; polymorphism; VDR

Introduction

Breast cancer is a heterogeneous disease regarding its morphology, invasive behavior, metastatic capacity, hormone receptor expression and clinical outcome. 10-15% of breast cancer cases have some family history of the disease but only 5% can be explained by rare highly penetrant mutations in genes such as BRCA1 and BRCA2. Although some of the familial risk may be due to the shared environment, there may be other common, low-penetrance genetic variants which alter the predisposition to breast cancer. Endogenous hormone exposure is known to affect breast cancer susceptibility and genes responsive to such hormones are plausible candidates for predisposition genes (Pharoah *et al*., 1997).

The steroid hormone 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) binds the vitamin D receptor (VDR) and the ligand/receptor complex regulates transcription of the genes involved in cell cycle, apoptosis and differentiation (Simboli-Campbell *et al*., 1996). In the normal mammary gland, $1,25(OH)_2D_3$ may function to regulate calcium transport during lactation or may act in concert with other hormones to maintain mammary cell differentiation and milk protein production (Bhattacharjee *et al*., 1987; Mezzetti *et al*., 1988). Dysregulation of VDR-mediated gene expression would alter mammary gland development or function and possibly predispose cells to transformation (Buras *et al*., 1994).

The VDR gene is located on the long arm of chromosome 12 (12q12-14) and is composed of 10 exons, the first of which is not transcribed (Tokita *et al*., 1996). A series of common polymorphisms in the vitamin D receptor gene were recently reported be associated with both circulating levels of active vitamin D and *in vitro* measures of gene expression (Morrison *et al*., 1992). Three of these polymorphisms can be distinguished by digestion with restriction enzymes (Hustmyer *et al.,* 1993). The presence or absence of a restriction site defines the specific allele. None of these polymorphisms change the translated protein. The *Bsm I* and *Apa I* polymorphisms are located in intron 8 of the VDR gene (Baker *et al*., 1988; Morrison *et al*., 1994). The *Taq I* polymorphism is located in exon 9 but leads to a silent codon change, with ATT and ATC both coding for isoleucine (Farrow, 1994).

Allelic variations of the VDR gene have been associated with the risk of osteoporosis in postmenopausal women (Jorgensen *et al*., 1996) and prostate cancer in men (Ingles *et al*., 1997). Presence of the tt genotype has been found to be less frequent in prostate cancer patients. Therefore, it has been suggested that the t allele might protect against prostate cancer (Taylor *et al*., 1996). In breast cancer, low vitamin D levels in serum are correlated with disease progression and bone metastases, a situation also

noted in prostate tumors and suggesting involvement of the VDR gene in breast carcinogenesis (Mawer *et al.*, 1997). However, the relationship between VDR polymorphisms and breast cancer is controversial (Ingles *et al.*, 2000; Hou *et al.,* 2002).

The purpose of this study was to investigate the association of the VDR gene *Taq I* and *Bsm I* polymorphisms with breast cancer.

Materials and Methods

Blood samples were obtained from 78 breast cancer patients and 27 healthy subjects. DNA for molecular analysis was isolated using standard procedures.

PCR amplification followed by restriction enzyme digestion was used to investigate the two (*Taq I* and *Bsm I*) polymorphisms at the VDR locus using 10 pmol each of the forward and reverse primers and 100 ng of genomic DNA. To analyze the intron 8 *Bsm I* polymorphism, we used the primer pair 5'CAACAAGACTACAAGTACCGCGTCAGTGA3', 5'AACCAGCGGGAAGAGGTCAAGGG3' and 30 amplification cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C 30 s. Final extension was allowed to proceed for 5 min at 72°C. In order to investigate the *Taq I* polymorphism, the primer pair used was 5'CAGAGCATGGACAGGGAGCAA3', 5'GCAACTCCTCATGGCTGAGGTTCT3' with 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, with a final extension for 5 min at 72°C. Amplification of the intron 8 and exon 9 polymorphic regions yielded products of 825 bp and 740 bp, respectively.

10 µl of amplification product was then digested with 10 U *Taq I* (MBI, Fermentas, Lithuania) or 2.5 U of *Bsm I* (Promega) for at least 3 h at 65°C and 37°C, respectively. The digestion products were subjected to electrophoresis on 2% agarose gels for 1 h at 100 V. The gels were evaluated in the video gel documentation system (Vilber-Lourmat, Cedex, France) using the BIOPROFIL 1D software and printed using the SONY UP 890 video graphic printer.

The χ^2 test was used to evaluate the variations of the genotype frequencies among the cases and the controls. The Odds ratios and the confidence intervals were calculated as an estimate of the relative risk. SPSS 7.5 professional statistics analysis (SPSS, Chicago) was used for the statistical calculations.

Results

The *Bsm I* and *Taq I* polymorphisms in the VDR gene were investigated by PCR-RFLP analysis in breast cancer patients. We also genotyped 27 healthy individuals as the control group. The frequencies of the alleles and the genotypes were in Hardy-Weinberg equilibrium among the patients and the controls.

