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Ethnicity and glutathione S-transferase (GSTM1/GSTT1) polymorphisms in a Brazilian population

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Abstract

The distribution of polymorphisms related to glutathione S-transferases (GST) has been described in different populations, mainly for white individuals. We evaluated the distribution of GST mu (GSTM1) and theta (GSTT1) genotypes in 594 individuals, by multiplex PCR-based methods, using amplification of the exon 7 of CYP1A1 gene as an internal control. In São Paulo, 233 whites, 87 mulattos, and 137 blacks, all healthy blood-donor volunteers, were tested. In Bahia, where black and mulatto populations are more numerous, 137 subjects were evaluated. The frequency of the GSTM1 null genotype was significantly higher among whites (55.4%) than among mulattos (41.4%; $P = 0.03$) and blacks (32.8%; $P < 0.0001$) from São Paulo, or Bahian subjects in general (35.7%; $P = 0.0003$). There was no statistically different distribution among any non-white groups. The distribution of GSTT1 null genotype among groups did not differ significantly. The agreement between self-reported and interviewer classification of skin color in the Bahian group was low. The interviewer classification indicated a gradient of distribution of the GSTM1 null genotype from whites (55.6%) to light mulattos (40.4%), dark mulattos (32.0%) and blacks (28.6%). However, any information about race or ethnicity should be considered with caution regarding the bias introduced by different data collection techniques, specially in countries where racial admixture is intense, and ethnic definition boundaries are loose. Because homozygous deletions of GST gene might be associated with cancer risk, a better understanding of chemical metabolizing gene distribution can contribute to risk assessment of humans exposed to environmental carcinogens.

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Key words

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- Glutathione S-transferase theta (GSTT1)
- Enzyme polymorphism
- Population frequency
- Ethnicity
- Brazil

Introduction

The glutathione S-transferases (GST) are an important enzymatic system of the cellular mechanism of detoxification that protects cells against reactive oxygen metabolites due to the conjugation of glutathione with electrophilic compounds. GST enzymes are involved in the metabolism of xenobiotics that include environmental carcinogens, reactive oxygen species and chemotherapeutic agents (1). The great majority of catalyzing reactions between glutathione and electrophilic compounds results in detoxification products, although in a few cases the metabolic product can be more reactive than the original one. Due to the detoxifying action, it has been suggested that these enzymes play an important role in cancer susceptibility (2-5).

At least seven distinct classes of soluble GST that are highly expressed in the mammalian liver have been already identified (6): alpha, mu, pi, sigma, theta, kappa, and zeta. The GSTM1 locus has been mapped on chromosome 1p13.3. Three different alleles have been identified in the same locus, including gene deletion, and two other mutations (GSTM1a and GSTM1b) that differ by C to G substitution at base position 534 (7). The human GSTT1 gene is 8.1 kb in length and it has been mapped on chromosome 22q11.2 (8,9). For GSTT1 two different alle-

les have been identified that confer functional properties to the gene. Subjects with at least one functional allele for GSTM1 and GSTT1 are grouped into the positive conjugator types, and called GSTM1-positive and GSTT1-positive, respectively (4). The deleted genotypes that lead to the inactive form of the enzymes were named "GSTM1-null" and "GSTT1-null".

Associations of GSTM1 and/or GSTT1 null genotypes with bladder, lung, and colorectal cancer, as well as head and neck squamous cell carcinoma have been reported and represent an area of intensive research (10-14). The relevance of the polymorphism of these enzymes regarding DNA adduct levels or urinary metabolites of xenobiotics has also been widely discussed (15-18).

The variability in the distribution of the null phenotypes of GSTM1 and GSTT1, due to total or partial gene deletion resulting in the lack of the active enzyme, has been reported in different populations, especially in ethnically well-defined groups (19-21). However, Brazil, as most of Latin American and Caribbean countries, is a multiracial society with no clearly defined differences among races, especially between blacks and whites. Brazilian history shows that since the mid-colonial era the population of mulattos was already numerous (22). However, differences between geographical regions can be observed (Table 1).

Table 1. Distribution of the population according to skin color in Brazil and in the States of São Paulo and Bahia, according to 2000 census data.

