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Steroid metabolism gene polymorphisms and their implications on breast and ovarian cancer prognosis

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Genet. Mol. Res. 16 (3): gmr16039691
Received April 4, 2017
Accepted May 31, 2017
Published July 6, 2017
DOI http://dx.doi.org/10.4238/gmr16039691

ABSTRACT. A role for estrogen in the etiology of breast and ovarian cancers has been suggested; therefore, genetic polymorphisms in steroid metabolism genes could be involved in the carcinogenesis of these tumors. We have aimed to investigate the role of *GSTP1* and *CYP17* polymorphisms and their correlation with MSI (microsatellite instability) and LOH (loss of heterozygosity) in *AR*, *ERβ* and *CYP19* genes in women from Espírito Santo State, Brazil. The study population consisted of 107 female breast and 24 ovarian tumors. *GSTP1* and *CYP17* polymorphisms were detected by polymerase chain reaction (PCR) amplification followed by restriction fragment length polymorphism (RFLP) analysis while MSI and LOH were analyzed by PCR. *GSTP1* and *CYP17* polymorphisms alone were not associated with an increased risk for breast or ovarian tumors. However, when combined with MSI/LOH in *AR*, *ERβ* and *CYP19* genes, we were able to detect significant associations with the *GSTP1* wild-type genotype in PR (progesterone receptor) negative breast cancers or the *CYP17* wild-type genotype in ER (estrogen receptor)

and PR-negative breast tumors. No associations with ovarian tumors were detected. Our results suggest that wild-type *GSTP1* or *CYP17* genes when combined with LOH/MSI in steroid metabolism genes may play a role in ER and/or PR negative breast cancers. These data support the hypothesis that genes related to steroid metabolism are important in the characterization of breast cancer and that the analysis of single polymorphisms may not be sufficient.

Key words: Breast/ovarian cancer; *GSTP1*; *CYP17*; STR markers; Polymorphisms

INTRODUCTION

Breast cancer is the most common malignancy among females and the most common cause of cancer death among women in Western countries (Imyanitov and Hanson, 2004; Antognelli et al., 2009) and ovarian cancer is the most lethal gynecological malignancy at present (Huan et al., 2008; Lurie et al., 2009).

Germline mutations in the so-called high penetrance genes of breast and ovarian cancer susceptibility, such as *BRCA1* and *BRCA2*, appear to account for the majority of hereditary breast and ovarian cancers, but they represent only 5 to 10% of breast cancer cases and up to 15% of ovarian cancers (Lynch et al., 2009; Ramalhinho et al., 2012).

Thus, polymorphisms in low penetrance genes can be linked with a significant percentage of breast and ovarian cancers. Low penetrance genes can be involved in a wide variety of functions including steroid hormone metabolism, detoxification of environmental carcinogens, DNA damage repair genes and tumor suppressor genes (Miyoshi and Noguchi, 2003; Delort et al., 2008; Ramalhinho et al., 2012).

Polymorphisms in the cytochrome P450 family (CYPs) and in the glutathione S-transferase (GSTs) enzymes have been of particular interest because these enzymes play an important role in the metabolism of environmental carcinogens and of estrogen (Torresan et al., 2008; Antognelli et al., 2009), which seems to be involved in breast and ovarian carcinogenesis. Genes of these families are highly polymorphic, presenting alleles with different enzymatic activities and possibly tumor risk (Torresan et al., 2008).

The *CYP17* gene encodes the cytochrome P450c17α enzyme, which is a key enzyme in the estradiol synthesis (Kristensen and Borresen-Dale, 2000), converting pregnenolone and progesterone to androgen and estrogen precursors (Torresan et al., 2008). The *T→C* polymorphism in the 5'-untranslated promoter region creates an additional SpI-type promoter site 34 bp upstream of the translation initiation site, which has been shown to be associated with *CYP17* expression levels and thus estrogen levels (Zhang et al., 2009).

Some groups have reported an association of the *A2* allele (variant allele *C*) with increased breast cancer risk because patients with the *A2* allele had higher levels of circulating estrogens than those with *A1* alleles (Torresan et al., 2008; Zhang et al., 2009), but other studies have failed to replicate these findings (Torresan et al., 2008).