The size of the PCR product for the *Bsm I* polymorphism was 825 bp. Following digestion two restriction fragments of 650 bp and 175 bp were observed for bb homozygotes. A single 825 bp band was obtained for BB homozygotes. Heterozygote individuals displayed all three bands (Figure 1).

57.7% of the patients were heterozygous for the *Bsm I* polymorphism, 19.3% were homozygous BB and 23% were homozygous bb. The respective frequencies in the control group were 63%, 18.5% and 18.5%. These frequencies are not significantly different from those in the breast cancer patients (χ^2= 0.28997, P = 0.86, df = 2). The frequencies of the B and b alleles were equal in both groups. The allelic and genotypic frequencies in the patients and controls for the *Bsm I* polymorphism are shown in Table 1. The size of the reaction product for the *Taq I* polymorphism was 740 bp, which on digestion with

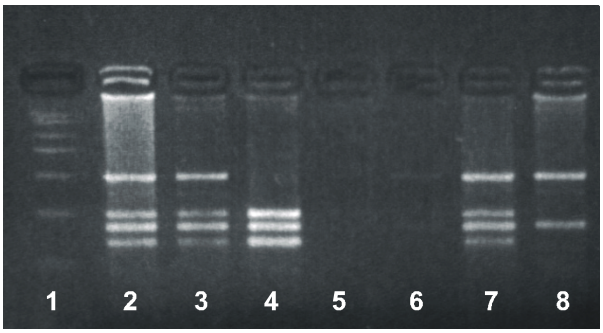

Figure 1. Ethidium bromide-stained 2% agarose gel showing PCR products of the VDR gene after digestion with *Taq I*. Lanes 2, 3 and 7; Tt, lane 4; tt and lanes 6 and 8; TT genotypes. Lane 1; molecular weight marker, lane 5; no template control.

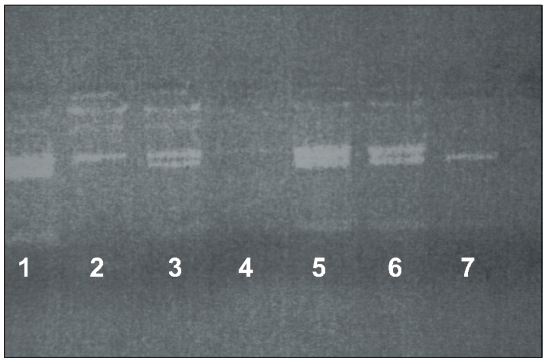

Figure 2. Detection of *Bsm I* polymorphism by PCR-RFLP. The upper bands represent allele B, the lower bands allele b. Lanes 1, 3, 5 and 6 Bb heterozygotes; lanes 2 and 4 BB homozygotes; and lane 7 bb homozygote.

Table 1. Genotypic and allelic frequencies of the *Taq I* polymorphism.

VDR *TaqI* genotypes	Breast cancer $n = 78$ (%)	Controls $n = 27$ (%)	OR (95% CI)
TT	26 (33.3%)	4 (14.8%)	1.04 (0.267-4.489) $P \geq 0.05$
Tt	44 (56.4%)	20 (74.0%)	1.486 (0.796-2.771) $P \geq 0.05$
tt	8 (10.3%)	3 (11.1%)	1 (reference)
Alleles			
T	96 (61.5%)	28 (52.0%)	
t	60 (38.4%)	26 (48.0%)	

Table 2. Genotypic and allelic frequencies of the *Bsm I* polymorphism.

VDR *BsmI* genotypes	Breast cancer $n = 78$ (%)	Controls $n = 27$ (%)	OR (95% CI)
BB	15 (19.2%)	5 (18.5%)	0.758 (0.250-2.294) $P \geq 0.05$
Bb	45 (57.7%)	17 (62.9%)	0.926 (0.498-1.722) $P \geq 0.05$
bb	18 (23.0%)	5 (18.5%)	1 (reference)
Alleles			
B	75 (48.1%)	27 (50.0%)	
b	81 (51.9%)	27 (50.0%)	

Taq I yielded three distinct patterns. Restriction of neither allele (TT) results in two fragments of 495 bp and 245 bp. Restriction of both alleles results in three fragments of 290 bp, 245 bp and 205 bp. Restriction of one allele only yields all four fragments of 495 bp, 290 bp, 245 bp and 205 bp. The 245 bp fragment is present among all genotypes, and is created by a nonpolymorphic *Taq I* site within the amplification product (Figure 2).

