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Frequencies of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in a Brazilian population

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ABSTRACT. The glutathione S-transferase (GST) family of enzymes has a vital role in phase II of biotransformation of environmental carcinogens, pollutants, drugs and other xenobiotics. GSTs are polymorphic, with the type and frequency of polymorphism being ethnic dependent. Polymorphisms in *GST* genes have been shown to be associated with susceptibility to disease and disease outcome. We determined the frequencies of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in 591 volunteers who had been residents of Rio de Janeiro for at least six months. Blood was collected and DNA extracted by proteinase K/SDS digestion. Information about social habits and health problems was also recorded. *GSTM1* and *GSTT1* polymorphisms were analyzed by a PCR-Multiplex procedure, whereas *GSTP1* polymorphism was analyzed by PCR-RFLP. We found that 42.1% (48.9% of whites and 34.2% of non-whites) of the individuals had the GSTM1 null genotype, whereas 25.4% (25.1% of whites and 25.7% of non-whites) had the GSTT1 null genotype. The genotypic distribution of GSTP1 was 49.7% I/I, 38.1% I/V, and 12.2% V/V, whereas the allelic frequencies were 0.69 for the Ile

allele, and 0.31 for the Val allele. The frequencies of GST polymorphisms in this Brazilian population were found to be different from those observed in other populations, particularly of other South American countries.

Key words: Glutathione S-transferase, Polymorphism, Brazil

INTRODUCTION

Glutathione S-transferases (GSTs) are a supergene family of enzymes involved in phase II of biotransformation, which is characterized by the conjugation of endogenous water-soluble compounds to lipophilic substrates. GSTs catalyze the conjugation of glutathione, a tripeptide consisting of glycine, glutamic acid and cysteine, to electrophilic compounds, resulting in less reactive and more easily excreted glutathione conjugates. Substrates of GST-catalyzed reactions include pre-carcinogens, such as polycyclic aromatic hydrocarbons, pharmacological drugs, including paracetamol, chemotherapeutic agents and free radicals generated during oxidative stress (Strange et al., 2001). Recently, GSTs have been shown to act as inhibitors of the jun kinase pathway, which is an important signaling mechanism for the activation of cytoprotective genes (Adler et al., 1999).

Human cytosolic GSTs have been well characterized, are polymorphic, and have ethnic-dependent polymorphism frequencies. The *GSTM1* gene is located on chromosome 1p13.3, and 20 to 50% of individuals do not express the enzyme due to a homozygous gene deletion, known as the *GSTM1*0*, or null allele (Seidgard et al., 1988). The percentage of individuals who do not express the enzyme is higher in Caucasians and Asians than in Africans (Bailey et al., 1998; Roth et al., 2000). *GSTM1* is involved in the detoxification of polycyclic aromatic hydrocarbons and other mutagens, and cells from *GSTM1* null individuals are more susceptible to DNA damage caused by these agents (Strange and Fryer, 1999).

The *GSTT1* gene is located on chromosome 22, and 20 to 60% of individuals do not express the enzyme, also due to a gene deletion, known as the *GSTT1*0* allele (Pemble et al., 1994). About 60% of Asians, 40% of Africans and 20% of Caucasians do not express this enzyme (Strange and Fryer, 1999). This polymorphism accounts for the variation in GST-catalyzed metabolism of halomethanes by human erythrocytes (Pemble et al., 1994).

The *GSTP1* gene is located on chromosome 11q13. The *GSTP1*A* allele (the wild type) contains adenine, whereas the *GSTP1*B* allele contains guanine at nucleotide + 313, producing Val^{105}, instead of Ile^{105} in the protein (Harries et al., 1997). The polymorphic enzyme has a 7-fold higher diol epoxide activity, and a 3-fold lower 1-chloro-2,4-dinitrobenzene activity, when compared to the wild-type protein (Harries et al., 1997).

Polymorphisms of *GSTM1*, *GSTT1*, and *GSTP1* have been associated with differences in susceptibility to various forms of cancer, particular those caused by cigarette smoking (Strange and Fryer, 1999), in resistance to chemotherapy treatment, in drug response (Hayes and Pulford, 1995), and in disease susceptibility and outcome (Lear et al., 1996; Fryer et al., 2000).

