JBUON 2014; 19(3): 633-642
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Association of FokI and PvuII polymorphisms with breast cancer staging and survival among Caucasian women: A prospective study

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Summary

Purpose: *Both vitamin D and estrogens play an important role in breast cell growth and differentiation. Therefore, we hypothesized that FokI polymorphism in the Vitamin D Receptor (VDR) gene, as well as PvuII polymorphism in the Estrogen Receptor (ESR) gene might be associated with progression of breast cancer. The aim of this study was to prospectively examine the association of these polymorphisms with histopathological features and prognosis among women with histologically proven breast cancer.*

Methods: *Patient characteristics, tumor histopathology, and genotyping of one VDR polymorphism variant (FokI) and one ER polymorphism variant (PvuII) were recorded. Patients were also routinely followed up.*

Results: *There was a significant difference regarding nodal stage (p<0.001) between the different genotypes of FokI polymorphisms (FF, Ff, ff), even though a trend was also detected in the frequency between ductal and lobular type, as well as tumor size (p=0.07). When further analysis was performed regarding patients whose polymorphism included the f allele, we found statistically significant differences in tumor size (p<0.001), nodal stage (p=0.03), tumor grade (p=0.04) and lymphovascular invasion (p<0.001), while no differences in nodal status, distant metastases and tumor stage were noticed. No significant associations were found between any of the PvuII polymorphism variants and tumor histopathology and stage. No statistical significance was proven between FokI polymorphism's variants or f allele and overall or progression-free survival. Statistically significant associations between overall and progression-free survival and PvuII polymorphism's variants was demonstrated (p<0.001).*

Conclusion: *The f allele was associated with the presence of lymphovascular invasion and poorly differentiated tumors, whereas the PP genotype was associated with increased overall and progression-free survival, suggesting that this variant is related to a more favorable prognosis.*

Key words: *apoptosis, breast cancer, FokI, polymorphism, PvuII*

Introduction

The onset and progression of breast cancer is multifactorial and still not fully defined. Apoptosis plays a pivotal role in the biological process of breast cancer development [1]. Apoptotic breast cancer cells that are detected in systemic circulation are a result of either an automatic mechanism or the combination of cytotoxic treatments, surgical therapy or even the detachment of epithelial cancer cells from the extracellular space in which they were attached [1,2]. The prognostic

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Received: 02/03/2014; Accepted: 14/03/2014

significance of detecting proapoptotic markers in peripheral blood regarding breast cancer progression remains unclear and there is sparse information in the current literature. Vitamin D is a known proapoptotic factor [3], whereas estrogens are regarded as an important antiapoptotic component [4].

Vitamin D endocrine system maintains calcium homeostasis and is involved in numerous biological processes, such as bone metabolism, modulation of immune response, and regulation of cell differentiation and proliferation [5].

In vivo and in vitro experimental studies have shown that vitamin D inhibits cellular proliferation, induces apoptosis and suppresses tumor growth [6,7]. The active form of vitamin D, 1, 25(OH)2D3 exerts its activity through the intracellular vitamin D receptor (VDR) [8,9]. VDR belongs to the steroid/thyroid hormone nuclear receptor family, and is expressed in different tissues, including normal and cancerous mammary epithelial cells [10,11]. Interestingly, several studies have demonstrated a decrease in VDR expression in breast cancer cells compared to normal breast cells [11].

VDR gene harbours several known polymorphisms [12], which have been investigated for their functional significance and potential effects on susceptibility to breast cancer [13,14]. The FokI polymorphism of VDR gene has been extensively investigated in breast cancer risk assessment studies and the conclusions have been inconsistent [14-23]. However, more recently, two reports with meta-analyses of multiple studies with large sample sizes provide evidence for a positive association between the FokI ff genotype and an augmented predisposition to the disease [19,20].

Estrogens play a crucial role in the pathogenesis and progression of breast cancer. The biological effects of estrogens are mediated primarily through high affinity binding to estrogen receptors (ERs) [24]. ERs are overexpressed in human breast cancer and associated with differentiated tumors and with a more favorable prognosis [25]. PvuII and XbaI polymorphisms of the ER-a gene were found to be correlated to breast cancer incidence at a younger age [26-29]. The association of ER polymorphisms with breast cancer risk indicates that these polymorphisms may also affect the survival of breast cancer patients.

