Polymorphisms of glutathione S-transferase Mu 1 (*GSTM1*), Theta 1 (*GSTT1*), and Pi 1 (*GSTP1*) genes and epithelial ovarian cancer risk

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Abstract. *Background:* Exposure of ovarian cells to estrogen, which is detoxified by glutathione S-transferases (GSTs), has been associated with epithelial ovarian cancer (EOC) development.

Objectives: We tested in this study whether the *GSTM1*, *GSTT1* and *GSTP1* Ile105Val polymorphisms alter the risk of EOC.

Materials and methods: Genomic DNA from 132 EOC patients and 132 controls was analyzed by polymerase chain reaction and restriction fragment length polymorphism methods. The differences between groups were analyzed by χ^2 or Fisher's exact test.

Results: The frequencies of *GSTP1* Ile/Ile (57.6% versus 45.5%, $P = 0.03$), *GSTM1* null plus *GSTP1* Ile/Ile (43.5% versus 25.8%; $P = 0.03$) and *GSTM1* null plus *GSTT1* null plus *GSTP1* Ile/Ile (30.3% versus 7.7%; $P = 0.007$) genotypes were higher in patients than in controls. Individuals with the respective genotypes had a 1.80 (95% CI: 1.06–3.06), 2.38 (95% CI: 1.08–5.24) and 11.28 (95% CI: 1.95–65.30)-fold increased risks of EOC than those with the remaining genotypes.

Conclusions: Our data present preliminary evidence that *GSTM1*, *GSTT1* and *GSTP1* polymorphisms, particularly in combination, constitute important inherited EOC determinants in individuals from Southeastern Brazil.

Keywords: Epithelial ovarian cancer, risk, *GSTM1*, *GSTT1*, *GSTP1*

1. Introduction

Estrogen and its metabolites act as initiators of genetic damage in ovarian cells by reactive oxygen species (ROS) [1] and are involved in epithelial ovarian cancer (EOC) development.

The detoxification of estrogen and ROS is performed by glutathione S-transferases M1, T1 and P1 [2]. *GSTM1*, *GSTT1* and *GSTP1* genes are polymorphic in humans. Genes are homozygously deleted in approximately 50% (*GSTM1*) and 20% (*GSTT1*) of Caucasians, resulting in loss of enzymatic activity [2]. Val/Val genotype of *GSTP1* Ile105Val polymorphism is identified in 0–15% of healthy individuals [3], and the protein encoded by Val allele has a defective enzymatic activity in protecting cells from oxidative damage [4].

The absence or reduction of M1, T1 and P1 enzymes has been associated with malignant transforma-

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tion of mammary and ovarian cells [5,6], but the roles of *GSTM1*, *GSTT1* and *GSTP1* Ile105Val polymorphisms, isolated or in combination, for the risk of EOC in Caucasians are still unclear [7–15].

The Brazilian population is highly heterogeneous, consisting of Amerindians and immigrants from Europe, Africa and Asia [16]. Thus, the identification of genotypes of aforementioned polymorphisms in healthy and EOC women was considered necessary to test their influences on the disease in our country.

2. Materials and methods

The case group comprised 132 consecutive EOC patients at diagnosis (median age: 53 years, range: 21–91; 126 Caucasians, 6 non-Caucasians: 58 with serous tumors and 74 with non-serous tumors) seen at the University Hospital and Amaral Carvalho Hospital from June 2007 to May 2010. The EOC was diagnosed according to the criteria of the World Health Organization [17]. One-hundred thirty-two female blood donors (median age: 50 years, range: 39–62; 126 Caucasians, 6 non-Caucasians), seen at the same Institution and period of study, served as controls to provide a representative group of the general population that seeks medical assistance in this region. Selection criteria for individuals were no evidence of any personal or family history of ovarian cancer. All procedures were carried out according to the Helsinki Declaration and written informed consent was obtained from each study participant.

Genomic DNA from peripheral blood of individuals was analyzed by polymerase chain reaction (PCR) multiplex for *GSTM1* and *GSTT1* [18] and PCR followed by enzymatic digestion for *GSTP1* Ile105Val (rs1695) genotyping [19].

