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Short Communication

CYP1A1, *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in an Afro-Brazilian group

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Abstract

Gene polymorphisms involved in the metabolism of drugs and chemical carcinogens seem to be responsible for differences in the susceptibility of individuals to cancer, but genetic population studies are needed to characterize these polymorphisms in different ethnic populations. We investigated polymorphisms of the cytochrome P450 (CYP) gene *CYP1A1* and the glutathione S-transferase (GSTs) genes *GSTM1*, *GSTT1* and *GSTP1* in a sample of Afro-Brazilians from the southern Brazilian city of Porto Alegre to verify if there were ethnic differences compared to the polymorphisms of the same genes in a previously described sample of Brazilians of European descent from the same city. The allele frequencies detected in the Afro-Brazilian population investigated in this study were *CYP1A1*2A* (30%) and *GSTP1*Val* (42%) while the frequency of the *GSTM1* null genotype was 34% and that of the *GSTT1* null genotype was 28%. Significant differences in genotype distribution and allelic frequencies were detected between Brazilians of African and of European descent from Porto Alegre in terms of the polymorphisms *CYP1A1*2A* ($p = 0.003$), *GSTP1-Ile105Val* ($p = 0.002$) and the *GSTM1* null genotype ($p = 0.01$) but there was no detectable significant difference in respect to *GSTT1* null genotype frequencies

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The metabolism of drugs and chemical carcinogens involves a variety of isoenzymes such as members of the Cytochrome P450 (CYP) and Glutathione S-transferase (GSTs) families, with the polymorphisms described in these genes appearing to be responsible for differences in individual susceptibility to cancer (Perera and Weinstein, 2000; Miller *et al*., 2002; Norppa, 2003). Environmental factors are important contributors to human carcinogenesis and the risk of cancer is strongly influenced by genetic differences related to ethnic propensity to develop cancer. Epidemiological studies have shown that some kinds of tumors (*e.g.* breast, lung, prostate, etc.) are more prevalent in European-derived population samples. This higher prevalence of tumors has also been reported in the population of the southernmost Brazilian state of Rio Grande do Sul (RS) according to data from the Rio Grande do Sul Health Secretariat Cancer Registry (Secretaria da Saúde do Estado do Rio Grande do Sul, Registro de câncer de base populacional de Porto Alegre, http://www.saude.rs.gov.br).

Genes involved in the metabolism of carcinogens may be used as markers of individual susceptibility to cancer since differential activity of these enzymes may increase or decrease the activity of xenobiotics. These enzymes are responsible for the activation (phase I reactions) or deactivation (phase II reactions) of carcinogens. Phase I enzymes such as those of the *CYP* gene family may convert harmless chemicals into electrophilic compound via oxidation reactions while phase II enzymes, such as those belonging to the GSTs gene family, detoxify carcinogenic metabolites to extractable hydrophilic products and remove the activated compound by way of conjugation reactions (Perera and Weinstein, 2000; Norppa, 2003).

The capital of Rio Grande do Sul state (population ≈ 11 million) is Porto Alegre, a city of about 3 million people of which the Brazilians of European descent are still mainly of Portuguese descent, although individuals of Italian, Spanish and German ancestry have also contributed to the gene pool. About 14% of the Porto Alegre population is constituted of Afro-Brazilians who are mainly descendants of slaves brought to Brazil between the 15th and 18th centuries, mainly from the West Coast of Africa but also from Angola and Mozambique (Bortolini *et al.*, 1997; IGBE, 2000). In Brazil skin color is often seen as equivalent to race, resulting in a complex and subjective evaluation of ethnicity. The Brazilian Institute of Geography and Statis-

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tics (Instituto Brasileiro de Geografia e Estatística, IBGE) classifies Brazilians into five ethnic groups according to the prevalence of the ethnic group in the general Brazilian population as 53% White, 38% Brown, 6% Black and 3% Yellow or Amerindian (IBGE, 2000). However, in Rio Grande do Sul the proportion is 87.5% White, 7% Brown, 5% Black and 0.2% Yellow or Amerindian (IBGE, 2000). According to Bortolini and Pena (2004) the expression of 'Afro-descendent' has recently been incorporated into the ethnic semantic concerning the extent of admixture in Brazil. These authors also point out that, independent of the criteria chose, it is still difficult to place people into distinct ethnic groups.

