

# Association of *CYP1A1* A4889G and T6235C polymorphisms with the risk of sporadic breast cancer in Brazilian women

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**OBJECTIVES:** We examined the influence of *CYP1A1* A4889G and T6235C polymorphisms on the risk of sporadic breast cancer.

**METHODS:** DNA from 742 sporadic breast cancer patients and 742 controls was analyzed using the polymerase chain reaction, followed by the restriction fragment length polymorphism technique.

**RESULTS:** More patients had the *CYP1A1* 4889AG+GG genotype compared to controls (29.0% versus 23.2%,  $p=0.004$ ). The G allele carriers had a 1.50-fold increased risk (95% CI: 1.14–1.97) of sporadic breast cancer compared to the other study participants. The frequency of the 4889AG+GG genotype among the Caucasian patients was higher than in the non-Caucasian patients (30.4% versus 20.2%,  $p=0.03$ ) and controls (30.4% versus 23.2%,  $p=0.002$ ). Caucasians and G allele carriers had a 1.61-fold increased risk (95% CI: 1.20–2.15) of sporadic breast cancer compared to other subjects. The *CYP1A1* 4889AG+GG genotype was more common among patients with a younger median age at first full-term pregnancy than among controls (33.8% versus 23.2%,  $p=0.001$ ) and subjects whose first full-term pregnancies occurred at an older age (33.8% versus 26.1%,  $p=0.03$ ). Women with the *CYP1A1* 4889AG+GG genotype and earlier first full-term pregnancies had a 1.87-fold (95% CI: 1.32–2.67) increased risk of sporadic breast cancer compared to the other study participants. Excess *CYP1A1* 4889AG+GG (39.8% versus 27.1%,  $p=0.01$ ) and 6235TC+CC (48.4% versus 35.9%,  $p=0.02$ ) genotypes were also observed in patients with grade I and II tumors compared to patients with grade III tumors and controls (39.8% versus 23.2%,  $p=0.04$ ; 48.4% versus 38.6%,  $p=0.04$ ). The G and C allele carriers had a 2.44-fold (95% CI: 1.48–4.02) and 1.67-fold (95% CI: 1.03–2.69) increased risk, respectively, of developing grade I and II tumors compared to other subjects.

**CONCLUSIONS:** The *CYP1A1* A4889G and T6235C polymorphisms may alter the risk of sporadic breast cancer in Brazilian women.

**KEYWORDS:** Sporadic Breast Cancer; *CYP1A1*; Genetic Polymorphism; Risk.

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## INTRODUCTION

High-penetrance mutations account for a small proportion of breast cancer (BC) cases (1) and several epidemiological studies have indicated the involvement of low-penetrance genetic polymorphisms in the pathophysiology of BC (2). In addition to these genetic factors, compelling evidence

supports the role of systemic hypertension, tobacco, obesity and exposure to polycyclic aromatic hydrocarbons (PAHs) in BC formation through the promotion of DNA damage and cell proliferation (3-6). Prolonged estrogen exposure is also widely accepted as a risk factor for BC due to its mitotic activity and increased mutation rates (7) and catechol estrogens can be carcinogen metabolites (8,9). Hence, some reproductive events, such as age at menarche, first-full term pregnancy (FFTP) and menopause, may play important roles in BC risk (10,11). Carriers of low-penetrance gene mutations, combined with the above-mentioned factors, might have a higher tumor risk (12,13).

The *CYP1A1* gene (14) encodes the P450-1A1 (*CYP1A1*) enzyme, which has aryl hydrocarbon hydroxylase activity. This enzyme converts PAHs to aryl epoxides carcinogens.

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The enzyme also participates in estrogen metabolism by catalyzing the 2-hydroxylation of estradiol, which results in free radical and DNA adduct production (15). Two polymorphisms in this gene have been studied in relation to BC: an A4889G substitution in exon 7 (rs1048943) and a T6235C substitution in the 3' untranslated region (rs4646903) (16). G and C allele variants yield enzymes with increased activities in PAHs activation (17) and estrogen metabolism (18). However, the role of these variants in BC risk in individuals from the United States of America, Europe and Asia is still controversial. Some studies have reported that G and C allele variants increase BC risk (5,19-29), while other studies have not observed main effects for *CYP1A1* variants (3,5,19-22,25,27-37). Regarding the clinical features and biological characteristics of tumors, the impact of these genetic polymorphisms varies (3,5,10,12,15,19-23,27,29-40). These contradictory findings might be explained by the ethnic variations among populations and small study sample sizes (41).

The Brazilian population is highly heterogeneous and consists of indigenous Amerindians and immigrants from Europe, Asia and Africa (42). Cancer is the second most common cause of death in the southeastern region of Brazil and BC represents the most common cause of tumor death among women (43). To the best of our knowledge, the role of *CYP1A1* A4889G and T6235C polymorphisms in the risk, clinical features and biological characteristics of BC tumors is unknown for people living in the Southern hemisphere. Because large numbers of participants are needed worldwide to examine the associations between a genetic polymorphism and cancer risk, we analyzed the frequencies of the *CYP1A1* A4889G and T6235C genotypes in large samples of BC patients and controls from the southeastern region of Brazil. We also investigated whether these genotypes influence the risk of BC tumors in Brazil.

