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Analysis of the ERalpha germline *Pvu*II marker in breast cancer risk

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Prolonged exposure to estrogens was found to be a risk factor for breast cancer. The molecular mechanism has been suggested to be the binding of estrogen receptors in mammary tissue, which promotes the proliferation of breast tissue. Different biomarkers mapping estrogen receptor alpha (ESR1) have been associated with breast cancer risk, although the size of the effect is not consistent among different reports. Variation in the estrogen receptor gene *Pvu*II has been associated with an increased risk of developing breast cancer. However, some studies suggest that its effect might be constrained to a definite subgroup of patients.

Material/Methods: In this study the involvement of *Pvu*II in breast cancer was analyzed in an independent sample of 444 unrelated breast cancer cases and 704 controls of Spanish origin. A case-control comparison was performed and the genotype distributions examined according to different tumor and population parameters.

Results: A trend towards association was observed in adjusted case-control association analysis (p=0.07). *Pvu*II was associated with the familial forms of breast cancer (OR=3.81, p=0.02). T allele frequency was higher among patients with highly differentiated tumors (p=0.02), positive for steroid receptors (p=0.06), and negative for p53 (p=0.02). However, the *Pvu*II genetic background did not affect disease-free survival time (p=0.65).

Conclusions: The *Pvu*II T allele may be a germline risk factor for familial forms of breast cancer and is associated with a specific subset of immunohistochemical tumor phenotype.

key words: **breast cancer • estrogen receptor • p53 • polymorphism**

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Background

Breast cancer (BC) is the leading cause of death among solid tumors in women. The estimated annual incidence of breast cancer is around one million cases, with approximately 200,000 in the United States and 320,000 in Europe [1]. There are several recognized epidemiological and genetic risk factors affecting breast cancer. However, there is no realistic option for primary prevention for most patients lacking germline mutations in the hereditary breast cancer-related genes [2]. Adjuvant chemotherapy and hormonal treatment significantly improve survival, but have potentially serious side effects and are costly. Therefore, this treatment should be given preferentially to high-risk patients. Since traditional prognostic factors, such as tumor size or lymph node status, are insufficiently accurate, great effort has been made to develop improved predictors of high risk and better treatment response [2–4]. Furthermore, some authors have highlighted the importance of defining common variants exerting intermediate risks (20–60%) due to their wide distribution and the possibility of synergic interaction between them. Thus, common low-penetration variants in genes such as cytochrome P450 genes, the glutathione S-transferase family, DNA repair genes, and steroid receptor genes have contributed to a more accurate evaluation of the overall risk of susceptibility to breast cancer [1].

Regardless of the genetic and environmental factors that influence the appearance of the initial neoplasia, the subsequent mutations and rearrangements occurring in the tumor will define the malignancy, it aggressiveness, and probably its further response to treatment. To date, HER-2, p53, Ki67, and steroid receptors such as estrogen and progesterone receptor expression are routinely assayed in paraffin-embedded biopsies. These, together with the tumor size, lymph node status, and histological tumor grade, guide oncologists in classifying and treating the malignancy. Particularly, estrogen receptor content is a well-known predictor of clinical outcome in human breast cancer due to its role as an important mediator of hormonal responses, such as proliferation in estrogen-sensitive tissues. Its role in regulating the effect of estrogens on breast epithelial cells supports its putative involvement in the etiology of breast cancer [5].

Within the ER gene there is a plethora of single-nucleotide polymorphisms that have been subjected to analysis in case-control studies (OMIM: 133430). Among these markers, the *Pvu*II T>C transition (rs2234693) has been analyzed in independent breast cancer cohorts [6–9]. In those studies showing a positive association to an increased risk attributed to the T allele, the magnitude of the effect varies between 20 to 50%. However, statistical significance is sometimes compromised after multivariate corrections. Some authors found a moderately increased risk associated with the T allele among postmenopausal breast cancer patients when adjusting for circulating estradiol levels [6]. Others found a correlation with worse prognosis in a five-year follow-up study for those women harboring the T allele [10]. A third report described a *Pvu*II-dependent increased breast cancer risk mainly among the familial forms of the disease [11]. Overall it seems that *Pvu*II might be involved in breast cancer heritability, but data suggest that it may be a risk factor in only a proportion of patients, which remains to be elucidated.

