GSTM1, *GSTT1*, and *GSTP1* polymorphisms, breast cancer risk factors and mammographic density in women submitted to breast cancer screening

Polimorfismos GSTM1, GSTT1 e GSTP1, fatores de risco para câncer de mama e densidade mamográfica em mulheres submetidas a rastreamento mamográfico

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Abstract

Genetic polymorphisms in genes related to the metabolism of xenobiotics, such as genes of the glutathione S-transferases (*GSTM1*, *GSTT1*, and *GSTP1*) superfamily have been associated with an increased risk for breast cancer (BC). Considering the high incidence of BC in the city of Porto Alegre in southern Brazil, the purpose of this study was to characterize genotypic and allelic frequencies of polymorphisms in *GSTM1*, *GSTT1*, and *GSTP1*, and correlate these molecular findings with established risk factors for breast cancer including mammographic density, in a sample of 750 asymptomatic women undergoing mammographic screening. Molecular tests were performed using the multiplex polymerase chain reaction (PCR) for *GSTM1* and *GSTT1*, and quantitative PCR for *GSTP1* polymorphisms. Overall, the frequencies of *GSTM1* and *GSTT1* null genotypes were 45% and 21%, respectively. For *GSTP1* polymorphism, genotypic frequencies were 44% for the Ile/Ile genotype, 44% for the Ile/Val genotype, and 12% for Val/Val genotype, with an allelic frequency of 66% for the wild type allele in this population, similar to results of previous international publications. There was a statistically significant association between the combined *GSTM1* and *GSTT1* null genotypes (M-/T-) and mammographic density in post menopausal women ($p = 0.031$). When the *GSTT1* null (T-) genotype was analyzed isolated, the association with mammographic density in post menopausal women and in the overall sample was also statistically significant ($p = 0.023$ and $p = 0.027$, respectively). These findings suggest an association of *GSTM1* and *GSTT1* null genotypes with mammographic density.

Keywords: Breast cancer. Risk factors. Genetic polymorphisms. GSTT1. GSTM1. GSTP1.

Resumo

Polimorfismos genéticos em genes relacionados com o metabolismo de xenobióticos, como os genes da superfamília das glutationa S-transferases (*GSTM1*, *GSTT1* e *GSTP1*) têm sido associados com o aumento do risco para câncer de mama (CM). Considerando a alta incidência de CM na cidade de Porto Alegre, região Sul do Brasil, a proposta deste estudo foi caracterizar genótipos e frequências alélicas dos polimorfismos *GSTM1, GSTT1* e *GSTP1*, e correlacionar esses achados moleculares com fatores de risco já estabelecidos para câncer de mama, incluindo densidade mamográfica, em uma amostra de 750 mulheres assintomáticas durante o rastreamento mamográfico. Para os testes moleculares foi utilizado multiplex da reação em cadeia de polimerase (PCR) para *GSTM1* e *GSTT1*, e PCR quantitativo para o polimorfismo *GSTP1*. As frequências dos genótipos *GSTM1* e *GSTT1* nulos foram 45% e 21%, respectivamente. Para o polimorfismo *GSTP1*, as frequências genotipicas foram: 44% para o genótipo Ile/Ile, 44% para o genótipo Ile/Val e 12% para o genótipo Val/Val. A frequência do alelo lle nesta população foi 66%, semelhante a outros estudos. Houve uma associação significativa entre a combinação dos genótipos (T-/M-) nulos e densidade mamográfica nas mulheres pós-menopáusicas (p = 0,031). Quando analisamos isoladamente o genótipo *GSTT1* nulo (T-) também encontramos uma associação significativa com a densidade mamográfica nas mulheres pós-menopáusicas (p = 0,027) e na amostra total. Estes achados sugerem uma associação dos genótipos (T-/M-) nulos com densidade mamográfica.

Palavras-chave: Câncer de mama. Fatores de risco. Polimorfismos genéticos. GSTT1. GSTM1. GSTP1.

Introduction

Breast cancer (BC) is the second most common type of malignancy worldwide and the first among women[1]. In Brazil, BC is a significant public health problem due to its morbidity and high incidence and mortality rates, representing the leading cause of cancer-related deaths in women of all ages. The state of Rio Grande do Sul (RS), for reasons still unknown, has one of the highest BC incidence and mortality rates in the country. In this state, BC is currently the leading cause of cancer deaths in young women (30-49 years of age)[2].