In ovarian cancer, presence of the *A2* variant has been associated with increased disease risk. Furthermore, the *A2* allele has also been associated with polycystic ovarian syndrome, a condition resulting from high androgen levels (Goodman et al., 2001).

GST enzymes have the capacity to detoxify reactive PAHs (polycyclic aromatic

hydrocarbons) metabolites, preventing them from becoming carcinogens. These enzymes are involved in DNA protection from oxidative damage, including free radicals and metabolites generated through estrogen metabolism (Torresan et al., 2008).

Single nucleotide substitutions in *GSTP1* exon 5 (*A313G*; *Ile105Val*) are in close proximity with the substrate binding site of GSTP1 and the *Val* variant has been demonstrated to have either lower or higher specific activity and affinity than the *Ile* variant, depending on the substrate (Ramalhinho et al., 2012).

Previous studies on the potential association of *GSTP1*polymorphisms and breast cancer have produced inconsistent results (Torresan et al., 2008; Antognelli et al., 2009) and few studies have investigated the association of this polymorphism with ovarian cancer.

Moreover, polymorphisms in short tandem repeat regions (STR), also known as microsatellites markers, have been extensively studied in tumors (Zhang and Yu, 2007). These polymorphisms can occur due to expansion or contraction of repeat sequences giving rise to what is referred to as MSI (Zhang and Yu, 2007; Yoon et al., 2008). MSI occurs in about 90% of hereditary non-polyposis colorectal cancer (HNPCC) and has also been observed in a variety of sporadic cancers, including colon, endometrium, pancreas, and bladder (Yoon et al., 2008). In breast cancer, there is no consensus regarding the frequency of MSI. Some studies have found that MSI is not associated with this neoplasm, while others have reported frequencies that vary from 5 to 40% (Shen et al., 2000). In ovarian cancer, MSI frequency ranges from 5 to 50% (Sood et al., 2001), being the higher frequencies observed in endometrioid, mucinous and clear cell ovarian tumors (Plisiecka-Hałasa et al., 2008). Analysis of the highly polymorphic microsatellite loci not only provides information about MSI, but also allows for the detection of LOH in tumor cells (Powierska-Czarny et al., 2003).

LOH is thought to indicate regions harboring tumor suppressor genes and this phenomenon may also reflect random chromosomal instability. Thus, a high frequency of LOH may indicate DNA damage or instability (Tokunaga et al., 2012).

In breast and ovarian cancer, frequent LOH has been detected on chromosomes 3p, 6q, 11p, 13q, 16q, 17p, 17q, and 18q, so that several tumor suppressor genes have been mapped to these regions (Ando et al., 2000; Plisiecka-Hałasa et al., 2008).

For all these reasons, our aim was to analyze the influence of *GSTP1* and *CYP17* polymorphisms in breast and ovarian cancer cases in Espírito Santo, Brazil, and their correlation with MSI/LOH in *AR*, *ERβ* and *CYP19* genes, which are involved in steroid biosynthesis.

MATERIAL AND METHODS

Ethics

This study was approved by the Ethics Committee of Universidade Federal do Espírito Santo, protocol No. 02/09, including the informed consent waiver for breast and ovarian cancer cases and control group volunteers.

Sample

The study population consisted of archival formalin-fixed paraffin-embedded tissues from 107 female breast and 24 ovarian tumors, obtained from Santa Rita de Cássia Hospital

Pathology Department, Espírito Santo State, Brazil, during years 2009 and 2010. Tumor cells were collected by a 3-mm punch in the tumor block, guided by the Pathologist. Briefly, histological slides were prepared from formalin-fixed paraffin-embedded tissues, stained with hematoxylin and eosin and allowed to air dry. Tumor tissue was marked in the hematoxylin-eosin stained slides by a Pathologist and slides were aligned with the block for punch extraction. Normal control tissue was obtained from the same block. General population *GSTP1* and *CYP17* gene polymorphism frequencies were studied from 61 healthy female blood donors at Espírito Santo Hemotherapy and Hematology Center with no previous personal or familial history of breast and ovarian cancer.

