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Glutathione S-Transferase Variants in a Brazilian Population

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Glutathione S-transferases · Pharmacogenetics · Metabolic enzymes

Abstract
Recent pharmacogenomic studies have revealed significant interethnic differences in glutathione S-transferase (GST) allelic frequencies among various ethnic groups. Therefore, we have investigated *GSTM1* (gene deletion), *GSTT1* (gene deletion) and *GSTP1* (rs1695) polymorphism frequencies in 3 Brazilian ethnic groups (n = 203). *GSTM1* and *GSTT1* polymorphism analyses were performed by multiplex polymerase chain reaction, and *GSTP1* (rs1695) analysis was done by polymerase chain reaction restriction fragment length polymorphism. *GSTM1*– polymorphism frequency was 33.2%, while *GSTT1* null *(GSTT1–)* was 30.2%. The valine *GSTP1*B* (rs1695) allele was present in 35.1% subjects, while the heterozygous form (isoleucine/valine) was the most prevalent genotype (46.6%). We found a statistically significant difference in genotype frequency among Amerindians versus Caucasians (p = 0.016) and among Amerindians versus African-Americans (p = 0.033). Considerable frequency variation was found in our study, even when compared with other studies showing phylogeographical heterogeneity to the genes studied in Brazilian populations.

Introduction

Characterization of naturally occurring variations in the human genome has evoked immense interest during recent years. Variations known as containing common functional allelic variants that affect gene expression or protein function have become increasingly popular markers in molecular genetics [1, 2]. Glutathione S-transferases (GSTs) are an enzymatic family consisting of numerous cytosolic, mitochondrial and microsomal proteins able to catalyze multiple reactions with endogenous and xenobiotic substrates [3]. They catalyze reduced glutathione conjugation to electrophilic centers via the sulfhydryl group on a wide variety of substrates [4–6].

Soluble GSTs exist as dimeric proteins (approximately 25 kDa) which are highly expressed, constituting up to 4% of the total soluble proteins in the liver [7]. At present, 8 soluble cytoplasmic mammalian GST distinct classes have been identified: alpha, kappa, mu, omega, pi, sigma, theta and zeta [8]. In general, GST-catalyzed reactions are considered detoxifying and serve to protect cellular macromolecules from damage caused by several environmental carcinogens found in food, tobacco smoke, air and medications, endogenous compounds such as peroxidized lipids [4], and inactivate products formed as secondary metabolites during oxidative stress [9–11]. GSTs

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are involved in gene-environment interactions, modifying individual predisposition to various diseases and they were shown to be able to influence treatment response to drugs such as glucocorticoids and alkylating agents [12–14].

It has been hypothesized that allelic variants are associated with less effective detoxification [4–6]. Genetic polymorphisms have been described in all classes of GSTs [14]. There are 3 important variants described at the *GSTM1* locus (1p13.3): *GSTM1*0* (a deletion) and 2 other polymorphisms (*GSTM1*A* and *GSTM1*B*) which differ by a substitution at base position C534G; there are no phenotype differences [15]. The theta GST class is encoded by the *GSTT1* gene (22q11.23) and it may present a deletion that results in a lack of functional gene product *(GSTT1*0)* [16]. Most *GSTM1* and *GSTT1* null variant studies have compared the double deletion with the genotypes containing at least 1 functional allele [14]. DNA adducts and cytogenetic endpoints analyzed have indicated an increased susceptibility of *GSTM1* and/or *GSTT1* null genotype to the genotoxicity of common low-dose chemicals [17, 18]. The most extensively studied *GSTP1* gene (11q13) variant, *GSTP1*B* (rs1695), is an A1578G change at the fifth exon (change of isoleucine to valine in the codon 105), which generally confers a lower metabolic activity [19, 20].

Ethnicity is an important variable influencing drug response, and pharmacogenetic studies have revealed significant interethnic differences in allelic frequencies of polymorphic genes encoding drug-metabolizing enzymes, drug transporters and drug targets [21–24]. Accordingly, *GSTM1, GSTT1* and *GSTP1* allelic frequencies are different among various ethnic groups and regions [25]. Brazilians form one of the most heterogeneous populations of the world, which is the result of 5 centuries of mixing between populations of colonizing Europeans, African slaves and native Amerindians [26]. Due to this high degree of miscegenation, the Brazilian population, the fifth largest in the world, is unique compared with other populations [27]. In this study, we have investigated *GSTM1, GSTT1* and *GSTP1* variant frequencies in Brazilian individuals residing in Bahia, a Brazilian northeast state with a highly admixed population. This work provides the basis for future clinical studies concerning variability in the response and/or toxicity to drugs known to be substrates for GSTs and we determined whether there are differences in this gene polymorphism among the 3 most prevalent Brazilian ethnic groups (African-Americans, Amerindians and Caucasians).