	Total	White	Black	Pardos	Asian	Amerindian
Brazil	169,799,170	90,647,461 (53.4%)	10,402,450 (6.1%)	66,016,783 (38.9%)	866,972 (0.51%)	701,462 (0.41%)
São Paulo	37,032,403 (21.8% of total)	26,067,368 (70.4%)	1,667,745 (4.5%)	8,480,871 (22.9%)	518,278 (1.40%)	62,018 (0.16%)
Bahia	13,070,250 (7.7% of total)	3,067,786 (23.5%)	1,700,531 (13.0%)	8,095,318 (61.9%)	23,868 (0.18%)	60,329 (0.46%)

Pardos = designates those of intermediate skin color between white and black. Data from Ref. 24 (www.ibge.gov.br).

In a recent cross-sectional study conducted in the interior of Bahia, a State in the northeast of Brazil, we investigated rural workers mainly exposed to wood smoke and diesel exhaust while cutting wood or executing their tasks in charcoal factories. Some enzyme polymorphisms were assessed in the study, as they were considered to be possible modulating factors of the biomarker levels among workers and also useful to identify higher susceptibility to potentially hazardous substances.

Therefore, we evaluated the distribution of GSTM1 and GSTT1 genotypes within the Bahian rural worker group and compared it to GST polymorphism frequency in groups of whites, mulattos, and blacks living in São Paulo city, located in southern Brazil.

Material and Methods

The GSTM1 and GSTT1 gene frequency was assessed in 594 individuals divided into four subgroups. The first group comprised 233 unrelated white individuals (mean age = 36.6 ± 13 years), the second group included 87 mulattos (mean age = 33.5 ± 10 years) and the third, 137 blacks (mean age = 37.5 ± 10 years) selected by personal interview in the city of São Paulo. Unrelated healthy blood donor volunteers for the study were selected at the Pró-Sangue Hemocentro of São Paulo Foundation.

Subjects were interviewed and asked about their ethnic group and also about their parents' and grandparents' groups, according to their own definition. Phenotype analysis (skin pigmentation in the inner part of the arm, face and hair characteristics) was performed by the interviewer. Subjects were classified as black when they defined themselves and their parents and all the grandparents as blacks and presented the characteristic phenotype.

The last group consisted of 137 male individuals (mean age = 34.1 ± 10 years) who worked in the rural area of Bahia State, Brazil.

They worked in seven different companies located in the northeastern counties, 100 to 150 km from the capital of the state. They were all born in Bahia or in the neighboring states of Alagoas and Sergipe. Socio-demographic data were obtained by interview by properly trained personnel. To verify ethnicity, the interviewees answered the question "what is your color?", structured as in the census questionnaire (23,24), and the interviewers classified volunteers according to phenotype.

The present studies were approved by the Research Ethics Committee, and all participating subjects signed a term of consent after being informed about the objectives of the study.

Genomic DNA for genotyping was isolated from 5 ml peripheral blood lymphocytes by a non-organic DNA extraction procedure (25). A single assay using a multiplex PCR was performed for simultaneous gene amplification (26). Briefly, 50 ng of DNA was amplified in a 50- μ l multiplex reaction mixture containing 30 pmol of each of the following GSTM1 primers (G1 - 5' GAA CTC CCT GAA AAG CTA AAG C 3' and G2 - 5' GTT GGG CTC AAA TAT ACG GTG G 3'), and of the following GSTT1 primers (T1 - 5' TTC CTT ACT GGT CCT CAC ATC TC 3' and T2 - 5' TCA CCG GAT CAT GGC CAG CA 3'). As an internal control, the exon 7 of the CYP1A1 gene was also amplified (5' GAA CTG CCA CTT CAG CTG TCT 3' and 5' CAG CTG CAT TTG GAA GTG CTC 3') in a medium consisting of 1.5 mM MgCl₂, 200 μ mol dNTPs, 5 μ l 10X PCR buffer (10 x 500 mM KCl, 100 mM Tris-HCl, pH 9.0), and 2 U TaqDNA polymerase (Promega, Madison, WI, USA). The PCR protocol included an initial melting temperature of 94°C (5 min) followed by 35 cycles of amplification (2 min at 94°C, 1 min at 59°C, and extension for 1 min at 72°C). A final 10-min extension step (72°C) terminated the process. The final PCR product from co-amplification of GSTM1 (215 bp) and GSTT1 (480 bp) was visualized on an ethidium bro-

mid-stained 2.0% agarose gel.