Because of the important role of both vitamin D and estrogens in breast cell growth and differentiation, the genetic heterogeneity of their receptors might influence the sensitivity of circulating tumor cells in the continuous and dynamic apoptotic process inducing a possible non metastatic potential to these cells. Therefore, we hypothesized that FokI polymorphism in the VDR gene, as well as PvuII polymorphism in ESR gene might be associated with progression of breast cancer.

The aim of this study was to prospectively examine the association of these polymorphisms with histopathological features and prognosis among women with histologically proven breast cancer.

Methods

Ethics Committee

This study approval was approved by the Aretaieion University Hospital's Ethics Committee. Written informed consent was obtained from all participants.

Study population

This was a prospective hospital-based study. Subjects were selected from the Breast Cancer Unit in the Division of General Surgery at Aretaieion University Hospital.

A total of 87 women who had been planned for treatment for a histologically proven invasive or in situ breast carcinoma or an excisional biopsy for a suspicious lesion were included in our study. Exclusion criteria were the presence of specific (inflammatory breast cancer) or nonspecific septic or non septic inflammatory disease, neutropenia, hematologic malignancy, and history of previous autoimmune disease or malignancy. Patients were recruited between January 2008 and December 2010.

Patient characteristics

Demographic details, clinical workup (liver function tests, alkaline phosphatase, diagnostic bilateral mammogram, breast ultrasound, pathology review, staging imaging, tumor markers (CA125, CA15.3) and the presence of distant metastasis were recorded.

Tumor characteristics

Tumor size, histologic grade, histologic subtype, lymphovascular invasion, and axillary lymph node status were also recorded.

Blood sampling

A total of 25ml of peripheral venous blood was obtained between 08.30 and 11.30 am after 12 hrs fasting prior to any treatment (surgical or medical) and equally distributed in one pre-frozen tube without anticoagulant, three pre-frozen tubes with EDTA as anticoagulant and one pre-frozen tube with EDTA and Trizol as

mRNA stabilizer.

Samples were used in order to detect the genetic heterogeneity in the VDR and ER genes.

DNA extraction

Genomic DNA was extracted from peripheral blood mononuclear cells using the commercial Qiagen DNA extraction kit according to the manufacturer's instructions.

Samples were first lysed in the supplied lysis buffer containing proteinase K, incubated for a suitable period and then the lysates were loaded onto the supplied genomic column. DNA was bound to the column while other cell constituents passed through. Following removal of the remaining contaminants by two wash steps, the purified high-molecular-weight DNA was eluted and precipi¬tated with isopropanol and dissolved in 50 μl distilled water. The quality of the isolated DNA was examined in 1% agarose gel stained with ethidium bromide. The DNA concentration was determined by a spectrophotometer, 2-10 ng of genomic DNA was used for each polymerase chain reaction (PCR).

Polymorphisms' detection

We examined the following polymorphisms: VDR - FokI (reference single-nucleotide polymorphic site- SNP 2228570), and ESR - PvuII (reference SNP 2234693).

VDR and ESR genotyping for all single nucleotide polymorphisms (SNPs) was performed using the Pyrosequencing technology (sequencing by synthesis). We used specially designed primers' sets, with one primer out of each set containing biotinylated edge. VDR-FokI polymorphic site was amplified using following primers: (Forward) 5'- GTGGCCTGCTTGCTGTTC - 3', (Reverse) 5'- ACACACCCCACAGATCCG - 3', and 5'- TTGCTGTTCTTACAGGG- 3' (Sequencinq). Primers used for analysis of ER-α genotypes were: 5'- TTCATCTGAGTTCCAAATGTCC -3' (forward), 5'- ACCATTAGAGACCAATGCTCATC -3' (reverse), and, 5'- . CTGAGTTCCAAATGTCC –3' (sequencing). PCR conditions were as follows: denaturation at 950 C for 5 min, followed by 30 cycles of PCR at 950C (30 sec), 580 C (30 sec), 72o C (30 sec), and 72o C (4 min). Annealing temperature for FokI and PvuII PCR was 580C (30 sec). Following PCR, aliquots of the amplified PCR products were digested with FokI and PvuII restriction endonucleases in accordance with the manufacturer's specifications (Qiagen Inc.). Sample analysis and results were processed by the PyroMark Q24 logismic. PCR products of FokI 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. Regarding the PvuII, P signified by the absence of restriction sites, yielded a 1.3-kb fragment, whereas p signified by the presence of PvuII restriction sites on the two alleles, was digested into two frag¬ments (0.85 and 0.45 kb).