Methods

Subjects

All 203 individuals included in this study were blood donors at the Hospital São José (Ilhéus, Brazil), who have reported no symptomatic, metabolic or genetic conditions (age 34.3 ± 10.2 years; males 61%; African-Americans 69.1%, Caucasians 15.7% and Amerindians 15.2%). Volunteers were classified by self-reported ancestry in Caucasians, African-Americans or Amerindians. Signed informed consent was obtained from each participant, and the study was conducted after being approved by the Human Ethics Committee from the Universidade Estadual de Santa Cruz.

Sample Collection and DNA Analyses

Peripheral blood (5 ml) was collected in EDTA vacutainer tubes from all participating individuals after obtaining their written consent. Genomic DNA extraction was performed from whole blood using the FlexiGene DNA Kit (Qiagen, Boston, Mass., USA). *GSTM1* and *GSTT1* polymorphism analyses were performed by multiplex polymerase chain reaction (PCR) [28], with the ubiquitous *β-globin* gene as an internal standard. Amplification was carried out using the following primers: *GSTT1* forward primer: 5′-TCT CCT TAC TGG TCC TCA CAT CTC-3′; *GSTT1* reverse primer: 5′-TCA CCG GAT CAT GGC CAG CA-3′; *GSTM1* forward primer: 5′-TCA CCG GAT CAT GGC CAG CA-3′; and *GSTM1* reverse primer: 5′-GTT GGG CTC AAA TAT ACG GTG G-3′. Each 25-μl PCR reaction contained 2.5 μl of 10× reaction buffer (Tris-HCl 10 mmol/l, pH 8.3, and KCl), 2 mmol/l $MgCl_2$, 200 μmol/l each of deoxynucleoside triphosphates, 10 pmol/l of each primer, 1 unit of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, Calif., USA), and 100 ng genomic DNA. Thermal cycling conditions for the PCRs were as follows: 15 min at 95°C, followed by 30 cycles of 95°C for 2 min, 60°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were visualized on a 2% (w/v) agarose gel (Pronadisa, Madrid, Spain), with electrophoresis at 100 V for 50 min. Two 480-bp bands for *GSTT1* and 215 bp for *GSTM1* were obtained for the *GSTT1+/GSTM1+* genotype. The *GSTT1+/GSTM1–* genotype showed 1 band of 480 bp, and the *GSTT1–/GSTM1+* genotype showed a band of 215 bp. For the *GSTT1–/GSTM1–* genotype (designated as null genotype), no bands were obtained, and thus, *β-globin* (268 pb) internal positive standard was necessary. *GSTP1* (rs1695) analysis was done as previously described by Harris et al. [29]. Briefly, the primers P105 F 5′-ACC CCA GGG CTC TAT GGG AA-3′ and P105 R 5′-TGA GGG CAC AAG AAG CCC CT-3′ were used to obtain a 176-bp fragment. PCR products (20 μl) were digested with 5 units *Bsm*AI restriction endonuclease (New England Biolabs, Beverly, Mass., USA) in a 25-μl reaction, and digestion products were separated by electrophoresis on 3.5% (w/v) agarose gel. Mutations resulted in 91- and 85-pb fragments, and a 176-pb fragment was seen for the wild-type form.

Statistical Analysis

Individual marker analyses comparing variant frequencies among ethnic groups were performed using χ^2 tests. Statistical programs used were the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Ill., USA) and UNPHASED [30]. A Hardy-Weinberg equilibrium test was also performed using χ^2 tests [31].

Table 1. *GSTT1, GSTM1* and *GSTP1* variant frequencies

Gene	Variant	Total %	Caucasian %	African-American, %	Amerindian %	Male %	Female %
GSTM1	*GSTM1–*	0.332	0.379	0.338	0.265	0.328	0.328
	GSTM1+	0.668	0.621	0.662	0.735	0.672	0.672
GSTT1	*GSTT1–*	0.302	0.276	0.289	0.382	0.292	0.328
	GSTT1+	0.698	0.724	0.711	0.618	0.708	0.672
GSTM1/GSTT1	*GSTM1–/GSTT1–*	0.122	0.138	0.113	0.147	0.117	0.148
	GSTM1+/GSTT1–	0.180	0.138	0.176	0.235	0.175	0.180
	GSTM1–/GSTT1+	0.210	0.241	0.225	0.118	0.212	0.180
	GSTM1+/GSTT1+	0.488	0.483	0.486	0.500	0.496	0.492
GSTP1 (rs1695)	Ile	0.649	0.625	0.611	0.741	0.644	0.620
	Val	0.351	0.375	0.389	0.259	0.356	0.375
	Ile/Ile	0.405	0.300	0.364	0.621	0.394	0.432
	Ile/Val	0.466	0.650	0.495	0.241	0.500	0.386
	Val/Val	0.128	0.050	0.141	0.138	0.106	0.182

Ile = Isoleucine allele; Val = valine allele.