The Hardy-Weinberg (HW) equilibrium was evaluated using the chi-square (χ^2) goodness of fit test. The differences between groups were analyzed by the χ^2 or Fisher's exact test. The statistical significance of the differences between median values of the groups was calculated by Mann-Whitney test. Multivariate analysis was performed using the logistic regression model and served to obtain age-adjusted odds ratio (ORs) and assess the associations between genotypes and EOC. Power analysis (PA) was used to calculate the minimum effect size that is likely to be detected in a study using a given sample size (DSS research Statistical Power Calculators: <http://www.dssresearch.com/KnowledgeCenter/toolkitcalculators/statisticalpowercalculators.aspx>). Statistical significance was established at a $P <$

Table 1
Clinical aspects of patients with epithelial ovarian cancer and controls and tumor characteristics of patients

Clinical aspect	Patients (%)	Controls (%)	P value
Median age (years)	53.5	49.8	0.003
range	21–91 years	39–62 years	
Ethnic origin			
Caucasian	126 (95.4)	126 (95.4)	1.00
Black	6 (4.6)	6 (4.6)	
Tumor characteristic			
Serous	58 (43.9)		
Endometrioid	22 (16.7)		
Mucinous	18 (13.6)		
Undifferentiated	15 (11.3)		
Clear cell	8 (6.1)		
Mixed	7 (5.3)		
Transitional	3 (2.3)		
Epidermóide	1 (0.8)		
Histologic grade			
I	45 (34.1)		
II	52 (39.4)		
III	35 (26.5)		
Stage of tumor			
I	52 (39.4)		
II	13 (9.8)		
III	58 (44.0)		
IV	9 (6.8)		

0.05 and testes were done using the SPSS 15.0 software (SPSS Incorporation, Chicago, IL, USA).

3. Results

The clinical aspects of participants and tumor characteristics are presented in Table 1. Differences in age of patients and controls were corrected in all statistical analyses.

Controls' samples were under HW equilibrium ($\chi^2 = 0.07$, $P = 0.79$) but patients' samples did not confirm the HW expectation at the *GSTP1* Ile105Val locus ($\chi^2 = 4.44$, $P = 0.03$).

Similar frequencies of isolated and combined *GSTM1* and *GSTT1* genotypes were seen in patients and controls. Individuals with distinct genotypes were under similar risks for the disease. In contrast, *GSTP1* Ile/Ile genotype was more common in patients than in controls (57.6% versus 45.5%). Carriers of the genotype were under a 1.80 (95% CI: 1.06–3.06)-fold increased risk for EOC than those with Ile/Val and Val/Val genotypes (power of analysis: 34%). Excesses of *GSTM1* null plus *GSTP1* Ile/Ile (43.5% versus 25.8%) and *GSTM1* null plus *GSTT1* null plus *GSTP1* Ile/Ile (30.3% versus 7.7%) combined genotypes were also more seen in patients than in controls. Individuals with the respective genotypes had a 2.38 (95% CI: 1.08–5.24)-fold (power

Table 2
GSTs genotypes in patients with epithelial ovarian cancer and controls

Genotypes	Number (%)		P-value	OR* (95% CI)	Power analysis
	Patients	Controls			
<i>GSTM1</i>					
Null	48 (36.4)	42 (31.8)	0.42	1.23 (0.73–2.07)	12%
Present	84 (63.6)	90 (68.2)		1.00 (reference)	
<i>GSTT1</i>					
Null	39 (29.5)	34 (25.7)	0.47	1.22 (0.70–2.12)	11%
Present	93 (70.5)	98 (74.3)		1.00 (reference)	
<i>GSTM1/GSTT1</i>					
One null	72 (54.5)	68 (51.5)	0.71	1.09 (0.66–1.79)	8%
Both null	15 (11.4)	8 (6.0)	0.08	2.35 (0.90–6.16)	21%
Both present	60 (45.5)	64 (48.5)		1.00 (reference)	
<i>GSTP1</i>					
Ile/Ile	76 (57.6)	60 (45.5)	0.03	1.80 (1.06–3.06)	34%
Ile/Val	42 (31.8)	59 (44.7)	0.62	1.23 (0.53–2.87)	32%
Val/Val	14 (10.6)	13 (9.8)		1.00 (reference)	
Ile/Ile+Ile/Val	118 (89.4)	119 (90.2)	0.89	1.23 (0.53–2.87)	6%
Val/Val	14 (10.6)	13 (9.8)		1.00 (reference)	
Ile/Ile	76 (57.6)	60 (45.5)	0.04	1.68 (1.02–2.77)	51%
Ile/Val+Val/Val	56 (42.4)	72 (54.5)		1.00 (reference)	
<i>GSTM1/GSTP1</i>					
Null + Ile/Ile+Ile/Val	42 (84.0)	37 (82.2)	0.80	1.14 (0.39–3.37)	6%
Present + Val/Val	8 (16.0)	8 (17.8)		1.00 (reference)	
Null + Ile/Ile	27 (43.5)	16 (25.8)	0.03	2.38 (1.08–5.24)	55%
Present + Ile/Val+Val/Val	35 (56.5)	46 (74.2)		1.00 (reference)	
<i>GSTT1/GSTP1</i>					
Null + Ile/Ile+Ile/Val	34 (79.0)	28 (80.0)	0.98	1.01 (0.31–3.22)	5%
Present + Val/Val	9 (21.0)	7 (20.0)		1.00 (reference)	
Null + Ile/Ile	22 (36.0)	19 (25.0)	0.12	1.81 (0.85–3.87)	29%
Present + Ile/Val+Val/Val	39 (64.0)	57 (75.0)		1.00 (reference)	
<i>GSTM1/GSTT1/GSTP1</i>					
Null + Null + Ile/Ile+Ile/Val	13 (72.2)	6 (60.0)	0.32	2.37 (0.42–13.36)	11%
Present + Present + Val/Val	5 (27.8)	4 (40.0)		1.00 (reference)	
Null + Null + Ile/Ile	10 (30.3)	3 (7.7)	0.007	11.28 (1.95–65.30)	70%
Present + Present + Ile/Val+Val/Val	23 (69.7)	36 (92.3)		1.00 (reference)	