Table 1. Patient and breast cancer characteristics

Characteristics	*Patients N (%)*
Tumor type	
Ductal	45 (61.6)
Lobular	22 (30.12)
Papillary	4 (5.5)
Mucinous	2 (2.7)
Tumor size (T)	
Tis	4 (5.6)
T1	22 (30.1)
T2	39 (53.4)
T3	4 (5.5)
T4	4 (5.5)
Nodal stage (N)	
N0	24 (32.9)
N1	23 (31.5)
N2	20 (27.4)
N3	6 (8.2)
Nodal status (N)	
N (positive)	49 (67.1)
N (negative)	24 (32.9)
Distant metastasis (M)	
M0	71 (97.3)
M1	2 (2.7)
TNM	
I	19 (26.0)
II	26 (35.6)
III	26 (35.6)
IV	2 (2.7)
Lymphovascular invasion	
Present	48 (65.8)
Absent	25 (34.2)

Follow- up/ Surveillance

We routinely used the same protocol for post-treatment surveillance. History and physical examinations were done every 6 months for 5 years, mammography was performed every 12 months, liver function tests and alkaline phosphatase levels were evaluated on a yearly basis. Patients were followed up until December 2013. Overall survival, progression-free survival (time until evidence of growth or worsening of disease at previously known sites of disease and/or the occurrence of new sites of metastatic disease) were estimated.

Statistics

Results were expressed as mean±SD. Comparison between groups was performed using the Chi-Square test for qualitative data and the unpaired t-test for quantitative data. Kaplan–Meier overall and progression-free survival curves were generated for each group and compared with log-rank test for proportional hazards test. A p-value less than 0.05 was considered as significant.

Results

A total of 87 patients were recruited for this study. Table 1 shows the characteristics of pa-

Table 2. Association of FokI polymorphism and tumor pathology

Tumor pathology	*FokI (N=71) N (%)*	*FF (N=11) N (%)*	*Ff (N=58) N (%)*	*ff (N=2) N (%)*	*p-value*
Tumor type					
Ductal	45 (67.2)	9 (81.8)	36 (66.7)	0 (0)	0.075
Lobular	22 (32.8)	2 (18.2)	18 (33.3)	2 (100)	
Tumor size (T)					
Tis	2 (2.8)	2 (28.6)	0 (0)	0 (0)	
T1	22 (31.0)	3 (28.6)	19 (32.8)	0 (0)	0.077
T2	39 (54.9)	6 (24.9)	31 (53.4)	2 (100)	
T3	4 (5.6)	0 (0)	4 (6.9)	0 (0)	
T4	4 (5.6)	0 (0)	4 (6.9)	0 (0)	
Nodal stage (N)					
N0	22 (31.0)	5 (45.5)	17 (29.3)	0 (0)	
N1	23 (32.4)	0 (0)	23 (39.7)	0 (0)	<0.001
N2	20 (28.3)	6 (54.5)	14 (24.1)	0 (0)	
N3	6 (8.5)	0 (0)	4 (6.9)	2 (100)	
Nodal status (N)					
N (positive)	49 (69.0)	6 (54.5)	41 (70.7)	2 (100)	0.359
N (negative)	22 (31.0)	5 (45.5)	17 (28.3)	0 (0)	
Distant metastasis (M)					
M0	69 (97.2)	11 (100)	56 (96.6)	2 (100)	0.794
M1	2 (2.8)	0 (0)	2 (3.4)	0 (0)	
Tumor grade					
G1	1 (1.4)	1 (9.1)	0 (0)	0 (0)	
G2	21 (29.6)	2 (18.2)	19 (32.8)	0 (0)	0.133
G3	49 (69.0)	8 (72.7)	39 (67.2)	2 (100)	
TNM					
I	17 (23.9)	3 (27.3)	14 (24.1)	0 (0)	
II	26 (36.6)	2 (18.2)	24 (41.4)	0 (0)	0.345
III	26 (36.6)	6 (54.5)	18 (31.0)	2 (100)	
IV	2 (2.8)	0 (0)	2 (3.4)	0 (0)	
Lymphovascular invasion					
Present	48 (67.6)	6 (54.5)	40 (69.0)	2 (100)	0.394
Absent	23 (32.4)	5 (45.5)	18 (31.0)	0 (0)	