Gaspar *et al.*, (2002) were the first to investigate polymorphisms related to carcinogen metabolism in Afro-Brazilians from Porto Alegre, when they compared samples of Brazilians of African and of European descent from Porto Alegre in respect to *CYP1A1*2C* polymorphisms and found high *CYP1A1*2C* allele frequencies in both groups as compared to populations from other parts of the world, although the differences between the Porto Alegre groups were not statistically significant.

The aim of this study described in our current paper was to investigate the frequency of *CYP1A1*2A*, *GSTP1-Ile105Val*, *GSTM1* and *GSTT1* gene polymorphisms in an Afro-Brazilian sample from Porto Alegre to ascertain if these polymorphisms show ethnic differences compared to data (Gaspar *et al.*, 2004) for Brazilians of European descent from Porto Alegre. We also compared the frequencies detected in this study with some other Brazilian populations. The UFRGS ethics committee approved this study and all subjects signed an informed consent form to participate in this investigation.

The sample of Afro-Brazilians (n = 100; mean age = 38 years; 68% males) investigated consisted of patients from the Central Laboratory of a general Public Hospital (*Santa Casa de Misericórdia de Porto Alegre* to which they had been referred to for routine blood examinations and who had been classified as Afro-Brazilians based on skin color and ancestry.

Genomic DNA was isolated from whole blood by the salting out method and four polymorphic markers investigated by genotyping using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The *CYP1A1*2A* allele was genotyped using the procedure of Hayashi *et al.* (1991) and detected after digestion with *MspI*. The *GSTM1*, *GSTT1* and *GSTP1* genes were genotyped using a multiplex PCR method and a reaction mixture consisting of 100 ng of genomic DNA, 15 pmol of each primer, 10 mM Tris HCl, 4.5 mM $MgCl_2$, 50 mM KCl, 100 mM dNTPs and 1 unit of *Taq* DNA polymerase in a total volume of 50μL. The amplification protocol consisted of initial denaturation at 94 °C for 5 min, 6 touchdown cycles of 1 min at 94 °C followed by 2 min at 59 °C (decreasing to 54 °C at a rate of 1 °C per cycle) and 1 min at 72 °C, and 30 cycles at 94 °C for 1 min followed by 1 min at 55 °C and 1 min at 72 °C, plus a final extension of 5 min at 72 °C. An aliquot of the amplification product was subjected to horizontal agarose gel (3.5%, w/v) electrophoreses to verify the presence or absence of *GSTM1* and *GSTT1* fragments, the *GSTP1* product being used as a control for this reaction. Primer sequences were those reported by Harries *et al.* (1991), Bell *et al.* (1993) and Pemble *et al.* (1994). A second aliquot of the amplified *GSTP1* product was digested with *BsmaI* as described by Harris *et al.* (1991).

Allelic frequencies were estimated by gene counting. Comparisons among allelic and genotype frequencies were performed using the PEPI software program (Abramson, 2004). Agreement of genotypic frequencies with Hardy-Weinberg expectations was evaluated using the χ^2 test of Roff and Bentzen (1989) and Fisher's exact test (Beiguelman, 1988). Haplotypes estimates and pairwise linkage disequilibrium tests were performed using the PHASE program version 2.0 (Stephens *et al*., 2001; Li and Stephens, 2003).

The genotype distributions and allele frequencies detected in this study are shown in Table 1. Although there was no deviation from the expected Hardy-Weinberg frequencies there were significant differences in genotype distribution and allelic frequencies between Afro-Brazilians and Brazilians of European descent in respect of the frequency of the *CYP1A1*2A* (p = 0.003) and *GSTP1-Ile105Val* (p = 0.002) polymorphisms and of the *GSTM1*

Table 1 - The *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* genotype and allele frequencies (%) in Euro-Brazilians and African-Brazilians from Porto Alegre, Rio Grande do Sul, Brazil. Allele frequencies are shown in bold.

