

## Glutathione S-transferase M1 (GSTM1) polymorphism in two Brazilian populations

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### Abstract

The distribution of GSTM1 phenotype frequencies was studied in two Brazilian samples composed of healthy and unrelated blood donors of both sexes ranging in age from 18 to 61 years. The first sample consisted of 658 individuals from Rio de Janeiro, and the second included 179 individuals from Brasília. The GSTM1 phenotypes were detected using PCR reactions and subsequent digestion by the restriction enzyme *Hae*II. The GSTM1 null phenotype frequency was 46% and 49% for Rio de Janeiro and Brasília samples, respectively. The GSTM1 phenotype distributions were not in agreement with Hardy-Weinberg equilibrium in either sample,  $\chi^2_1 = 11.49$  ( $P < 0.001$ ) for Rio de Janeiro and  $\chi^2_1 = 6.77$  ( $P < 0.01$ ) for Brasília. This deviation from Hardy-Weinberg equilibrium may be due to factors such as selection, errors in the phenotype determination or incomplete panmixia of the Brazilian population, whose main racial components are Caucasians, Africans and Indians.

### INTRODUCTION

The glutathione S-transferase (GST) enzyme system constitutes a family of multifunctional enzymes which play an important role in biotransformation and detoxification of many different xenobiotic and endogenous substances (Wolkoff, 1980; Mannervick, 1985). GSTM1, a mu class gene of the GST family is polymorphic and has three alleles: two of them are active, GSTM1\*A and GSTM1\*B, and the third, GSTM1\*0, is a null allele. The GSTM1\*0 allele is a deletion and homozygotes for this allele (null phenotype) do not produce any protein product. The GSTM1\*A and GSTM1\*B alleles code for the GSTM1 A and GSTM1 B proteins (Seidegard *et al.*, 1988). These proteins are functionally identical (Widersten *et al.*, 1991) and differ only in a single amino acid. The GSTM1 A protein contains lysine at position number 172 (DeJong *et al.*, 1988), whereas the GSTM1 B protein contains asparagine at this position (Seidegard *et al.*, 1988). The products from these two genes combine with each other to form homo- and hetero-dimeric active enzymes (Seidegard *et al.*, 1988; DeJong *et al.*, 1991).

This locus is interesting because of its polymorphic characteristics and the high frequency of the null phenotype (40–60%) in most of the populations studied, which varies among ethnic groups (Board, 1981; Harada *et al.*, 1987; Groppi *et al.*, 1991; Mikelsaar *et al.*, 1994). Reports of a lower incidence of the null allele in Nigerians and Indians contrast with a higher incidence in Chinese and some Micronesians (Board *et al.*, 1990; Zhao *et al.*, 1994; Lin *et al.*, 1994). There are also differences in the other alleles: the frequency of GSTM1\*A is higher and that of GSTM1\*B is lower in Indians, French, English and Nigerians than in Chi-

nese and Japanese populations (Laisney *et al.*, 1984; Harada *et al.*, 1987; Board *et al.*, 1990; Zhao *et al.*, 1994; Heagerty *et al.*, 1996). In addition, homozygosity for GSTM1\*0 is associated with an increased risk of several pathologies, including some malignancies (Strange, 1993; Baranov *et al.*, 1996; Helzlsouer *et al.*, 1998). Thus, GSTM1 polymorphism could be used, possibly in combination with other detoxifying enzyme polymorphisms, to identify high-risk individuals in clinical surveillance programmes (Strange, 1993). This is complicated, however, by the substantial differences in the GSTM1 allele frequencies observed in different ethnic groups. These frequencies are known in many populations but there are limited data from Brazilian populations.

In this work our aim was to study GSTM1 gene polymorphism in the Brazilian population, since it is a special case of a tri-racial mixture of Caucasian, African, and Indian genes (Krieger *et al.*, 1965).

### MATERIAL AND METHODS

Blood samples from unrelated adult volunteers of both sexes, with ages ranging from 18 to 61 years were collected from the Clementino Fraga Filho Hospital Blood Bank (Rio de Janeiro) and from the Brasília Blood Bank. The Rio de Janeiro sample consisted of 658 subjects (392 males and 266 females) and the Brasília sample was comprised of 179 subjects (154 males and 25 females). DNA was extracted from 5 ml of venous blood collected in EDTA Vacutainer tubes and stored at 4°C (Lahiri and Nurnberger Jr., 1991).