tients (histopathological features) included in our study. The median patient age was 59.7 years (range 32-81). A histologically proven malignancy was detected in 73 (84%) patients, whereas 14 (16%) patients were found to have a benign breast lesion. The majority of the patients had infiltrating ductal carcinoma (61.6%). Among the cases, approximately 62% had stage I/II, and 38% had stage III/ IV disease.

FokI polymorphism of the VDR gene and PvuII polymorphism of the ER gene were genotyped in 71 patients, since we weren't able to extract DNA during the laboratory testing. The associations between FokI polymorphism (FF,Ff,ff) and breast tumor histopathology and stage are summarized in Table 2, whereas Table 3 shows the associations when comparing patients bearing the f allele (Ff and ff genotype) in their polymorphism with tumor characteristics and disease stage. There was a significant difference regarding nodal stage (p<0.001) between FokI polymorphisms, even though a trend was also detected in the frequency between ductal and lobular type, as well as the tumor size. No significant differences were found between FokI polymorphism's variants and presence of distant metastases, tumor stage, tumor grade and the presence of lymphovascular invasion.

When further analysis was performed regarding patients whose polymorphism included the f allele, statistically significant differences were noticed in tumor size, nodal stage, tumor grade and lymphovascular invasion, while no differences in nodal status, distant metastases and tumor stage were registered.

Associations between PvuII polymorphism and histopathological features of the tumor and disease stage were examined. No significant associations were found between any of the PvuII polymorphism variants and tumor histopathology

Table 3. Association of FF and f (Ff+ff) alleles and tumor pathology

Tumor pathology	*FF (N=11) N (%)*	*f allele(Ff+ff) (N=60) N (%)*	*p-value*
Tumor size (T)			
Tis	2 (18.2)	0 (0)	
T1	3 (27.3)	19 (31.7)	<0.001
T2	6 (54.5)	33 (55.0)	
T3	0 (0)	4 (6.7)	
T4	0 (0)	4 (6.7)	
Nodal stage (N)			
N0	5 (45.5)	17 (28.3)	
N1	0 (0)	23 (38.3)	0.032
N2	6 (54.5)	14 (23.3)	
N3	0 (0)	6 (10.0)	
Nodal status (N)			
N (positive)	6 (54.5)	43 (71.7)	0.066
N (negative)	5 (45.5)	17 (28.3)	
Distant metastasis (M)			
M0	11 (100)	58 (96.7)	0.800
M1	0(0)	2 (3.3)	
Tumor grade			
G1	1 (9.1)	0 (0)	
G2	2 (18.2)	19 (31.7)	0.049
G3	8 (72.7)	41 (68.3)	
TNM			
I	3(27.3)	14 (23.3)	
II	2 (18.2)	24 (40.0)	0.192
III	6 (54.5)	20 (33.3)	
IV	0 (0)	2 (3.3)	
Lymphovascular invasion			
Present	6 (54.5)	42 (70.0)	<0.001
Absent	5 (45.5)	18 (30.0)	

and stage (Table 4).

Patient overall and progression-free survival in association with FokI and PvuII polymorphisms are shown in Tables 5 and 6, respectively. No statistical significance was depicted between FokI polymorphism's variants and overall or progression-free survival (Figure 1). There was no difference when comparing separately patients with the f allele (Figure 2). Statistically significant associations between overall and progression-free survival and PvuII polymorphism's variants was demonstrated ($p<0.001$ for overall and progression-free survival, respectively; Figure 3).