## ■ MATERIALS AND METHODS

### Study population

We analyzed 742 consecutive female patients with sporadic breast cancer (SBC) at diagnosis (median age, 53 years; range, 22–91 years; 638 Caucasians and 104 non-Caucasians). The patients were treated at the Center of Integral Attention to Women's Health of the University of Campinas, Campinas/SP, Brazil, from March 2004 to March 2007. The clinical features were determined from a questionnaire, including age at diagnosis, ethnic origin, age at menarche, age at FFTP and age at menopause, lactation status, smoking habits, oral contraceptives use and presence or absence of systemic hypertension. The patients were considered to be smokers if they kept smoking until the moment of diagnosis and the patients were classified as non-smokers if they had never smoked. The body mass index (BMI) was calculated according to the World Health Organization classification and the patients were classified into four groups: underweight, normal, pre-obese and obese (44). BC was diagnosed through the histological evaluation of tumor biopsies. The nuclear and histological grades (45) and tumor stages (46) were established according to conventional criteria. The expression levels of estrogen and progesterone receptors were analyzed through immunohistochemistry using the streptavidin-biotin complex method (47) with estrogen receptor-clone 6F11 and progesterone receptor-clone 6F16 antibodies (Novocastra Laboratories, Tyne and Wear, Newcastle, UK).

The control group was composed of 742 healthy female blood donors (median age, 40 years; range, 18–65 years; 638 Caucasians and 104 non-Caucasians) from the Centro de Hematologia e Hemoterapia of the Universidade de Campinas (during the same study period). These patients were selected to represent the general population seeking medical assistance in our region. The control group patients were matched with the BC patients according to age and ethnicity. Patients and controls with evidence of a personal or family history of BC and patients who chose not to participate in the study were excluded from the analyses. All procedures were performed according to the Declaration of Helsinki and all subjects provided written informed consent. The study was approved by the local ethics committee (process number 581/2002).

### Polymorphism analysis

The *CYP1A1* A4889G (rs1048943) and T6235C (rs4646903) genotypes were identified in genomic DNA from the peripheral blood samples of subjects using the polymerase chain reaction followed by restriction fragment length polymorphism, as previously reported (48).

### Statistical analyses

The Hardy-Weinberg (HW) equilibrium was tested using chi-squared ( $\chi^2$ ) statistics for the goodness-of-fit (one degree of freedom). The pairwise linkage disequilibrium (LD) analysis was performed using Haploview 4.2 software ([www.broad.mit.edu/mpg/haploview](http://www.broad.mit.edu/mpg/haploview)) to ensure that the markers were appropriate for inclusion in the haplotype estimates. The LD was measured using the disequilibrium coefficient ( $D'$ ) and strong LD significance was considered as  $D' \geq 80\%$ . Between-group differences were analyzed with  $\chi^2$  or Fisher exact tests. A multivariate analysis using the logistic regression model was used to obtain the age and ethnic origin adjusted crude odds ratios (ORs) with 95% confidence intervals (CI) and to assess the associations between the genotypes and BC. A power of analysis (PA) was used to calculate the minimum effect size likely to be detected in a study using a given sample size, according to Pocock (49) and Hulley et al. (50) in analyses involving patients and controls and according to the DSS Research statistical power calculators (51) when considering the patients stratified by clinical and tumor characteristics. The data analyses were performed with SPSS 15.0 software (SPSS Incorporation, Chicago, IL, USA). For all statistical tests, the level of significance was 2-sided at  $p < 0.05$ .

## ■ RESULTS

The patient and control samples were in HW equilibrium at the *CYP1A1* A4889G ( $\chi^2=0.15$ ,  $p=0.70$ ;  $\chi^2=1.15$ ,  $p=0.28$ ) and T6235C ( $\chi^2=2.65$ ,  $p=0.10$ ;  $\chi^2=1.93$ ,  $p=0.16$ ) loci, respectively. The LD analyses revealed a moderate LD between the A4889G and T6235C polymorphisms of *CYP1A1* ( $D'=73\%$ ), and the referred polymorphisms were considered for further analysis of the haplotypes.

More patients had the *CYP1A1* 4889AG (PA=87.0%) and *CYP1A1* 4889AG+GG (PA=93.0%) genotypes compared with the controls. Compared with the other study participants, carriers of the *CYP1A1* 4889AG genotype had a 1.45-fold increased risk of SBC and carriers of the *CYP1A1* 4889AG + GG genotypes had a 1.50-fold increased risk. Similar frequencies of the *CYP1A1* T6235C genotypes and *CYP1A1* haplotypes were



observed in the patients and controls enrolled in the study (Table 1).