Therefore our first aim was to elucidate whether the *Pvu*II variation within ER was associated with breast cancer risk in our sample and, secondly, to determine whether this increased risk was associated with a defined clinicopathological tumor profile. Thus we designed a case-control association study of 1148 women of Spanish origin, consisting of 444 unrelated breast cancer patients and 704 women from the general population. We analyzed the involvement of the *Pvu*II variation in breast cancer risk in our sample together with different immunohistochemical parameters such as ER/PR, p53, HER-2, and Ki67 status, among others.

Material and methods

Patients and controls

The study included 1182 individuals, 1148 of whom were satisfactorily genotyped (444 cases and 704 controls, call ratio: 97%). The cases were recruited from three independent hospitals from central and southern Spain: Hospital Universitario 12 de Octubre (Madrid), Hospital Ramón y Cajal (Madrid), and Hospital Universitario Virgen del Rocio (Sevilla). Controls from the general population were all Mediterranean Caucasians from the same geographical areas. The study population is briefly described in Supplementary Table 1. All subjects provided written informed consent together with a blood sample and a completed questionnaire regarding individual, environmental, and familial variables and pharmacological treatment. Among those families reporting more than one breast cancer patient, only one index was included in the study. Institutional review boards from the referral centers approved the protocol used in this study. We considered the existence of a history of breast cancer when at least one first- or second-degree relative also had breast cancer. Familial aggregation of cases indicates at least three independent cases of breast cancer were confirmed in the first- or second-degree relatives. Nuclear cancer was defined as at least one first-degree relative who was also diagnosed with breast cancer.

DNA extraction and SNP detection

To isolate germline DNA from leukocytes, we obtained 5 ml of peripheral blood from all of the patients. DNA extraction was performed automatically according to standard procedures using the Magnapure DNA isolation system (Roche, Germany). To perform polymerase chain reactions (PCRs), we prepared aliquots of DNA at a concentration of 5 ng/µl. The rest of the stock was cryopreserved at –20°C. The primers used for the amplification were forward: 5'-ACT GAT ATC CAG GGT TAT GTG G-3' and reverse: Biotin-CAG AAC CAT TAG AGA CCA ATG C-3'. PCR reactions were performed at a final volume of 20 µl, containing 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 2U of Taq Polymerase, and 5 pmol of each primer. The amplification cycles were as follows: 5 min at 95°C, 45 cycles at 95°C for 15 sec, 65°C for 30 sec, and 72°C for 15 sec, and a final extension of 72°C for 3 min. Genotyping was performed using a PSQ96 Pyrosequencer (Biotage AB, Uppsala, Sweden) and the sequencing primer 5'-TCC AAA TGT CCC AGC-3' according to the manufacture's instructions.

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Statistical analysis

For the statistical analysis of the genotype distribution, the test for deviation from Hardy-Weinberg equilibrium or case-control association studies, we employed tests adapted from Sasieni [12]. These calculations were performed using the online resource at the Institute for Human Genetics, Munich, Germany (*http://ihg.gsf.de*). To compare allelic frequencies between groups and linearity between parameters (degree of co-appearance), chi-squared tests were also performed according to Sasieni algorithm-based software and confirmed using Statcalc (EpiInfo 5.1, Centers for Disease Control, Atlanta, GA, USA). In order to obtain adjusted odds ratio (*OR*) estimates, we fitted unconditional logistic regression models using Stata 8.2 (College station, TX, USA).

Results

A summary of the genotype distribution according to the different clinicopathological parameters is presented in Table 1. The allele frequency of *Pvu*II (C: 0.42) was concordant with those previously documented with regard to other European populations [6,13–15]. The genotype distribution was in accordance with Hardy-Weinberg equilibrium for cases and controls (p=0.49 and 0.45, respectively). Furthermore, we found that the *Pvu*II T allele was more frequent among breast cancer patients than among the control population (Table 2). However, when adjusted for age, BMI, and menopause status, the adjusted odds ratio did not reach statistical significance, although a trend towards association was observed (p=0.07).

