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Short Communication

Polymorphisms in *GSTM1*, *GSTT1*, *GSTP1*, and *GSTM3* genes and breast cancer risk in northeastern Mexico

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ABSTRACT. Glutathione *S*-transferases (GSTs) are a family of phase II metabolizing enzymes involved in carcinogen detoxification and the metabolism of various bioactive compounds. Several genes that code for these enzymes are polymorphic in an ethnicity-dependent manner, with particular genotypes previously associated with an increased risk of breast cancer. The purpose of this study was to determine the frequencies of polymorphisms in the genes *GSTM1*, *GSTT1*, *GSTP1*, and *GSTM3* and to investigate whether an association exists between these genes and breast cancer risk in subjects from northeastern Mexico. Genotypes were determined for 243 women with histologically

confirmed breast cancer and 118 control subjects. Gene polymorphisms were analyzed using a DNA microarray. We found an increased breast cancer risk associated with the *GSTM1* gene deletion polymorphism (OR = 2.19; 95%CI = 1.50-3.21; P = 0.001). No associations between the *GSTT1*, *GSTP1*, and *GSTM3* genotypes and neoplasia risk were observed. In conclusion, we determined the genotype distribution of *GST* polymorphisms in control subjects and breast cancer patients from northeastern Mexico. The *GSTM1* null genotype was associated with breast cancer risk. Our findings may be used to individualize breast cancer screening and therapeutic intervention in our population, which displays ethnic characteristics that differentiate it from other populations in Mexico.

Key words: Breast cancer; Ethnicity; Glutathione *S*-transferases; Northeastern Mexico; Polymorphisms

INTRODUCTION

Breast cancer is the most frequently diagnosed malignancy and the leading cause of cancer death in females in both developed and developing countries (Jemal et al., 2011). Although the mechanism of breast carcinogenesis is not fully understood, it is clear that both genetic and environmental factors play a role in this disease. Up to 5% of all breast cancers arise from germ-line mutations in high-penetrance breast cancer susceptibility genes such as *BRCA1* and *BRCA2*. Therefore, the interaction between polymorphic, low-penetrance genes, and lifestyle or environmental risk factors likely accounts for a much higher proportion of breast cancer cases (Sarmanová et al., 2004).

Low-penetrance genes can participate in the detoxification of environmental carcinogens, steroid hormone metabolism, and DNA damage repair pathways (Mitrunen and Hirvonen, 2003). Glutathione *S*-transferases (GSTs) are a family of phase II metabolizing enzymes involved in carcinogen detoxification and the metabolism of various bioactive compounds (Coles and Kadlubar, 2003). Functional polymorphisms in *GSTM1*, *GSTT1*, and *GSTP1* have been investigated for their associations with breast cancer in a large number of studies (Egan et al., 2004; Syamala et al., 2008; Van Emburgh et al., 2008; Saxena et al., 2009; Ramalhinho et al., 2011; Reding et al., 2012; Sohail et al., 2013). In contrast, studies analyzing the relationship between *GSTM3* and breast cancer are limited (Mitrunen et al., 2001). Identification of inter-individual variability in *GST* polymorphisms may be useful for individualizing breast cancer screening and therapeutic intervention.

Most studies previously were carried out in Caucasians, African-Americans, and Asians. In Mexico, only one related study has been carried out, in which subjects in the country's central zone were analyzed (Martínez-Ramírez et al., 2013). Thus, the purpose of this study was to investigate the genotypic and allelic frequencies of polymorphisms in *GSTM1*, *GSTT1*, *GSTP1*, and *GSTM3* to determine whether an association exists between polymorphisms in these genes and the risk of breast cancer in subjects from northeastern Mexico.

MATERIAL AND METHODS

Biological samples

The patient population and methods have been described in detail in a previous report (Alcazar-González et al., 2013). The case subjects included 243 women with histologically confirmed breast cancer who received chemotherapy at the University Cancer Center of the University Hospital "Dr José E González" of the Autonomous University of Nuevo Leon and the Hospital of Specialities number 25 of the Mexican Institute of Social Security, both located in Monterrey, Nuevo León, Mexico. Both are reference centers for breast cancer patients from throughout the northeastern area of Mexico, including the states of Zacatecas, San Luis Potosí, Tamaulipas, Coahuila, and Nuevo León. A total of 118 controls with no previous history of any type of cancer or other vital disease were also studied. This study conformed to the Declaration of Helsinki, was approved by the local ethics committee (registration HU BI10-002), and all participants provided informed written consent.

Genotyping

Genomic DNA was obtained from peripheral blood samples either using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer protocol or using TSNT lysis buffer (1% Triton, 1% sodium dodecyl sulfate, 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) followed by phenol-chloroform extraction and ethanol precipitation.