In Brazil, approximately 75% of the population has access to health care only through the Brazilian Public Health System (SUS, Sistema Único de Saúde), which is responsible for the provision of breast health care to the majority of the population. Regarding mammographic screening, a national survey conducted in 2003 confirms the lack of a structured screening program in the country, showing that 49.7% of women above the age of 50 have never been submitted to a mammography[3].

Several epidemiologic studies have suggested that environmental carcinogens contribute to the risk for BC and that genetic differences in the metabolism of those carcinogens may be associated with individual variations in susceptibility to this tumor[4,5]. Genes involved in the metabolism of carcinogens have been used as markers of individual susceptibility to cancer. Their products, detoxicating enzymes, may exacerbate or suppress the activity of xenobiotics[6,7]. Thus, changes in the balance between activation and detoxification of carcinogens may explain part of the individual variations in response to exposure to these agents[8].

Glutathione S-transferases (GST), which are involved in phase II of biotransformation, acting on carcinogens, environmental pollutants, drugs, and other xenobiotics, has been implicated as key detoxification enzymes. A significant effect on tolerance to carcinogens has been demonstrated

when there is deficiency of specific isozymes of this family[9]. The three major genes of the GST family are *GSTM1, GSTT1*, and *GSTP1*[10]. *GSTM1* is located on chromosome 1p13.3, and 20% to 50% of the population has a homozygous deletion of the gene, not expressing its product, the gstm1 enzyme[11]. *GSTM1* is involved in the detoxification of polycyclic aromatic hydrocarbons and other carcinogens, and the cells of individuals with *GSTM1* null genotypes are more susceptible to damage to DNA caused by these agents[12]. *GSTT1* is located on chromosome 22q11.2, similarly polymorphic, and its null allele has been observed at a frequency of 20 to 60%, in different human populations[12]. *GSTP1* is located on chromosome 11q13, and the presence of a polymorphism at codon 105 (substitution of isoleucine for valine, rs1695) results in reduced activity of the gstp1 enzyme[13]. The *GSTP1* Ile105Val polymorphism has been associated with increased susceptibility to various forms of cancer, particularly those related to tobacco use and BC[12,13]. There are few previous studies on the prevalence of these polymorphisms in a region with high incidence and mortality rates for BC, and therefore, the purpose of this study was to determine the frequency of *GSTM1, GSTT1,* and *GSTP1* polymorphisms in a sample of BC unaffected-women undergoing routine mammographic screening.

Methods

Recruitment

A convenient subsample of 890 breast cancer-unaffected women (ages 40-69 years) enrolled in a mammography screening program (Núcleo Mama Porto Alegre - NMPOA cohort) in the city of Porto Alegre was recruited for this study during routine mammographic visits between November 2005 and March 2006[14]. Invitation to participate in the study was consecutive among women that scheduled their screening mammographies during this period. The refusal rate was 15.7%. A total of 750 women were included in the analysis. Sample size was calculated to estimate the frequencies of polymorphisms in *GSTM1, GSTT1*, and *GSTP1* with a 95% confidence level taking into consideration the allelic frequencies described in previously published papers. In accordance to this, we used the minimum allele frequency (MAF) of each polymorphism to obtain the sample size. Of note, since this sample included only women from the NMPOA cohort, we can not say that it is representative of the general population. For this reason we preferred to use the term "frequency" instead of "prevalence".

Collection of demographic and clinical data

Clinical data and information on BC risk factors were gathered from anamnesis sheets and medical history recorded in patient charts. Estimates on the lifetime risk of developing BC were obtained for all women using the Gail model[15] and results of the mammographic examination and mammographic density used the *BIRADS* and breast density categories of the American College of Radiology[16]. As for ethnicity, the self-designation of participants in the cohort was considered at enrollment, with categorization between whites and non-whites.

Molecular Analysis

Genomic DNA was obtained from peripheral blood samples using the standard method[17]. *GSTP1* Ile105Val polymorphism was studied by allelic discrimination using a commercially available TaqMan assay method; fluorescence was measured on an ABI 7500 Sequence Detector (Applied Biosystems, Foster City, CA). *GSTM1* and *GSTT1* polymorphisms were analyzed concurrently by multiplex-PCR and the region of interest of the *GSTP1* gene (amplicon of 176bp) was added to this reaction to provide an internal control for double null allele genotypes, as described previously[18]. The amplified products were resolved by

electrophoresis on 2% agarose gels and viewed in ultraviolet light. The presence or absence of the *GSTM1* and *GSTT1* genes was assessed by the detection of amplification products of 215bp and 480bp, respectively; distinction between homozygotes and heterozygotes was not possible with this method. Throughout the manuscript, genotyping results are presented as follows: genotypic frequencies in percentage of individuals and allelic frequencies as percentage of alleles.