The CYP1A1 4889AG and 4889AG+GG genotypes were more common in the Caucasian SBC patients than in the non-Caucasian patients (PA=73.0% and PA=57.3%, respectively) and controls (27.9% versus 21.1%,  $p=0.002$ , PA=98% and 30.4% versus 23.2%,  $p=0.002$ , PA=98%, respectively). Caucasians with the CYP1A1 4889AG and 4889AG+GG genotypes had a 1.61-fold and 1.61-fold increased risk of tumors, respectively, than Caucasians with the remaining genotypes, respectively. An excess of the CYP1A1 6235CC genotype was observed in SBC patients with a lower median age of menarche (younger than 13 years of age) compared to patients who experienced menarche at a higher median age (13 years of age and older, PA=55.3%). The CYP1A1 4889AG+GG genotype was more common among patients with a lower median age at FFTP (younger than 22 years of age) than among patients who experienced FFTP at a higher median age (22 years of age and older) PA=58.6%). The frequency of the CYP1A1 4889AG+GG genotype was higher among patients with an earlier FFTP than among the controls (33.8% versus 23.2%,  $p=0.001$ , PA>99.0%). Women with the CYP1A1 4889AG+GG genotype and FFTP occurring earlier in life had a 1.87-fold increased risk of SBC compared to subjects with the AA genotype. The frequency of the CYP1A1 6235CC genotype in pre-obese and obese patients was higher than in normal and underweight patients (PA=32.1%). Moreover, the CYP1A1 4889AG (PA=77.1%), CYP1A1 4889AG+GG (PA=69.3%) and CYP1A1 6235TC+CC (PA=63.0%) genotypes were more common among patients with I and II histological grade tumors than among patients with grade III tumors. The frequencies of the respective genotypes in patients from the first group were also higher than those observed in the controls (37.6% versus 21.2%,  $p<0.001$ , PA>99.0%; 39.8% versus 23.2%,  $p<0.001$ , PA>99.0%; 48.4% versus 38.6%,  $p=0.04$ , PA=95.0%). Individuals with the CYP1A1 4889AG, CYP1A1AG+GG, and CYP1A1 6235TC+CC genotypes had 2.48-, 2.44- and 1.67-fold

increased risks, respectively, for SBC compared to study participants with the remaining genotypes (Table 2). Similar frequencies of the CYP1A1 A4889G and T6235C genotypes were observed among patients stratified by age at diagnosis, age at menarche (younger than 12 years versus 12 years and older), age at menopause and FFTP (younger than 30 years versus 30 years and older), oral contraceptive use, lactation, cigarette smoking habit, systemic hypertension, tumor histology, stage and estrogen and progesterone receptor patterns. The frequencies of the CYP1A1 haplotypes did not differ among patients stratified by clinical and tumor features (data not shown).

## DISCUSSION

In this case-control study, we examined the influence of the CYP1A1 A4889G and T6235C polymorphisms on the risk of SBC. We also investigated whether these polymorphisms are associated with the clinical features of SBC patients and with the biological tumor characteristics.

We initially found that the CYP1A1 4889AG and 4889AG+GG genotypes were associated with a higher risk of BC in our cohort. Our results were consistent with the findings of Chacko et al. (23), Singh et al. (36) and Surekha et al. (27) (studies conducted in India). Ambrosone et al. (19) and Ishibe et al. (5) also reported an increased risk of BC among G variant allele carriers (with smoking habits) from the United States of America. In fact, cytochrome P450 enzymes encoded by the CYP1A1 gene play an important role in the phase I bioactivation of xenobiotics and in the metabolism of estrogen (converting the metabolites into carcinogens) (15). G variant allele carriers of the CYP1A1 A4889G polymorphism have a higher catalytic enzyme activity (17,18), which results in a higher level of carcinogens in breast tissue and, consequently, a higher level of DNA adducts, which may serve as the initial step in BC development. In contrast with our study, the CYP1A1 4889AG genotype was associated

**Table 1 - CYP1A1 genotypes among sporadic breast cancer patients and controls.**

Genotypes	Patients Number (%)	Controls Number (%)	p-value	OR* (95% CI <sup>a</sup> )
<b>A4889G</b>				
AA	527 (71.0)	570 (76.8)		reference
AG	195 (26.3)	157 (21.2)	<b>0.01</b>	<b>1.45 (1.10-1.93)</b>
GG	20 (2.7)	15 (2.0)	0.09	2.05 (0.89-4.71)
AA	527 (71.0)	570 (76.8)		reference
AG+GG	215 (29.0)	172 (23.2)	<b>0.004</b>	<b>1.50 (1.14-1.97)</b>
AA+AG	722 (97.3)	727 (98.0)		reference
GG	20 (2.7)	15 (2.0)	0.14	1.84 (0.81-4.20)
<b>T6235C</b>				
TT	464 (62.5)	456 (61.5)		reference
TC	236 (31.8)	243 (32.7)	1.00	1.00 (0.77-1.30)
CC	42 (5.7)	43 (5.8)	0.74	1.09 (0.65-1.83)
TT	464 (62.5)	456 (61.4)		reference
TC+CC	278 (37.5)	286 (38.6)	0.90	1.01 (0.79-1.30)
TT+TC	700 (94.3)	699 (94.2)		reference
CC	42(5.7)	43 (5.8)	0.70	1.10 (0.66-1.83)
<b>A4889G/T6235C</b>				
AA+TT	413 (74.5)	438 (76.3)		reference
AG+TC	128 (23.1)	123 (21.4)	0.18	1.25 (0.90-1.74)
GG+CC	13 (2.4)	13 (2.3)	0.34	1.59 (0.61-4.15)
AA+TT	413 (71.6)	438 (74.0)		reference
AG+GG+TC+CC	164 (28.4)	154 (26.0)	0.14	1.26 (0.93-1.70)
AA+AG+TT+TC	693 (98.1)	697 (98.2)		reference
GG+CC	13 (1.9)	13 (1.8)	0.44	1.45 (0.56-3.74)

\*: Adjusted odds ratio for age and ethnic origin; <sup>a</sup>: confidence interval. The significant results are shown in a bold font.