Discussion

Multiple factors have been associated with increased risk of developing breast cancer, such as genetic predisposition, environmental factors, and family history. Ever since apoptosis was first described by Kerr et al, it has been linked to the elimination of potentially malignant cells, hyperplasia and tumor progression [30]. One interesting observation is that circulating tumor cells are detected in many breast cancer patients with favorable outcome. This phenomenon suggests that some of the detected cells may lack metastatic potential. Apoptotic cells, occurring spontaneously or induced by cytotoxic therapy, surgery, or due to the loss of cell-matrix adherence in epithelial cells might be an possible interpretation to these findings [1,2].

Several studies have shown an important effect of VitaminD in the modulation of proliferation and differentiation of numerous malignant cell types [5,6,31]. At the cellular level, VDR induces cell-cycle arrest, differentiation and apoptosis in a variety of normal and transformed cell lines, including osteoblasts, epidermal keratinocytes and mammary epithelial cells [32]. Interestingly, several studies have demonstrated a decrease in VDR expression in breast cancer cells compared to normal breast cells [11]. This phenomenon could be attributed to VDR gene allelic variants (polymorphisms) [13], and/or DNA methylation [33-35].

One of the most studied VDR polymorphisms is the start codon polymorphism (FokI) in exon 2

Table 4. Association of PvuII polymorphism and tumor pathology

Tumor pathology	*PvuII (N=71) N (%)*	*PP (N=58) N (%)*	*Pp (N=13) N (%)*	*pp (N=0) N (%)*	*p-value*
Tumor type					
Ductal	45 (67.2)	36 (66.7)	9 (69.2)	0 (0)	0.860
Lobular	22 (32.8)	18 (33.3)	4 (30.8)	0 (0)	
Tumor size (T)					
Tis	2 (2.8)	2 (3.4)	0 (0)	0 (0)	
T1	22 (31.0)	19 (32.8)	3 (23.1)	0 (0)	0.345
T2	49 (54.9)	31 (53.4)	8 (61.5)	0 (0)	
T3	4 (5.6)	2 (3.4)	2 (15.4)	0 (0)	
T4	4 (5.6)	4 (6.9)	0 (0)	0 (0)	
Nodal stage (N)					
N0	22 (31.0)	19 (32.8)	3 (23.1)	0 (0)	
N1	23 (32.4)	17 (29.3)	6 (46.2)	0 (0)	0.379
N2	20 (28.2)	18 (31.0)	2 (15.4)	0 (0)	
N3	6 (8.5)	4 (6.9)	2 (15.4)	0 (0)	
Distant metastasis (M)					
M0	69 (97.2)	56 (6.6)	13 (100)	0 (0)	0.497
M1	2 (2.8)	2 (3.4)	0 (0)	0 (0)	
Tumor grade					
G1	1 (1.4)	1 (1.7)	0 (0)	0 (0)	
G2	21 (29.6)	16 (27.6)	5 (38.5)	0 (0)	0.678
G3	49 (69.0)	41(70.7)	8 (61.5)	0 (0)	
TNM					
I	17 (23.9)	14(24.1)	3 (23.1)	0 (0)	
II	26 (36.6)	22(37.9)	4 (30.8)	0 (0)	0.802
III	26 (36.6)	20 (34.5)	6 (46.2)	0 (0)	
IV	2 (2.8)	2 (3.4)	0 (0)	0 (0)	
Lymphovascular invasion					
Present	48 (67.6)	38 (65.5)	10 (76.9)	0 (0)	0.427
Absent	23 (32.4)	20 (34.5)	3 (23.1)	0 (0)	

Table 5. Distribution of overall and progression-free survival in relation to FokI polymorphism

FokI polymorphisms	*Overall survival, months (mean±SD)*	*p-value*	*Progression-free survival, months (mean±SD)*	*p-value*
FokI	57±2.2		55.7.8±2.6	
FF	60.8±3		60.7±3.3	
Ff	55.9.1±2.7		55.2±3.2	
ff	56.5±3.5	0.205	35.5±2.5	0.083
f allele	55.8±2.6	0.509	54.4±3.1	0.509

Table 6. Distribution of overall and progression-free survival in relation to PvuII polymorphism