The subjects were classified as either positive (when at least one copy of the gene was present) or null genotypes. Heterozygous and homozygous individuals for GSTM1 (GSTM1 +/0 and GSTM1 +/+) or GSTT1 (GSTT1 +/0 and GSTT1 +/+) have been reported to present similar enzyme expression levels (27) and were considered together for statistical analysis. The Mantel-Haenzel test or Fisher exact test was used to determine the differences between groups and the kappa statistic was used to assess the agreement between racial classifications.

Results and Discussion

The distribution of the GSTM1 null genotype (Table 2) was more frequent among white individuals (55.4%) than among mulattos (41.4%) and blacks (32.8%) from São Paulo, or Bahian rural workers in general (35.7%). The frequency of the GSTM1 null genotype observed in all four groups were statistically different when whites were compared to mulattos ($P = 0.03$), to blacks ($P < 0.0001$), or to Bahian rural workers ($P = 0.0003$). For GSTT1 null type, blacks showed the highest frequency (26.3%) followed by

Bahian rural workers (23.8%), whites (22.3%), and mulattos (17.2%); there was no statistically significant difference between groups (Table 2). Figure 1 illustrates the PCR results of GSTM1 and GSTT1 genotypes in comparison to the internal standard control.

The distribution of the Bahian population according to the interviewers and the respondents' classification of skin color is shown in Table 3. This population is essentially non-white, containing less than 10% white individuals. The majority of subjects classified themselves as "morenos", while the interviewers classified them mostly as mulattos. The word "moreno" was not suggested by the interviewers and was reported despite options such as "mulatto" and "black" were given. "Moreno" means tanned, darker skin or dark hair, can be applied to individuals of any ethnic origin, and is not an official term used to classify ethnic groups. Mulattos or "pardos" are terms officially applied to those of mixed African and European origin.

The State of Bahia was the first area of colonization in Brazil, which was started by the Portuguese and followed by Africans brought as slaves. At the present time, the Bahian population consists of 75% individu-

Table 2. Distribution of GSTM1 and GSTT1 null genotypes according to ethnicity in volunteers from São Paulo and Bahia States, Brazil.

Skin color	N	GSTM1 null (%)	GSTT1 null (%)	GSTM1 null/ GSTT1 null (%)
Total	594	261 (43.9)	137 (23.1)	54 (9.1)
São Paulo group				
White	233	129 (55.4)*	52 (22.3)	23 (9.9)
Mulatto	87	36 (41.4)	15 (17.2)	5 (5.8)
Black	137	45 (32.8)	36 (26.3)	13 (9.5)
Bahia group				
All workers	137	51 (35.7)	34 (23.8)	13 (9.1)
Self-reported ethnic classification				
Moreno	77	28 (36.4)	15 (19.5)	7 (9.1)
Moreno and mulatto	106	38 (34.5)	23 (21.7)	9 (8.5)
Interviewers' ethnic classification				
Mulatto	89	32 (36.0)	17 (19.1)	7 (7.9)

GST = glutathione S-transferase; GSTM1 = GST mu; GSTT1 = GST theta.

* $P < 0.05$ compared to the other groups (Fisher exact test).

als of African origin (Table 1). In São Paulo State, African descendants are less numerous (27.5%) (24). In addition, Bahia State is located much closer to the Equator than São Paulo State, receiving higher sunlight levels. Consequently, workers, especially those who execute their tasks outdoors, have deeply tanned skin color.

The proportion of racial admixture differs according to the region of the country, and the terminology used to denote ethnic groups might also vary (23). Furthermore, racial categories are often based on respondents' self-identification of skin color (23, 24), and "accurate color terminology is especially difficult when discussing Brazil" (22). Therefore, we assessed the comparability of the self-reported categorization with the interviewer-rated classification within

the rural worker group, and also compared this group to other groups in São Paulo.

It was clear that "moreno" corresponded mainly to the mulatto and black classification given by the interviewer, but also encompassed a wider range of possibilities (Table 3). And there was also a smaller group of respondents who identified themselves as mulattos. Due to this fact, the agreement between the interviewer's classification and the interviewee's self-reported race categorization was relatively low (weighted kappa = 0.4142; P < 0.05).