Table 2. Logistic regression analysis of the *GSTM1, GSTT1* and *GSTP1* polymorphisms among ethnic groups for risk of mutant allele

Variant	African-American OR	p	Caucasian OR	p	Amerindian OR	p
GSTM1–	1.153 (0.407–2.261)	0.789	1.483 (0.414–5.313)	0.545	0.793 (0.312–2.018)	0.626
GSTT1–	0.562 (0.184–1.722)	0.313	0.479 (0.129–1.777)	0.271	1.148 (0.472–2.790)	0.761
*GSTP1*Val*	0.212 (0.035–1.270)	0.089	0.095 (0.006–1.584)	0.101	0.120 (0.015–0.961)	0.046[a]

The reference group is *GSTM1+, GSTT1+* and *GSTP1*Ile,* respectively.
Odds ratio (OR) and 95% confidence intervals in parentheses for each reference allele compared are shown. [a] $p < 0.05$.

Population stratification was accessed using Structure 2.2 [32], and multiple logistic regression analysis was carried out to evaluate ethnic influences on the polymorphism frequency. Statistical significance was assumed as $p < 0.05$.

Results

GSTM1 and *GSTT1* variant frequencies among the 3 ethnic groups and gender are shown in table 1. The *GSTM1–* allele was found in 33.2% of the population, while the *GSTT1–* obtained a frequency of 30.2%. When both variants were analyzed together, the most frequent form observed in the total sample was *GSTM1+/GSTT1+* (48.8%), and this pattern was found for all conditions (ethnic groups and gender). Null variant frequencies showed that in Caucasians and African-Americans, *GSTM1–* was overrepresented when compared with Amerindians, while in Amerindians, *GSTT1–* was more frequently represented than in African-Americans or Caucasians. There were no significant statistical differences among the ethnic groups studied and gender (fig. 1).

The *GSTP1* (rs1695) most frequent genotype was the heterozygous form, with 46.6% (table 1), and this pattern was found in all conditions, except for Amerindians who had a higher prevalence of homozygous isoleucine genotype (62.1%). Indeed, we found a statistically significant difference when comparing *GSTP1* (rs1695) frequency in Amerindians and Caucasians ($p = 0.016$) or Amerindians and African-Americans ($p = 0.033$) (fig. 1). The isoleucine allele was the most frequent, with 64.9%. Using multiple logistic regression analysis, it was further confirmed that Amerindians are characterized for lower *GSTP1*B*