Next we proceeded to analyze our samples taking into account the family history of cancer. We found 383 cases lacking a recognized history of breast cancer and these were consequently defined as sporadic cases. In contrast, sixty-one patients presented at least one additional case of breast cancer in first- or second-degree relatives (14%), which is in accordance with previous epidemiological studies [1]. Fifty-two of these were in a nuclear form of the disease whilst ten exhibited an aggregation form of breast cancer. Both the sporadic and the familial subgroups did not differ in age, menopausal status, BMI, or age at menarche (data not shown). When genotype distribution was analyzed, we found a significant over-representation of the T allele among those cases with familial antecedents of breast cancer compared with controls (0.53 vs. 0.65, p=0.011) or to sporadic cases (data not shown). Thus the *Pvu*II-attributed risk was statistically significant among familial cases (*OR*=3.81, p_{trend}=0.02). In addition, the statistical significance of this association persisted among those patients exhibiting a nuclear form of breast cancer (at least 2 cases in first-degree relatives, *OR*=1.68, p_{trend} 0.049). It is worth noting the size of the effect in these significant associations, i.e. the increased relative risk among heterozygous intermediates values compared with those calculated for the homozygous ones (Table 2). These data suggest an additive genetic model. This behavior has been observed with similar directionality by different authors, regardless of the statistical significance of their studies [6,9,16]. These results would suggest that a proportion of hereditary predisposition to breast cancer can be accounted for by ER *Pvu*II polymorphism.

Familial forms of cancer are frequently characterized by an earlier development of the tumor. For this reason, we stratified the patients according to their age at diagnosis. In our cohort, 104 patients were diagnosed under 50 years of age and 258 over 50 years. The corresponding analysis revealed an increased risk of those TT subjects with an early (<50 years) development of the tumor (Table 2). No correlation could be found between *Pvu*II and the traditional prognostic markers such as lymph node status or tumor size (data not shown). In contrast, we found that the *Pvu*II T allele was strongly over-represented among those patients showing highly differentiated (grade I) biopsies (Table 3, *OR*=3.72, p=0.02). Next we concentrated on analyzing whether *Pvu*II distribution correlated with the immunohistochemical profile or the "signature" obtained from the tumor biopsies. In our cohort, 73.5% of them were positive for ER and 65.2% for PR. These ratios are in accordance with previous ones in other independent series [17]. On the other hand, 47% of the biopsies displayed a positive (moderate or high) expression of HER-2 and 46% were positive for p53 (Table 1). We observed that 47.6% of the biopsies expressed levels higher than 20% of Ki67. Univariate analysis revealed that *Pvu*II was associated with ER-positive (p=0.009), progesterone receptor-positive (p=0.05), and p53-negative tumors (p=0.01), but not to Ki67 or HER-2 expression (data not shown). Thus when linearity was assayed between ER, PR, and p53 status, we found that ER+ tumors tended to be also PR+ (84%) rather than PR– (16%) in a significant manner (p<0.001). Moreover, the ER+ tumors also tended to be p53– (58%) instead of p53+ (p=0.038). When the genotype distribution was studied according to these parameters, we found that the T allele was significantly over-represented among those patients whose biopsies were positive for ER and PR and negative for p53 (0.53 vs. 0.65, combined unadjusted *OR*=1.65, p=0.029). In addition, we analyzed the relationship between the histological differentiation grade and the genetic profile. We found that highly differentiated tumors (grade I) tended to be ER+ (p=0.005), PR+ (p=0.009), and negative for p53 (p=0.006), which made the association consistent.

Finally, in order to determine whether any *Pvu*II genotype could be used as an independent prognostic factor, we performed a different disease-survival time analysis using Kaplan-Meier tests. The results are summarized in Figure 1. For our analysis we excluded those cases with initial metastasis (Mx, n=17) and stratified our cases according to ER status. After these corrections, we failed to correlate *Pvu*II with a differential prognosis.

Discussion

Breast cancer has become the most common malignancy among the female population, affecting one in eight women, and is one of the main causes of female mortality in Western countries [18]. The importance of several molecular markers in breast cancer has been of considerable interest during recent years, not only as prognostic markers, but also as predictors of response to therapy [19,20]. On the other hand, great effort is currently being made to identify common variants that would serve as susceptibility factors. However, little is known about the connection between these susceptibility factors and those clinicopathological parameters used in the daily clinical routine. Estrogen receptor content is an established predictor of clinical outcome in human breast cancer due to

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Table 1. Distribution of the *Pvu*II genotypes according to the different clinicopathological parameters.