	PvuII	*PP*	*Pp*	*p-value*
Overall survival, months (mean±SD)	57±2.2	59.7±2.3	42.5±5.4	<0.001
Progression-free survival, months (mean±SD)	55.7±2.6	60.3±2.4	29±6.1	<0.001

[15]. The FokI site dictates which of two potential translation initiation sites will be used. Individuals that lack the FokI restriction site (f allele) start translation at the first site and express the full-length VDR, which consists of 427 amino acids. In contrast, those with the FokI restriction site (F allele) use a second ATG site and produce a VDR protein of 424 amino acids [13]. It is very important to note that FokI polymorphism has been found to be functional [36] and that the 424 amino acid VDR variant is somewhat more active than the 427 amino acid variant in terms of its transactivation capacity as a transcription factor [37].

Previous investigations of VDR FokI polymorphism and breast cancer risk have produced inconsistent results. Most of the studies, which are case control studies with a great number of cases, reported no association between VDR FokI polymorphism and breast cancer risk [16,21,22], even

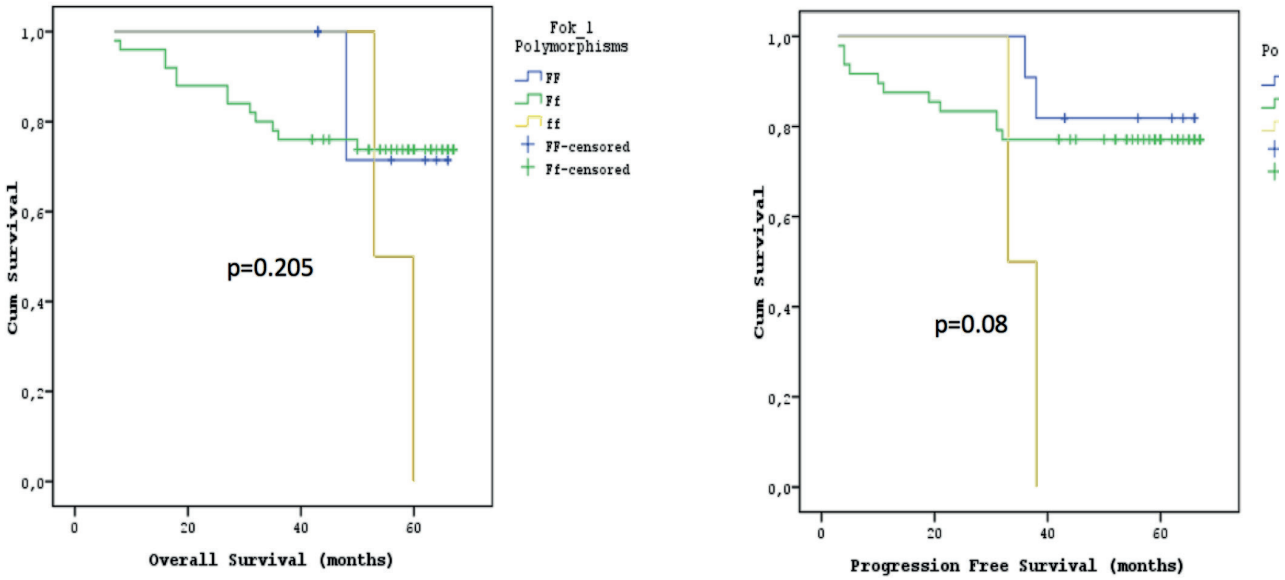

Figure 1. Kaplan-Meier curves depicting overall and progression-free survival regarding FokI polymorphism.