**Table 2** - CYP1A1 genotypes among sporadic breast cancer patients stratified by the clinical features and biological aspects of the tumor

Features	No <sup>a</sup>	CYP1A1 A4889G polymorphism						
		AA	AG	GG	AA + AG	GG	AA	AG + GG
<b>Ethnical origin</b>	742							
Caucasian	638	444 (69.6)	<b>178 (27.9)</b>	16 (2.5)	622 (97.5)	16 (2.5)	<b>444 (69.6)</b>	<b>194 (30.4)</b>
Non-Caucasian	104	83 (79.8)	<b>17 (16.3)</b>	4 (3.9)	100 (96.1)	4 (3.9)	<b>83 (79.8)</b>	<b>21 (20.2)</b>
p-value			<b>0.01</b>	0.64	0.47		<b>0.03</b>	
<b>MAM<sup>b</sup></b>	693 <sup>c</sup>							
younger than 13 years	298	202 (67.8)	89 (29.9)	7 (2.3)	291 (97.7)	7 (2.3)	202 (67.8)	96 (32.2)
13 years and older	395	289 (73.1)	93 (23.5)	13 (3.4)	382 (96.6)	13 (3.4)	289 (73.1)	106 (26.9)
p-value			0.12	0.58	0.48		0.19	
<b>MAFFTP<sup>d</sup></b>	692 <sup>c</sup>							
younger than 22 years	275	182 (66.2)	82 (29.8)	11 (4.0)	264 (96.0)	11 (4.0)	<b>182 (66.2)</b>	<b>93 (33.8)</b>
22 years and older	417	306 (73.4)	103 (24.7)	8 (1.9)	409 (98.1)	8 (1.9)	<b>306 (73.4)</b>	<b>111 (26.1)</b>
p-value			0.08	0.08	0.11		<b>0.03</b>	
<b>Body mass index</b>	708 <sup>c</sup>							
Under + normal	253	185 (73.1)	63 (24.9)	5 (2.0)	248 (98.0)	5 (2.0)	185 (73.1)	68 (26.9)
Pre-obese + obese	455	316 (69.5)	125 (27.5)	14 (3.0)	441 (97.0)	14 (3.0)	306 (69.5)	139 (30.5)
p-value			0.29	0.29	0.33		0.21	
<b>Histological grade</b>	642 <sup>c</sup>							
I + II	93	<b>56 (60.2)</b>	<b>35 (37.6)</b>	2 (2.2)	91 (97.8)	2 (2.2)	<b>56 (60.2)</b>	<b>37 (39.8)</b>
III	549	<b>400 (72.9)</b>	<b>132 (24.0)</b>	17 (3.1)	532 (96.9)	17 (3.1)	<b>400 (72.9)</b>	<b>149 (27.1)</b>
p-value			<b>0.008</b>	0.83	0.62		<b>0.01</b>	
Features	No <sup>a</sup>	CYP1A1 T6235C polymorphism						
		TT	TC	CC	TT + TC	CC	TT	TC + CC
<b>Ethnical origin</b>	742							
Caucasian	638	403 (63.2)	202 (31.7)	33 (5.1)	605 (94.9)	33 (5.1)	403 (63.2)	235 (36.8)
Non-Caucasian	104	61 (58.7)	34 (32.7)	9 (8.6)	95 (91.4)	9 (8.6)	61 (58.7)	43 (41.3)
p-value			0.70	0.21	0.20		0.45	
<b>MAM<sup>b</sup></b>	693 <sup>c</sup>							
younger than 13 years	298	181 (60.7)	94 (31.6)	<b>23 (7.7)</b>	275 (92.3)	23 (7.7)	181 (60.7)	117 (39.3)
13 years and older	395	255 (64.6)	124 (31.4)	<b>16 (4.0)</b>	379 (96.0)	16 (4.0)	255 (64.6)	140 (35.4)
p-value			0.78	<b>0.04</b>	0.05		0.37	
<b>MAFFTP<sup>d</sup></b>	692 <sup>c</sup>							
younger than 22 years	275	165 (60.0)	94 (34.1)	16 (5.9)	259 (94.1)	16 (5.9)	165 (60.0)	110 (40.0)
22 years and older	417	263 (63.1)	129 (30.9)	25 (6.0)	392 (94.0)	25 (6.0)	263 (63.1)	154 (36.9)
p-value			0.39	0.93	0.87		0.44	
<b>Body mass index</b>	708 <sup>c</sup>							
Under + normal	253	159 (62.8)	84 (33.2)	10 (4.0)	<b>243 (96.0)</b>	<b>10 (4.0)</b>	159 (62.8)	94 (37.2)
Pre-obese + obese	455	287 (63.1)	137 (30.1)	31 (6.8)	<b>424 (93.2)</b>	<b>31 (6.8)</b>	287 (63.1)	168 (36.9)
p-value			0.72	0.05	<b>0.04</b>		0.76	
<b>Histological grade</b>	642 <sup>c</sup>							
I + II	93	48 (51.6)	37 (39.8)	8 (8.6)	85 (91.4)	8 (8.6)	<b>48 (51.6)</b>	<b>45 (48.4)</b>
III	549	352 (64.1)	169 (30.8)	28 (5.1)	521 (94.9)	28 (5.1)	<b>352 (64.1)</b>	<b>197 (35.9)</b>
p-value			0.05	0.07	0.18		<b>0.02</b>	

<sup>a</sup>: number; <sup>b</sup>: Median age at menarche; <sup>c</sup>: The total number differs from the total (742) number quoted in the study because it was not possible to obtain information for some individuals; <sup>d</sup>: Median age at first full-term pregnancy. The significant results are presented in a bold font.

with a lower risk of BC among Japanese (40) patients. Furthermore, no association between the CYP1A1 4889AG polymorphism and BC risk was observed among North Americans (19-22,30,33,35), Chinese (21,34), Europeans (31), or Indians (25,28). These discrepant results seem to be due to ethnic variations among the studied populations because the CYP1A1 4889AG + GG genotype was both underrepresented (21,25,28,34,40) and overrepresented (19,20,22,30,31,33,35) in our controls compared with controls from the other studies.