DNA extraction

Breast and ovarian cancer genomic DNA was extracted from the punch fragment by slicing and incubating at 58°C for five days in 0.5 M Tris, 0.02 M EDTA, 0.01 M NaCl, 1 mg/mL proteinase K, and 2% SDS solution. Lysates were subsequently submitted to organic extraction with phenol-chloroform. Healthy control DNA was isolated from peripheral blood using a phenol-chloroform organic extraction.

Genotyping analysis

GSTP1 Ile-Val polymorphism was determined by PCR-RFLP (Harries et al., 1997). PCR products were digested with 1 U *Bsm*A1 restriction enzyme for 16 h at 55°C and visualized in silver stained 10% polyacrylamide gels. The wild-type allele was identified by the presence of a 176-bp size fragment. Variant alleles were restricted and yield two fragments of 91 and 85 bp. The presence of three fragments (176, 91 and 85 bp) indicated heterozigosity. *CYP17* genotypes were also determined by PCR-RFLP (McKean-Cowdin et al., 2001). PCR product digestion was carried for 16 h at 37°C with 1 U of *Msp*A1I restriction enzyme and products were visualized in silver stained 7% polyacrylamide gels. The wild-type allele was identified by a 414-bp size fragment. Variant alleles produced two fragments of 290 and 124 bp. The presence of three fragments (414, 290 and 124 bp) indicated heterozigosity. Microsatellite markers were analyzed by PCR in simplex reactions with a final volume of 15 µL and using cycling conditions specific for each primer pair. PCR mixtures containing 1X PCR buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4) of the Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 4-10 ng genomic DNA, 0.5-1.0 µM of each primer, 0.2 mM of each dinucleotide triphosphate, 1.5 mM $MgCl_2$, and 0.6 U Platinum® Taq DNA Polymerase (Invitrogen). Amplified fragments were resolved in 15% polyacrylamide gels at 160 V for 15 h and visualized after silver staining.

Statistical analysis

GSTP1 and *CYP17* polymorphism frequencies were analyzed using contingency tables to calculate odds ratio (OR) with a confidence interval (CI) of 95%. A Fisher exact test significance value of $P < 0.05$ was considered, using the EpiInfo version 3.5.1 software. Hardy-Weinberg equilibrium (HWE) was estimated using the Arlequin software, version 3.11 (Excoffier et al., 2005). Quantitative variables (mean age, median and standard deviation) were analyzed by SPSS version 17.0.

RESULTS

Histopathological characteristics of breast and ovarian tumors were summarized in Table 1. Mean and median ages for breast cancer were, respectively, 55.91 and 56.00 years (SD = 13.1; range 28 to 89 years); as for ovarian cancer, they were 55 and 54.5 years, respectively (SD = 13.8; range 30 to 84 years), whereas for healthy female controls, mean age was 31.8 and median age was 28 years (SD = 10.8; range 18 to 56).

Table 1. Clinical and histopathological features of breast and ovarian cancers.

	Characteristics	Total	
		N	(%)
Breast cancer	Histological type		
	Infiltrating ductal carcinoma	93	(86.9)
	Other carcinomas	14	(13.1)
	Histological grade[a]		
	Grade I	5	(5.4)
	Grade II	57	(61.3)
	Grade III	31	(33.3)
	ER status[a]		
	Negative	16	(17.2)
	Positive	77	(82.8)
	PR status[a]		
	Negative	25	(26.9)
	Positive	65	(69.9)
	Missing	3	(3.2)
	HER2 status[a]		
	Negative	88	(94.6)
	Positive	5	(5.4)
	Ki67 status[a]		
	Negative	0	(0.0)
	Positive	91	(97.8)
	Missing	2	(2.2)
	Age at diagnosis		
	≤45 years	22	(20.6)
	≥46 years	85	(79.4)
Ovarian cancer	Histological type		
	Serous cystadenocarcinoma	7	(29.2)
	Mucinous cystadenocarcinoma	1	(4.2)
	Other malignant	7	(29.2)
	Metastatic tumor	5	(20.8)
	Borderline tumor	4	(16.7)
	Age at diagnosis		
	≤50 years	9	(37.5)
	≥51 years	15	(62.5)

[a]Infiltrating ductal carcinomas only.