We analyzed the frequency of the *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in a multi-ethnic urban Brazilian population, since polymorphism in these low-penetrance genes may predispose Brazilians to certain adverse drug reactions or disease occurrence.

MATERIAL AND METHODS

The population studied

The population that took part in the present study was composed of outpatients recruited at the Hospital Universitário Pedro Ernesto (HUPE-UERJ) during 1999, who had had different pathologies; an effort was made to avoid selecting a specific group of volunteers. Those who had a history of cancer were not included in this study. They signed an informed consent and information was obtained by a standardized questionnaire, including data on social habits and health problems. All the volunteers had been residents of the metropolitan area of Rio de Janeiro for at least six months. Ethnic selection was based on skin color. Among the group of individuals eligible for the study (591), 319 were white and 272 were non-white (among these, 140 were mulatto and 132 were black individuals who were analyzed separately). The study proposal and the ethics procedures were approved by the Ethics Committee of HUPE-UERJ.

DNA extraction and genotyping of *GSTM1*, *GSTP1* and *GSTT1*

Blood was collected in EDTA-containing tubes and DNA was extracted from the lymphocytes by proteinase K/SDS digestion as described by Miller et al. (1988).

The polymorphisms of *GSTM1* and *GSTT1* were analyzed by a polymerase chain reaction (PCR)-multiplex procedure, as previously described (Amorim et al., 2002).

The polymorphism of *GSTP1* (Ile105Val) was studied by PCR-RFLP (Harries et al., 1997). Genomic DNA (100 ng) was used as a DNA template in 50 µl of total volume reaction. The PCR products were digested in 25 µl for 2 h at 37°C with 5 U Alw261 (MBI Fermentas, Lithuania). The digestion products were separated on a 3.5% agarose gel stained with ethidium bromide or on a 10% polyacrylamide gel that was silver stained (Sanguinetti et al., 1994). The presence of a 176-pb fragment indicated the wild type genotype (I/I), whereas 85- and 91-bp fragments indicated the homozygous polymorphic genotype (V/V). Heterozygous individuals had all three types of fragments.

Statistical analysis

Statistical analysis was done using GraphPad Instat (CA, USA) and Genepop (Genepop web version 3.1) software.

RESULTS

The Rio de Janeiro resident volunteers who took part in this study were being treated for diverse pathologies (Table 1). This is an important consideration since GST polymorphisms can predispose to some diseases, and patients who have been treated for a particular disease may have a specific GST genotype, which would otherwise lead to spurious results. The volunteers had a mean age of 50.4 years old, ranging from 18 to 89 years old. When the individuals were grouped according to gender or skin color, there were no significant differences in the mean age of the individuals.

Table 1. Characteristics of the Pedro Ernesto University Hospital (Rio de Janeiro) population.

Color	Men		Women		Total	
	N	age	N	age	N	age
Black	28	50.6	104	56.5	132	55.3
Mulatto	33	48.3	107	49.5	140	49.3
Non-white	61	49.3	211	52.9	272	52.2
White	100	44.5	219	51.1	319	48.9
Total	161	46.1	430	52.0	591	50.4

Non-white includes mulatto and black individuals.

The null *GSTM1* and GSTT1 genotypes were found for 42.1 and 25.4% of the individuals, respectively, whereas 9.8% had the null genotype for both genes (Table 2). When the individuals were grouped according to skin color, a significantly higher percentage (48.9%) of white individuals had the *GSTM1* null genotype than non-whites (34.2%, P = 0.0003). Although there was no significant difference in the frequency of the *GSTT1* null genotype between white and non-white individuals, white individuals had a significantly higher frequency of *GSTM1* and *GSTT1* null genotypes than non-white individuals (P = 0.0004). This difference was even greater when white individuals were compared with blacks; only 4.5% of the latter had the double null genotype. There were no significant differences between men and women in the frequency of *GSTM1* or *GSTT1* null genotypes, both in white and in non-white individuals.

Table 2. Frequencies (number and (%) of individuals) of *GSTM1* and *GSTT1* null genotypes.