Statistical analyses

For descriptive analyses, categorical variables were described by their absolute and/or relative frequencies and quantitative variables were expressed as mean and standard deviation (SD). For statistical data inference, the *t test* for independent variables and ANOVA were used to compare mean values of the quantitative variables. The existence of an association between categorical variables, the comparison of genotype frequencies and the deviation of genotype frequencies from those expected were examined by the chi-square test. In all analyses, a significance level of 0.05 was adopted. Comparative analysis of genotypic frequencies between the present study and other studies was done using the WINPEPI (PEPI-for-Windows)[19]. SPSS version 14.0 was used for data handling and further statistical analyses.

Ethical Aspects

Study approval was obtained from the ethics committees of participating institutions (Hospital de Clínicas de Porto Alegre and Hospital Moinhos de Vento) and all women recruited for the study signed the informed consent.

Results

The average age at inclusion was 51 years (SD = 7.6 years) and 421 women (56.1%) reported being postmenopausal. For the sample as a whole, the average lifetime risk of developing BC, estimated by the Gail model, was 7.8% (SD = 3.3%). Overall, 36.4% and 41.1% of the women had a body mass index (BMI) of 25-29.99 or ≥ 30, respectively. Seven hundred and thirty two women (97.6%) had mammography results in the BIRADS 1 or 2 categories, 9 (1.2%) had BIRADS 3 mammographic results and 5 (1.1%) had a mammographic result corresponding to the BIRADS 4 category. Four hundred and thirteen (55.1%) women had fatty breast tissue or moderately fatty breast tissue, < 25% and 26-50% of glandular area, respectively; 304 (40.5%) had moderately dense, dense or heterogeneity dense tissue, 57-75%, > 75% and 50-75% of glandular area, respectively; and 33 (4.4%) women did not have mammographic density available. A detailed description of BC risk factors is presented in Table 1.

The frequencies of *GSTM1* and *GSTT1* null genotypes were 45% and 21%, respectively, and 10% of the women had combined *GSTM1* and *GSTT1* null genotypes. The genotypic frequencies of the *GSTP1* polymorphism were: 44% for the Ile/Ile genotype, 44% for the Ile/Val genotype, and 12% for the Val/Val genotype. The frequency of the Ile allele in this sample was 66%. When we categorized the group under study in white and non-white women, we observed that the *GSTP1* homozygous Val/Val genotype was more frequent in non-white women. Furthermore, the *GSTP1* genotype frequencies were not in Hardy-Weinberg equilibrium (p = 0.005). These results are presented in Table 2.

When assessing the association of *GSTM1, GSTT1* and *GSTP1* genotypes with established risk factors for BC, there was no association with age, age at menarche, age at menopause or body mass index (data not shown). However, there was a difference between the combined *GSTM1* and *GSTT1* null genotypes and mammographic density in post-menopausal women (p=0.031). In individual analyses, the *GSTT1* null genotype was also associated with mammographic density in post menopausal women (p = 0.023), and in the overall sample (p

Table 1 - Distribution of breast cancer risk factors among patients studied.
Tabela 1 - *Distribuição de fatores de risco para câncer de mama entre as pacientes estudadas.*

Variable	n (%)	Mean	SD
Age at assessment (years)	---	51.0	7.6
Age at menarche (years)	---	13.0	1.8
Age at first childbirth (years)	---	22.0	5.3
Nulliparous	31 (4)	---	---
Postmenopausal (%)	421 (56)	---	---
Surgical menopause	45 (6)		
Age at menopause (years)	---	47.0	5.5
Use of hormone replacement (ever)	118 (28)	---	---
Use of hormone replacement for ≥ 5 ys	26 (3)		
Body mass index (kg/m^2)		29.6	5.8
≤18.4	6 (0.8)	---	---
18.5-24.99	158 (21)	---	---
25-29.99	273 (36)	---	---
≥30	308 (41)	---	---
Previous breast biopsy	40 (5)	---	---

Table 2 - Genotypic and allelic frequencies of *GSTP1* (Ile105Val) and genotypic frequency *of GSTM1/GSTT1* null genes by self-reported skin color.
Tabela 2 - *Frequências genotípicas e alélicas do polimorfismo* GSTP1 *(Ile105Val) e frequências genotípicas dos genes* GSTM1/GSTT1 *nulos de acordo com cor da pele autorreferida*.