The GSTM1 polymorphisms were identified by the use of site-directed mutagenesis of allele-specific PCR primers. All PCR reactions were performed using three primers

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to GSTM1. These comprised, firstly, a common GSTM1-specific primer to intron 6 (GSTM1I6) and secondly, primers to exon 7, one specific to GSTM1\*A (GSTM1E7A) and another (GSTM17B) specific to GSTM1\*B. In pilot studies using DNA from subjects with known GSTM1 A or GSTM1 B phenotypes GSTM1E7A did not anneal to GSTM1\*B and vice-versa (Fryer *et al.*, 1993). The finding of a 268-bp DNA fragment ( $\beta$ -globin) confirmed the success of amplification (Saiki *et al.*, 1988). Negative controls were included in every run to test possible contamination.

Ten microliters of the PCR product was analyzed on 2% agarose gels stained with ethidium bromide. At this stage two groups of individuals were determined: one with positive GSTM1 alleles (GSTM1+) and another without them (GSTM1-). This amplified material was subsequently digested by *Hae*II for 4 h at 37°C, to differentiate GSTM1\*A and GSTM1\*B because the GSTM1\*A primer differs from the GSTM1\*B sequence by an A→G substitution at the 3' end of the primer, thus introducing a *Hae*II restriction site into the amplified DNA. DNA from subjects with positive GSTM1 alleles allowed amplification of a 132-bp fragment. However, while GSTM1E7A and GSTM17B effected amplification of a fragment of the same size from GSTM1\*A and GSTM1\*B, respectively, the sequence of the PCR product differed by two bases, introducing a *Hae*II restriction site into the amplified DNA. Digestion by *Hae*II produced 112- and 20-bp fragments (corresponding to GSTM1 A). DNA fragments were resolved by electrophoresis in 4% agarose gels, which were ethidium bromide stained and photographed under UV light.

## RESULTS AND DISCUSSION

It is apparent from the GSTM1 phenotype distribution (Table I) that gene frequencies of the two samples are

very similar. The GSTM1 null phenotype frequencies were 46 and 49%, for Rio de Janeiro and Brasília, respectively. These values are similar to the data found in the literature for most human populations (Table II).

Both samples were also tested in order to check if there were differences in the GSTM1 phenotype frequencies according to age, sex or ethnic group (White versus Black), but no significant differences were observed. A tendency towards a decline in GSTM1\*0 frequencies for the Black group in Brasília and Rio de Janeiro samples was observed, but it is not statistically significant (Tables III and IV).

The difference in the GSTM1\*0 frequencies observed among races in the Rio de Janeiro and Brasília samples was not significant, however, it was borderline in the Rio de Janeiro sample, in which allelic frequencies were 42% in the Black group and 49% in the White group ( $P = 0.06$ ). Previous studies have shown that the admixture proportion in Rio de Janeiro is 40.18% Caucasian, 52.06% Black, and 0.08% Native (Lopez-Camelo *et al.*, 1996). This preponderance of black genes could be responsible for the GSTM1\*0 frequency (46%) in the Rio de Janeiro sample which is slightly lower than Brasília (49%). The racial admixture proportion of the Brasília population is unknown, but the proportion of black genes is certainly lower than in the Rio de Janeiro population, based on historical data. This is probably due to the recent founding of the Brasília population by migrants from many regions of the country, mainly from the Northeast, which is a tri-racial mixture with gene proportions of Caucasian = 0.586, Black = 0.301, and Indian = 0.113 (Krieger *et al.*, 1965).

Studies of African samples have shown that the frequency of the GSTM1 null phenotype is lower (22-38%) than in other populations (Bell *et al.*, 1993; Lin *et al.*, 1994; Zhao *et al.*, 1994; Mukanganyama *et al.*, 1997; Arruda *et*

**Table I** - Test for Hardy-Weinberg equilibrium of two Brazilian populations (Rio de Janeiro and Brasília).

Phenotype	Rio de Janeiro			Brasília		
	No. of individuals		$\chi^2$	No. of individuals		$\chi^2$
	Obs.	Exp.		Obs.	Exp.	
GSTM1 0	305	291.73	0.603	87	81.63	0.354
GSTM1 A	197	211.58	1.004	43	48.96	0.726
GSTM1 B	105	120.24	1.932	33	39.07	0.944
GSTM1 AB	51	34.45	7.951	16	9.34	4.749
Total	658	658.00	11.491	179	179.00	6.773
P-value	<0.001 (1 d.f.)			P-value <0.01 (1 d.f.)		
Alleles	Frequencies		SE	Alleles	Frequencies	SE
GSTM1*A	0.2087		0.0120	GSTM1*A	0.1788	0.0217
GSTM1*B	0.1254		0.0095	GSTM1*B	0.1459	0.0198
GSTM1*O	0.6659		0.0131	GSTM1*O	0.6753	0.0242

Obs. - Observed; Exp. - expected; SE - standard error of the mean.