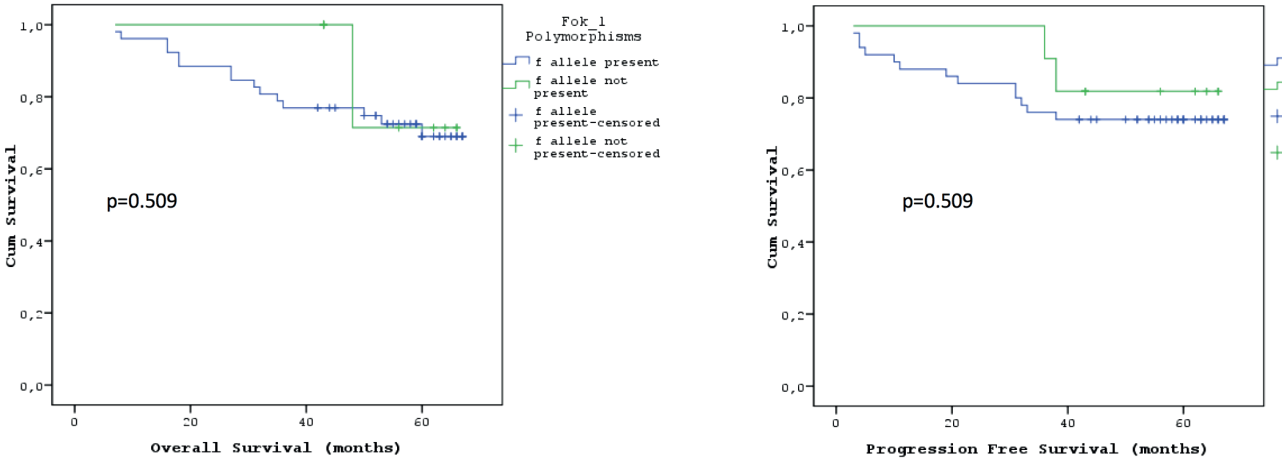

Figure 2. Kaplan-Meier curves depicting overall and progression- freee survival regarding FF genotype and f (Ff+ff) allele

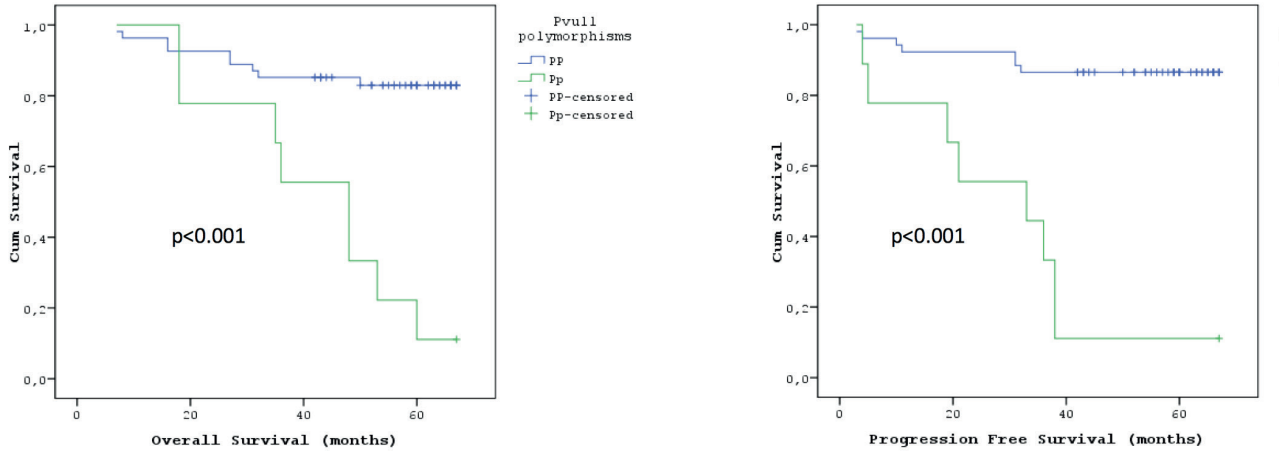

Figure 3. Kaplan-Meier curves depicting overall and progression- free survival regarding PvuII polymorphism

in the group of patients with male breast cancer [38]. Interestingly, a significantly increased risk of breast cancer was observed in one large study conducted by Chen et al. (1234 cases, 1676 controls) among carriers of the ff genotype of FokI (multivariate OR=1.34) compared with those with the FF genotype [17]. Moreover, two recent meta-analyses of multiple studies with large sample sizes provide evidence for a positive association between the FokI ff genotype and a predisposition to the disease [5,6]. These findings were further enhanced by a study by Mishra et al. who identified a positive association between the VDR-FokI ff genotype and/or f allele and breast cancer among African-American women [23].