No statistically significant differences in the distribution of GSTM1 and GSTT1 null genotypes were observed when 137 workers were stratified according to interviewers' or interviewees' classification of skin color (Table 2). Also, no difference was observed

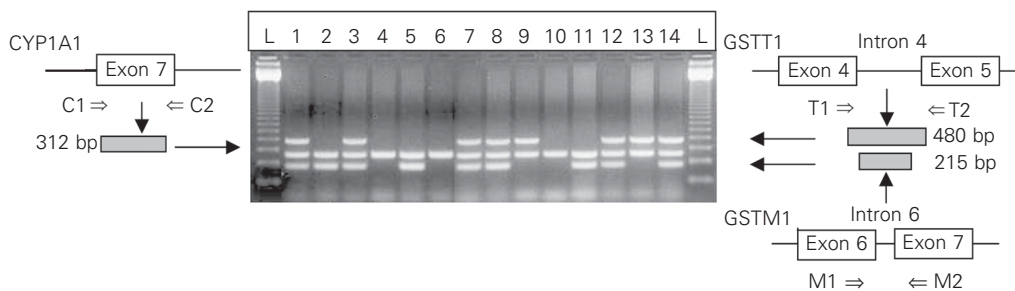


Figure 1. PCR products from the co-amplification of GSTM1 (215 bp), GSTT1 (480 bp) and the internal control, the CYP1A1 gene (312 bp). The gel was ethidium-bromide-stained 2% agarose gel (lanes 1, 3, 7, 8, 12 and 14, GSTM1+/GSTT1+ genotype; lanes 2, 5 and 11, GSTM1+/GSTT1 null genotype; lanes 9 and 13, GSTM1 null/GSTT1+ genotypes; lanes 4, 6 and 10, GSTM1 and GSTT1 null genotype). L = ladder.

Table 3. Comparison of the distribution of the Bahian volunteers according to self-reported skin color and the interviewers' categorization.

Respondent's categories	Interviewer's categories					Total of respondent's categories (%)
	White	Light mulatto	Dark mulatto	Black	Others	
White	5	0	0	0	0	5 (3.57)
Moreno	3	31	22	19	2	77 (55.00)
Mulatto	0	13	12	4	0	29 (20.7)
Black	0	0	2	11	0	13 (9.29)
Pardo	2	0	5	3	0	10 (7.14)
Others	0	3	3	0	0	6 (4.2)
Total of interviewer's categories (%)	10 (7.14)	47 (33.57)	44 (31.43)	37 (26.43)	2 (1.43)	140 (100)

Pardo = designates those of intermediate skin color between white and black.

when comparing the same categories in both classifications, and between morenos from respondents' classification and mulattos from the interviewers' classification.

The Bahian morenos and the interviewer-classified mulatto groups, as well the mulatto group from São Paulo, presented rather lower frequencies of the GSTT1 null genotype. The mulattos from São Paulo showed a lower prevalence of both GSTM1 and GSTT1 null types, albeit not statistically different from other groups. Apparently, there was no specific reason for the observed frequency, which might be attributed to a small sample size and to chance. Further investigation with larger samples needs to be conducted to better investigate these discrepancies.

The distribution of GSTM1 and GSTT1 null genotypes varies among different ethnic groups. Several population-based studies have reported a prevalence ranging from 47 to 58% for the GSTM1 deletion genotype, from 13 to 25% for the GSTT1-null genotype among white Europeans and 27.6% among the US white population (7,19). Relatively few data exist about GSTM1 polymor-

phism in black and mulatto populations (28-30). Garte and collaborators (19) evaluated database information on over 15,000 people, identifying only seven published papers about GSTM1 distribution among African and African-Americans resulting from the analysis of 479 individuals. The reported GSTT1 null genotype among African-Americans ranged from 20 to 24% (26,29).

In our study, the lowest frequency of the GSTM1 null genotype was observed among blacks (32.8%) and was similar to that previously reported for other populations of African descent. The white group presented an estimated GSTM1 null frequency similar to that reported for the Portuguese population by Moreira et al. (31). The null genotype frequencies observed for GSTM1 and GSTT1 in this report were in agreement with data reported by Arruda et al. (32), Hatagima et al. (33) and Gattás and Soares-Vieira (34) for some Brazilian groups.