*et al.*, 1998). These results corroborate our finding of a lower frequency of this allele in our Black group.

The distribution of the GSTM1 null phenotype varies in the different ethnic groups (Table II). Despite the fact that the methods to determine GSTM1 phenotypes were different (electrophoresis in starch gel or S-GEF and PCR) the results were similar. Most of these populations are in Hardy-Weinberg equilibrium, except for the Brazilian samples ( $\chi^2_1 = 11.49$ ;  $P < 0.001$  and  $\chi^2_1 = 6.77$ ;  $P < 0.01$ , for Rio de Janeiro and Brasília, respectively). This departure from Hardy-Weinberg equilibrium had already been observed in a previous study of a sample from the city of Ribeirão Preto (Klautau-Guimarães *et al.*, 1993). In the three Brazilian samples the deviation of the equilibrium proportions is mainly due to an excess of GSTM1 A/B heterozygotes.

Three factors might explain the deviation from Hardy-Weinberg equilibrium proportions. Firstly racial mixture, because GSTM1 frequency varies in the different ethnic groups and our samples are basically tri-hybrids. Brazilian populations are a product of admixture (Krieger *et al.*, 1965) among Caucasian (mainly Portuguese), Black (Bantu and Sudanese origin) and native Indians. We did not find significant differences among the ethnic groups, although the results were borderline. This could be explained by the fact that our race classifications were relatively subjective.

**Table II** - Distribution of glutathione S-transferase M1 phenotype frequencies in different populations.

Samples	Phenotype GSTM1				$\chi^2$ (d.f. = 1)	Methods
	A	B	A/B	0 (Null)		
Brazilian (RJ) <sup>a</sup>	197	105	51	305 (46.0%)	11.49	PCR
Brazilian (DF) <sup>a</sup>	43	33	16	87 (48.6%)	6.77	PCR
Brazilian (SP) <sup>b</sup>	21	10	7	35 (47.9%)	3.98	S-GEF
Nigerians <sup>c</sup>	49	4	1	15 (21.7%)	0.09	PCR
Japanese <sup>d</sup>	13	68	5	80 (48.2%)	0.04	S-GEF
Chinese <sup>e</sup>	10	28	2	56 (58.3%)	0.01	S-GEF
Indians <sup>e</sup>	15	7	6	15 (35.0%)	1.93	S-GEF
French <sup>f</sup>	24	5	3	24 (42.9%)	0.31	S-GEF
English <sup>g</sup>	158	74	23	306 (54.5%)	1.96	PCR

d.f. = Degrees of freedom. S-GEF = Starch gel electrophoresis and PCR = polymerase chain reaction. <sup>a</sup>Present paper. <sup>b</sup>Klautau-Guimarães, 1993. <sup>c</sup>Zhao *et al.*, 1994. <sup>d</sup>Harada *et al.*, 1987. <sup>e</sup>Board *et al.*, 1990. <sup>f</sup>Laisney *et al.*, 1984. <sup>g</sup>Heagerty *et al.*, 1996.

Secondly, the association of GSTM1 polymorphism with many types of diseases is documented by several studies, which indicate that individuals homozygous for the GSTM1\*0 allele seem to have a higher risk for some types of lung cancer (Seidegard *et al.*, 1986), stomach cancer (Harada *et al.*, 1992), bladder cancer (Abdel-Rahman *et al.*, 1998), cystic fibrosis (Baranov *et al.*, 1996) and atherosclerosis (Pessah-Rasmussen *et al.*, 1992). The higher frequencies of the GSTM1\*0 allele, in spite of its association with some diseases, could be explained by the fact that most of these diseases are manifested at a mature age when most GSTM1 0/0 homozygous individuals have already transmitted the GSTM1\*0 gene to their descendents (Board *et al.*, 1990). It has also been observed that in individuals with gastrointestinal, brain and skin cancer the GSTM1 A/B genotype is less common than in controls (Strange *et al.*, 1991; Strange, 1993; Heagerty *et al.*, 1994), suggesting that this polymorphism is going through some kind of selection that is favorable to the GSTM1 A/B heterozygous and/or disadvantageous to GSTM1 0/0 homozygous individuals. When we consider that GSTM1 0/0 homozygous individuals are deficient in GST, a carcinogen detoxification enzyme, it is reasonable to suggest that these individuals have higher risks when exposed to carcinogens. GSTM1 A/B heterozygous individuals, in turn, express both alleles and have higher enzyme activity (Brockmüller *et al.*, 1992, 1994), and are consequently better protected and more effective in the process of detoxification. In the specific case of GSTM1, since the null allele frequency is high, the Hardy-Weinberg model predicts that most of the GSTM1 A and GSTM1 B phenotypes are heterozygous for GSTM1\*0. These two phenotypes have a lower average activity than that of GSTM1 A/B heterozygous individuals. This selection, which is favorable to GSTM1 A/B heterozygotes and/or unfavorable to GSTM1 0/0 homozygotes, could have taken place recently because of increased industrialization and the exposure to toxic substances, such as insecticides, cigarettes and other chemical pollutants and carcinogens. In our study the observed frequency of GSTM1 A/B heterozygotes is higher than the expected frequency and is the main reason for the departure of our data from Hardy-Weinberg equilibrium.