Our study revealed no significant differences in the prevalence of the alleles and the genotypes in the patients and the control group. In our study group, the genotype frequencies were 33.3% vs. 14.8% TT, 56.4% vs. 74.0% Tt and 10.3% vs. 11.2% tt in the breast cancer patients and the controls, respectively. We observed the t allele in 38.5% and 48%, and the T allele in 61.5% and 52.0% in the patients and the control group, respectively. ($\chi^2 = 3.37$, $P = 0.18$, $df = 2$). The frequencies of *Taq I* genotypes and alleles were not significantly different in the patients and the controls ($\chi^2 = 1.56$, $P = 0.2$, $df = 2$) (Table 2).

Our data indicate that, polymorphisms in the VDR gene do not affect breast cancer susceptibility.

Discussion

There are substantial numbers of reports that have investigated candidate genes for breast cancer susceptibility. Breast cancer risk is strongly related to endogenous hormone exposure and genes responsive to such hormones are therefore plausible candidates for being susceptibility genes. The VDR is a member of the steroid hormone receptor superfamily and regulates gene transcription through interaction with hormone response elements in the promoter region of target genes (Christakos *et al.*, 1996). The presence of VDR in breast cancer has been documented both in cell lines (Demirpence *et al.*, 1994; James *et al.*, 1994; Vink-van Wijngaarden *et al.*, 1994; Love-Schimenti *et al.*, 1996) and in tumor samples (Swedish Breast Cancer Cooperative Group, 1996). Both the 5' and 3'ends of the VDR gene are polymorphic. A polymorphism in the first of the two possible translation start codons produces variants differing in size and activity (Miyamoto *et al.*, 1997). Allelic variation in the 3' end of the VDR gene, although less clearly related to its function, appears to have phenotypic consequences for the calcium (Dawson-Hughes *et al.*, 1995; Wishart *et al.*, 1997) and vitamin D metabolism

(Morrison *et al*., 1994; Ma *et al*., 1998), bone mineral density (Morrison *et al*., 1998) and osteoporosis (Ingles *et al*., 1997a), while the 5' polymorphisms are involved in peak bone density (Harris *et al*., 1997).

The *TaqI* polymorphisms have been investigated most frequently in prostate cancer and presence of the T alleles has been associated with increased prostate cancer risk. However, the issue is still controversial since there are also reports that do not confirm such an association. Studies investigating VDR polymorphisms in breast cancer are rare and inconsistent. Two studies (Ruggiero *et al*., 1998; Ingles *et al*., 2000) have revealed a 4-fold higher risk of metastatic spread in breast cancer patients carrying the *BsmI* restriction site in the VDR gene. Bretherton-Watt *et al*. (2001) also reported an association between the bb genotype and grade II and III tumors. On the other hand, an association between the bb allele and breast cancer has been refuted in other reports (Mocherla *et al.,* 1997; Gross *et al.,* 1998; Hou *et al.,* 2002). In our study, no difference in the frequency of the b allele was observed in the breast cancer group. Statistical analysis did not reveal any significant difference in the prevalance of the *BsmI* polymorphism in breast cancer patients when compared with controls. Genotype frequencies were also similar in women with breast cancer and in the control population. Our data are consistent with reports on breast (Schndorf *et al*., 2003) and prostate cancer (Ma *et al*., 1998; Suzuki *et al*., 2003).

It has been reported that individuals homozygous for the t allele are significantly underrepresented among prostate cancer patients, illustrating a protective role for the t allele in prostate carcinogenesis (Taylor *et al*., 1996; Hamasaki *et al.,* 1999). However, these findings have not been corroborated in later studies (Blazer *et al.,* 2000; Habuchi *et al.,* 2000). Regarding breast cancer, both a tendency towards a decreased mortality rate in tt homozygote breast cancer patients (Lundin *et al*., 1999) as well as decreased risk in patients with the TT genotype (Schöndorf *et al*., 2003) have been reported. In our study, investigation of the *TaqI* site between affected and control individuals, however, did not reveal a difference between the frequencies of the genotypes. Lack of association between the *TaqI* alleles and breast cancer is in agreement with earlier (Dunning *et al.,* 1999; Lundin *et al.,* 1999) and recent reports (Hou *et al.,* 2002; Newcomb *et al*., 2002; Schndorf *et al.,* 2003). The genotype frequencies in our breast cancer group are in concordance with the reports in the literature. However, the frequency of tt homozygotes in our control group is lower than reported by Schöndorf *et al*., 2003. This difference may be due to the ethnic origin of populations.

The frequencies of T and t alleles were 61.5% and 38.5% (P = 0.058) in our patient group, while Curran *et al*. have reported allele frequencies of 64.0% and 36.0% (P = 0.053) and concluded that this indicated a significant difference between the patients and the control groups. However, in our opinion statistical significance at the 0.05 level provides no evidence that this polymorphism is associated with incidence of breast cancer.

In conclusion, we have not found any evidence that differences in the oncogenic properties of the VDR gene *TaqI* and *BsmI* alleles could confer a genetic predisposition to breast carcinogenesis. Comparing the patient group with the controls revealed no significantly increased risk for breast cancer patients carrying the TT or bb genotypes.

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