Gene	Genotypic frequencies n (%)			Allelic frequencies** (%)		**p-value**
GSTP1	**Ile/Ile**	**Ile/Val**	**Val /Val**	**Ile**	**Val**	
Overall	330 (44)	329 (44)	91 (12)	66	34	
Whites	268 (45)	270 (45)	61 (10)	67	33	0.005
Non-whites	62 (41)	59 (39)	30 (20)	61	39	
GSTM1	***M1 -***	***M1+***				
Overall	339 (45)	411 (55)				
Whites	281 (47)	318 (53)				0.061
Non-whites	58 (38)	93 (62)				
GSTT1	***T1 -***	***T1+***				
Overall	158 (21)	592 (79)				
Whites	119 (20)	480 (80)				0.108
Non-whites	39 (26)	112 (74)				
GSTT1 /M1	***T1 -/M1 –***	***T1+/M+***				
Total	76 (10)	674 (90)				
Whites	64 (11)	535 (89)				0.319
Non-whites	12 (8)	139 (92)				

Number of subjects studied: Overall = 750; whites = 599; non-white = 151.
*p-value = whites X non-whites **allelic frequencies are presented as percentages of all alleles. Common alleles: *GSTT1* present (*T1+*), *GSTM1* present (*M1+*), *GSTP*1 (Ile/Ile). Variant alleles *GSTT1* null (*T1-*), *GSTM1* null (M1-), *GSTP1* (Ile/Val or Val/Val).
*Número de sujeitos estudados: Total = 750; Brancas= 599; Não-Brancas= 151 * Valor-p = Brancas X Não-Brancas ** frequências alélicas são apresentadas como percentagem de todos alelos.*
Alelos comuns: GSTT1 presente (T1+), GSTM1 presente (M1+), GSTP1 (Ile/Ile). Alelos variantes: GSTT1 nulo (T1-), GSTM1 nulo (M1-), GSTP1 (Ile/Val ou Val/Val).

= 0.027). As for ethnicity, considering the classification based on self-designation, there was an association between T- and breast density between white and non-white women (p = 0.028). There was no association between *GSTP1* alleles and genotypes with mammographic density. These results are presented in Table 3.

Table 3 - Association between *GSTM1/GSTT1* null genotypes and *GSTP1* (Ile105Val) and mammographic density in overall sample, and according to menopausal status and self-reported skin color.

Tabela 3 - *Associação entre os genes GSTM1/GSTT1 nulos e genótipos de GSTP1 (Ile105Val) e densidade mamográfica considerando a amostra total e por status da menopausa e cor da pele.*

Genotype	Total* (n=717)		Menopausal status				Self-denominated skin color			
			Premenopausal (n=270)		Postmenopausal (n=402)		White (n=575)		Non-white (n=142)	
	Mammographic density		Mammographic density		Mammographic density		Mammographic density		Mammographic density	
	≤ 50% (n=413) n(%)	> 50% (n=304) n(%)	≤ 50% (n=118) n(%)	> 50% (n=152) n(%)	≤ 50% (n=275) n(%)	> 50% (n=127) n(%)	≤ 50% (n=337) n(%)	> 50% (n=238) n(%)	≤ 50% (n=76) n(%)	> 50% (n=66) n(%)
T1-	77(18.6)	78(25.7)	25(21.2)	36(23.7)	47(17.1)	35(27.6)	58(17.2)	59(24.8)	19(25.0)	19(25.0)
p	0.027		0.662		0.023		0.028		0.705	
M1-	188(45.5)	134(44.1)	45(38.1)	58(38.2)	134(48.7)	66(52.0)	160(47.5)	108(45.4)	28(36.8)	26(39.4)
p	0.705		>0.999		0.592		0.671		0.863	
T1-M1-	34(8.2)	39(12.8)	10(8.5)	18(11.8)	21(7.6)	19(15.0)	29(8.6)	33(13.9)	5(6.6)	6(9.1)
p	0.046		0.425		0.031		0.055		0.755	
GSTP1 (Ile /Val+ Val/Val)	235(56.9)	161(53)	50(42.4)	67(44.1)	119(43.3)	63(49.6)	144(42.7)	118(49.6)	34(44.7)	25(37.9)
p	0.323		0.805		0.238		0.107		0.495	