Thirdly, there may be an error in the determination of the GSTM1 phenotypes. We can not differentiate GSTM1 A/A from GSTM1 A/0 individuals, so we consider all of

**Table III** - Distribution of glutathione S-transferase M1 allele frequencies in racial sub-groups of the Brasília sample.

Alleles	Allele frequencies				Black (N = 5)
	White (N = 53)	Light Mulatto (N = 54)	Medium Mulatto (N = 51)	Dark Mulatto (N = 9)	
A	0.174 ± 0.039	0.193 ± 0.040	0.180 ± 0.039	0.155 ± 0.098	0.216 ± 0.150
B	0.141 ± 0.035	0.127 ± 0.033	0.149 ± 0.036	0.214 ± 0.113	0.216 ± 0.150
O	0.685 ± 0.045	0.680 ± 0.045	0.670 ± 0.042	0.630 ± 0.093	0.570 ± 0.143

**Table IV** - Distribution of glutathione S-transferase M1 allele frequencies in racial sub-groups of the Rio de Janeiro sample.

Allele	Allele frequencies		
	White (N = 429)	Mulatto (N = 134)	Black (N = 95)
A	0.186±0.014	0.212±0.027	0.313±0.038
B	0.130±0.012	0.110±0.020	0.127±0.025
O	0.684±0.016	0.678±0.029	0.560±0.037

them as class GSTM1 A. The same is true for the GSTM1 B phenotype. GSTM1 A/O and GSTM1 B/O heterozygous genotypes could give a weaker reaction in the electrophoresis gel, which would increase the probability of these individuals being considered as null homozygotes and consequently would overestimate the proportion of this phenotype and decrease the frequency of the GSTM1 A and GSTM1 B phenotypes. On the other hand, our results do not show a significant difference in the expected values of these classes. A recent study (Arruda *et al.*, 1998) on three distinct ethnic groups of Brazilians indicated GSTM1 null phenotype frequencies of 55% in Caucasian, 33% in descendants of Africans and 20% in Indian lineages. Taking into account the racial admixture in our samples our results are in agreement with those found by Arruda *et al.* (1998). The deviation from Hardy-Weinberg equilibrium verified in our study could be explained by selection, incomplete panmixia of these populations or other unknown factors.

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#### RESUMO

A distribuição das frequências fenotípicas do gene GSTM1 de duas amostras brasileiras, compostas de doadores sanguíneos de ambos os sexos com idade entre 18 e 61 anos, foi estudada. A primeira amostra é composta de 658 indivíduos provenientes do Rio de Janeiro e a segunda de 179 indivíduos de Brasília. Os fenótipos da GSTM1 foram determinados utilizando-se a técnica da reação em cadeia da polimerase e subsequente digestão com a enzima de restrição *Hae*II. As frequências observadas para o fenótipo GSTM1 nulo foram de 46,4% e 48,6% para as amostras do Rio de Janeiro e Brasília, respectivamente. Os resultados também mostram que a distribuição fenotípica da GSTM1 não está em equilíbrio de Hardy-Weinberg para ambas as amostras:  $\chi^2_1 = 11,49$  ( $P < 0,001$ ) para o Rio de Janeiro e  $\chi^2_1 = 6,77$  ( $P < 0,01$ ) para Brasília. Este desequilíbrio pode ser atribuído a fatores como seleção, erros na determinação fenotípica ou panmixia ainda incompleta da população brasileira, cujos componentes raciais são caucasóides, africanos e índios.

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