*Pvu*II	Controls	Cases	Cases (%)					
			Immunohistochemistry					
			ER		PR		p53	
			+	–	+	–	+	–
TT	193 (27.4)	153 (34.6)	92 (35.7)	28 (30.1)	77 (34.7)	40 (33.9)	21 (26.9)	37 (40.2)
TC	361 (51.3)	209 (47.3)	124 (48.1)	43 (46.2)	104 (47.2)	55 (46.6)	40 (51.3)	41 (44.6)
CC	150 (21.3)	82 (18.6)	42 (16.3)	22 (23.7)	40 (18.1)	23 (19.5)	17 (21.8)	14 (15.2)

*Pvu*II	Cases (%)							
	Immunohistochemistry					Tumot type		
	HER-2		Ki67 (%)					
	M/H*	A/L*	≤20	20–45	≥45	Ductal	Lobular	Medular
TT	49 (33.3)	56 (34.3)	29 (32.9)	19 (40.4)	6 (12.2)	106 (32.9)	11 (42.3)	2 (66.6)
TC	73 (49.7)	78 (47.9)	42 (47.7)	20 (42.6)	19 (57.6)	153 (47.5)	13 (50.0)	1 (33.3)
CC	25 (17.0)	29 (17.8)	17 (19.3)	8 (17.0)	8 (24.2)	63 (19.6)	2 (7.7)	0 (0.0)

*Pvu*II	Controls	Cases	Histological grade			Lymph node status			
			I	II	III	N0	N1	N2	N3
TT	193 (27.4)	153 (34.6)	22 (40.7)	47 (33.8)	39 (29.5)	48 (32.2)	41 (28.9)	14 (37.8)	10 (76.9)
TC	361 (51.3)	209 (47.3)	26 (48.1)	64 (46.0)	65 (49.3)	77 (51.7)	73 (51.4)	17 (45.9)	2 (15.4)
CC	150 (21.3)	82 (18.6)	4 (7.4)	28 (20.2)	28 (21.2)	24 (16.1)	28 (19.7)	6 (16.2)	1 (7.7)

	Infiltration		Tumor Size (mm)		
	Vascular	Lymph	≤20	20<∅<40	≥40
TT	48 (33.8)	13 (37.1)	16 (28.6)	20 (31.3)	7 (30.4)
TC	63 (44.4)	12 (34.3)	27 (48.2)	31 (48.4)	9 (39.1)
CC	31 (21.8)	10 (28.6)	13 (23.2)	13 (20.3)	7 (4.3)

* M stands for moderate and H for high, A for absent and L for low. In brackets we represent percentages.

Suplementary Table 1. General demographic parameters.

	Controls		Cases	
Age (years)	58.32±9.77	[34–82]	57.16±11.58	[26–86]
BMI (m/kg^2)	27.5±4.48	[16.7–47]	28.5±5.46	[18.2–51]
Smoking (ever)	184/506	(36.4%)	110/367	(30.8%)
Menopause (age)	47.87±5.39	[19–59]	47.94±5.58	[17–67]
Menarche (age)	12.86±1.58	[8–18]	12.64±1.62	[9–17]
Breast cancer antecedents	–		13.7%	

its role as an important mediator of hormonal responses, such as proliferation in estrogen-sensitive tissues. Here we show that the ER *Pvu*II T allele is associated with a moderately increased risk of developing breast cancer among the Spanish population (Table 2). This association was more apparent when the patients analyzed were under 50 years of age. Furthermore, we also detected a significant over-representation of the *Pvu*II T allele among the familial cases of breast cancer. This observation was previously suggested by Shen et al. [11], but the role of this ER variant in the heritability of breast cancer remains to be confirmed.

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Table 2. Genotype distribution of *Pvu*II according to different epidemiological characteristic.

