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MDR1 C3435T polymorphism in Mexican patients with breast cancer

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ABSTRACT. We investigated whether the *MDR1* C3435T polymorphism is associated with fibrocystic changes (FCC), infiltrating ductal breast cancer (IDBC), and/or clinical-pathological features of IDBC in Mexican patients. Samples from women who received surgical treatment in 2007 at the Centro Médico de Occidente (México) were included in the analysis. Genotyping was performed by polymerase chain reaction-restricted fragment length polymorphisms in 64 paraffin-embedded breast samples with IDBC, 64 samples with FCC, and 183 peripheral blood samples of healthy females designated as the healthy group (HG). The frequency of the T allele was 41, 45, and 52% for the FCC, IDBC, and HG samples, respectively. Significant differences were only found between the FCC

and HG samples [odds ratio (OR) = 0.64, 95% confidence interval (CI) = 0.43-0.96; P = 0.032]. The prevalence of the T/T genotype was 8, 13, and 24% for FCC, IDBC, and HG samples, respectively. Again, statistical differences were only found between FCC and HG samples for the T/T genotype (OR = 0.28, 95%CI = 0.106-0.77; P = 0.009). Although the T allele and the T/T genotype were less frequent in the IDBC group than in the HG, the differences were not significant. Furthermore, no associations were found between the C3435T polymorphism and clinical-pathological features of the IDBC group. Both the FCC and IDBC groups had a high frequency of the C allele relative to the HG in this sample of women from Western Mexico.

Key words: Breast Cancer; Malignant breast lesions; Fibrocystic changes; P-glycoprotein; *MDR1*

INTRODUCTION

The mammalian adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of transporters includes a large number of diverse transmembrane proteins. The multi-drug resistance 1 (*MDR1*) gene is a member of the ABC family and encodes a membrane-bound phosphoglycoprotein (P-gp), which acts as an efflux pump and provides cell protection against various substances such as organic cations, carbohydrates, amino acids, some antibiotics, polysaccharides, and proteins (Hoffmeyer, 2000; Marzolini et al., 2004; Ieiri, 2012). Accordingly, P-gp is highly expressed in the kidney, adrenal gland, liver, blood-brain barrier, placenta, and testis (Fojo et al., 1987; Sugawara et al., 1989; Fung and Gottesman, 2009). In each tissue, P-gp directly influences drug efficacy, and in cancer cells, its expression determines the degree of chemotherapy resistance. It has been suggested that synonymous single nucleotide polymorphisms (SNPs), such as C3435T of *MDR1* (rs1045642), affect protein expression and function via impaired stability of the mRNA (Sauna et al., 2007); however, this remains to be fully clarified. Several clinical studies have examined whether the C3435T polymorphism is a protective or a risk factor to tumor development, including breast cancer, as well as its effect on clinical outcome and anti-cancer drugs; however, conflicting results have been reported (Sheng et al., 2012; Wang et al., 2012). The aim of this study was to analyze the association of the C3435T polymorphism of *MDR1* in Mexican women with either benign fibrocystic changes (FCC) or infiltrating ductal breast cancer (IDBC).

MATERIAL AND METHODS

Study population

Paraffin-embedded samples from patients with either IDBC (N = 64) or FCC (N = 64) were collected from the histopathology archives of the Centro Médico Nacional de Occidente (México) from January to December 2007. The tissues of IDBC patients were primary breast tumors selected at diagnosis and before treatment. From the IDBC group, we also recorded several clinical-pathological features at the time of diagnosis: age, tumor size, metastasis to axilar nodes, and tumor grade according to the Scarff-Bloom-Richardson (SBR) classification. As a control group, we included 183 peripheral blood samples from healthy women without a family history of breast or other cancers, with a median age of 45 years (range = 30-84 years), who also lived in Western Mexico. This group was designated as the healthy group (HG).