The Brazilian population resulted basically from the racial mixture between whites from the Iberian Peninsula and Africans of various ethnic groups, with a small contribution by native Amerindians. Blacks from Bahia descend predominantly from those brought as slaves from the Gulf of Guinea. In the south, descendants from the Bantu tribe, originally from Angola and the Congo, are more numerous (35,36). In the official census the word "pardo" has been used to designate those with a wide range of skin color between white and black. The term does not only mean mulatto, but can also refer to other admixtures. The population of pardos has been increasing and comprises about 40% of the national population (22,24).

There was no statistical difference within the Bahian group or between any non-white groups (Table 2) in our study. Lack of statistical power to show a difference due to relatively small sample sizes of some groups is one possible reason. However, this may also represent the lack of a rigid distinction between races and the intense admixture that

Table 4. Distribution of GSTM1 and GSTT1 null genotypes in Bahian volunteers, Brazil.

	GSTT1 null	GSTM1 null	Total
Self-reported skin color			
White	0 (0%)	3 (60%)	5
Moreno	15 (20%)	28 (35%)	77
Mulatto	8 (28%)	10 (35%)	29
Black	6 (50%)	3 (25%)	12
Brown	5 (25%)	3 (38%)	8
Others	1 (17%)	2 (33%)	6
Total	32 (23%)	49 (36%)	137
Interviewer-classified skin color			
White	2 (22%)	5 (56%)	9
Light mulatto	8 (17%)	19 (40%)	47
Dark mulatto	9 (21%)	13 (31%)	42
All mulatto	17 (19%)	32 (36%)	89
Black	13 (37%)	10 (29%)	35
Others	0 (0%)	2 (100%)	2
Total	32 (24%)	47 (35%)	135

GST = glutathione S-transferase; GSTM1 = GST mu; GSTT1 = GST theta.

has been occurring in this country.

Telles and Lim (37) have previously shown discrepancies between self-reported information and interviewer classification of race. In our study, the frequencies of GSTM1 and GSTT1 null types in the moreno group and in the interviewer-classified mulattos from Bahia, as well as in the mulatto group from São Paulo were very similar and reached intermediate values between those found for white and black groups for GSTM1 (Table 2). In the interviewer-classified Bahian group it was possible to notice a gradient. The presence of the GSTM1 null genotype was detected in 55.6% of whites, 40.4% of light mulattos, 32.0% of dark mulattos, and 28.6% of blacks (Table 4).

The loose boundaries of the ethnic definition also cause changes in self-identification. Surrat and Inciardi (38), in a health behavior study conducted in Rio de Janeiro at different time points, reported that 106 (12.5%) of their clients changed their racial categories. They also questioned the use of racial comparison of health risks within their setting, since they consider racial identities in Brazil as “dynamic concepts, which can only be understood if situated and explored within appropriate cultural context”. Telles and Lim (37) suggested that interviewer classification might be more appropriate for studying social inequalities, since it reflects how others classify one’s race.

The perception of skin color differences and the cultural context of the words denoting race or ethnicity might differ from North to South in Brazil, between urban and rural areas, as well as from the interviewer to the interviewed (23). In our study, we did not

investigate differences in social or cultural context. However, it was important to assess whether the Bahian group could be comparable to other previously studied populations regarding genetic polymorphisms, and also if there was any difference between morenos and mulattos. The fact that the interviewer classification was able to detect a trend from whites to blacks for the GSTM1 null genotype is an indication that it can be more useful than the self-identification of race in molecular epidemiology studies.

Any information about race, skin color, or ethnicity should be considered with caution in Brazil, and data collection as well as interpretation of the information should be conducted within the appropriate cultural/social setting. Comparison of studies, especially those conducted in different regions or countries, should evaluate the weight of the bias introduced by different data collection techniques.

We have presented here the genotype distribution of two susceptibility genes of the GST family observed in samples from the São Paulo and Bahia population in Brazil. The mulatto groups showed intermediate values between whites and blacks, and there were no statistically different distributions among any non-white groups. A better understanding of polymorphic chemical metabolizing genes can contribute, in the future, to preventive actions or to risk assessment of humans exposed to environmental carcinogens. For this purpose, however, it is important to take into account the different characteristics and cultural contexts of each population.

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