Number of subjects studied: Overall = 750 *(33 women did not have mammographic density available). Common alleles: *GSTT1* present (*T1+*), *GSTM1* present (*M1+*), *GSTP*1 (Ile/Ile). Variant alleles *GSTT1* null (*T1-*), *GSTM1* null (M1-), *GSTP1* (Ile/Val or Val/Val).

*Número de sujeitos estudados: Total = 750 *(33 mulheres tinham densidade mamográfica não avaliada). Alelos comuns: GSTT1 presente (T1+), GSTM1 presente (M1+), GSTP1 (Ile/Ile). Alelos variantes: GSTT1 nulo (T1-), GSTM1 nulo (M1-), GSTP1 (Ile/Val ou Val/Val).*

Discussion

The frequencies of *GSTM1* and *GSTT1* null genotypes found in this study were not different from those described in previous publications involving subjects from southern Brazil[18,20] (Supplemental Materials, Tables S1 and S2). The *GSTM1* null genotype frequency was comparable to those reported in other studies with Brazilian and non-Brazilian populations[9,21-25]. Similarly, the *GSTP1* polymorphism genotype frequencies was similar to those found in other Brazilian studies[20,23], and in studies in non-Brazilian populations predominantly with European ancestry[26,27], but considerably different from those described in studies of China and Australia[28,29]. Finally, the frequency of the *GSTT1* null gene in this sample was much lower than previously found in other Western countries[4,28].

Established reproductive risk factors for BC were not frequent in the sample studied: mean age at menarche was relatively late, and mean age at birth of the first child and at menopause were early. A small number of women were nulliparous and were users of hormone replacement therapy for more than 5 years. However, there were frequent reports of first-degree family history of BC, and a very significant proportion of women were overweight and/or obese. In a study of 3,665 women not affected by BC, from the same mammographic screening cohort (Núcleo Mama Porto Alegre - NMPOA cohort), Reyes et al.[30] described a high prevalence of increased BMI (69% of women with BMI ≥ 25) and a low frequency of BC risk factors traditionally included in the Gail model. As expected for a population-based sample, both in the study of Reyes et al. and in this study, estimates of the lifetime risk for developing BC using the Gail model were not higher than expected for the general population. However, Reyes et al. observed that there was a statistically significant difference between categories of mammographic density and estimation of risk by the Gail model: increase of mammographic density and increase of estimated lifetime risk of developing cancer. Considering that BMI and mammographic density are risk factors for BC, Reyes et al. suggested, as others publications, that the inclusion of these variables

could improve risk estimation models in certain populations[31,32,33]. Gail et al. (2008, 2009)[34,35] also attempted to include to the model, data on seven single-nucleotide polymorphisms (SNPs) previously associated with risk of BC, but found only small differences from the estimates obtained with the original variables, possibly due to the choice of SNPs used in the study. The polymorphisms studied by Gail in 7 genes or regions of risk did not include the genes studied here, but the initiative shows that the inclusion of genotyping in risk models may contribute to improving the accuracy and results of such models.

Several pieces of evidence indicate an association between *GSTM1* and *GSTT1* null genotypes and greater susceptibility to a number of tumors (colon, breast, bladder, head, and neck). Specifically in relation to risk for BC, the results are somewhat controversial. In a previous study from Brazil (Amorim *et al.* 2002)[36], and in some studies of white and African-American women in other countries[37-39], there are reports that show no association between *GSTT1* and *GSTM1* null alleles and risk for BC. On the other hand, an increased risk for BC has been observed in women with combined *GSTM1* and *GSTT1* null genotypes in a few studies[3,40]. There are also prior reports of an association between *GSTT1* and *GSTM1* null genotypes and increased mammographic density, a recognized risk factor for BC. In a Brazilian study, Morais et al. (2008)[21] found that women with *GSTM1* deletions had dense breast patterns more often.