Subjects		Cases	OR unadjusted (95% CI)	OR adjusted* (95% CI)
All cases	CC	82	1.00 (reference)	1.00 (reference)
	TC	209	1.06 (0.77–1.46)	1.13 (0.78–1.63)
	TT	153	1.45 (1.30–2.04)	1.42 (0.95–2.12)
			p_{trend} 0.02	p_{trend} 0.07
Familial cases	CC	5	1.00 (reference)	1.00 (reference)
	TC	33	2.74 (1.05–7.16)	3.16 (1.08–9.23)
	TT	23	3.58 (1.33–9.65)	3.81 (1.25–11.6)
			p_{trend} 0.01	p_{trend} 0.02
Sporadic cases	CC	77	1.00 (reference)	1.00 (reference)
	TC	176	0.95 (0.68–1.39)	1.00 (0.69–1.47)
	TT	130	1.31 (0.92–1.87)	1.25 (0.83–1.91)
			p_{trend} 0.08	p_{trend} 0.24
Breast cancer <50	CC	22	1.00 (reference)	1.00 (reference)
	TC	31	0.6 (0.33–1.05)	0.37 (0.17–0.83)
	TT	51	1.80 (1.05–3.10)	1.5 (0.69–3.27)
			p_{trend} 0.003	p_{trend} 0.06
Breast cancer ≥50	CC	44	1.00 (reference)	1.00 (reference)
	TC	144	1.36 (0.92–2.00)	1.48 (0.97–2.25)
	TT	70	1.24 (0.80–1.91)	1.31 (0.82–2.09)
			p_{trend} 0.42	p_{trend} 0.36
Postmenopausal women	CC	44	1.00 (reference)	1.00 (reference)
	TC	131	1.24 (0.84–1.83)	1.39 (0.02–2.10)
	TT	76	1.34 (0.88–2.00)	1.54 (0.98–2.42)
			p_{trend} 0.19	p_{trend} 0.07
Premenopausal women	CC	23	1.00 (reference)	1.00 (reference)
	TC	45	0.81 (0.48-1.39)	0.27 (0.94-0.76)
	TT	44	1.49 (0.86-2.57)	0.60 (0.20-1.84)
			p_{trend} 0.08	p_{trend} 0.71

* Adjusted by age, BMI, and menopause status.

Suplementary Table 2. Linearity of different immunohistochemical parameters under study.

Tumor stage	ER		PR		p53		HER-2			
	+	–	+	–	+	–	Absent	Low	Moderate	High
Grade I	**45 (90%)***	5 (10%)	**40 (82%)***	9 (18%)	3 (14%)	**16 (84%)***	17 (40%)	10 (24%)	8 (19%)	7 (16%)
Grade II	107(83%)*	22 (17%)	94 (75%)*	31 (25%)	31 (49%)	32 (51%)	37 (31%)	29 (24%)	25 (21%)	29 (24%)
Grade III	71 (51%)*	63 (49%)	55 (43%)*	73 (57%)	41 (57%)	31 (43%)	40 (34%)	17 (14%)	28 (24%)	33 (29%)
Total (reference)	223(73%)	90 (27%)	189(65%)	113(25%)	79 (49%)	75 (51%)	94 (34%)	56 (20%)	61 (22%)	69 (25%)

* $p<0.05$ compared with the reference distribution according to the Mantel-Haenszel test. In bold are the parameters found together in a statistically significant ratio.

Nonetheless, we conducted an analysis taking into account the different genetic markers routinely assayed in tumor biopsies. We observed that the over-representation of the *Pvu*II T allele found among breast cancer cases fits better with certain histologically differentiated tumors (grade I, ER/PR+ and p53–), which would suggest an improved prognosis according to several authors [3,21,22]. To this end, we performed disease-free survival analysis in our series. According to our data, disease-free survival time was significantly lower among those cases initially diagnosed with metastasis (Figure 1A, log-rank $p<0.001$) and also significantly different between ER+ and ER– cases (Figure 1B, log-rank $p<0.001$). Thus, ini-

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Table 3. Genotype distribution of *Pvu*II according to different tumour classification.