Regarding ethnicity, breast cancer patients were considered 22.4% (24/107) caucasoids and 56.1% (60/107) mulattos, reflecting the mixed ethnicity present in this state (Perrone and Moreira, 2003). For ovarian tumor patients and healthy controls, this information was not possible to collect.

GSTP1 and *CYP17* genotypic and allelic distribution in breast/ovarian cases and controls were summarized in Table 2. We did not find any significant increase of breast and ovarian cancer risk associated with genotypes and/or alleles of these genes.

Healthy control genotypic frequencies were in HWE. Associations between breast and ovarian cancer clinicopathological characteristics with *GSTP1* and *CYP17* genotypic frequencies were shown in Tables 3 and 4. We found a significant statistical association between the *CYP17 A2/A2* genotype and PR-positive breast cancer (P = 0.049). Histological

type and grade, ER and HER2 status and age at diagnosis of breast cancer did not show significant correlations with genotypes. The *CYP17 A2/A2* genotype was significantly more present in the ovarian cancer age group ≤50 years (P = 0.027).

Table 2. *GSTP1* and *CYP17* genotypic and allelic frequencies.

	Controls		Breast cases			Ovarian cases		
GSTP1								
Genotype	N = 59	% (95%CI)	N = 106	% (95%CI)	OR (95%CI)	N = 21	% (95%CI)	OR (95%CI)
Ile/Ile	19	32.2 (20.3-44.1)	45	42.5 (33.1-51.9)	1.0	10	47.6 (26.3-69.0)	1.0
Ile/Val	29	49.2 (36.4-61.9)	49	46.2 (36.7-55.7)	0.71 (0.33-1.53)	9	42.9 (21.6-64.1)	0.59 (0.18-1.95)
Val/Val	11	18.6 (8.7-28.6)	12	11.3 (5.3-17.3)	0.46 (0.15-1.36)	2	9.5 (0.0-22.1)	0.35 (0.04-2.22)
Ile/Val+Val/Val	40	67.8 (55.9-79.7)	61	57.5 (48.1-66.9)	0.64 (0.31-1.32)	11	52.4 (31.0-73.8)	0.52 (0.17-1.62)
Allele	N = 118		N = 212			N = 42		
Ile	67	56.8 (47.8-65.7)	139	65.6 (59.2-72.0)	1.0	29	69.0 (55.1-83.0)	1.0
Val	51	43.2 (34.3-52.2)	73	34.4 (28.0-40.8)	0.69 (0.42-1.12)	13	31.0 (17.0-44.9)	0.59 (0.26-1.32)
CYP17								
Genotype	N = 61		N = 69			N = 12		
A1/A1	21	34.4 (22.5-46.3)	26	37.7 (26.3-49.1)	1.0	4	33.3 (6.6-60.0)	1.0
A1/A2	31	50.8 (38.3-63.3)	36	52.2 (40.4-64.0)	0.94 (0.41-2.12)	5	41.7 (13.8-69.6)	0.85 (0.17-4.35)
A2/A2	9	14.8 (5.8-23.6)	7	10.1 (3.0-17.2)	0.63 (0.17-2.26)	3	25.0 (0.5-49.5)	1.75 (0.24-12.46)
A1/A2 + A2/A2	40	65.6 (53.7-77.5)	43	62.3 (50.9-73.7)	0.87 (0.40-1.89)	8	66.7 (40.0-93.4)	1.05 (0.24-4.76)
Allele	N = 122		N = 138			N = 24		
A1	73	59.8 (51.1-68.5)	88	63.8 (55.8-71.8)	1.0	13	54.2 (34.3-74.1)	1.0
A2	49	40.2 (31.5-48.9)	50	36.2 (28.2-44.2)	0.85 (0.50-1.44)	11	45.8 (25.9-65.7)	1.26 (0.48-3.31)

Table 3. *GSTP1* and *CYP17* genotype distribution according to breast tumor features.