In the present study, we identified a statistically significant association between *GSTM1* and *GSTT1* null genotypes and mammographic density (≤ 50% or > 50% dense) in post-menopausal women ($p = 0.031$). The *GSTT1* null genotype alone, was also statistically associated with mammographic density in post-menopausal women ($p = 0.023$) and the overall sample ($p = 0.027$). These findings confirm results from previous studies from our group[14] and other authors, and suggest that the inclusion of specific genotypic analyses and mammographic density may improve the estimation of BC risk in specific populations.

Competing interests: The authors declare that they have no competing interests.

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References

1. Available in http://www.inca.gov.br/estimativa/2010/ (URL accessed in August, 24, 2011).
2. Câncer no Brasil – Dados dos registros de base populacional. volume IV; 2010.
3. Lima-Costa MF, Matos DL. Prevalence and factors associated with mammograms in the 50–69-year age group: a study based on the Brazilian National Household Sample Survey (PNAD-2003) Cad Saude Publica 2007; 23: 1665-73.
4. Park SK, Kang D, Noh DY, Lee KM, Kim SU, Choi JY, et al. Reproductive factors, glutathione S-transferase M1 and T1 genetic polymorphism and breast cancer risk. *Breast Cancer Res Treat* 2003; 78: 89-96.
5. Dunning AM, Healey CS, Pharoah PDP, Teare MD, Ponder BAJ, Easton DF, et al. A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiol Biomar Prev* 1999; 8: 843-54.
6. Norppa H. Genetic susceptibility, biomarkers response, and cancer. *Mutat Res* 2003; 544: 339-48.
7. Miller PD, Liu G, De Vivo I, Lynch TJ, Wain JC, Su L, et al. Combination of the variant genotype of GSTP1, GSTM1 and, p53 are associated with an increased lung cancer risk. *Cancer Res* 2002; 62: 2819-23.
8. Hirvonen A. Genetic factors in individual responses to environmental exposures. *J Occup Med* 1995; 37: 37-41.

9. Zheng W, Wen WQ , Gustafson DR , Gross M , Cerhan JR, Folsom AR. GSTM1 and GSTT1 polymorphisms and postmenopausal breast cancer risk. *Breast Cancer Res Treat* 2002; 74: 9-16.

10. Lancaster JM, Berhuck A, Carney ME, Wiseman R, Taylor JA. Progesterone receptor gene polymorphism and risk for breast and ovarian cancer. *Br J Cancer* 1998; 78: 227-78.

11. Rossit ARB, Cabral IR, Conforti-Froes NDT. Avaliação das frequências alélicas de genes do biometabolismo em uma população brasileira. Genet Mol Biol 1999; 22(Suppl).

12. Strange RC, Fryer AA. The glutathione S-tranferases: influence of polymorphism on cancer susceptibility. *IARC Sci Publ* 1999; 148: 231-49.

13. Fryer AA, Bianco A, Hepple M, Peter WJ, Strange RC, Spiteri MA. Polymorphism at the Glutathione S-transferase GSTP1 Locus. *Am J Respir Crit Care Med* 2000; 161: 1437-42.

14. Giacomazzi J, Aguiar E, Palmero EI, Schmidt AV, Skonieski G, Duarte Filho D, et al. Prevalence of the STK15 F31I polymorphism and its relationship with mammographic density. *Braz J Med Biol Res* 2011; 44: 291-6.

15. Gail MH, Brinton LA, Byar DP, Sylvan BC. Schairer GC, Mulvihill JJ. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst* 1998; 81: 1879-86.

16. American College of Radiology. *Illustrated breast imaging reporting and data system (BIRADS)*. 3th ed. Reston: American College of Radiology; 1998.

17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; 16: 1215.

18. Gaspar PA, Moreira J, Kvitko K, Torres MR, Moreira A and Weimer TA. CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1, and TP53 polymorphisms: Do they indicate susceptibility to chronic obstructive pulmonary disease and non small- cell lung cancer. *Genet Mol Biol* 2004; 27: 133-8.

19. Abramson JH. WINPEPI (PEPI-for-Windows): Computer programs for epidemiologists. *Epidemiol Perspect Innov* 2004; 1(6): 1-10.