In our study, the CYP1A1 T6235C polymorphism was not associated with SBC risk. Although a large number of subjects were evaluated, the statistical analyses were characterized by a low statistical power. In addition, the CYP1A1 T6235C polymorphism did not alter the risk of BC among African-Americans (30,33), North-Americans (5,21,30,33), Chinese (34), Indians (25,36), or Brazilians (37). In contrast, Miyoshi et al. (40)

and da Fonte de Amorim et al. (32) reported a lower risk of BC among Japanese and Brazilian carriers of the C variant allele. Moreover, an association between the CYP1A1 T6235 C allele and a high BC risk was previously described by Taioli et al. (20,22), Li et al. (33), Chacko et al. (23), Shen et al. (24), Gulyaeva et al. (26), Naushad et al. (28) and Khvostova et al. (29). Discrepancies between the results of our study and the da Fonte de Amorim study (32) (both studies were conducted among Brazilians) and discrepancies between the results of our study and those of Taioli (20,22) and Naushad (28) (African-American and Indian women, respectively) may be attributed to different sample sizes. We analyzed 742 SBC patients. The studies by da Fonte de Amorim, Taioli and Naushad analyzed significantly smaller numbers of patients (n=128, n=57 and n=342, respectively). Conversely, the discrepancies between our study and the remaining





discordant studies may be explained by ethnic variation within the populations because the frequency of the *CYP1A1* 6235TC + CC genotype was lower (40) or higher (23,24,26,29,33) among our controls compared to their controls.

Additionally, in our study, the *CYP1A1* 4889AG and 4889AG + GG genotypes were overrepresented in Caucasian Brazilian women with BC compared with non-Caucasian women. The frequencies of the referred genotypes were also higher among our Caucasian SBC patients than in our control group. Therefore, Caucasian women carrying the G allele had a higher risk of SBC development. Our finding is consistent with the Sergentanis & Economopoulos meta-analysis (52), which included Caucasian, Chinese and African populations from 29 eligible studies. We also observed similar frequencies of the distinct *CYP1A1* T6235C polymorphisms among our Caucasian and non-Caucasian women. To the best of our knowledge, the association between the *CYP1A1* T6235 C allele and a high risk BC was only observed in a small study of North-Americans, which was conducted by Taioli et al. (20,22).

We identified a relationship between the *CYP1A1* 6235CC genotype and SBC in women who experienced an earlier age at menarche compared to women who experienced a later age at menarche, as previously described by Huang et al. (21,39). In fact, women with early menarche are exposed to estrogen for a longer period of time, which might amplify the effect of the *CYP1A1* 6235CC variant product on breast tissue. We also surprisingly found that the *CYP1A1* 4889AG + GG genotype was overrepresented in SBC patients who experienced their FFTP earlier in life compared to women who experienced a later FFTP. An early age at FFTP is well-known to be highly protective against the onset of BC (11,53,54) and the *CYP1A1* A4889G genotype is associated with an increased BC risk in women with an older age at FFTP (55,56) because of the prolonged estrogen exposure of the breast epithelium (6,10). However, Brazilian women are characterized by a low average FFTP age and also by multiparity (57), which seem to be protective factors (11). However, Kobayashi et al. (58) reported that each pregnancy, including the first one, increases the risk of early onset BC due to the reaction cascade triggered by chorionic gonadotropin hormone (hCG) during the first trimester of pregnancy. This cascade results in the morphological and functional development of breast tissue. The high progesterone conditions induced by hCG seem to be essential to pregnancy-associated breast development, presumably as a result of mammary epithelial stem cell expansion. This fact, associated with the high estrogen metabolism among multiparous women with the G variant allele, may explain our results. However, we did not determine how many pregnancies each woman had experienced until BC was diagnosed.

In our study and Huang et al. (21,39), BC risk was higher among women with a high BMI. In Huang et al., the CC variant genotype of the *CYP1A1* T6235C polymorphism was overrepresented in pre-obese and obese women, suggesting that the CC variant might affect BC susceptibility. One plausible mechanism for this association is that obese women have a higher circulating estrogen level caused by the conversion of androgen to estrogen in adipose tissue. Apart from this possibility, obese women have a higher formation rate of genotoxic compounds that may lead to DNA adduct formation in mammary cells (10,11).

Finally, we observed that the G and C variant alleles of both polymorphisms were positively associated with lower

histological grade tumors, thereby indicating their roles in the progression of mammary carcinogenesis (even compared to controls). Thus, carriers of the respective variant alleles of both polymorphisms have an increased risk of developing histological grade I and II BC. Conflicting results regarding the roles of *CYP1A1* A4889G and T6235C in the histological grade of BC have been previously observed in small studies conducted by Miyoshi et al. (40) (n=195) and Singh et al. (36) (n=150).