	Black	Mulatto	Non-white	White	Total
Men					
N	28	33	61	100	161
GSTM1-	6 (21.4)	14 (42.4)	20 (32.8)	45 (45.0)	65 (40.4)
GSTT1-	10 (35.7)	8 (24.2)	18 (29.5)	31 (31.0)	49 (30.4)
GSTM1-/T1-	3 (10.7)	1 (3.0)	4 (6.6)	18 (18.0)	22 (13.7)
Women					
N	104	107	211	219	430
GSTM1-	31 (29.8)	42 (39.3)	73 (34.6)	111 (50.7)	184 (42.8)
GSTT1-	27 (26.0)	25 (23.4)	52 (24.6)	49 (22.4)	101 (23.5)
GSTM1-/T1-	3 (2.9)	7 (6.5)	10 (4.7)	26 (11.9)	36 (8.4)
Total					
N	132	140	272	319	591
GSTM1-	37 (28.0)	56 (40.0)	93 (34.2)	156 (48.9)	249 (42.1)
GSTT1-	37 (28.0)	33 (23.6)	70 (25.7)	80 (25.1)	150 (25.4)
GSTM1-/T1-	6 (4.5)	8 (5.7)	14 (5.1)	44 (13.8)	58 (9.8)

Non-whites include mulatto and black individuals.

The *GSTP1* polymorphism frequencies in these Brazilian individuals were 49.7% for the I/I genotype, 38.1% for the I/V genotype, and 12.2% for the V/V genotype (Table 3). Among white individuals, the frequencies were 51.4% I/I, 34.2% I/V, and 14.4% V/V, whereas among non-white individuals, 47.8% were I/I, 42.6% were I/V, and 9.6% were V/V. Therefore,

although the *GSTP1* homozygous genotype (V/V) was more frequent in white than in non-white individuals, the former had a lower frequency of the heterozygous genotype. The *GSTP1* genotype frequencies in this white Brazilian population are not in Hardy-Weinberg equilibrium (Table 4), with a lower than expected frequency of heterozygous individuals. There was no significant difference between men and women in the frequency of the *GSTP1* genotype, both in white and in non-white individuals. The Ile allele frequency in this Brazilian population was 0.687.

Table 3. Genotypic frequencies (number and (%) of individuals) of *GSTP1* polymorphisms.

	Black	Mulatto	Non-white	White	Total
Men					
N	28	33	61	100	161
I/I	12 (42.9)	15 (45.5)	27 (44.3)	49 (49.0)	76 (47.2)
I/V	14 (50)	14 (42.4)	28 (45.9)	35 (35.0)	63 (39.1)
V/V	2 (7.1)	4 (12.1)	6 (9.8)	16 (16.0)	22 (13.7)
Women					
N	104	107	211	219	430
I/I	49 (47.1)	54 (50.5)	103 (48.8)	115 (52.5)	218 (50.7)
I/V	46 (44.2)	42 (39.3)	88 (41.7)	74 (33.8)	162 (37.7)
V/V	9 (8.7)	11 (10.3)	20 (9.5)	30 (13.7)	50 (11.6)
Total					
N	132	140	272	319	591
I/I	61 (46.2)	69 (49.3)	130 (47.8)	164 (51.4)	294 (49.7)
I/V	60 (45.5)	56 (40.0)	116 (42.6)	109 (34.2)	225 (38.1)
V/V	11 (8.3)	15 (10.7)	26 (9.6)	46 (14.4)	72 (12.2)

Non-whites include mulatto and black individuals.

Table 4. *GSTP1* genotypes and allelic frequencies in white and non-white individuals.

Population	I/I	I/V	V/V	N	Allelic frequency		χ^2	P
					I	V		
White								
Observed	164	109	46	319	0.683	0.317	15.06	0.0001
Expected	149	138	32					
Non-white								
Observed	130	116	26	272	0.691	0.309	0	1.000
Expected	130	116	26					
Total								
Observed	294	225	72	591	0.687	0.313	7.65	0.046
Expected	279	254	58					

Non-whites include mulatto and black individuals.

DISCUSSION

The Brazilian population is multi-ethnic, being mainly composed of people from Iberian, African and South-Amerindian origins, with a smaller number of individuals having Caucasian

origins, such as descendants from Germany, Italy and other European countries. A recent study has shown that most of the current population is composed of descendants of Portuguese men and African and South Amerindian women (Carvalho-Silva et al., 2001). Therefore, the Brazilian population may be unique in the types and frequencies of genetic polymorphisms of drug metabolizing enzymes, which have so far untill now only been analyzed in ethnic groups that are not so strongly mixed. Polymorphisms in *GST* genes can lead either to a lack of expression or to the expression of GST enzymes that possess a different catalytic activity than the wild-type protein. Since GST enzymes play a vital role in cellular defense against environmentally toxic compounds, such as carcinogens, polymorphism of *GST* genes in Brazilians may predispose them to diseases caused by such xenobiotics.