20. Kvitko K, Gaspar PA, Torres MR, Hutz MH. CYP1A1, GSTM1, GSTT1 and GSTP1 polymorphisms in an Afro-Brazilian group. *Genet Mol Biol* 2006; 29: 613-16

21. Morais LMTS, Filho CC, Lourenço GJ, Shinzato JY, Zeferino LC, Lima CSP, et al. Características Mamográficas do Câncer de Mama Associadas aos Polimorfismos GSTM1 E GSTT1, *Rev Assoc Méd Bras* 2008; 54 (1): 61-6.

22. Gattás GJF, Kato M, Soares-Vieira JA, Siraque MS, Kohler P, Gomes L, et al. Ethnicity and glutatione S-transferase (GSTM1/GSTT1) polymorphisms in a Brazilian population. *Braz J Med Biol Res* 2004; 37: 451-8.

23. Rossini A, Rapozo DCM, Amorim LMF, Macedo JMB, Medina R, Neto JFN, et al. Frequencies of GSTM1, GSTT1 and GSTP1 polymorphisms in a Brazilian population. Genet *Mol Res* 2002; 1: 233-40.

24. Van Der Hel OL, Bueno-De-Mesquita HB, Van Gils CH, Roest M, Slothouber B, et al. Cumulative genetic defects in carcinogen metabolism may increase breast cancer risk. *Cancer Causes Control* 2005; 16: 675-81.

25. Milikan R, Pittman G, Tse C, Savitz DA, Newman B, Bell D. Glutathione S-transferase M1, T1, and P1 and breast cancer. *Cancer Epidemiol Biomarkers Prev* 2000; 9: 567-73.

26. Samson M, Swaminathan R, Rama R, Sridevi V, Nancy KN, et al. Role of GSTM1 (Null/Present), GSTP1 Ile105Val) and P53 (Arg72Pro) genetic polymorphisms and the risk of breast cancer: a case control study from South India. *Asian Pac J Cancer Prev* 2007; 8: 253-7.

27. Gudmundsdottir K, Tryggvadottir L, and Eyfjord JE. GSTM1, GSTT1, and GSTP1 Genotypes in Relation to Breast Cancer Risk and Frequency of Mutations in the p53 Gene. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 1169-73.

28. Egan KM, Kai Q, Shu XO, Jin F, Zhu TL, Dai Q, et al. Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai Breast Cancer Study and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2004; 13: 197-204.

29. Curran, JE, Weinstein SR and Griffiths LR. Polymorphisms of glutathione S-transferase genes (GSTM1, GSTP1 and GSTT1) and breast cancer susceptibility. *Cancer Lett* 2000; 153: 113-20.

30. Reyes VB. Estimativa de risco de câncer de mama, segundo o modelo de GAIL, em uma população submetida a rastreamento mamográfico em Porto Alegre [dissertação de mestrado]. Rio Grande do Sul: Universidade Federal do Rio Grande do Sul; 2009. Available in http://hdl.handle.net/10183/16768.

31. Chen J, Pee D, Ayyagari R, Graubard B, Schairer C, Byrne C, et al. Projecting absolute invasive breast cancer risk in White women with a model that includes mammographic density. *J Natl Cancer Inst* 2006, 98: 1215-26.

32. Barlow WE, White E, Ballard-Barbash R, Vacek PM, Titus-Ernstoff L, Carney PA, et al. Prospective breast cancer risk prediction model for women undergoing screening mammography. *J Natl Cancer Inst* 2006; 98: 1204-14.

33. Tyrer J, Duffy SW, Cuzick J.A breast cancer prediction model incorporating familial and personal risk factors. *Stat Med* 2004; 15(23): 1111-30.

34. Gail MH. Value of Adding Single-Nucleotide Polymorphism Genotypes to a Breast Cancer Risk Model. *J Natl Cancer Inst* 2009; 101: 959-963.

35. Gail MH. Discriminatory accuracy from single-nucleotide polymorphisms in models to predict breast cancer risk. *J Natl Cancer Inst* 2008; 100: 1037-41.

36. Amorim LMF, 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: 179-86.

37. Ambrosone CB, Coles BF, Freudenheim JL, Shields PG. Glutathione S-transferase (GSTM1) genetic polymorphisms do not affect human breast cancer risk, regardless of dietary antioxidants. *J Nutr* 1999; S129: 565-68.

38. Bailey LR, Roodi N, Verrier CS, Yee CJ, Dupont WD, Parl FF. Breast cancer risk and CYP1A1, GSTM1, and GSTT1 polymorphisms: evidence of a lack of association in Caucasians and African Americans. *Cancer Res* 1998; 58: 65-70.