Genotyping

DNA of paraffin-embedded tissues was extracted with Chelex-100, following manufacturer protocols. The DNA extraction of the HG samples was carried out by the phenol-chloroform method. The C3435T *MDR1* polymorphism was identified by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. For DNA amplifications, the following primers were used: (sense: 5'-TGTTTTCAGCTGCTTGATGG-3' and antisense: 5'-AAGGCATGTATGTTGGCCTC-3'), leading to a 197-bp fragment. The amplification program consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; moreover, a final elongation was performed at 72°C for 7 min. The amplified product (197 bp) was cleaved with the restriction enzyme *Mbo*I for 16 h at 37°C; the resulting fragments were 197 bp (wild allele, C), 158, and 39 bp, which were observed on a 12% polyacrylamide gel stained with $AgNO_3$ (Droździk et al., 2003).

Statistical analysis

Statistical analysis was performed with the package SPSS 15.0 v software for Windows (SPSS Incorporation, Chicago, IL, USA). The allele and genotype frequencies were determined by the Fisher exact test. Associations with the different clinical-pathological parameters were determined by calculating odds ratio (ORs) and 95% confidence intervals (CIs).

RESULTS

The clinical-pathological features of the IDBC group are summarized in Table 1. The frequency of the T allele was 41, 45, and 52% for the FCC, IDBC, and HG samples, respectively (Table 2). A significant difference was only found between the FCC and HG samples (OR = 0.28; 95%CI = 0.106-0.77, P = 0.009, for the TT genotype and OR = 0.64; 95%CI = 0.43-0.96, P = 0.032, for the T allele). The genotype frequencies in the three groups were similar with no significant intergroup differences observed (Table 2). Likewise, no significant difference was found when genotypes were analyzed in relation to the clinical-pathological features in the IDBC group (Table 3).

Table 1. Clinical-pathological features of samples with infiltranting ductal breast cancer.

Clinical-pathological feature	N	%
Age (years)		
≤50	22	34
>50	42	66
Mama affected		
Right	35	55
Left	28	44
Both	1	1
Tumor size (cm)		
≤5	48	75
>5	16	25
Tumor grade[a]		
1	6	9
2	36	56
3	22	35
Lymph nodes		
Positive	38	59
Negative	26	41

[a]Scarff-Bloom-Richardson (SBR) classification.

Table 2. Genotype and allele frequencies of the C3435T *MDR1* polymorphism in the healthy group and patients with fibrocystic changes and infiltranting ductal breast cancer.

Genotype allele	Controls [N = 183 (%)]	Patients [N = 64 (%)]	OR (95%CI)	P value[1]
	HG	FCC		
C/C	37 (20)	17 (26)	1.0 (Reference)	
C/T	103 (56)	42 (66)	0.89 (0.45-1.75)	0.73
T/T	43 (24)	5 (8)	0.28 (0.106-0.77)	0.009
C	177 (48)	76 (59)	1.0 (Reference)	
T	189 (52)	52 (41)	0.64 (0.43-0.96)	0.032
	HG	IDBC		
C/C	37 (20)	15 (23)	1.0 (Reference)	
C/T	103 (56)	41 (64)	0.98 (0.49-1.98)	0.96
T/T	43 (24)	8 (13)	0.46 (0.20-1.07)	0.07
C	177 (48)	71 (55)	1.0 (Reference)	
T	189 (52)	57 (45)	0.75 (0.50-1.13)	0.17
	FCC	IDBC		
C/C	17 (26)	15 (23)	1.0 (Reference)	
C/T	42 (66)	41 (64)	1.11 (0.49-2.50)	0.808
T/T	5 (8)	8 (13)	1.63 (0.49-5.42)	0.41
C	76 (59)	71 (55)	1.0 (Reference)	
T	52 (41)	57 (45)	1.17 (0.72-1.93)	0.53

[1]Fisher exact test. OR = odds ratio; HG = healthy group; FCC = fibrocystic changes; IDBC = infiltranting ductal breast cancer.

Table 3. Genotype frequencies and size tumor, Scarff-Bloom-Richardson classification and lymph nodes in patients with infiltranting ductal breast cancer.

	Genotype frequencies		
	T/T	C/T	C/C
Tumor size (cm)			
≤5 (N = 48)	6	31	11
>5 (N = 16)	2	10	4
OR (95%CI)	1	0.84 (0.145-4.91)	0.75 (0.98-5.76)
P value[1]		0.85	0.78
Tumor grade[2]			
1,2 (N = 42)	4	25	13
3 (N = 22)	2	17	3
OR (95%CI)	1	2.15 (0.382-11.82)	0.75 (0.98-5.76)
P value[1]		0.45	0.78
Lymph nodes			
Nodes (+, N = 37)	6	20	11
Nodes (-, N = 27)	2	21	4
OR (95%CI)	1	2.87 (0.15-15.85)	1.01 (0.15-7.82)
P value[1]		0.26	0.93

OR = odds ratio. [1]Fisher exact test. [2]Tumor classifications according to Scarff-Bloom-Richardson.