	GSTP1			P	*CYP17*			P
	Ile/Ile	*Ile/Val*	*Val/Val*		*A1/A1*	*A1/A2*	*A2/A2*	
Histological type	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
IDC[a]	35 (38.0)	46 (50.0)	11 (12.0)	0.061	22 (36.7)	31 (51.7)	7 (11.7)	0.549
Other carcinomas	10 (71.5)	3 (21.5)	1 (7.0)		4 (44.4)	5 (55.6)	0 (0.0)	
Grade								
I	3 (60.0)	3 (20.0)	1 (20.0)	0.517	1 (33.0)	2 (67.0)	0 (0.0)	0.709
II	20 (35.0)	30 (53.0)	7 (12.0)		11 (30.0)	19 (53.0)	6 (17.0)	
III	12 (40.0)	15 (50.0)	3 (10.0)		10 (48.0)	10 (48)	1(4.0)	
ER status								
ER+	26 (34.0)	40 (53.0)	10 (13.0)	0.244	17 (35.0)	25 (52.0)	6 (13.0)	0.881
ER-	9 (56.0)	6 (38.0)	1 (6.0)		5 (42.0)	6 (50.0)	1 (8.0)	
PR status								
PR+	25 (39.0)	33 (52.0)	6 (9.0)	0.388	17 (41.5)	17 (41.5)	7 (17.0)	0.049
PR-	9 (36.0)	11 (44.0)	5 (20.0)		5 (28.0)	13 (72.0)	0 (0.0)	
HER2 status								
HER+	2 (40.0)	3 (60.0)	0 (0.0)	0.690	3 (75.0)	0 (0.0)	1 (25.0)	0.101
HER-	33 (38.0)	43 (49.0)	11 (13.0)		19 (34.0)	31 (55.0)	6 (11.0)	
Age at diagnosis								
≤45 years	8 (36.0)	12 (55.0)	2 (9.0)	0.667	6 (43.0)	7 (50.0)	1 (7.0)	0.862
≥46 years	37 (44.0)	37 (44.0)	10 (12.0)		20 (36.0)	29 (53.0)	6 (11.0)	

[a]Infiltrating ductal carcinoma.

Table 4. *GSTP1* and *CYP17* genotype distribution according to ovarian tumor features.

	GSTP1 genotypes			P	*CYP17* genotypes			P
	Ile/Ile	*Ile/Val*	*Val/Val*		*A1/A1*	*A1/A2*	*A2/A2*	
Histologic type	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
Ser cystadenoc[a]	2 (29.0)	4 (57.0)	1 (14.0)	0.346	1 (25.0)	3 (75.0)	0 (0.0)	0.198
Muc cystadenoc[b]	1 (100.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	
Others malignant	2 (40.0)	2 (40.0)	1 (20.0)		2 (67.0)	1 (33.0)	0 (0.0)	
Metastatic tumors	4 (100.0)	0 (0.0)	0 (0.0)		1 (50.0)	0 (0.0)	1 (50.0)	
Borderline tumor	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	1 (33.0)	2 (67.0)	
Age at diagnosis								
≤50 years	3 (43.0)	4 (57.0)	0 (0.0)	0.461	0 (0.0)	0 (0.0)	2 (100.0)	0.027
≥51 years	7 (50.0)	5 (36.0)	2 (14.0)		4 (40.0)	5 (50.0)	1 (10.0)	

[a]Serous cystadenocarcinoma. [b]Mucinous cystadenocarcinoma.

To further investigate if *GSTP1* and *CYP17* might be related with breast and ovarian cancer, we analyzed their association with polymorphisms in steroid metabolism genes *AR*, *ERβ* and *CYP19*. Initially, we analyzed the combination of *GSTP1* with MSI/LOH in each gene individually. Later on, we performed a combinatorial analysis of *GSTP1* genotypes with MSI/LOH in *AR+ERβ*, *AR+CYP19* or *ERβ+CYP19*. Finally, we analyzed the association of *GSTP1* genotypes with MSI/LOH in *AR+ERβ+CYP19* all together. The same analyses were performed for *CYP17*. Associations of *GSTP1* genotypes and MSI/LOH were shown in Tables 5 and 6. The *Ile/Val* genotype showed a correlation with *ERβ* MSI/LOH in ER-negative breast cancers (P = 0.028), as well as the *Ile/Ile* genotype with *AR+CYP19* (P = 0.021) and *AR+ERβ+CYP19* (P = 0.036) MSI/LOH in PR-negative breast cancers. *CYP17 A1/A1* genotype was associated with *AR+ERβ* and *AR+ERβ+CYP19* MSI/LOH in ER- or PR-negative breast tumors (P = 0.039). No associations were detected for ovarian tumors (data not shown).