With regard to relevance of various VDR FokI polymorphism and breast tumor histopathology, tumor stage, and receptor status there is little information available in order to reach safe conclusions. Abbas et al. [21] found no statistically significant interaction between the FokI polymorphism and the ER/PR status of the tumor in a large retrospective case control study. Furthermore, Mishra et al. [23] identified no significant associations between any of the polymorphic VDR variants and tumor stage, tumor differentiation, and ER/PR/HER2 receptor status.

Our study represents a prospective hospital-based clinical study in which a number of Caucasian female patients was included. To date, this is the first study analyzing associations of two different gene polymorphisms (FokI and PvuII) with various tumor characteristics and survival in European Caucasian women. As opposed to the aforementioned studies, we found a significant difference regarding nodal stage between FokI polymorphisms. Moreover, a trend for statistical significance was observed with regard to the frequency of lobular vs ductal type, and tumor size. In our study we chose to further stratify Fokl polymorphisms according to the presence of the f allele or not. This was done in order to compare the wild type (FF) polymorphism with the other genotypes of the FokI polymorphisms. In addition, as previously shown, the f allele has been shown to have an association with early onset of breast cancer, whereas the FF genotype has been found to play a role in tumor progression and patient outcome [23]. In our stydy, the f allele was associated with the presence of lymphovascular invasion and poorly differentiated tumors. These findings are consistent with the study by Alimirah et al. [39] who found increased expression of pro-inflammatory genes such as COX2, IL-8 and CCL2, which may characterize the VDRff variant in breast cancer cells as a possible clinical marker for aggressive tumors. It is important to note that VDRff itself does not cause the aggressive phenotype, but due to its increased transcriptional activity of genes implicated in an aggressive phenotype, the VDRff genotype may fail to protect normal cells from oncogenic insults over time. Furthermore, upregulation of BIRC-3 mRNA was observed in VDRff cells indicating that breast cancer cells expressing this genotype may be resistant to apoptosis, potentially contributing to an unfavorable prognosis.

Estrogens have been found to contribute greatly in breast cancer pathogenesis and progression. Their biological effects, such as growth stimulation and differentiation of normal mammary tissue, are mediated primarily through high-affinity binding to ERs [24]. There are two types of ERs: ERα, whose gene is localized on chromosome 6q25.1 and acts as a transcription factor, and ERβ, whose gene is located on chromosome 14q22-24, and regulates genes that function as tumor suppressors [26]. The association between genetic polymorphisms in the ERα gene and the risk of breast cancer has been a subject of increasing interest. Several studies have shown that among ERα genotypes assessed by PvuII restriction fragment-length polymorphism, the PP genotype showed higher bone mineral density than the Pp and pp genotypes [40], and adolescent boys with the PP genotype reached greater body height than the others [41]. These findings may suggest that the local estrogenic action is more potent in those carrying a PP genotype compared to those carrying the Pp and pp genotypes. In some studies, the PP genotype has been found to be significantly elevated in breast cancer patients, suggesting that this genotype confers a risk for the development of breast cancer [42]. These results could be explained by the fact that PP genotype is related to a stronger estrogenic action on the one hand, and estrogens are known to induce cell proliferation on the other hand.

In the present study, no significant associations were found between any of the PvuII polymorphism variants and tumor histopathology and stage. However, with regard to survival, the PP genotype was associated with increased overall and progression-free survival, suggesting that this variant is related to a more favorable prognosis. Our findings are in consistency with the results in the study by Surekha et al. [42]. When the size and stage of the disease were considered,

Pp and pp genotype frequencies were increased in patients with larger tumor size and advanced stage of the disease; there was a corresponding elevation of p allele frequency in advanced-stage disease, suggesting that the presence of the p allele might confer a risk for an aggressive disease form. It is possible that individuals with the p allele have a lower expression of the ERα receptor or lower estrogen affinity and, therefore, are not controlled by endocrine therapy, resulting in greater tumor aggressiveness and poor prognosis.

This is the first prospective study of a Caucasian female population comparing VDR and ER polymorphisms to multiple histopathological tumor characteristics and survival. Limitation of our study is its sample size, which results in sparse information especially regarding specific gene variants. Furthermore, our findings are not representative for non-Caucasian populations because women in our population are primarily Caucasian and allele frequencies vary widely among populations of different ethnic origin.

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