39. Maugard CM, Charrier J, Bignon Y-J. Allelic deletion at glutathione S-transferase M1 locus and its association with breast cancer susceptibility. *Chem Biol Interact* 1998; 111: 365-75.

40. Mitrunen K, Kataja V, Eskelinen M, Kosma VM, Kang D, Benhamou S, et al. Combined COMT and GST genotypes and hormone replacement therapy associate breast cancer risk. *Pharmacogenetics* 2002; 12: 67-72.

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Supplementary tables
Tabelas suplementares

Table S1 - *GSTT1* (-) and *GSTM1* (-) polymorphisms in breast cancer-unaffected women in the present study and in other studies.
Tabela S1 - *Polimorfismos* GSTT1(-), GSTM1(-) *em mulheres sem câncer de mama neste e em outros estudos*.

Reference	Country	Subjects enrolled	Genotypic frequencies			
			GSTT1 (-) n (%)	p-value	*GSTM1* (-) n (%)	p-value
Present study	Brazil	750	158 (21)		339 (45)	
Gattás *et al.* 2000	Brazil	292			160 (55)	0.005
Rossini *et al.* 2002	Brazil	591	150 (25)	0.062	249 (42)	0.261
Amorim *et al.* 2002	Brazil	256	65 (25)	0.150	103 (40)	0.167
Gaspar *et al.* 2004	Brazil	90	19 (21)	0.992	45 (50)	0.388
Gattás *et al.* 2004	Brazil	594	137 (23)	0.380	261 (44)	0.644
Linhares *et al.* 2005	Brazil	278			104 (37)	0.025
Kvitko *et al.* 2006	Brazil	190	49 (26)	0.161	84 (44)	0.807
Morais *et al.* 2008	Brazil	169	33 (19)	0.656	75 (44)	0.846
Torresan *et al*. 2008	Brazil	102	33 (30)	0.010	56 (55)	0.065
Bailey *et al.* 1997	USA	221	61 (28)	0.041	124 (56)	0.004
Millikan *et al.* 2000	USA	663	104 (16)	0.009	264 (40)	0.041
Curran *et al.* 2000	Australia	129	20 (16)	0.146	72 (56)	0.026
Zheng *et al.*2002	USA	481	62 (13)	<0.001	249 (52)	0.024
Egan *et al.* 2003	China	1221	596 (49)	<0.001	683 (57)	<0.001
Van der Hel *et al.* 2004	Holland	263	50 (19)	0.478	129 (49)	0.281
Park *et al.* 2004	Korea	289	121 (42)	<0.001	152 (54)	0.032

(-) null allele; (-) *alelo nulo*

Table S2 - *GSTP1* (Ile105Val) polymorphism in breast cancer-unaffected women in the present study and in other studies.
Tabela S2 - *Polimorfismo* GSTP1 *(Ile105Val) em mulheres sem câncer de mama neste e em outros estudos.*

Reference	Country	Subjects enrolled	Genotypic frequencies			Allelic frequencies		p-value
			II genotype n (%)	IV genotype n (%)	VV genotype n (%)	I allele (%)	V alelle (%)	
Present study	Brazil	750	330 (44)	329 (44)	91 (12)	0.66	0.34	
Rossini *et al.* 2002	Brazil	591	294 (50)	225 (38)	72 (12)	0.69	0.31	0.081
Kvitiko *et al.* 2006	Brazil	190	76 (40)	94 (49)	20 (11)	0.65	0.35	0.377
Torresan *et al.* 2008	Brazil	102	61 (59)	38 (37)	3 (4)	0.78	0.22	0.002
Millikan *et al.* 2000	USA	663	195 (33)	304 (51)	96 (16)	0.58	0.42	<0.001
Curran *et al.* 2000	Australia	129	59 (46)	64 (50)	6 (4)	0.70	0.30	0.039
Gudmundsdottir *et al.* 2001	Iceland	395	177 (45)	172 (44)	46 (11)	0.67	0.33	0.953
Egan *et al.* 2003	China	1221	809 (67)	371 (31)	31 (2)	0.82	0.18	<0.001
Syamala *et al.* 2007	India	250	125 (50)	109 (44)	16 (6)	0.72	0.28	0.027
Samson *et al.* 2007	India	500	230 (46)	219 (44)	51 (10)	0.68	0.32	0.534