DISCUSSION

P-gp is the most widely studied membrane protein of the cassette family of membrane transporters. Although the *MDR1* mechanism of transcription is surprisingly complex and poorly understood, it has been suggested that impaired *MDR1* expression and protein con-

formation can result in several cancer types (Trock et al., 1997; Tatari et al., 2009; Rao et al., 2010; Sabahi et al., 2010; Mhaidat et al., 2011).

Several studies have investigated the association of the C3435T polymorphism with breast cancer development, prognosis, and treatment outcome; however, the inconsistent and contradicting evidence has led to a confused picture (Turgut et al., 2007; Huang et al., 2008; Rodrigues et al., 2008; George et al., 2009; Taheri et al., 2010; Chen et al., 2012). In the present study, we analyzed the *MDR1* C3435T polymorphism in Mexican women with IDBC or FCC as compared with healthy women. We found a greater frequency of the C allele in the FCC and IDBC groups; by contrast, the T allele was more frequent in the HG. We previously reported that the T allele predominates in the healthy Mexican population but not in patients with acute lymphoblastic leukemia (ALL) (Leal-Ugarte et al., 2008); indeed, these and the present results allow us to conclude that the T allele is unrelated to ALL and breast cancer.

The allele frequencies observed in the HG are very similar to those found in other control populations (George et al., 2009; Taheri et al., 2010). However, allele and genotype frequencies differ among diverse populations with breast cancer (Ashariati, 2008; Rodrigues et al., 2008; George et al., 2009; Henríquez-Hernández et al., 2009; Taheri et al., 2010; Fang et al., 2013). In addition, we observed that the T/T genotype appears to have a protective effect in the FCC group, whereas no association was found with IDBC.

It is important to remark that many factors could account for the wide variability of *MDR1* C3435T polymorphism results, including ethnicity, the tissue analyzed, time of sampling, gender and estrogen receptor (ER), and method and sample size. With respect to ethnicity, a meta-analysis focused on the C3435T *MDR1* polymorphism showed that the T allele is a risk factor for the development of hematological malignances, breast cancer, and renal cancer in Caucasian individuals but not in other races (Wang et al., 2012); at the same time, this can be explained by the different frequencies of the polymorphism among diverse populations (www.ncbi.nlm.nih.gov/snp). Most reports about this polymorphism and breast cancer have directly analyzed the affected tissue; however, a few studies have analyzed the surrounding tissue or peripheral blood (mainly for reference groups as in this study). Previous reports suggest that P-gp protein expression may vary even in different regions of certain organs, which implies that several factors could be derived from the analyzed tissue. The time of sampling (before, during, or after treatment) is relevant because some drugs can modify the P-gp protein (Haenisch et al., 2007). In addition, estrogenic activity was shown to down-regulate P-gp expression via post-transcriptional processes in ER-α-positive cell lines, leading to increased cellular uptake of P-gp substrates; in this sense, it is significant that most breast cancer cells are ER-α-positive (Mutoh et al., 2006). Finally, in spite of the small sample, our study suggests a possible protective effect of the T/T genotype in the FCC group.

In conclusion, our data point to the T/T genotype as a protective factor in the FCC group but did not support any association between the C3435T *MDR1* polymorphism and clinical-pathological features in Mexican women with IDBC. Future studies should consider interethnic variations as well as protein interactions in order to provide more conclusive information about the role of the *MDR1* C3435T polymorphism in the course and outcome of breast cancer. Although this polymorphism by itself is not causative, there are nearly 50 other SNPs in the *MDR1* gene, including 30 that lead to amino acid substitutions (Fung and Gottesman, 2009). Moreover, other genes may also be implicated in the etiology of sporadic breast cancer. Therefore, it is necessary to carry out studies that include additional polymorphisms of

MDR1 and other genes to obtain more information regarding the influence of common genetic variants in breast cancer development.

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