Analysis of gene polymorphisms was performed using the PHARMAchip® DNA microarray following manufacturer protocols (Progenika Biopharma SA, Derio, Spain). Deletion polymorphisms in *GSTM1* and *GSTT1* and a base transition polymorphism at codon 105 (Ile/Val) in *GSTP1* were analyzed. In the *GSTM3* gene, the *GSTM3*A* wild-type and *GSTM3*B* variant allele, which differed by a deletion of 3 base pairs in intron 6, were analyzed.

Statistical analysis

To identify significant differences in polymorphism frequencies between case and control groups, allele and genotype frequencies were compared statistically using the R x C contingency-table exact test. Hardy-Weinberg equilibrium (HWE) for *GSTP1* and *GSTM3* was estimated using the Chi-square test. HWE was not evaluated for *GSTM1* and *GSTT1* because the genotyping method did not distinguish heterozygous and homozygous genotypes. The data were input to SPSS, version 22.0 (SPSS Inc., Chicago, IL, USA) for handling and further statistical analyses. ORs and 95%CI were calculated to determine significant associations using the Epi Info program (version 7.1.3, CDC, Atlanta, GA, USA). In all analyses, a significance level of 0.05 was adopted.

RESULTS

Genotypes and allele distributions of *GSTM1*, *GSTT1*, *GSTP1*, and *GSTM3* polymorphisms in cases and controls are summarized in Table 1. Thirty-four (30.1%) of the control subjects had the *GSTM1* null genotype. The observed frequency of the null genotype in breast cancer patients was 48.5%. The *GSTM1* null genotype was associated with a 2.19-fold

(95%CI = 1.50-3.21) increase in the risk of developing breast cancer. This association was statistically significant (P = 0.001).

Table 1. Genotype and allele frequencies of glutathione *S*-transferase (GST) genes polymorphisms in breast cancer patients and healthy controls from northeastern Mexico.

Gene	Frequencies	Cases [N[a] (%)]	Controls [N[a] (%)]	P value[b]
GSTM1	Genotype			
	Present	124 (51.5)	79 (69.9)	0.001
	Null	117 (48.5)	34 (30.1)	
	Total	241 (100)	113 (100)	
GSTT1	Genotype			
	Present	211 (86.8)	92 (80.9)	0.156
	Null	32 (13.2)	22 (19.1)	
	Total	243 (100)	114 (100)	
GSTP1	Genotype			
	Ile/Ile	58 (24.0)	35 (29.7)	0.301
	Ile/Val	105 (43.4)	53 (44.9)	
	Val/Val	79 (32.6)	30 (25.4)	
	Total	242 (100)	118 (100)	
	χ^2	3.82[c]	1.18[d]	
	Allele			
	Ile	0.4570	0.5212	
	Val	0.5430	0.4788	
GSTM3	Genotype			
	*A/*A	198 (83.9)	102 (87.2)	0.329
	*A/*B	34 (14.4)	15 (12.8)	
	*B/*B	4 (1.7)	0 (0.0)	
	Total	236 (100)	117 (100)	
	χ^2	2.93[d]	0.54[d]	
	Allele			
	*A	0.9110	0.9359	
	*B	0.0890	0.0641	

χ^2 = Hardy-Weinberg equilibrium. [a]Numbers may not sum to totals due to missing data. [b]R x C with 50,000 simulations. [c]P = 0.05. [d]P > 0.05

The *GSTT1* null genotype was observed in 13.2 and 19.1% of cases and controls, respectively. In all, 43.4% of cases and 44.9% of controls were heterozygous and 32.6% of cases and 25.4% of controls were homozygous for the *GSTP1* Ile105Val polymorphism. Finally, when the *GSTM3* gene was analyzed, 14.4% of cases and 12.8% of controls carried the heterozygous *A/*B genotype, while 1.7% of cases and 0.0% of controls carried the homozygous *B/*B genotype. The distribution of genotype frequencies of *GSTP1* and *GSTM3* were in HWE, with the exception of *GSTP1* frequencies in breast cancer cases. No associations between *GSTT1*, *GSTP1*, and *GSTM3* genotypes and breast cancer risk were observed.

DISCUSSION

Enzymes involved in carcinogen detoxification and metabolism of bioactive compounds have been shown to be polymorphic in an ethnicity-dependent manner. In the present study, genetic polymorphisms of *GSTs* were investigated in subjects from northeastern Mexico, and their possible association with breast cancer risk was evaluated.