*age-adjusted odds ratio; CI: confidence interval.

of analysis: 55%) and 11.28-fold (95% CI: 1.95–65.30) (power of analysis: 70%) increased risks for EOC than those with the remaining genotypes (Table 2).

Similar frequencies of isolated and combined *GSTM1*, *GSTT1* and *GSTP1* genotypes were seen in patients stratified by age, ethnical origin, histological subtypes, grade and stage of the tumor (data not shown).

4. Discussion

We have investigated the influence of *GSTM1*, *GSTT1*, and *GSTP1* Ile105Val polymorphisms on susceptibility and tumor characteristics of EOC in a case-control study conducted in Southeastern Brazil.

We initially observed similar frequencies of *GSTM1* and *GSTT1* genotypes in patients and controls, as previously seen in England [7], Germany [8], United States [9], Australia [11], Brazil [12] and France [13].

Baxter et al. [10] found an excess of *GSTM1* null genotype in Caucasian EOC patients when compared with controls from England. Association of *GSTT1* null genotype with EOC was also seen only in a particular women group with genital talc use by Gates et al. [13]. Our results, as well as those of a recent meta-analysis of previous studies conducted by Economopoulos et al. [15], advocated that *GSTM1* and *GSTT1* genotypes do not confer additional risk for EOC.

Secondly, we observed that *GSTP1* Ile/Ile wild genotype was associated with an increased risk of EOC (power of analysis: 34%), in disaccord with studies conducted in Australia [11] and France [14]. No influence of *GSTP1* Ile105Val polymorphism for the EOC risk in Caucasians was also seen in a recent meta-analysis [15]. In fact, the association between *GSTP1* Ile/Ile genotype and increased risk of EOC in our cases was unexpected because the protein encoded by wild Ile allele seems to be more efficient in protecting cells

from oxidative damage than that produced by variant Val allele [4]. Otherwise, Holley et al. [20] demonstrated that the wild and variant alleles of the *GSTP1* Ile105Val polymorphism protect cells against apoptosis through JNK-independent and JNK-mediated mechanisms, respectively. Thus, the association of *GSTP1* wild genotype and EOC increased risk in our study might be attributed to the effects of the protein encoded by Ile allele for favoring ovarian cell survival by a JNK-independent mechanism.

Thirdly, we observed associations between *GSTM1* null plus *GSTP1* Ile/Ile and *GSTM1/GSTT1* null plus *GSTP1* Ile/Ile with increased risk of EOC (power of analysis 55% and 70% respectively). According to Gates et al. [13], associations of *GSTT1* null and combined *GSTM1/GSTT1* null genotypes with EOC were seen in women using talc in their genital. In contrast, Spurdle et al. [11] and Morari et al. [12] found no association of combined *GSTM1/GSTT1* null genotypes with EOC risk. Thus, we reported for the first time that *GSTM1* null, *GSTT1* null and *GSTP1* Ile/Ile genotypes in combination increase consistently the disease risk in our population, suggesting that GSTs can act synergistically in EOC development. In fact, *GSTM1*, *GSTT1*, and *GSTP1* act on estrogen and ROS metabolism involved in EOC [1,4–6], and inherited homozygous deletions or mutation of the genes lead to the complete absence or reduced activity of enzymes [2,4] and possibly to malignant transformation of ovarian cells [5].

Finally, we observed no associations of *GSTM1*, *GSTT1* and *GSTP1* genotypes with age, ethnical origin, histological type, tumor grade and stage in this study, as previously reported [8,15]. Endometrioid/clear cell subtypes had an increased frequency of *GSTM1* null, according to Baxter's [10] and Spurdle's (2001) [11] studies, suggesting that EOC of different histological types may have different etiologies [21].

The data present preliminary evidence that *GSTM1*, *GSTT1*, and *GSTP1*, particularly in combination, alter the risk of EOC in a mixed population from South-eastern Brazil. We recognize that our conclusions are based on relatively small numbers of individuals and will require verification from additional larger independent studies. Moreover, functional analyses of the proteins encoded by the wild and variant alleles of *GSTP1* gene on ovarian cell survival are also required for clarifying the roles of the gene in EOC development.

Acknowledgments

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Con-

selho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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