Subjects		Cases	OR unadjusted (95% CI)	OR adjusted* (95% CI)
Grade I	CC	4	1.00 (reference)	1.00 (reference)
	TC	26	2.70 (0.93–7.87)	2.84 (0.96–8.41)
	TT	22	4.25 (1.44–12.67)	3.72 (1.21–11.4)
			p_{trend} 0.004	p_{trend} 0.02
Grade II	CC	28	1.00 (reference)	1.00 (reference)
	TC	64	0.95 (0.59–1.54)	1.06 (0.60–1.87)
	TT	47	1.30 (0.78–2.18)	1.47 (0.81–2.67)
			p_{trend} 0.24	p_{trend} 0.16
Grade III	CC	28	1.00 (reference)	1.00 (reference)
	TC	65	0.97 (0.59–1.56)	0.93 (0.55–1.59)
	TT	39	1.08 (0.64–1.84)	0.96 (0.53–1.74)
			p_{trend} 0.74	p_{trend} 0.92
p53 –	CC	14	1.00 (reference)	1.00 (reference)
	TC	41	1.22 (0.64–2.30)	1.18 (0.60–2.33)
	TT	37	2.05 (1.07–3.93)	2.13 (1.07–4.25)
			p_{trend} 0.01	p_{trend} 0.02
	CC	17	1.00 (reference)	1.00 (reference)
p53 +	TC	40	0.96 (0.54–1.78)	1.29 (0.63–2.67)
	TT	21	0.96 (0.49–1.88)	0.96 (0.42–2.18)
			p_{trend} 0.91	p_{trend} 0.84
ER +	CC	42	1.00 (reference)	1.00 (reference)
	TC	124	1.23 (0.82–1.83)	1.15 (0.74–1.79)
	TT	92	1.70 (1.12–2.60)	1.52 (0.95–2.43)
			p_{trend} 0.01	p_{trend} 0.06
ER –	CC	22	1.00 (reference)	1.00 (reference)
	TC	43	0.81 (0.47–1.41)	0.86 (0.46–1.58)
	TT	28	0.99 (0.54–1.80)	1.04 (0.54–2.02)
			p_{trend} 0.96	p_{trend} 0.22
PR +	CC	40	1.00 (reference)	1.00 (reference)
	TC	104	1.08 (0.72–1.63)	1.11 (0.69–1.76)
	TT	77	1.50 (0.97–2.32)	1.44 (0.87–2.37)
			p_{trend} 0.049	p_{trend} 0.13
PR –	CC	23	1.00 (reference)	1.00 (reference)
	TC	55	1.00 (0.60–1.68)	1.10 (0.61–1.98)
	TT	40	1.35 (0.76–2.36)	1.43 (0.77–2.66)
			p_{trend} 0.23	p_{trend} 0.23
HER-2 A/L	CC	29	1.00 (reference)	1.00 (reference)
	TC	78	1.12 (0.70–1.78)	1.15 (0.68–1.92)
	TT	56	1.50 (0.91–2.47)	1.45 (0.84–2.53)
			p_{trend} 0.08	p_{trend} 0.16
HER-2 M/H	CC	25	1.00 (reference)	1.00 (reference)
	TC	73	1.21 (0.74–1.99)	1.22 (0.70–2.13)
	TT	49	1.52 (0.90–2.58)	1.42 (0.78–2.58)
			p_{trend} 0.105	p_{trend} 0.25

* Adjusted by age, BMI, and menopause status.

tially metastatic cases were excluded from the survival time analysis and the rest were stratified according to ER status. As can be seen in Figure 1, we failed to correlate *Pvu*II as an independent prognostic factor. On the other hand, the subgroup with ER/PR– p53+ with high levels of HER-2 has been associated with a much less favorable prognosis in different studies [23,24]. Moreover, we detected a correlation between the proliferation index Ki67, which is higher in those tumors negative for ER/PR ($p<0.001$), and moderate to high expression of HER-2 ($p=0.044$) and those tumors positive for p53 ($p=0.027$) (Supplementary Table 2).

Despite all the data available from different sources, the molecular nature of the effect involving these signaling