Table 5. *GSTP1* and *CYP17* association with MSI and LOH in *AR*, *ERβ* and *CYP19* genes, stratified by ER/PR status in breast cancer.

GSTP1 vs AR								*CYP17 vs AR*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	0/2	0.077	und[b]	0/2	0.082	und[b]	*A1/A1*	+	0/1	0.238	und[b]	0/1	0.238	und[b]
	-	22/7			21/7				-	16/4			16/4		
Ile/Val	+	1/0	0.900	0.0 (0.0-200.0)	1/0	0.790	0.0 (0.0-72.9)	*A1/A2*	+	0/1	0.185	und[b]	0/1	0.423	und[b]
	-	35/4			29/8				-	22/4			15/10		
Val/Val	+	0/1	0.111	und[b]	0/1	0.555	und[b]	*A2/A2*	+	0/0	und[b]	und[b]	0/0	und[b]	und[b]
	-	8/0			4/4				-	5/1					
GSTP1 vs ERβ								*CYP17 vs ERβ*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	0/1	0.381	und[b]	0/1	0.350	und[b]	*A1/A1*	+	0/1	0.210	und[b]	0/1	0.210	und[b]
	-	13/7			13/6				-	15/3			15/3		
Ile/Val	+	0/2	0.028	und[b]	0/2	0.097	und[b]	*A1/A2*	+	0/1	0.250	und[b]	0/1	0.521	und[b]
	-	27/4			21/8				-	18/5			11/11		
Val/Val	+	0/0	und[b]	und[b]	0/0	und[b]	und[b]	*A2/A2*	+	0/0	und[b]	und[b]	0/1	und[b]	und[b]
	-	4/0			3/1				-	4/1			5/0		
GSTP1 vs CYP19								*CYP17 vs CYP19*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	1/0	0.727	0.0 (0.0-51.0)	0/1	0.281	und	*A1/A1*	+	0/0	und[b]	und[b]	0/0	und[b]	und[b]
	-	23/9			23/8				-	17/5			17/5		
Ile/Val	+	1/1	0.251	7.60 (0.0-346.2)	1/1	0.451	3.1 (0.0-127.9)	*A1/A2*	+	1/0	0.800	0.0 (0.0-81.1)	0/1	0.448	und[b]
	-	38/5			31/10				-	23/6			16/12		
Val/Val	+	0/1	0.111	und[b]	0/1	0.555	und[b]	*A2/A2*	+	1/0	0.857	0.0 (0.0-636.3)	1/0	und [b]	und[b]
	-	8/0			4/4				-	5/1			6/0		

[a]MSI+LOH. [b]Undetermined.

Table 6. *GST1* and *CYP17* association with *AR*, *ERβ* and *CYP19*, stratified by ER/PR status in breast cancer.