The genotype distributions for *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in control subjects were in agreement with the results of previous reports in Mexicans subjects (Pérez-

Morales et al., 2011; Martínez-Rodríguez et al., 2013). The frequency of the *GSTM1* null genotype found in this study was 30%, which is similar to the values observed in Asians and African Americans (~27%), but not similar to the value in Caucasians (~50%). The frequencies of the Ile/Ile (29%) and Val/Val (25%) genotypes of *GSTP1* found in this study were more similar to those in African Americans (~29 and ~20%, respectively) that in Caucasians (~44 and ~10%) and Asians (~49 and ~7%). The *GSTT1* null genotype frequency (19%) was similar to that in most populations analyzed thus far (Egan et al., 2004; Syamala et al., 2008; Van Emburgh et al., 2008; Saxena et al., 2009; Ramalhinho et al., 2011; Reding et al., 2012; Sohail et al., 2013). Finally, we determined the frequencies of the *GSTM3* genotypes in Mexican subjects. Previous reports regarding *GSTM3* genetic polymorphisms frequencies are very limited in the literature (Mitrunen et al., 2001).

A homozygous deletion (null genotype) of *GSTM1* or *GSTT1* was found to be associated with a lack of the corresponding enzyme activity. The presence of the substitution of isoleucine for valine results in reduced activity of the *GSTP1* enzyme. Relatively little is known about the role of *GSTM3* in the metabolism of carcinogenic compounds, but the *GSTM3 *A* and **B* alleles may be expressed at different levels with different efficiencies in the metabolism of harmful agents (Mitrunen et al., 2001). This may compromise an individual's ability to deactivate carcinogens, thus increasing the risk of cancer (Mitrunen and Hirvonen, 2003). In the current study, only the *GSTM1* null genotype was associated with breast cancer risk. We found no significant increase in breast cancer risk associated with the *GSTT1*, *GSTP1*, and *GSTM3* genotypes.

A large number of studies have examined the relationship between *GST* polymorphisms and breast cancer risk. Overall, no clear pattern has emerged. Studies in which an association between individual or combined *GST* genotypes and breast cancer was observed were not confirmed by other studies and were refuted (Mitrunen et al., 2001; Egan et al., 2004; Syamala et al., 2008; Van Emburgh et al., 2008; Saxena et al., 2009; Ramalhinho et al., 2011; Reding et al., 2012; Sohail et al., 2013; also see for reviews and meta-analyses: Mitrunen and Hirvonen, 2003; Sergentanis and Economopoulos, 2010; Chen et al., 2011). The discrepancies among studies may be due to several factors, including differences in ethnicity, environmental exposure to carcinogens, and diet. Alternatively, these differences may be attributable to methodological issues, such as the study design, sample size, and analysis method.

In the only previous related study carried out in Mexico, *GSTP1* polymorphisms were associated with an increased breast cancer risk; no association was observed for *GSTM1* and *GSTT1* (Martínez-Ramírez et al., 2013). The differences between this study and our results are likely related to ethnicity and methodological factors.

The admixture among Amerindians, Europeans, and Africans resulted in Mestizos, who represents more than 90% of the Mexican population (Instituto Nacional de Estadística y Geografía, 2010). However, the Mexican population exhibits high genetic variability. Rubi-Castellanos et al. (2009) analyzed the genetic data of 13 combined DNA index system-short tandem repeats in subjects representing population samples from different regions of Mexico. They found significant genetic differentiation among Mestizos from different Mexican regions, mainly between the North and the Central and South regions. Furthermore, they observed genetic heterogeneity or asymmetric admixture throughout Mexico, displaying an increasing North-to-South gradient of Amerindian ancestry, and vice versa regarding the European component. Our study examined subjects from northeastern Mexico, whereas in the

previous study, subjects of the country's central zone were analyzed. Thus, ethnic diversity may have contributed to the discrepancies observed between these studies.

However, the differences in results between both studies may be related to methodological issues. In our study, the genotype frequencies of *GSTP1* deviated from the HWE in the case group (Table 1). Because other polymorphisms did not deviate from HWE, our sample set was appropriately ascertained (Wittke et al., 2005). Deviation from HWE may have resulted from random selection, likely because of sample size. Thus, an analysis of a larger sample size of our population may reveal an association between the *GSTP1* genotype and breast cancer risk, which would coincide with the results of the other study carried out in Mexico.

In conclusion, we determined the genotype distribution of *GSTM1*, *GSTT1*, *GSTP1*, and *GSTM3* polymorphisms in control subjects and breast cancer patients from northeastern Mexico. Only the *GSTM1* null genotype was associated with breast cancer risk. Analysis of a larger sample, inclusion of matched controls, and the consideration of gene-environment interactions could augment our understanding of the association between *GSTs* and breast cancer risk in our population.

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