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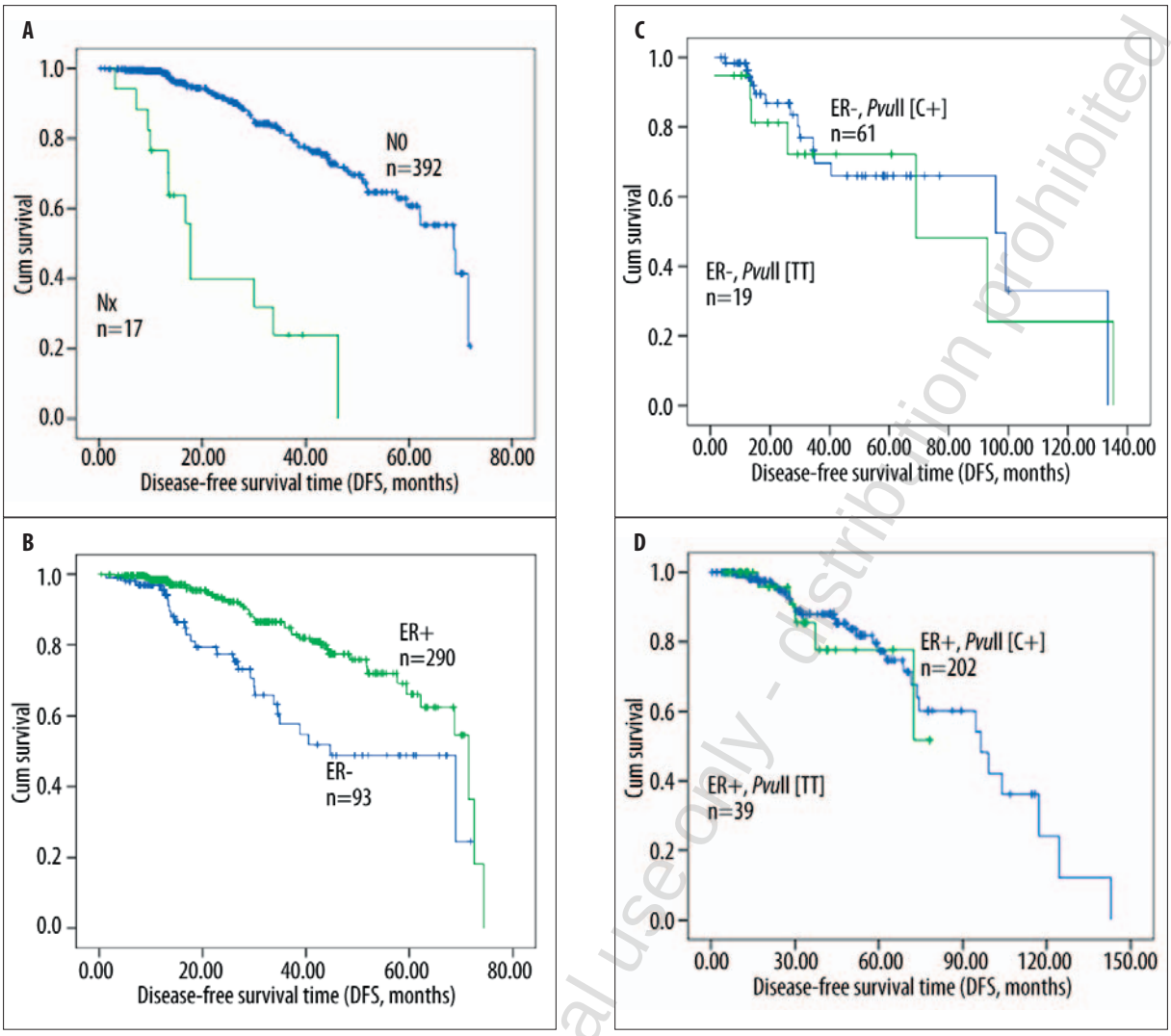

Figure 1. Kaplan-Meier analysis of disease-free survival time expressed in months of (**A**) patients with (Nx) or without (N0) affected lymph nodes at diagnosis (Mantel-Cox test *p*-value <0.001), (**B**) patients with ER+ vs. ER– biopsies ($p<0.001$), (**C**) N0 patients with ER- biopsies, stratified according to the *Pvu*II genotype ($p=0.71$), and (**D**) N0 patients with ER+ biopsies, stratified according to the *Pvu*II genotype ($p=0.65$).

pathways remains to be fully elucidated. Interestingly, a recent study found that ER plays a crucial role in regulating p53 activity. ER binding to p53 resulting in functional inactivity of wild-type p53 could be one of the reasons for the inability of wild-type p53 to restrain tumor growth and metastasis in ER-positive breast cancers [25]. We believe that understanding how common susceptibility factors influence tumor progression would certainly help in the development of novel strategies focused on its prevention and treatment.

Conclusions

According to our data, *Pvu*II is certainly a susceptibility factor for breast cancer among the Spanish population. Furthermore, we found that the nature of this association leads to the development of preferably highly differentiated tumors, characterized by being ER/PR+ and expressing the wild-type form of p53. Therefore, tumor cell growth is suitable to be repressed while keeping (a priori) their response to apoptotic-inducing agents. Further independent analysis should be performed in order to elucidate the molecular mechanisms underlying this effect.

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