GSTP1 vs AR + ERβ								*CYP17 vs AR + ERβ*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	0/2	0.147	und[b]	0/2	0.122	und[b]	*A1/A1*	+	0/2	0.039	und[b]	0/2	0.039	und[b]
	-	12/6			12/5				-	14/2			14/2		
Ile/Val	+	1/2	0.060	16.7 (0.8-686.7)	1/2	0.176	6.7 (0.4-228.1)	*A1/A2*	+	0/1	0.238	und[b]	0/1	0.500	und[b]
	-	25/3			20/6				-	16/4			10/9		
Val/Val	+	0/1	0.200	und[b]	0/1	0.400	und[b]	*A2/A2*	+	0/0	und[b]	und[b]	0/0	und[b]	und[b]
	-	4/0			3/1				-	4/1			5/0		
GSTP1 vs AR + CYP19								*CYP17 vs AR + CYP19*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	1/2	0.195	6.0 (0.3-199.8)	0/3	0.021	und[b]	*A1/A1*	+	0/1	0.238	und[b]	0/1	0.238	und[b]
	-	21/7			21/6				-	16/4			16/4		
Ile/Val	+	2/1	0.337	4.1 (0.0-89.8)	2/1	0.567	1.69 (0.0-29.8)	*A1/A2*	+	1/1	0.342	5.3 (0.0-257.8)	0/2	0.169	und[b]
	-	33/4			27/8				-	21/4			15/9		
Val/Val	+	0/1	0.111	und[b]	0/1	0.555	und[b]	*A2/A2*	+	1/0	0.833	0.0 (0.0-527.4)	1/0	und[b]	und[b]
	-	8/0			4/4				-	4/1			5/0		
GSTP1 vs ERβ + CYP19								*CYP17 vs ERβ + CYP19*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	1/1	0.653	1.6 (0.0-71.2)	0/2	0.123	und[b]	*A1/A1*	+	0/1	0.210	und[b]	0/1	0.210	und[b]
	-	11/7			12/5				-	15/3			15/3		
Ile/Val	+	1/2	0.083	12.5 (0.6-465.2)	1/2	0.251	4.8 (0.3-156.0)	*A1/A2*	+	1/1	0.462	3.2 (0.0-150.4)	0/2	0.286	und[b]
	-	25/4			19/8				-	16/5			10/10		
Val/Val	+	0/1	0.200	und[b]	0/1	0.400	und[b]	*A2/A2*	+	1/0	0.800	0.0 (0.0-418.5)	1/0	und[b]	und[b]
	-	4/0			3/1				-	3/1			4/0		
GSTP1 vs AR + ERβ + CYP19								*CYP17 vs AR + ERβ + CYP19*							
Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)	Genotype	M/L[a]	ER+/ER-	P	OR (95%CI)	PR+/PR-	P	OR (95%CI)
Ile/Ile	+	1/2	0.344	3.7 (0.2-129.9)	0/3	0.036	und[b]	*A1/A1*	+	0/2	0.039	und[b]	0/2	0.039	und[b]
	-	11/6			12/4				-	14/2			14/2		
Ile/Val	+	2/2	0.119	7.7 (0.5-143.0)	2/2	0.318	3.0 (0.2-41.3)	*A1/A2*	+	1/1	0.428	3.8 (0.0-187.2)	0/2	0.237	und[b]
	-	23/3			18/6				-	15/4			10/8		
Val/Val	+	0/1	0.200	und[b]	0/1	0.400	und[b]	*A2/A2*	+	1/0	0.800	0.0 (0.0-418.5)	1/0	und[b]	und[b]
	-	4/0			3/1				-	3/1			4/0		

[a]MSI+LOH. [b]Undetermined.

DISCUSSION

There is substantial evidence that steroid hormones play an important role in the etiology of breast and ovarian cancer (Lurie et al., 2009; Dumas and Diorio, 2011). Therefore, there has been recent interest in steroid metabolism genes, because the activity of these gene products may affect long-term estrogen levels and its potentially carcinogenic metabolites, influencing breast and ovarian cancer risk (Garner et al., 2002).

In most studies, estrogen biosynthesis and metabolism gene polymorphisms are considered separately (Ramalhinho et al., 2012), finding a small effect of low-penetrance breast and ovarian cancer risk gene polymorphisms (Zhang et al., 2009). In our study, we have analyzed SNPs in two low-penetrance genes and their association with MSI/LOH in steroid metabolism genes. Specific associations between gene polymorphisms and MSI/LOH could result in high-risk profiles, by influencing lifetime estrogen levels and therefore breast and ovarian cancer risk (Ramalhinho et al., 2012).

We performed a case-control study of Brazilian women to investigate the association of *GSTP1* and *CYP17* gene polymorphisms with breast and ovarian cancer, as well as with MSI/LOH in three genes implicated in steroid metabolism (*AR*, *ERβ* and *CYP19*). When tested alone, the *GSTP1* gene showed no significant association with breast and ovarian cancer risk. Most previous studies on the potential association of *GSTP1* polymorphisms and breast and ovarian cancer risk produced inconsistent results. Spurdle et al. (2001), Delort et al. (2008) and Ramalhinho et al. (2011) showed results similar to ours, whereas an association between *GSTP1* genotypes and breast cancer risk was observed by Torresan et al. (2008) in Euro-descendent women from Southern Brazil and by Antognelli et al. (2009), which found that the frequency of the *Val* allele was significantly lower in the breast cancer population. Inconsistencies may be partly due to differences in populations and their exposures to different environmental risk factors (Torresan et al., 2008).