We found that 42.1% of this Brazilian population were homozygotic for the *GSTM1* deletion. This frequency is similar to that seen in two other studies that analyzed *GSTM1* polymorphism in Brazilians (Arruda et al., 1998; Hatagima et al., 2000). The frequency of *GSTM1* null was also similar to that found in studies made on some other populations. The *GSTM1* homozygotic deletion is present in 46% of Americans (Bailey et al., 1998), in 49% of Polish (Szklarz et al., 1999), and in 51% of Swedish people (Zhang et al., 1999). However, the frequency of Brazilians who possess the *GSTM1* deletion is much higher than that of Chileans (21.4%) (Quinones et al., 1999). This difference can probably be explained by the ethnic mixture that makes up Brazilian and Chilean populations, with the former having a greater influence of individuals of African origin, whereas the latter has a higher number of individuals of South Amerindian origin.

We observed that 25.4% of these Brazilians were homozygotic for the gene deletion *GSTT1*. This frequency is higher than that observed in some other populations, found in 14% of Americans (Bailey et al., 1998) and 20% of Swedish (Zhang et al., 1999). Although the frequencies of *GSTM1* and *GSTT1* gene deletions in our population appear to be higher than or similar to the frequency of these polymorphisms in other Western countries, they are lower than those found in Asia. In a Chinese study, 64 and 63% of individuals were found to have *GSTM1* and *GSTT1* deletions, respectively (Tan et al., 2000). It has been suggested that the high frequencies observed are associated with the high incidence of esophageal cancer in China (Tan et al., 2000).

When we grouped the Brazilian population according to skin color, we observed that the *GSTM1* gene deletion was more frequent in white (48.9%) than in non-white Brazilians (34.2%), whereas the *GSTT1* polymorphisms were equally frequent in non-whites (25.7%) and in whites (25.1%). The North American population also has a higher frequency of *GSTM1* gene deletion in Caucasians (62%) than in African-Americans (41%) (Bailey et al., 1998), though these frequencies are slightly higher than those found in both of the ethnic groups of our Brazilian population. Additionally, the frequency of *GSTT1* polymorphism was also equally represented in both ethnic groups in the American population (28%) (Bailey et al., 1998).

In our examination of the *GSTP1* polymorphism, we found that 49.7% had the I/I genotype, 38.1% had the I/V genotype, and 12.2% had the V/V genotype, with an allelic frequency of 0.687 for the Ile allele. The *GSTP1* homozygous polymorphic genotype (V/V) was more frequent in white than in non-white individuals. These results presented a similar allelic frequency, but a different genotypic distribution than those obtained in a previous study with the North American population, in which Watson et al. (1998) found that among the Euro-American population, 42% were I/I, 51% were I/V, and 7% were V/V. Among the Afro-Ameri-

can individuals, 35% were I/I, 46% were I/V, and 19% were V/V. In the North American population as a whole, the Ile allele frequency was 0.67 (Watson et al., 1998).

The analysis of *GSTP1* polymorphism in our Brazilian population revealed that there is a higher frequency of homozygotes than heterozygotes, particularly among white individuals. This particular group is not in Hardy-Weinberg equilibrium. Another study that analyzed the frequency of the *GSTM1* genotype in the Brazilian population of Rio de Janeiro and Brasília, also showed that these groups are not in Hardy-Weinberg equilibrium (Hatagima et al., 2000). This lack of equilibrium does not seem to be unique to Brazilians. A recent study showed that the European-American population was not in Hardy-Weinberg equilibrium for the *GSTP1* polymorphism (Watson et al., 1998).

Nevertheless, we observed that the frequencies of *GSTM1*, *GSTT1*, and *GSTP1* polymorphisms in this Brazilian population were different from those found in populations with a lower degree of ethnic mixture. They were greatly different from those found in other South American populations. We suggest that studies on the impact of these low penetrance genes on disease susceptibility and outcome should be in Brazil, rather than extrapolating results obtained from other populations.

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