Allele (in bold) or genotype	Ethnicity	
	Afro-Brazilians (n = 100)	Euro-Brazilians[1] (n = 90)
*CYP1A1*2A*		
**1A/*1A*	47.0	71.1
**1A/*2A*	45.0	23.3
**2A/*2A*	8.0	5.6
CYP1A1*2A	**0.30**[2]	**0.166**[2]
GSTM1 null genotype	34.0[3]	50.0[3]
GSTT1 null genotype	28.0	21.1
GSTP1 genotypes		
Ile/Ile	29.0	52.2
Ile/Val	58.0	40.0
Val/Val	13.0	7.8
GSTP1*Val	**0.420**[4]	**0.278**[4]

[1]From Gaspar *et al*. (2004).
[2]Allelic and genotype frequencies differ (χ^2 test, p = 0.003).
[3]Genotype frequency differs (χ^2 test, p = 0.01).
[4]Allelic and genotype frequencies differ (χ^2 test, p = 0.002).

null genotype (p = 0.01) but there were no detectable difference between the two groups for the *GSTT1* null genotype. Some studies have pointed out ethnic differences in genotype frequencies of *CYP1A1* and *GST*s family polymorphisms and these polymorphisms may be responsible for differences in individual susceptibility to carcinogens (Perera and Weinstein, 2000, Norppa, 2003).

Polymorphisms of the *CYP1A1* gene have been detected in some population studies and the frequency and type of polymorphism have been associated with susceptibility to cancer and have shown ethnic correlations (Garte, 1998). The *CYP1A1*2A* homozygous variant genotype has been detected at higher frequencies in Asians (11-13%) and African-Americans (6-7%) but at lower frequencies in Northern Europeans and European-Americans (0-2%) (Crofts *et al*., 1993; Raunio *et al*., 1995). In our study, 8% of the Porto Alegre Afro-Brazilians studied showed the rare *CYP1A1*2A* homozygous genotype as compared to the 5.6% reported by Gaspar *et al*. (2004) for Brazilians of European descent, these ethnic differences being similar to those detected when Europeans, European-Americans and African-Americans populations were compared (Crofts *et al*., 1993; Raunio *et al*., 1995). Furthermore, in a breast cancer study Amorim *et al.* (2002) also described higher frequencies of the variant *CYP1A1*2A* genotype in a non-white ethnic sample than in an ethnically white sample from the Brazilian city of Rio de Janeiro.

The higher frequencies of *CYP1A1*2A* polymorphism detected in our two Porto Alegre groups compared to frequencies of the same polymorphism described for populations of African (6-7%, Crofts *et al*., 1993) and European (0-2%; Raunio *et al*., 1995) descent may have been due to the ethnic admixture described for Brazilian populations because our Porto Alegre Afro-Brazilian group showed 59% European admixture while our European-Brazilian group showed 7.8% African admixture based on the reports of various studies (Bortolini *et al*., 1997; Bortolini and Penna, 2004; Franco *et al*., 1982). The *CYP1A1*2C* polymorphism studied by Gaspar *et al*. (2002) also showed higher frequencies in Porto Alegre than that described for other populations.

The *GSTM1* and *GSTT1* null genotype frequencies for our Porto Alegre Afro-Brazilian sample were similar to those described for other Afro-Brazilian populations (Amorim *et al*., 2002; Rossini *et al.*, 2002; Gattás *et al*., 2004) which themselves are similar to the frequencies described for other (non-Brazilian) Afro-descend populations (Harries *et al*., 1991, Bailey *et al*., 1998). These studies have also detected significant differences in the frequency of *GSTM1* polymorphisms among Afro-Brazilians and Brazilians of European descent (Amorim *et al*., 2002; Rossini *et al.*, 2002; Gattás *et al*., 2004).

We also analyzed the frequency of *CYP1A1*2B* haplotypes (*i.e.* haplotypes involving combinations of the **2A/*2C* polymorphism. See http:// www.imm.ki.se/CYPalleles/cyp1a1.htm for *CYP1A1* allele nomenclature) in Afro-Brazilians and Brazilians of European descent and found linkage disequilibrium (D) to be 0.85 for Brazilians of European descent but zero (*i.e.* no linkage) the Afro-Brazilian group.

The *GSTP1-Ile105Val* polymorphism frequency described in this article is similar to those described for other populations by Harris *et al*. (1991). In our study we also detected ethnic differences in genotype distribution and allelic frequencies between Afro-Brazilians and Brazilians of European descent in respect of this polymorphism. However, Rossini *et al*., (2002) studied Rio de Janeiro samples and detected the GSTP1*Val/Val* homozygous genotype more frequently in ethnically white individuals than in non-white individuals, which is at variance with our results and merits further investigation.

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