Our data are useful for predicting BC risk. Our results showed that *CYP1A1* A4889G and T6235C polymorphisms alter the risk and clinical or tumor characteristics of BC among a heterogeneous population from Southeastern Brazil. Although this study is not the first study to investigate SBC in Brazil (32,37), the large sample size provides more reliable conclusions regarding SBC among Brazilians.

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## ■ AUTHOR CONTRIBUTIONS

Lima CS was responsible for the study concepts, study design, quality control of the data and algorithms, data analysis, data interpretation, statistical analyses and manuscript review. Gurgel MS was responsible for the study design and quality control of the data and algorithms. Cardoso-Filho C and Bossi LS were responsible for data acquisition. Oliveira CM was responsible for data acquisition, data analysis, data interpretation, statistical analyses, manuscript preparation and manuscript editing. Lourenço GJ was responsible for statistical analyses.

## ■ REFERENCES

1. Scheuner MT, McNeel TS, Freedman AN. Population prevalence of familial cancer and common hereditary cancer syndromes; The 2005 California Health Interview Survey. *Genet Med*. 2010;12(11):726-35, <http://dx.doi.org/10.1097/GIM.0b013e3181f30e9e>.
2. Johnson-Thompson MC, Guthrie J. Ongoing research to identify environmental risk factors in breast carcinoma. *Cancer*. 2000;88(5 Suppl):1224-9.
3. Rebbeck T, Rosvold E, Duggan D, Zhang J, Buetow K. Genetics of *CYP1A1*: coamplification of specific alleles by polymerase chain reaction and association with breast cancer. *Cancer Epidemiol Biomarkers Prev*. 1994;3(6):511-4.
4. Perera FP, Estabrook A, Hewer A, Channing K, Rundle A, Mooney LA et al. Carcinogen-DNA adducts in human breast tissue. *Cancer Epidemiol Biomarkers Prev*. 1995;4(3):233-8.
5. Ishibe N, Hankinson SE, Colditz GA, Spiegelman D, Willett WC, Speizer FE et al. Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer risk in the Nurses' Health Study. *Cancer Res*. 1998;58(4):667-71.
6. Dumitrescu RG, Cotarla I. Understanding breast cancer risk – where do we stand in 2005? *J Cell Mol Med*. 2005;9(1):208-21, <http://dx.doi.org/10.1111/j.1582-4934.2005.tb00350.x>.
7. Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. *Cancer Epidemiol Biomarkers Prev*. 2000;9(6):575-9.
8. Page DL, Dupont WD, Rogers LW, Rados MS. Atypical hyperplastic lesions of the female breast: a longterm follow-up study. *Cancer*. 1985;55(11):2698-708, [http://dx.doi.org/10.1002/1097-0142\(19850601\)55:11<2698::AID-CNCR2820551127>3.0.CO;2-A](http://dx.doi.org/10.1002/1097-0142(19850601)55:11<2698::AID-CNCR2820551127>3.0.CO;2-A).
9. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. *J Natl Cancer Inst Monogr*. 2000;(27):67-73.
10. Mitrunen K, Hirvonen A. Molecular epidemiology of sporadic breast cancer. The role of polymorphic genes involved in oestrogen biosynthesis and metabolism. *Mutat Res*. 2003;544(1):9-41, [http://dx.doi.org/10.1016/S1383-5742\(03\)00016-4](http://dx.doi.org/10.1016/S1383-5742(03)00016-4).
11. Yang XR, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL, et al. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. *J Natl Cancer Inst*. 2011;103(3):250-63, <http://dx.doi.org/10.1093/jnci/djq526>.
12. de Jong MM, Nolte IM, te Meerman GJ, van der Graaf WT, Oosterwijk JC, Kleibeuker JH, et al. Genes other than BRCA1 and BRCA2