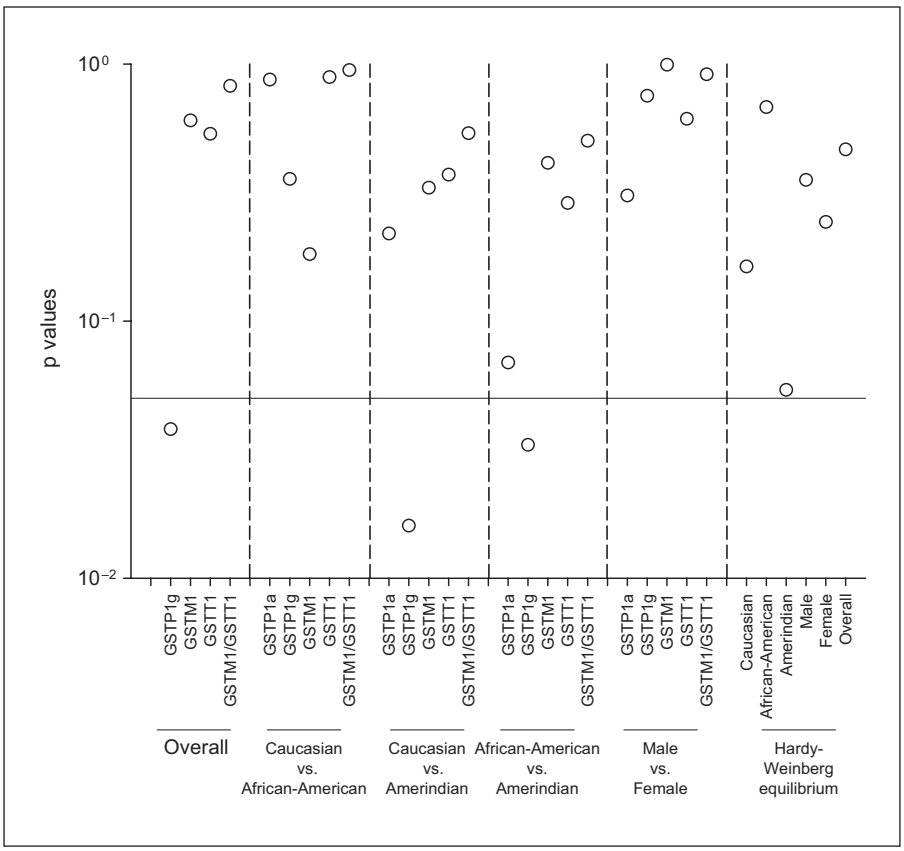

Fig. 1. Statistical analysis results. 'a' = Allelic association test; 'g' = genotypic association test. The solid line parallel to the x-axis represents p = 0.05. Any point plotted below this line represents a statistically significant difference among compared groups.

frequency after corrections for age and gender (OR = 0.120, 95% CI 0.015–0.961; p = 0.046) (table 2).

Statistical analysis showed no significant differences for genotypic or allelic frequency among gender, and observed frequencies of all variants have followed the Hardy-Weinberg equilibrium ($p > 0.05$) (fig. 1). No significant population stratification was observed when analyzing all 3 variants on Structure 2.2 (data not shown).

Discussion

Metabolic genes form a genetic marker group that has been extensively examined, especially in case-control studies in complex diseases, such as cancer or drug therapy response. The identification of genetic variants with high frequencies in genes involved with carcinogenic metabolism, as found in our study (35.1% to *GSTP1*B*), has allowed hypotheses that attempt to explain the high degree of individual variability in susceptibility to cancer [33].

In addition, pharmacogenetics has recently taken an important role showing significant differences, among and within populations, in metabolism, efficacy and toxicity of drugs [34], and this fact varies between regions studied. The Brazilian population, especially in the northeast, offers a great research potential because it presents a unique admixture and diversity of variant combinations in different loci enabling gene interaction studies [35]. The southeast of the Bahia region presents one of the most heterogeneous Brazilian populations [36].

This study aimed to access *GSTM1, GSTT1* and *GSTP1* variant frequencies and, for the first time, determine whether there are differences in these among ethnic groups of northeastern Brazil. Although the African-American gender ratio was not homogeneous (data not shown), genetic variant distribution studied in this sample was homogeneous across gender. These results confirm data from other studies that found no statistically significant differences either, when frequencies were compared across gender [33]. Subjects' age was another factor without influence on variant frequencies (data not

shown), corroborating with another study [33], and therefore, allows us to exclude that the possibilities of these variants are crucial for the overall survival of individuals.

Interestingly, we found a significant difference in the variant distribution of *GSTM1* and *GSTT1* in our sample, when compared with other studies conducted in Brazil. The *GSTM1*– frequency (33.2%) is the lowest, corroborating with other studies. Moreover, the frequency of *GSTT1*–, with 30.2%, is the highest [37, 38].

The heterozygote frequency of the *GSTP1* gene, which is a measure of genetic diversity, was shown to be high (46.6%), suggesting a possible trend towards heterozygosis for the variant in the northeast population. To date, this is the first study that has performed *GSTP1* (rs1695) analysis in a Brazilian Amerindian population.

GSTP1 (rs1695) frequencies were statistically different in Amerindians from Caucasians and African-Americans ($p = 0.016$ and 0.033, respectively). The genetic drift may be explained for the *GSTP1* (rs1695) homozygote high frequencies among Amerindians (62.1%), as compared with African-Americans (36.4%) and Caucasians (30.0%), beyond natural selection in response to environmental changes, since these genes encode enzymes responsible for metabolizing a large number of xenobiotics.

Multiple logistic regression analysis was carried out and confirmed that there is a significant difference in allelic distribution among the ethnic groups. This finding supports that *GSTP1* allelic distribution is differently present among the ethnic populations. However, these findings need to be interpreted with restrictions because there is no molecular analysis of ethnic background in order to confirm the information raised by the volunteer's self-identification of ethnicity, especially considering the intense ethnic admixture in this population.

Our results shown in Brazilian individuals genotyped for *GSTM1, GSTT1* and *GSTP1* allelic variants provided a considerable finding of phylogeographical heterogeneity, reporting further evidence for ethnic variability in the metabolism of drugs and xenobiotics. We hope that our results will aid in understanding the Brazilian population ethnic diversity and offer a basis for more rational use of drugs that are substrates for those variants.

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