A combinatorial analysis of *GSTP1* genotypes and MSI/LOH in *AR*, *ERβ* or *CYP19* enabled us to detect that the *Ile/Ile* genotype is related to MSI/LOH in *AR+CYP19* or *AR+ERβ+CYP19* in PR-negative breast tumors (Table 6), suggesting that this genotype when combined with MSI/LOH may be associated with poor prognosis of breast cancer.

According to Ramalhinho et al. (2011), the *GSTP1 Ile/Ile* genotype increases breast cancer risk when associated with *GSTM1/GSTT1* null genotypes, suggesting that the *Val* allele tends to act as a protective, rather than a risk factor.

Regarding *CYP17* polymorphism distribution in breast and ovarian tumors, there are also controversial evidences. Early reports suggested an association of *CYP17 A2* with an increased breast cancer risk; however, subsequent studies failed to confirm this association (Garner et al., 2002; Miyoshi and Noguchi, 2003). As for ovarian cancer risk, Goodman et al. (2001) and Spurdle et al. (2001) reported no evidence for an association between *CYP17* polymorphisms and ovarian cancer risk, while Garner et al. (2002) noted an increased risk for ovarian cancer in *A2* variant women >50 years.

Our results did not show a significant association between *CYP17* polymorphisms and breast and ovarian cancer risk. However, when we analyzed the relation between *CYP17* genotypes and histopatological characteristics of breast tumors, a statistically significant frequency of the *A2/A2* genotype in patients with PR-positive breast cancers was found (Table 3). For ovarian cancer, a significant frequency of the *A2/A2* genotype was observed in the age at diagnostic group ≤50 years (Table 4).

Because the *A2* variant allele creates an additional putative Sp-1 binding site (CCACC) in the *CYP17* promoter region, it is speculated that the *C* allele enhances gene transcription, leading to increased estrogen synthesis in breast and ovarian tumors (Garner et al., 2002; Zhang et al., 2009).

A combinatorial analysis of *CYP17* genotypes with MSI/LOH in *AR*, *ERβ* or *CYP19* genes allowed for the detection of a correlation between the *A1/A1* genotype and a higher frequency of MSI/LOH in *AR+ERβ* or *AR+ERβ+CYP19* in ER-negative and PR-negative tumors (Table 6). Similarly, the *CYP17* wild-type genotype when combined with MSI/LOH was associated with poor prognosis of breast cancer.

AR, *ERβ* and *CYP19* genes participate in the androgen hormone pathway. The androgen receptor (*AR*) has a polymorphic polyglutamine repeat (CAG repeat) in the amino-terminal domain while the *ERβ* gene contains a polymorphic dinucleotide CA repeat in the non-coding 3'-portion of the gene. Polymorphic repeats appear to influence function and alter androgen serum levels in premenopausal women (Westberg et al., 2001), because sex hormone receptors (*AR* and *ERβ*) mediate hormone response at breast tissue level, having a possible pathological role in breast cancer development (Anghel et al., 2006). *CYP19* (aromatase) is involved in the last stage of androgen to estrogen conversion (androstenedione into estrone and testosterone into estradiol) (Miyoshi and Noguchi, 2003; Zhang et al., 2009) and its activity helps determines local estrogen levels. MSI in intron 5 polymorphic tetranucleotide repeat has been observed in human breast, ovary, soft tissue and brain carcinomas. This aromatase gene region may be involved in splice site determination (Kristensen and Borresen-Dale, 2000; Huber et al., 2002).

To our knowledge, this is the first study to analyze the potential role of *GSTP1* and *CYP17* genotypes in combination with *AR*, *ERβ* and *CYP19* MSI/LOH in Brazilian women with breast or ovarian tumors. We observed that wild-type *GSTP1* and *CYP17* genotypes when combined with MSI/LOH in steroid metabolism genes is associated with ER-negative or PR-negative breast cancers. These results support the hypothesis that estrogen metabolism genes can be helpful in the characterization of breast cancer prognosis (Ramalhinho et al., 2012).

Conflicts of interest

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Research partly supported by Fibria Celulose. E.V.W. dos Santos was supported by a CNPq doctorate scholarship. L.N.R. Alves was supported by a CNPq scholarship.

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