- involved in breast cancer susceptibility. *J Med Genet.* 2002;39(4):225-42, <http://dx.doi.org/10.1136/jmg.39.4.225>.
13. Masson LF, Sharp L, Cotton SC, Little J. *Am J Epidemiol.* 2005;161(10):901-15.
  14. Hildebrand CE, Gonzalez FJ, McBride OW, Nebert DM. Assignment of the human 2,4,7,8-tetrachlorodibenzo-p-dioxin-inducible cytochrome P1-450 gene to chromosome 15. *Nucleic Acids Res.* 1985;13(6):2009-16, <http://dx.doi.org/10.1093/nar/13.6.2009>.
  15. Han W, Kang D, Park IA, Kim SW, Bae JY, Chung KW, et al. Associations between breast cancer susceptibility gene polymorphisms and clinicopathological features. *Clin Cancer Res.* 2004;10(1 Pt 1):124-30.
  16. Harth V, Brüning T, Abel J, Koch B, Berg I, Sachinidis A, et al. Real-time genotyping of cytochrome P4501A1 A4889G and T6235C polymorphisms. *Mol Cell Probes.* 2001;15(2):93-7, <http://dx.doi.org/10.1006/mcpr.2001.0349>.
  17. Guengerich FP, Shimada T. Activation of procarcinogens by human cytochrome P450 enzymes. *Mutat Res.* 1998;400(1-2):201-13, [http://dx.doi.org/10.1016/S0027-5107\(98\)00037-2](http://dx.doi.org/10.1016/S0027-5107(98)00037-2).
  18. Landi MT, Bertazzi PA, Shields PG, Clark G, Lucier GW, Garte SJ, et al. Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics.* 1994;4(5):242-6, <http://dx.doi.org/10.1097/00008571-199410000-00002>.
  19. Ambrosone C, Freudenheim J, Graham S, Marshall J, Vena J, Brasure J, et al. Cytochrome p450 1A1 and glutathione S-transferase (M1) genetic polymorphisms and postmenopausal breast cancer risk. *Cancer Res.* 1995;55(16):3483-5.
  20. Taioli E, Trachman J, Chen X, Toniolo P, Garte S. A CYP1A1 restriction fragment length polymorphism is associated with breast cancer in African-American women. *Cancer Res.* 1995;55(17):3757-8.
  21. Huang CS, Shen CY, Chang KJ, Hsu SM, Chern HD. Cytochrome P4501A1 polymorphism as a susceptibility factor for breast cancer in postmenopausal Chinese women in Taiwan. *Br J Cancer.* 1999;80(11):1838-43, <http://dx.doi.org/10.1038/sj.bjc.6690608>.
  22. Taioli E, Bradlow HL, Garbers SV, Sepkovic DW, Osborne MP, Trachman J, et al. Role of estradiol metabolism and CYP1A1 polymorphisms in breast cancer risk. *Cancer Detect Prev.* 1999;23(3):232-7, <http://dx.doi.org/10.1046/j.1525-1500.1999.09912.x>.
  23. Chacko P, Joseph T, Mathew BS, Rajan B, Pillai MR. Role of xenobiotic metabolizing gene polymorphisms in breast cancer susceptibility and treatment outcome. *Mutat Res.* 2005;581(1-2):153-63, <http://dx.doi.org/10.1016/j.mrgentox.2004.11.018>.
  24. Shen CY, Li DK, Wy J, Zhang Z, Gao E. Joint effects of the CYP1A1 MspI, ERalpha PvuII, and ERalpha XbaI polymorphisms on the risk of breast cancer: results from a population-based case-control study in Shanghai, China. *Cancer Epidemiol Biomarkers Prev.* 2006;15(2):342-7, <http://dx.doi.org/10.1158/1055-9965.EPI-05-0485>.
  25. Singh V, Rastogi N, Sinha A, Kumar A, Mathur N, Singh MP. A study on the association of cytochrome-P450 A1A polymorphism and breast cancer risk in North Indian women. *Breast Cancer Res Treat.* 2007;101(1):73-81, <http://dx.doi.org/10.1007/s10549-006-9264-2>.
  26. Gulyaeva LF, Mikhailova ON, Pustylnyak VO, Kim IV, Gerasimov AV, Krasilnikov SE et al. Comparative analysis of SNP in estrogen-metabolizing enzymes for ovarian, endometrial, and breast cancers in Novosibirsk, Russia. *Adv Exp Med Biol.* 2008;617:359-66, <http://dx.doi.org/10.1007/978-0-387-69080-3>.
  27. Surekha D, Sailaja K, Rao DN, Padma T, Raghunadharao D, Vishnupriya S. Association of CYP1A1\*2 polymorphisms with breast cancer risk: a case control study. *Indian J Med Sci.* 2009;63(1):13-20, <http://dx.doi.org/10.4103/0019-5359.49077>.
  28. Naushad SM, Reddy CA, Rupasree AP, Digumarti RR, Gottumukkala SR, Kuppasamy P, et al. Cross-talk between one-carbon metabolism and xenobiotics metabolism: implications on oxidative DNA damage and susceptibility to breast cancer. *Cell Biochem Biophys.* 2011;61(3):715-23, <http://dx.doi.org/10.1007/s12013-011-9245-x>.
  29. Khvostova EP, Pustylnyak VO, Gulyaeva LF. Genetic polymorphism of estrogen metabolizing enzymes in Siberian women with breast cancer. *Genet Test Mol Biomarkers.* 2012;16(3):167-73, <http://dx.doi.org/10.1089/gtmb.2011.0131>.
  30. Bailey LR, Roodi N, Verrier CS, Yee CJ, Dupont WD, Parl FF. Breast cancer and CYP1A1, GSTM1, and GSTT1 polymorphisms: evidence of a lack of association in Caucasians and African Americans. *Cancer Res.* 1998;58(1):65-70.
  31. Basham VM, Pharoah PDP, Healey CS, Luben RN, Day NE, Easton DF, et al. Polymorphisms in CYP1A1 and smoking: no association with breast cancer risk. *Carcinogenesis.* 2001;22(11):1797-800, <http://dx.doi.org/10.1093/carcin/22.11.1797>.
  32. Da Fonte de Amorim LM, Rossini A, Mendonça GAS, Lotsch PF, Simão TA, Gallo CVM, et al. CYP1A1, GSTM1, and GSTT1 polymorphisms and breast cancer risk in Brazilian women. *Cancer Lett.* 2002;181(2):179-86, [http://dx.doi.org/10.1016/S0304-3835\(02\)00058-7](http://dx.doi.org/10.1016/S0304-3835(02)00058-7).
  33. Li Y, Millikan RC, Bell DA, Cui L, Tse CKJ, Newman B, et al. Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer among African-American and white women. *Breast Cancer Res.* 2004;6(4):R460-73, <http://dx.doi.org/10.1186/bcr814>.
  34. Boyapati SM, Shu XO, Gao YT, Cai Q, Jin F, Zheng W. Polymorphisms in CYP1A1 and breast carcinoma risk in a population-based case-control study in Chinese women. *Cancer.* 2005;103(11):2228-35, <http://dx.doi.org/10.1002/cncr.21056>.
  35. Modugno F, Zmuda JM, Potter D, Cai C, Ziv E, Cummings SR, et al. Estrogen metabolizing polymorphisms and breast cancer risk among older white women. *Breast Cancer Res Treat.* 2005;93(3):261-70, <http://dx.doi.org/10.1007/s10549-005-5347-8>.
  36. Singh N, Mitra AK, Garg VK, Agarwal A, Sharma M, Chaturvedi R, et al. Association of CYP1A1 Polymorphisms with Breast Cancer in North Indian Women. *Oncol Res.* 2007;16(12):587-97, <http://dx.doi.org/10.3727/000000007783629972>.
  37. Torresan C, Oliveira MMC, Torrezan GT, Oliveira SFV, Abuázar CS, Losi-Guembarovski R, et al. Genetic polymorphisms in oestrogen metabolic pathway and breast cancer: a positive association with combined CYP/GST genotypes. *Clin Exp Med.* 2008;8(2):65-71, <http://dx.doi.org/10.1007/s10238-008-0159-x>.
  38. Garte S. The role of ethnicity in cancer susceptibility gene polymorphisms: the example of CYP1A1. *Carcinogenesis.* 1998;19(8):1329-32, <http://dx.doi.org/10.1093/carcin/19.8.1329>.
  39. Huang CS, Chern HD, Chang KJ, Cheng CW, Hsu SM, Shen CY. Breast cancer risk associated with genotype polymorphism of the estrogen-metabolizing genes CYP17, CYP1A1, and COMT: a multigenic study on cancer susceptibility. *Cancer Res.* 1999;59(19):4870-5.
  40. Miyoshi Y, Takahashi Y, Egawa C, Noguchi S. Breast cancer risk associated with CYP1A1 genetic polymorphisms in Japanese women. *Breast J.* 2002;8(4):209-15, <http://dx.doi.org/10.1046/j.1524-4741.2002.08404.x>.
  41. Singh V, Parmar D, Singh MP. Do single nucleotide polymorphisms in xenobiotic metabolizing genes determine breast cancer susceptibility and treatment outcomes? *Cancer Invest.* 2008;26(8):769-83, <http://dx.doi.org/10.1080/07357900801953196>.
  42. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One.* 2011;6(2):e17063, <http://dx.doi.org/10.1371/journal.pone.0017063>.
  43. Instituto Nacional de Câncer (INCA), Brasil. Incidência de Câncer no Brasil. Estimativa 2014. Ministério da Saúde 2013; <http://www.inca.gov.br/estimativa/2014>. Accessed on June 1st, 2015.
  44. World Health Organization (WHO). BMI Classification 2004; [http://apps.who.int/bmi/index.jsp?introPage=intro\\_3.html](http://apps.who.int/bmi/index.jsp?introPage=intro_3.html). Accessed on May 25<sup>th</sup>, 2015.
  45. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology.* 1991;19(5):403-10, <http://dx.doi.org/10.1111/j.1365-2559.1991.tb00229.x>.
  46. Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, et al. *Breast. In: Balch CM (editor). American Joint Committee on Cancer; Cancer staging manual, 6th edition. New York: Springer, 2002. p. 223-240.*
  47. Hsu SM, Raine L, Fanger H. A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am J Clin Pathol.* 1981;75(5):734-8.
  48. Cascorbi J, Brockmüller J, Roots I. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res.* 1996;56(21):4965-9.
  49. Pocock SJ. *Clinical Trials: A Practical Approach.* John Wiley and Sons, New York. 1983.
  50. Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB. *Designing Clinical Research.* Lippincott Williams and Wilkins, Philadelphia. 1988.
  51. Decision Support Systems. DSS Research Statistical Power Calculators 2013; <http://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx>. Accessed on May 15<sup>th</sup>, 2015.
  52. Sergentanis TN, Economopoulos KP. Four polymorphisms in cytochrome P450 1A1 (CYP1A1) gene and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat.* 2010;122(2):459-69, <http://dx.doi.org/10.1007/s10549-009-0694-5>.
  53. Ma H, Bernstein L, Pike MC, Ursin G. Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies. *Breast Cancer Res.* 2006;8(4):R43, <http://dx.doi.org/10.1186/bcr1525>.
  54. Lord SJ, Bernstein L, Johnson KA, Malone KE, McDonald JA, Marchbanks PA, et al. Breast cancer risk and hormone receptor status in older women by parity, age of first birth, and breastfeeding - a case-control study. *Cancer Epidemiol Biomarkers Prev.* 2008;17(7):1723-30, <http://dx.doi.org/10.1158/1055-9965.EPI-07-2824>.
  55. McPherson K, Steel CM, Dizon JM. ABC of breast cancer diseases. Breast cancer-epidemiology, risk factors, and genetics. *BMJ.* 2000;321(7261):624-8, <http://dx.doi.org/10.1136/bmj.321.7261.624>.
  56. Bernstein L. Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia.* 2002;7(1):3-15, <http://dx.doi.org/10.1023/A:1015714305420>.
  57. Mirra AP, Cole P, MacMahon B. Breast Cancer in an Area of High Parity: São Paulo, Brazil. *Cancer Res.* 1971;31(2):77-83.
  58. Kobayashi S, Sugiura H, Ando Y, Shiraki N, Yanagi T, Yamashita H, et al. Reproductive history and breast cancer risk. *Breast Cancer.* 2012;19(4):302-8, <http://dx.doi.org/10.1007/s12282-012-0384-8>.